JOURNAL OF AGRICULTURAL SCIENCES

TARIM BİLİMLERİ DERGİSİ

ANKARA UNIVERSITY FACULTY OF AGRICULTURE

e-ISSN 2148-9297

JAS

Year 22

Volume 28

Issue 04

Ankara University
Faculty of Agriculture

JOURNAL OF AGRICULTURAL SCIENCES

TARIM BILIMLERI DERGISI

e-ISSN: 2148-9297

Ankara - TURKEY

Year **2022** Volume **28** Issue **4**

JOURNAL OF AGRICULTURAL SCIENCES
TARIM BİLİMLERİ DERGİSİ
ANKARA UNIVERSITY FACULTY OF AGRICULTURE
e-ISSN 2148-9297

Product Information

Publisher	Ankara University, Faculty of Agriculture
Owner (On Behalf of Faculty)	Prof. Dr. Hasan Huseyin ATAR
Editor-in-Chief	Prof. Dr. Halit APAYDIN
In Charge of Publication Unit	Agricultural Engineer Asim GOKKAYA
Journal Administrator	Salih OZAYDIN
Library Coordinator	Dr. Can BESIMOGLU
IT Coordinator	Lecturer Murat KOSECAVUS
Graphic Design	Ismet KARAASLAN
Date of Online Publication	15.10.2022
Frequency	Published four times a year
Type of Publication	Double-blind peer-reviewed, widely distributed periodical
Aims and Scope	JAS publishes high quality original research articles that contain innovation or emerging technology in all fields of agricultural sciences for the development of agriculture.
Indexed and Abstracted in	Clarivate Science Citation Index Expanded (SCIE) Elsevier-Scopus TUBITAK-ULAKBIM-TRDizin CAB International FAO-AGRIS SOBIAD OpenAire
Management Address	Journal of Agricultural Sciences Tarım Bilimleri Dergisi Ankara University Faculty of Agriculture Publication Department 06110 Diskapi/Ankara-TURKEY Telephone : +90 312 596 14 24 Fax : +90 312 317 67 24 E-mail: tbdeditor@ankara.edu.tr http://jas.ankara.edu.tr/

JOURNAL OF AGRICULTURAL SCIENCES
TARIM BİLİMLERİ DERGİSİ
ANKARA UNIVERSITY FACULTY OF AGRICULTURE
e-ISSN 2148-9297

JOURNAL OF AGRICULTURAL SCIENCES
TARIM BİLİMLERİ DERGİSİ
ANKARA UNIVERSITY FACULTY OF AGRICULTURE
e-ISSN 2148-9297

CONTENTS **2022, 28(4)**

Research articles:

Effects of Melatonin on *Morus nigra* cv. 'Eksi Kara' Exposed to Drought Stress **555**
Duygu ÖZELÇİ, Gülçin BEKER AKBULUT, Emel YİĞİT

Pelleting with Puperabsorbent, Chitosan, and Phosphorus Fertilizer as a New Method to Improve Growth, Yield, and Physiological Attributes of Potato mini-tuber **570**
Sevda GHASEMI GERMI, Morteza BARMAKI, Salim FARZANEH, Mandana AMIRI

Genoprotective Role of Purslane Methanol Extract Against Somatic Mutations Induced by Bifenthrin, a third Generation Prethyroid Insecticide **583**
Halit KIZILET, Handan UYSAL

Effects of Sequential Hydrogen Peroxide Applications on Salt Stress Tolerance in Bread Wheat Varieties **592**
Elif Saadet ARICAN, Sefer DEMIRBAS

Effects of Foliar Applications on Nutrient Concentrations of Kernel, Pomological Properties and Yield of 'Chandler' Walnut Variety at Different Altitudes **603**
Nihal ACARSOY BILGIN

Investigation of The Roles of Hydrogen Peroxide and NADPH Oxidase in The Regulation of Polyamine Metabolism in Maize Plants under Drought Stress Conditions **613**
Mehmet DEMİRALAY, Aykut SAĞLAM, Fuat YETİŞSİN, Asım KADIOĞLU

Microsatellite Diversity and Restriction Enzyme-based Polymorphisms of MHC Loci in Some Native Turkish Goats **626**
Mehmet ASLAN, Eymen DEMİR, Taki KARSLI

A Novel Machine Learning Approach: Soil Temperature Ordinal Classification (STOC) **635**
Cansel KUCUK, Derya BIRANT, Pelin YILDIRIM TASER

Influence of 24-Epibrassinolide on Physiological Characteristics of Tomato Seedlings Infested with Root-knot Nematode *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) **650**
Çiğdem GÖZEL, Zeliha GÖKBAYRAK

Determination of Pneumatic Conveying Characteristics of Canola Seeds **656**
Refi Ratip ÖZLÜ, Metin GÜNER

Exploitable Potential of Biomass Energy in Electrical Energy Production in the Mediterranean Region of Turkey **666**
Mehmet Emin BİLGİLİ

Risk Analysis Using Geographic Information Systems by Determining the Factors Affecting Yield in Plant Production: A case study from Ankara, Turkey **677**
Emre YENIAY, Aydın ŞIK

Effects of Moisture Contents and Storage Temperatures on the Physical, Chemical and Microbiological Qualities of Non-Sulfitted Dried Apricots **691**
Nihal GÜZEL, Şeref TAĞI, Mehmet ÖZKAN

Investigation of Flight Activity and Damage Status of European Grapevine Moth *Lobesia botrana* (Denis & Schiffermuller) (Lepidoptera: Tortricidae) **704**
Ayşe ÖZDEM, Arzu AYDAR, Yasemin SABAHOĞLU

Determination of Germination Threshold Value of Chickpea Varieties with GGE Biplot Method Under Different Irrigation Water Salinity Conditions **711**
Ismail TAS, Tulay TUTENOCAKLI, Yalcin COSKUN, Mevlut AKCURA

Secondary Metabolite Changes in Tedara (*Bituminaria bituminosa* L.) Genotypes in Different Growing Period **723**
Zeki ACAR, Erdem GÜLÜMSER, Sema LEBLEBİCİ, İlknur AYAN, Cihan DARCAN

Effects of Row Spacing and Sowing Rate on Quality Performance of Alfalfa (Medicago sativa L.) Under Tokat Ecological Conditions **732**
Mahir OZKURT, Yaşar KARADAG

Transplanting and Seed Hydro-priming Affects Yield, Water Use Efficiency, and Grain Quality of Maize Cultivars under Delayed Planting **740**
Amir GHALKHANI, Farzad PAKNEJAD, Ali MAHROKH, Mohammad Reza ARDAKANI, Farid GOLZARDI

Association of Satellite RNA with *Grapevine fanleaf virus* in its Geographical Origin and Sequence Characteristics **751**
Afsaneh DELPASAND KHABBAZI, Nemat SOKHANDAN BASHIR, Shaheen NOURINEJHAD ZARGHANI

Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

Effects of Melatonin on *Morus nigra* cv. 'Eksi Kara' Exposed to Drought Stress

Duygu ÖZELÇİ[a], Gülçin BEKER AKBULUT[b*], Emel YİĞİT[a]

[a] *Inonu University, Science and Art Faculty, Department of Biology, Malatya, TURKEY*

[b] *Malatya Turgut Ozal University, Battalgazi Vocational School, Department of Park and Garden Plants, Malatya, TURKEY*

ARTICLE INFO

Research Article

Corresponding Author: Gülçin BEKER AKBULUT, E-mail: gulcin.akbulut@ozal.edu.tr

Received: 17 June 2021 / Revised: 18 October 2021 / Accepted: 18 October 2021 / Online: 15 October 2022

Cite this article

ÖZELÇİ D, AKBULUT BEKER G, YİĞİT E (2022). Effects of Melatonin on *Morus nigra* cv. 'Eksi Kara' Exposed to Drought Stress. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 28(4):555-569. DOI: 10.15832/ankutbd.953558

ABSTRACT

Today, drought stress threatens the world seriously. Determining the effects of some exogenous stimulators in acquiring resistance against stress will contribute to agriculture under drought stress. In this regard, we investigated the effects of melatonin (MEL) on *Morus nigra* cv. 'Eksi Kara' (black mulberry) in challenging drought. To reach this object, we reproduced 'Eksi Kara', which is registered in Turkey and has economic importance, in tissue culture by using the meristem culture method. Plants were then transferred in a medium containing polyethylene glycol (PEG) 8000, which causes -1.5 MPa drought stress, and 20 µl MEL has applied. Leaf samples were taken on the 5th, 10th, and 15th days after treatments in groups of plants grown in a different medium (Control, Control+MEL, PEG and PEG+MEL). The changes in the pigment system, relative water content (RWC) and antioxidant system were evaluated comparatively between the groups to assess plants' growth and determine their roles in coping with stress. Our findings showed that RWC decreased in leaves under drought. Exogenous MEL added in MS medium had a mitigation effect on stress. The reduction was detected in the chlorophyll and carotenoid content of leaves. Moreover, MEL+PEG combination improved the chlorophyll level. It was seen that exogenous MEL application promoted the plant defence mechanism of *M. nigra* plants, which exposed to drought stress, by increasing the accumulation of non-enzymatic antioxidants; total glutathione (GSH), total phenolic, proline) and activities of antioxidant enzymes; catalase (CAT), superoxide dismutase (SOD), Glutathione-S-transferase (GST), glutathione reductase (GR), peroxidase (POD), ascorbate peroxidase (APX). This study also indicates that the application of MEL+PEG composition partially prevented membrane lipid peroxidation by decreasing (malondialdehyde) MDA content.

Keywords: Black mulberry, Malondialdehyde, Pigment, Proline, Relative water Content, Total phenolic

1. Introduction

Abiotic stress is the primary cause of yield loss worldwide, and it corresponds to more than 50% of yield loss in high-yield cultivated plants (Wang et al. 2004). Drought is considered one of the significant abiotic stresses, and it alters the average growth balance in plants negatively. It also damages plants' growth and productivity by causing a series of morphological, physiological, biochemical, and molecular changes (Pandey et al. 2017). Researches on obtaining drought-resistant plant varieties are critical in challenging drought.

Morus nigra L. (black mulberry) is in Urticales order Moraceae family and *Morus* genus. *Morus* is widely cultivated, particularly in East, West, Southeast Asia, Southern Europe, the south of North America, the northwest of South America, and some parts of Africa (Datta 2002). Mulberry can be grown in temperate and subtropical climates due to its high ability to adapt to different climatic and soil conditions. Therefore, it has been reported that mulberry will have significant potential bio-energy plants in a rapidly changing global climate under drought conditions (Sekhar et al. 2017).

Mulberry in Turkey is cultivated as a closure and mixed garden or border plant. The fact that urbanization damages the genetic diversity of the mulberry made the protection of varieties critical (Vijayan et al. 2011). Narrowing of agricultural lands and the inverse effect of stress on environmental conditions are reasons for decreased productivity. Thus, it is very substantial to increase productivity in vegetative production, which is achieved by breeding or selecting resistant plants to stress conditions (Arici & Eraslan 2012). Sugar, organic acids, minerals, anthocyanins, and vitamins included in black mulberry make it an essential source of nourishment. Most of the daily calcium, iron, B, and C vitamin requirements can be met by eating black mulberry (Hepsag et al. 2012; Wang et al. 2021). It has also been used in traditional herbal medicine for both animals and humans. It exhibited anti-inflammatory, antimicrobial, anti-diabetic, anti-obesity, and anticancer properties (Lim & Choi 2019;

Erden 2021; Mehta & Kumar 2021). In this regard, it is essential to determine the drought resistance of *M. nigra*, which has medical and economic importance.

Avoidance and tolerance are two primary defence mechanisms that allow the plant to survive under drought stress (Zheng et al. 2017a). Drought tolerance is the ability to keep the plant tissue's physiological and metabolic activities at lower levels when the water potential within the plant decreases (Blum & Ebercon 1981). Plants under drought stress have an effective defence system that uses enzymatic antioxidant systems consisting of antioxidants such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase (GR) and Glutathione-S-transferase (GST) as well as a non-enzymatic antioxidant system including some secondary metabolites such as ascorbic acid, GSH, flavonoids, and total phenols to mitigate the deleterious effects of reactive oxygen species (ROS) (Cheng et al. 2018).

Melatonin (MEL) has long been considered an important antioxidant or hormone in animals (Reiter 1991). Recently, it has been proven that endogenous MEL is widely found in bacteria, fungi, animals, and plants (Lin et al. 2019; Qiao et al. 2019). Also, MEL is recognized as a potential growth stimulator in plants (Arnao & Hernández-Ruiz 2019; 2020). MEL acts as the primary regulator by increasing tolerance to biotic and abiotic stresses such as salinity, drought, extreme temperatures, high light intensity, herbicides, ultraviolet radiation (Zhang et al. 2012; Wang et al. 2017; Jahan et al. 2019; Ahammed et al. 2020a; Moustafa-Farag et al. 2020). A compound's qualification as a plant hormone It must be formed within the plant, transported from where it is formed to another location, and be able to manage or regulate various life events in the location where it is transported. It must be able to show these effects even at very low concentrations (Kaynak & Ersoy 1997). MEL can dissolve both in water and lipids and easily reach all intracellular components (Posmyk & Janas 2009). It can effectively protect the cell membrane, organelles, and nucleus from free radicals' devastating effect. Poeggeler et al. (2002) reported that MEL is also a more potent antioxidant than vitamins C, E, and K. According to our literature exploration on the effects of MEL on the antioxidant system, we could not find any study of applications to plants in the form of trees. However, there were many kinds of research conducted with herbaceous plants.

In this study, *M. nigra* cv. 'Eksi Kara', which has economic and medical importance, was reproduced in tissue culture, and groups were formed, in which MEL and drought stress (-1.5 MPa) was applied, *in vitro*. Also, pigment content, RWC, GSH, total phenol, proline content, and antioxidant enzyme activities (CAT, SOD, GST, GR, POD, and APX) were evaluated comparatively in both groups.

2. Material and Methods

2.1. Plant growth in tissue culture

The *M. nigra* cv. 'Eksi Kara' explants used in the research were obtained from the National Mulberry Gene Resources parcel located in in the Republic of Turkey, Ministry of Agriculture and Forestry, Malatya Apricot Research Institute. 'Eksi Kara' is the only *M. nigra* variety registered in the Seed Registration and Certification Center, and its fruits are economically and pharmacologically important. Explants were taken from nodal buds on one-year shoots in June, July, and August in 2017-2019. Explants were reproduced in MS medium (Murashige & Skoog 1962, M0222 Duchefa) after surface sterilization. In the first phase of culture, 0.75 mg L^{-1} benzyl amino purine (BAP) was used, then 1 mg.L^{-1} BAP for shoot propagation, and 1.5 mg.L^{-1} indole butyric acid for rooting.

2.2. Drought and melatonin application

Plants were grown over a period of 5 months prior to application. Following applications were made to the plants rooted *in vitro* tissue culture: **Control:** Liquid (agar-free) MS medium was used. **Control+MEL:** The plants rooted under *in vitro* conditions were transferred to a liquid MS medium containing 20 µM MEL. The MEL dose was chosen as a result of preliminary evaluations based on reference studies (Korkmaz et al. 2016; Han et al. 2017; Zheng et al. 2017b). **PEG group:** Plants were transferred to a liquid MS medium containing PEG 8000, a polymer that retains water, to provide the drought condition. To reach -1.5 MPa drought, 355 g.L^{-1} PEG 8000 was added to the liquid MS medium according to the formula developed by Michel (1983). **PEG+MEL group:** Plants were transferred to liquid MS medium containing 20 µM MEL and 355 g L^{-1} PEG 8000.

$$PEG = \frac{4 - \sqrt{(5.16\,\Psi\,T - 560\,\Psi + 16)}}{(2.58\;T - 280)} \times 1000$$

Ψ= -1.5 MPa T= 25 ºC

2.3. Samples for analysis

The leaves to be used in the analysis were taken on the 5th, 10th, and 15th days after the applications. Samples were frozen in liquid nitrogen and preserved at -40 °C to be used for analysis. They were studied as soon as possible to eliminate the risk of loss of enzymatic activity. Fresh leaves were used without freezing to evaluate the relative water content.

2.4. Determination of relative water content

Fresh weights (FW) of the leaf samples were determined. Then, these leaves were kept in distilled water for 4 hours, and their turgor weights (TW) were determined. After drying for 48 hours in a 70 °C drying oven, the dry weights (DW) of samples were weighed. (Sanchez et al. 2004; Demiral & Turkan 2005).

2.5. Determination of total chlorophyll and carotenoid content

The method of De Kok & Graham (1980) was used for pigment content. In the first step, 1 g sample taken from the leaf was homogenized in 50 mL acetone (100% Merck) and kept at +4 °C for 24 hours. Homogenates were then centrifuged, and their absorbance values were calculated according to Lichtenthaler & Welburn (1983) with spectrophotometric reading (Biochrom Libra S22) at 662, 645, 470 nm.

2.6. Extraction for enzyme analysis

To analyze enzyme, 0.5 g leaves were homogenized with 2.5 mL 0.1 M, pH 7.5, Tris-HCl buffer, 2.5 mL 0.1mM EDTA and 0.5 mL 1% PVP. The homogenate was centrifuged at +4 °C and 18.000 rpm for 30 minutes. Then, the supernatant was stored at -40 °C until analysis (Andrews et al. 2005). Enzyme values were calculated in terms of specific activity by dividing by total protein.

2.7. CAT activity

The method of Luck (1963) was used to determine CAT enzyme activity. CAT enzyme activity was defined as the absorbance change obtained in 1 minute by reading at 240 nm in the spectrophotometer (molar extinction coefficient for H_2O_2 is 0.0396 $cm^2.\mu mol^{-1}$).

2.8. SOD activity

SOD enzyme activity determination was made using the method of McCord & Fridovich (1969). The SOD activity was measured spectrophotometrically at 550 nm.

2.9. GST activity

The method of Habig et al. (1974) used to assign GST enzyme activity. Enzyme activity was determined as 344 nm in the spectrophotometer (the extinction coefficient of CDNB is 9.6 $mM^{-1}.cm^{-1}$).

2.10. GR activity

Determination of GR activity was made according to Carlberg & Mannervik (1985) method. Enzyme activity was measured in the spectrophotometer at 340 nm wavelength. NADPH was calculated with an extinction coefficient of 6.2 $mM^{-1}.cm^{-1}$.

2.11. GSH content

Assignation of total glutathione content was made according to the method of Akerboom & Sies (1981). One-minute absorbance change was determined at 412 nm.

2.12. POD activity

The method of Peters et al. (1989) and MacAdam et al. (1992) was performed for POD activity determined with modification. The change in enzyme activity in the first minute was measured at the wavelength of 436 nm (Guaicol's extinction coefficient is 26.6 $mM^{-1}.cm^{-1}$).

2.13. APX activity

The methods of Nakano & Asada (1981) and Cakmak (1994) were used for APX activity determination. Enzyme activity was determined at 290 nm in the spectrophotometer, and APX activity was calculated with an extinction coefficient of 2.8 $mM^{-1}.cm^{-1}$.

2.14. Determination of MDA content

MDA analysis was made according to the method of Heath & Packer (1968). The absorbance of the supernatant was measured at 532 and 600 nm. The content of MDA was calculated with an extinction coefficient of 155 $mM^{-1}.cm^{-1}$.

2.15. Determination of total phenolic

Slinkard & Singleton's (1977) and Chandler & Dodds' (1983) method used to determine the total phenolic determination. It was measured at 760 nm in the spectrophotometer, and a standard curve was prepared with the gallic acid solution.

2.16. Determination of proline content

Proline determination was made spectrophotometrically by the acid-ninhydrin method according to Bates et al. (1973). The absorbance was measured at a wavelength of 520 nm. The same method was repeated using proline, and a standard graphic was created.

2.17. Total protein content

Total protein content was determined using the method of Bradford (1976). 50 µL of homogenate prepared from leaves was added onto 1000 µL Bradford reagent. It is measured in a spectrophotometer at a wavelength of 595 nm.

2.18. Statistical analysis

SPSS (Statistical Program in Social Sciences) V.25 software was used to evaluate this study's data. The data's compliance with normal distribution was checked with the Shapiro – Wilk test, and the homogeneity of variances was controlled with the LEVENE test at the 5% threshold. Duncan's multiple range test (Duncan 1955) was performed for paired comparisons of groups, and variance analysis (ANOVA) was made. Differences between the means were showed with different letters (P>0.05). Statistically same groups were showed with the same letters (P>0.05). Lower case letters show the comparison between averages in days (within groups), upper case letters show the comparison between groups. Each stage (5th, 10th, and 15th days) was compared within itself with Duncan's multiple range test.

3. Results

3.1. Relative water content

It was found that RWC decreased in the application of PEG to *M. nigra* leaves compared to the Control (P<0.05). The highest RWC was 64.11% in the Control+MEL group, while the lowest RWC was 21.11% in the PEG group. Moreover, there was a substantial decrease in RWC in different PEG group days (P<0.05) (Figure 1).

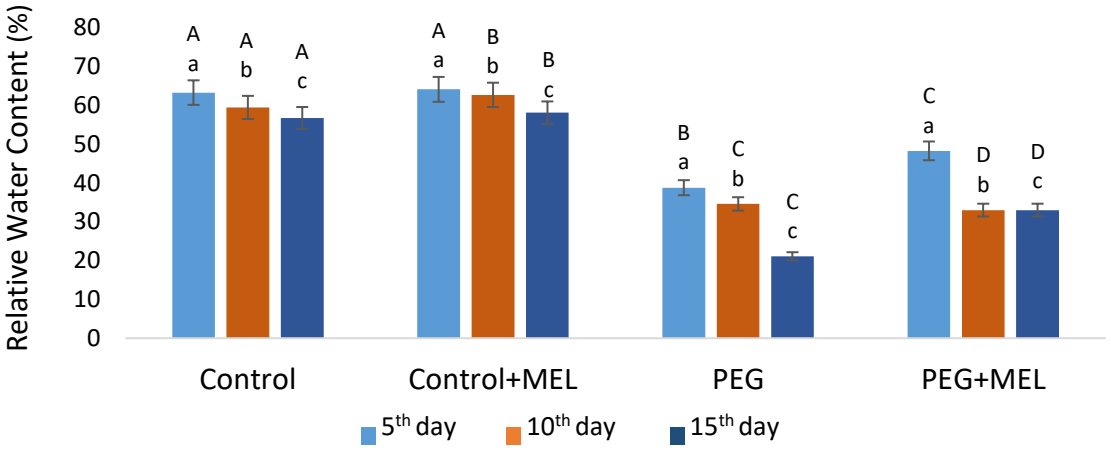


Figure 1- Relative water content changes in *M. nigra* leaves depending on groups and days

3.2. Total chlorophyll and carotenoid content

The total chlorophyll content was lower in all treatment groups than in the Control (P<0.05). The highest total chlorophyll content was 11.43 µg.g^{-1} in the control group, while the lowest was 4.54 µg.g^{-1} in the PEG group. Higher total chlorophyll content was observed in the PEG+MEL group compared to the PEG group. These differences were found to be statistically significant (P<0.05) (Figure 2). The carotenoid content was lower in all treatment groups than in the control group (P<0.05). The highest carotenoid content was observed as 2.57 µg.g^{-1} in the Control+MEL group, while the lowest was 1.46 µg.g^{-1} in PEG+MEL group. PEG+MEL group were determined lower than the PEG group in terms of carotenoid content (P<0.05) (Figure 3).

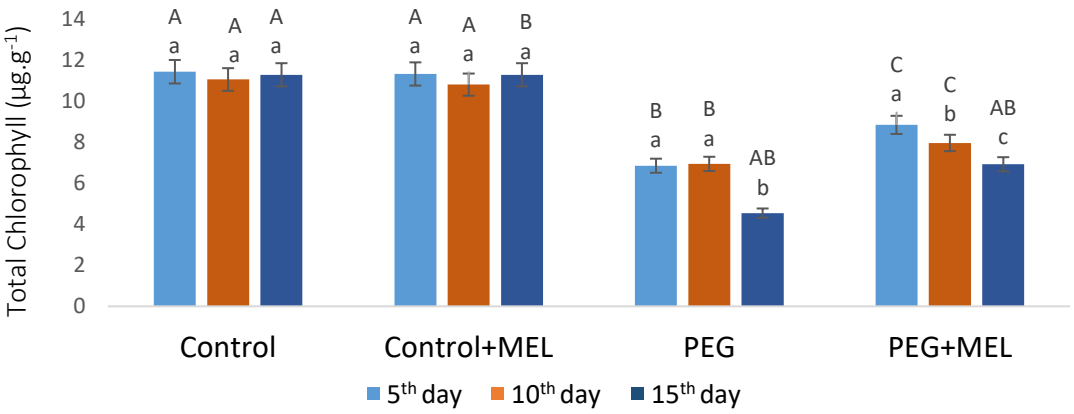


Figure 2- Variation of total chlorophyll content of M. nigra leaves depending on groups and days

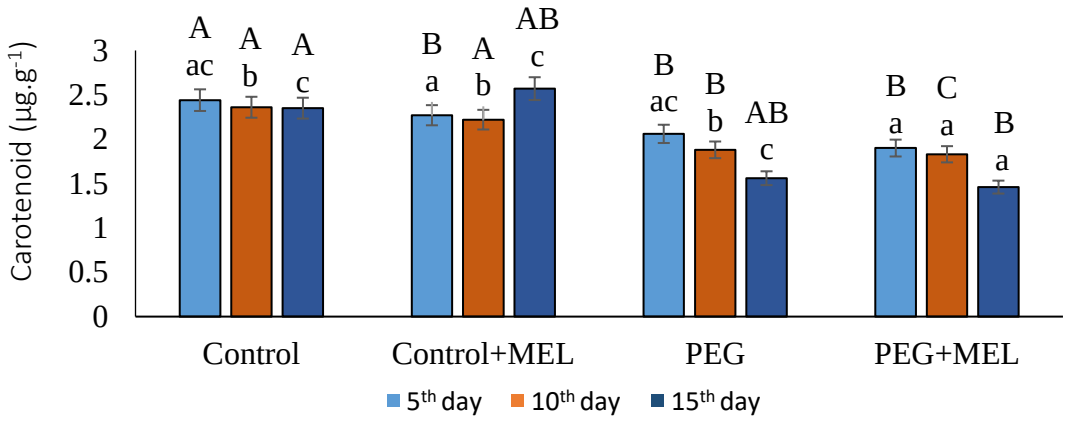


Figure 3- Variation of carotenoid content of M. nigra leaves depending on groups and days

3.3. CAT activity

CAT activity increased in all groups treated than the Control group (P<0.05). The highest enhancement in PEG+MEL group was found by 40% on the 10th day and 57% on the 15th day than the PEG group (P<0.05). On the other hand, CAT activity improved by 19% on the 5th day and 39% on the 10th day in Control+MEL group compared to the Control group (P<0.05) (Figure 4).

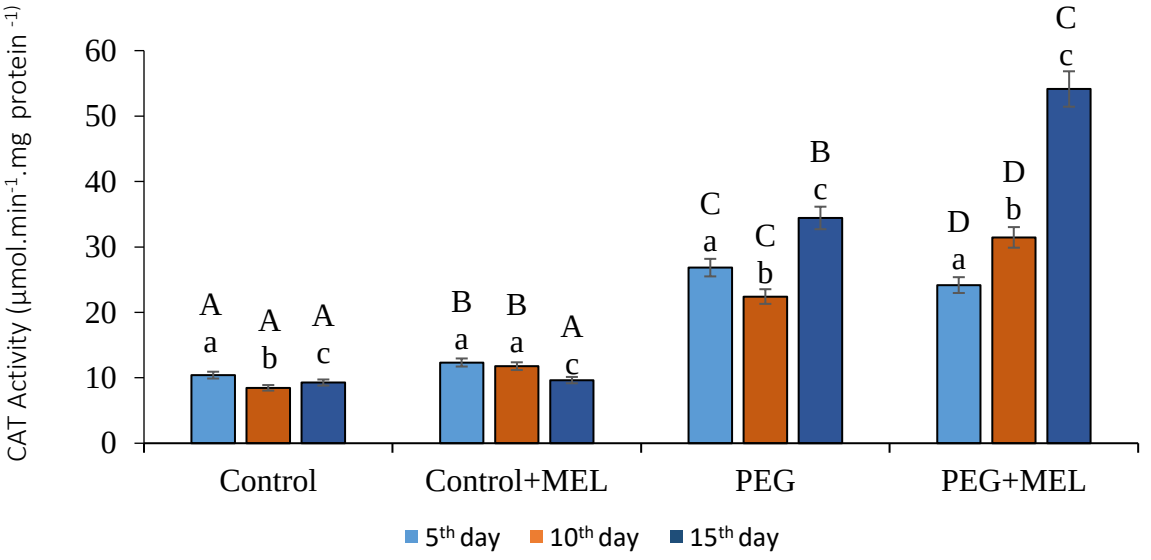


Figure 4- Variation of CAT activity in *M. nigra* leaves depending on groups and days

3.4. SOD activity

We determined improvement in SOD activity in all treatments, excluding the Control group (P<0.05). The highest SOD activity was found in PEG+MEL group as 97.06 µmol.min^{-1}.mg protein^{-1}. We also observed that SOD activity increased 100% on the 10th day and 92% on the 15th day in the PEG+MEL group compared to the PEG group (P<0.05). Also, MEL supplementation to the Control group enhanced SOD activity by 52% on the 10th day and 384% on the 15th day (P<0.05) (Figure 5).

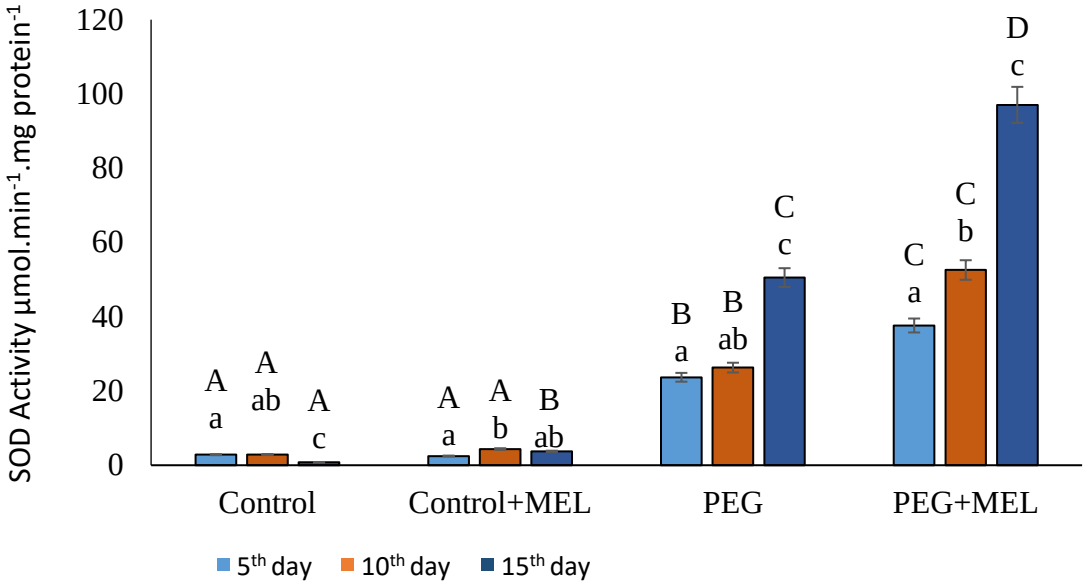


Figure 5- Variation of SOD activity in M. nigra leaves depending on groups and days

3.5. GST activity

It was determined that the GST activity increased in all the treatment groups compared to the Control (P<0.05). The highest GST activity was 66.57 µmol.min^{-1}.mg protein^{-1} in PEG+MEL group, and increasing by 125% was found compared to the 15th day of the PEG group (Figure 6).

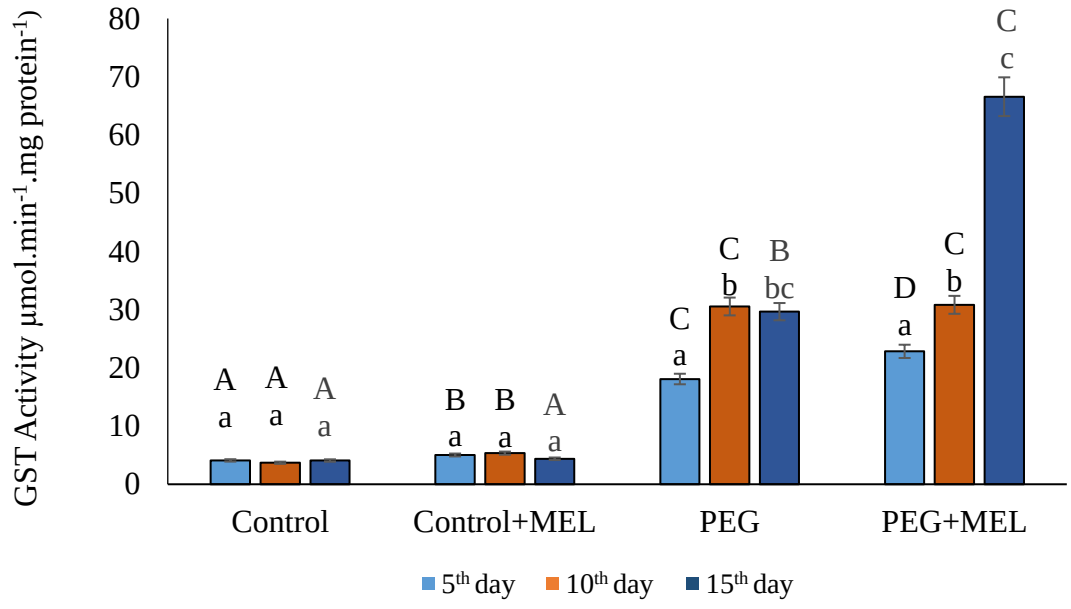


Figure 6- Variation of GST activity in M. nigra leaves depending on groups and days

3.6. GR activity

GR activity increased in all treatment groups compared to the Control ($P<0.05$). The highest GR activity was found in PEG+MEL group as 124.48 µmol.min^{-1}.mg protein^{-1}. Compared to the PEG group, GR activity in PEG+MEL group decreased 12% on the 5th day, while increased 28% on the 10th day and 55% on the 15th day ($P<0.05$) (Figure 7).

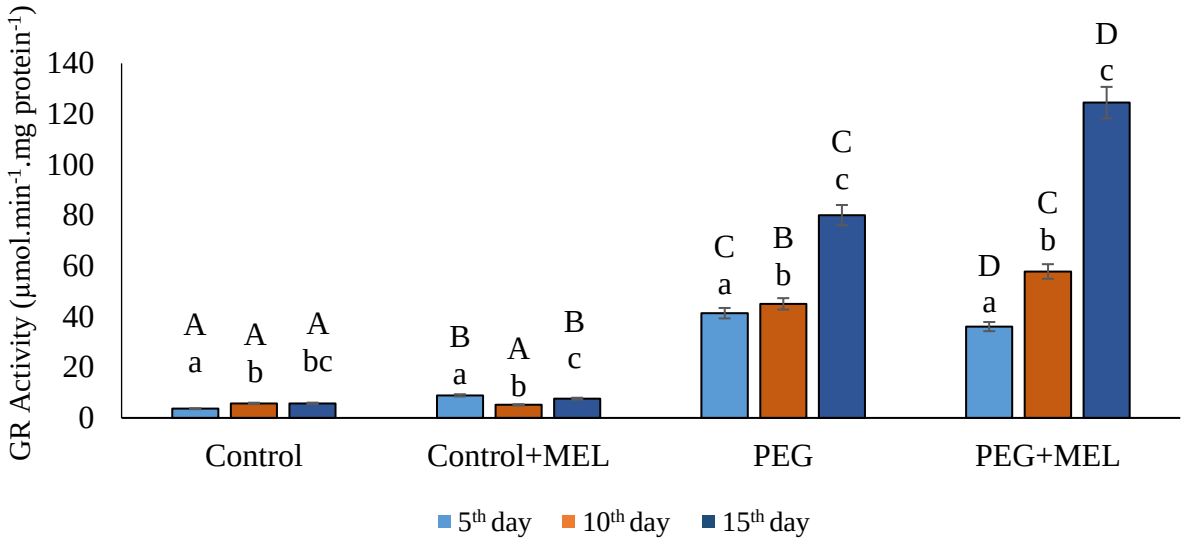


Figure 7- Variation of GR activity in *M. nigra* leaves depending on groups and days

3.7. GSH content

It was seen that treatment groups, excluding Control, positively impacted GSH content ($P<0.05$). The highest GSH content detected in the PEG+MEL group was 2.34 µmol.min^{-1}.mg protein^{-1}. It was found that the PEG+MEL group improved, compared to the PEG group, 38% on the 10th day and 93% on the 15th day ($P<0.05$) (Figure 8).

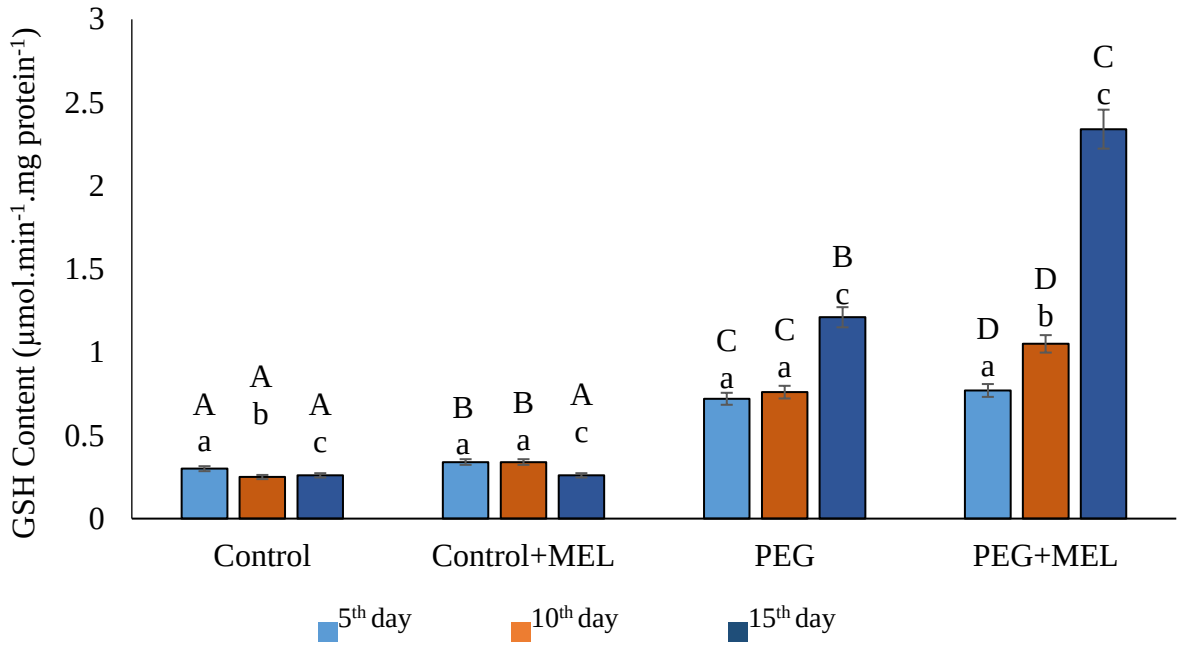


Figure 8- Variation of GSH content in *M. nigra* leaves depending on groups and days

3.8. POD activity

Our findings showed that PEG application increased POD activity in *M. nigra* leaves compared Control group on all days ($P<0.05$). The highest POD activity was observed in the PEG+MEL group with 3.92 µmol.min^{-1}.mg protein^{-1}. The PEG+MEL group enhanced POD activity by 48% on the 5th and 10th days and by 92% on the 15th day when compared to the PEG group ($P<0.05$) (Figure 9).

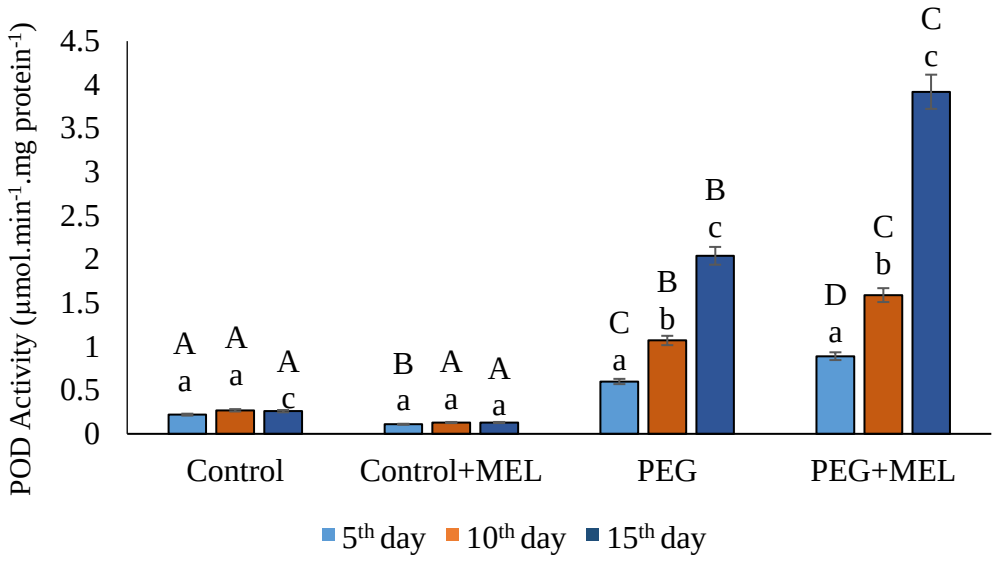


Figure 9- Variation of POD activity in *M. nigra* leaves depending on groups and days

3.9. APX activity

It was seen that APX activity in *M. nigra* leaves increased along with PEG treatment compared to the Control group on all days ($P<0.05$). The highest APX activity was found in the PEG+MEL group with 317.59 µmol.min^{-1}.mg protein^{-1}. In this context, it was observed that improvement was 84% on the 10th and 15th days in PEG+MEL group compared to the PEG group ($P<0.05$) (Figure 10).

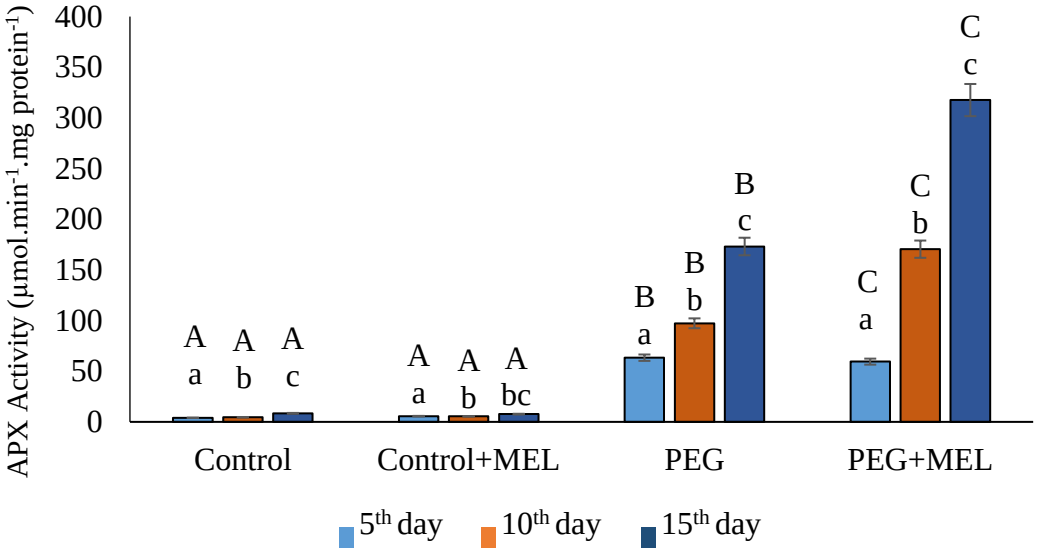


Figure 10- Variation of APX activity in *M. nigra* leaves depending on groups and days

3.10. MDA content

When compared with the Control group, it was determined that the MDA content of *M. nigra* leaves increased on all days with PEG application (P<0.05). On the other hand, the highest MDA content was examined as 27.75 µmol MDA.g^{-1} FW in the PEG group. (P>0.05) (Figure 11).

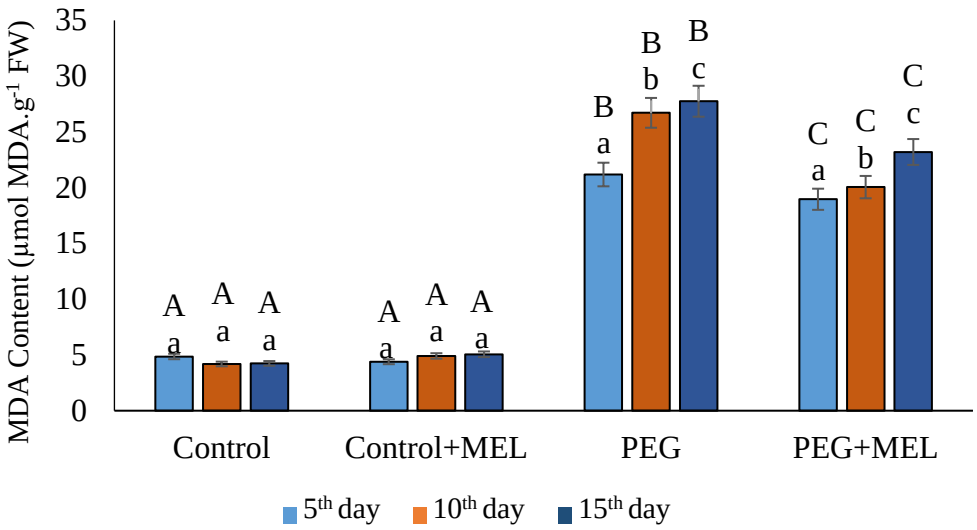


Figure 11- Variation of MDA content in M. nigra leaves depending on groups and days

3.11. Total phenolic content

The highest total phenolic content was found as 13.5 µg.g $^{-1}$ FW in the PEG+MEL group. On the other hand, it was observed that PEG+MEL group enhanced total phenolic content by 17% on the 5th day, 12% on the 10th day, and 53% on the 15th day compared with the PEG group (P<0.05). Analysis results indicate that there was no difference between the Control+MEL and Control group in terms of total phenolic content (P>0.05) (Figure 12).

3.12. Proline content

Our findings indicate that proline content was higher in all treatment groups than in the Control group (P<0.05). The highest proline content was found as 115.11 µg.g^{-1} FW in PEG+MEL group. Exogenous MEL application to the PEG group improved proline content by 32% on the 5th day, 20% on the 10th day, and 17% on the 15th day (P<0.05) (Figure 13).

4. Discussion

Drought has become the most critical problem limiting agricultural production. Moreover, it ranks first according to evaluating the adverse effects of natural disasters in the world (Marchin et al. 2020). In this context, to find solutions to yield losses caused by drought, understanding plants' mechanisms that could adapt to deficient water conditions is crucial. Studies on different plant species have shown that PEG can successfully provide drought stress conditions to plants (Zhu et al. 2005; Rouhi et al. 2006; Caruso et al. 2008; Chen et al. 2010; Ipek 2015). Also, PEG has a substantial impact on plants' water uptake by affecting its environment's osmotic potential. Hence, the intensity of use of PEG causes different levels of drought stress.

The soil's water potential is, on average, -1.5 MPa at the permanent wilting point (Kocacaliskan 2008). Leaf water content reflects the ability of the plant leaves to maintain water balance. On the other hand, RWC in plant leaves is reduced as soil moisture decreases (Marshall et al. 2000; Chen et al. 2012). It has been reported by Korkmaz et al. (2016) that chilling injury stress caused a diminishment in RWC in the leaves of pepper seedlings while MEL treatment enhanced it. In this study, although RWC was observed to be lower in the PEG group compared to the Control group, PEG+MEL composition improved RWC. Also, MEL was potent in protecting RWC and water potential in *M. nigra* exposed to drought (Figure 1).

According to Santos (2004), decreasing chlorophyll biosynthesis or increase in chlorophyll fragmentation is the cause of degradation in chlorophyll content in plants. On the other hand, Fracheboud et al. (2004) stated that drought stress might reduce chlorophyll and carotenoid content. They also reported that it is closely related to the carbon exchange ratio. Drought prevents photosynthesis by causing inconsistency in the light-harvesting complex, which triggers oxidative stress (Smirnoff 1993). Our

findings showed that PEG treatment in *M. nigra* negatively affected the pigment system. The enhancement of the total chlorophyll content in the PEG+MEL group indicates that MEL positively affects the pigment system (Figure 2).

Carotenoids are a photoprotective agent used to mitigate the deleterious effects of light and oxygen. They protect all photosynthetic organisms against light damage by transforming the light into heat or detoxifying ROTs, which leads to a reduction in lipid peroxidation (Collins 2001). Sharma et al. (2020) determined that MEL's application to *Carya cathayensis* (Chinese hickory) plants under drought stress conditions regulates metabolic pathways such as phenylpropanoid, chlorophyll, and carotenoid biosynthesis, and carbon fixation. Our findings regarding carotenoid content indicate that the decrease in PEG and PEG+MEL groups' carotenoid content may be due to the consumption of carotenoids in the plant during challenges with drought stress (Figure 3).

Free radical-induced peroxidation of lipid membranes is both the reflection and measurement of stress-induced damage at the cellular level (Jain et al. 2001). MDA is the end-product of membrane lipid peroxidation, and its accumulation demonstrates that plant cells have high levels of lipid peroxides (Zhao & Tan 2005; Upadhyaya et al. 2007; Liu et al. 2014; Ju et al. 2018). On the other hand, plant resistance can be assessed by measuring the MDA content (Gao et al. 2020). According to our findings, the MDA content was the highest in the PEG group's drought stress compared to the Control group. It was also observed that MEL+PEG group, interestingly, had lower MDA content compared to the PEG group. In this context, it is thought that MEL prevented membrane lipid peroxidation, while drought stress increased MDA content as well as the simultaneous application of MEL and PEG decreased MDA content (Figure 11). According to some studies, the MDA content decreased in groups that applied melatonin under stress (Li et al. 2019; Imran et al. 2021). The obtained findings indicate that MEL might alleviate abiotic stress-triggered ROS accumulation and cell membrane damage.

CAT is one of the antioxidant enzymes that affect cell life as it contributes to coping with ROS, which forms in oxidative stress (Volkert et al. 1994). In the study of Huihui et al. (2020), it was reported that SOD and CAT activity was increased in leaves of mulberry (*M. alba* L.) seedlings exposed to NaCl stress, and O_2 accumulation was not observed. Liu et al. (2015) reported that exogenous MEL application (0.1 mM) to the roots of tomato (*Lycopersicum esculentum*) under drought stress conditions promoted CAT, SOD, POD enzyme activities, and AsA levels. It has been stated that this status is related to the alleviation of damage in the membranes and thus the enhancement in drought tolerance. We observed that our findings are coherent with this statement (Figures 4, 5, 9).

It has been reported that SOD plays a role in the antioxidant defence mechanism of plants during oxidative stress and increases the activity of this enzyme as a reaction to stress (Jacoby et al. 2010). Plant GSTs detoxify ROSs, which form as consequences of various stresses, and can reduce peroxides along with GSH (Gill & Tuteja 2010). Kaya & Doğanlar (2019) reported that proline, GSH, and MDA contents and APX, GST, and GR activities increased in peppers (*Capsicum annuum*) exposed to pendimethalin and drought stress. They also found that exogenous MEL application reduced the plants' MDA content compared to control plants, while the proline content, GSH, and antioxidant enzymes (APX, GST, and GR) activities increased. Oxidized glutathione and NADPH transform GSH again with the reaction catalyzed by GR reductase (GR), and GR is the only enzyme that catalyzes this recovery (Karuppanapandian et al. 2011; Guller et al. 2020). On the other hand, increasing GR activity in plants promotes GSH accumulation. Thus, resistance and tolerance can be formed in plants against oxidative stresses (Shereefa & Kumaraswamy 2016). GR is the only enzyme that catalyzes the recovery of reduced glutathione from oxidized glutathione using NADPH as a reducing agent. In the research conducted by Xia et al. (2020), it is reported that exogenous MEL application to seedlings grown under drought stress led to the enhancement of ascorbic acid-glutathione (AsA-GSH) cycle, carotenoid biosynthesis, and antioxidant enzymes activities. It has been stated that the increase in POD activity under drought stress conditions is associated with the drought tolerance of the plant and that high POD activity will strengthen drought stress tolerance in the plant (Sairam & Saxena 2000). In another study, Campos et al. (2019) reported that MEL application (300 µM) increased the enzymatic and non-enzymatic antioxidants activity and reduced lipid peroxidation in coffee plants under water stress. Our findings indicate that SOD, GST, GR, POD, and APX enzyme activities and GSH content were increased in the PEG group compared to the Control group. Moreover, these parameters raised more in PEG+MEL group (Figures 5-10). We observed that MEL enhanced these enzymes, which are prominent in defence, and it demonstrates that MEL has a positive effect on the antioxidant system. These findings are in accordance with the literature.

Flavonoids and phenolic acids function as scavengers of free radicals and play a role in chelating ROS-producing metals by the Fenton reaction (Zhang et al. 2016). Gao et al. (2020), who experimented on two ornamental plants (*Adonis amurensis* and *A. pseudoamurensis*), reported that drought stress conditions improved flavonoid and total phenol content remarkably. Researchers also stated that plants mitigated damage caused by drought stress through soluble sugar and proline accumulation. Our findings indicate that drought stress increased the total phenolic content in *M. nigra* by 53% in PEG+MEL treatment than in the PEG group (Figure 12). Literature results suggest that exogenous MEL application in drought stress conditions contributes to plants' defence mechanism in stress tolerance by enhancing the phenolic content.

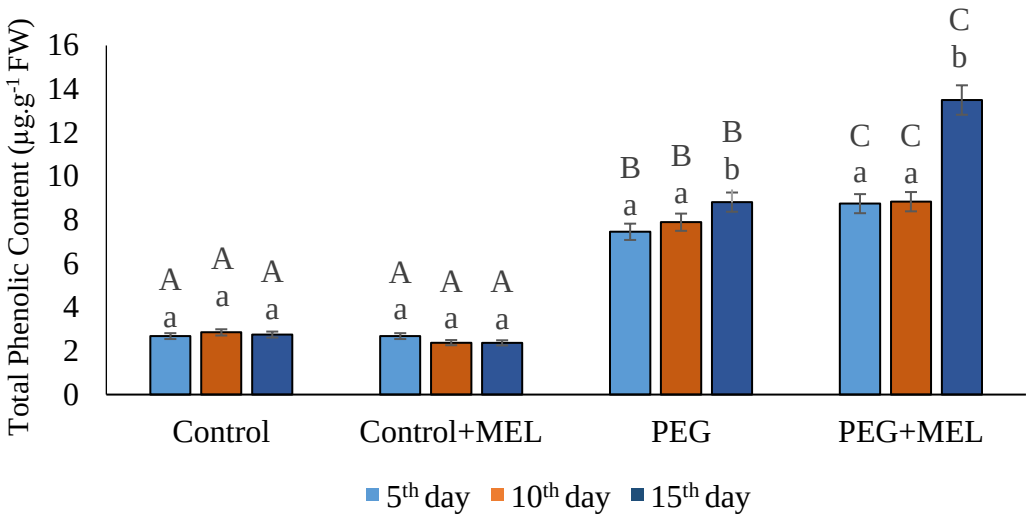


Figure 12- Variation of total phenolic content in *M. nigra* leaves depending on groups and days

Proline accumulation in plants preserves the moisture in the tissue, assists the absorption of water from the environment, and supports the cells to maintain their normal physiological and biochemical activities. Many studies have confirmed this finding in different plants under drought stress conditions (Karimi et al. 2012; Rostami & Rahami 2013; Bolat et al. 2014; Ipek 2015). Besides, many researchers reported soluble sugar and protein increment also seen under drought stress conditions as a protective mechanism of plants (Sarker & Oba 2018). Relative to the results of the study conducted by Ding et al. (2017), it was observed that exogenous MEL application to the leaves of tomato seedlings increased antioxidant enzyme activity, sugar, and proline content. Our findings demonstrated a similar trend in the *M. nigra* plant, and proline was higher, especially in the MEL group (Figure 13).

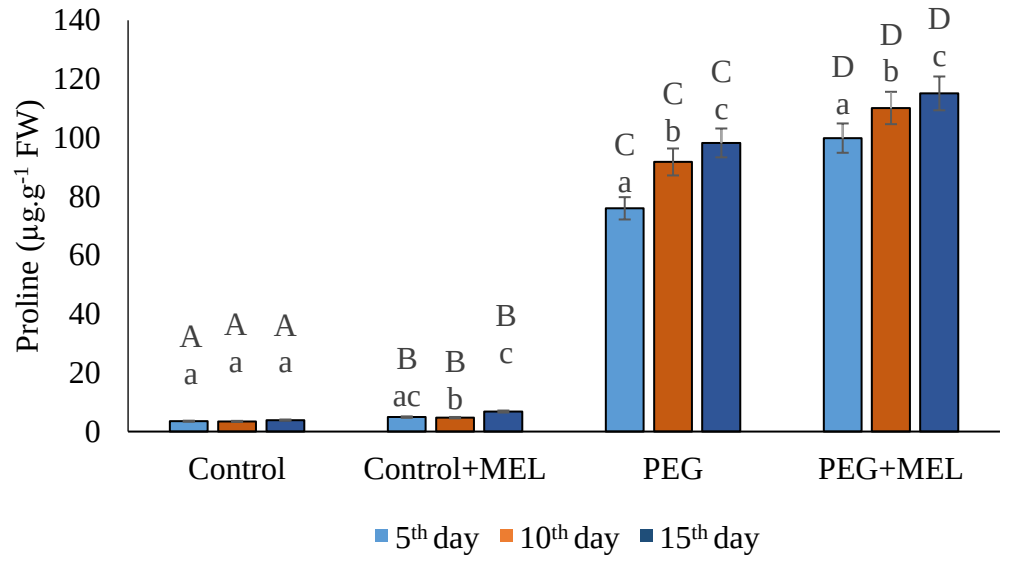


Figure 13- Variation of proline content in *M. nigra* leaves depending on groups and days

5. Conclusions

This study determined that MEL mitigates drought stress damage in *M. nigra* plants and could use as a protector. Moreover, our findings have shown that MEL positively impacts the pigment system, RWC, MDA content, non-enzymatic (GSH, proline, carotenoid), and enzymatic antioxidants (SOD, CAT, GST, GR, POD, APX). Hence, this study suggests significant findings to *in vitro* and *in vivo* research in the future.

Acknowledgements

This research is a part of the doctoral thesis of Duygu ÖZELÇİ. We want to thank Inonu University Scientific Research Project Unit (Project No FDK-2017-682) for supporting the research, and Apricot Research Institute for their contribution in providing laboratory facilities and samples.

References

Ahammed G J, Wang Y, Mao Q, Wu M, Yan Y, Ren J, Wang X, Liu A & Chen S (2020). Dopamine alleviates bisphenol A-induced phytotoxicity by enhancing antioxidant and detoxification potential in cucumber. Environmental Pollution 259: 113957. https://doi.org/10.1016/j.envpol.2020.113957

Akerboom T P & Sies H (1981). Assay of glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples. In Methods in enzymology 77: 373-382 Academic Press. https://doi.org/10.1016/s0076-6879(81)77050-2

Andrews C J, Cummins I, Skipsey M, Grundy N M, Jepson I, Townson J & Edwards R (2005). Purification and characterisation of a family of glutathione transferases with roles in herbicide detoxification in soybean (*Glycine max* L.); selective enhancement by herbicides and herbicide safeners. Pesticide Biochemistry and Physiology 82(3): 205-219. https://doi.org/10.1016/j.pestbp.2004.11.009

Arici E S & Eraslan F (2012). The effect of salt stress on colt (*Prunus avium* X *Prunus psudocerasus*) cherry rootstock cultured in vitro conditions. *Journal of Süleyman Demirel University Faculty of Agriculture* 7(2):41-48 (In Turkish).

Arnao M B & Hernández-Ruiz J (2019). Melatonin: a new plant hormone and/or a plant master regulator? Trends in Plant Science 24(1): 38-48. https://doi.org/10.1016/j.tplants.2018.10.010

Arnao M B & Hernández-Ruiz J (2020). Is phytomelatonin a new plant hormone? Agronomy 10(1): 95. https://doi.org/10.3390/agronomy10010095

Bates L S, Waldren, R P & Teare I D (1973). Rapid determination of free proline for water-stress studies. Plant and Soil 39(1): 205-207. https://doi.org/10.1007/BF00018060

Blum A & Ebercon A (1981). Cell membrane siability as a measure of drought and heat tolerance in wheat. Crop Sci 21: 43-47. https://doi.org/10.2135/cropsci1981.0011183X002100010013x

Bolat I, Dikilitas M, Ercisli S, Ikinci A & Tonkaz T (2014). The effect of water stress on some morphological, physiological, and biochemical characteristics and bud success on apple and quince rootstocks. *The Scientific World Journal*. https://doi.org/10.1155/2014/769732

Bradford M M (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72(1-2): 248-254. https://doi.org/10.1006/abio.1976.9999

Cakmak I (1994). Activity of ascorbate-dependent H_2O_2-scavenging enzymes and leaf chlorosis are enhanced in magnesium-and potassium-deficient leaves, but not in phosphorus-deficient leaves. *Journal of Experimental Botany* 45(9): 1259-1266. https://doi.org/10.1093/jxb/45.9.1259

Campos C N, ÁvilaRG de Souza K R D, Azevedo L M & Alves J D (2019). Melatonin reduces oxidative stress and promotes drought tolerance in young *Coffea arabica* L. plants. Agricultural Water Management 211: 37-47. https://doi.org/10.1016/j.agwat.2018.09.025

Carlberg I & Mannervik B (1985) Glutathione reductase. In Methods in Enzymology 113: 484-490. Academic press. https://doi.org/10.1016/s0076-6879(85)13062-4

Caruso A, Chefdor F, Carpin S, Depierreux C, Delmotte F M, Kahlem G & Morabito D (2008). Physiological characterization and identification of genes differentially expressed in response to drought induced by PEG 6000 in *Populus canadensis* leaves. *Journal of Plant Physiology* 165(9): 932-941. https://doi.org/ 10.1016/j.jplph.2007.04.006

Chandler S F & Dodds J H (1983). The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasodine in callus cultures of *Solanum laciniatum*. Plant Cell Reports 2(4): 105-108. https://doi.org/10.1007/BF00270105

Chen J, Wu W, Zheng Y, Hou K, Xu Y & Zai J (2010). Drought resistance of Angelica dahurica during seedling stage under polyethylene glycol (PEG-6000)-simulated drought stress. Zhongguo Zhong yao za zhi= Zhongguo Zhongyao Zazhi= China *Journal of Chinese Materia Medica* 35(2): 149-153. https://doi.org/10.4268/cjcmm20100205

Chen Y, Chen F, Liu L & Zhu S (2012). Physiological responses of Leucaena leucocephala seedlings to drought stress. *Procedia Engineering* 28: 110-116. https://doi.org/10.1016/j.proeng.2012.01.691

Cheng L, Han M, Yang L M, Li Y, Sun Z & Zhang T (2018). Changes in the physiological characteristics and baicalin biosynthesis metabolism of *Scutellaria baicalensis* Georgi under drought stress. Industrial Crops and Products 122: 473-482. https://doi.org/10.1016/j.indcrop.2018.06.030

Collins A R (2001). Carotenoids and genomic stability. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 475(1-2): 21-28. https://doi.org/10.1016/s0027-5107(01)00071-9

Datta R K (2002). Mulberry cultivation and utilization in India. Mulberry for animal production. FAO Animal Production and Health Paper 147: 45-62

De Kok L J & Graham M (1980). Levels of pigments, soluble proteins, amino acids and sulhydryl compounds in foliar tissue of Arabidopsis thaliana during dark-induced and natural senescence. *Plant Physiology and Biochemistry* (Paris) 27(2): 133-142.

Demiral T & Turkan I (2005). Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. *Environmental and Experimental Botany* 53(3): 247-257. https://doi.org/ 10.1016/j.envexpbot.2004.03.017

Ding F, Liu B & Zhang S (2017). Exogenous melatonin ameliorates cold-induced damage in tomato plants. Scientia Horticulturae 219: 264-271. https://doi.org/ 10.1016/j.scienta.2017.03.029

Duncan D B (1955). Multiple range and multiple F tests biometrics. International Biometric Society 11(1): 1-42. https://doi.org/10.2307/3001478

Erden Y (2021). Sour black mulberry (*Morus nigra* L.) causes cell death by decreasing mutant p53 expression in HT-29 human colon cancer cells. Food Bioscience 42: 101113. https://doi.org/10.1016/j.fbio.2021.101113

Fracheboud Y, Jompuk C, Ribaut J M, Stamp J & Leipner P (2004). Genetic analysis of cold-tolerance of photosynthesis in maize. Plant Molecular Biology 56(2): 241-253. https://doi.org/10.1007/s11103-004-3353-6

Gao S, Wang Y, Yu S, Huang Y, Liu H, Chen W & He X (2020). Effects of drought stress on growth, physiology and secondary metabolites of two Adonis species in Northeast China. Scientia Horticulturae 259: 108795. https://doi.org/ 10.1016/j.scienta.2019.108795

Gill S S &Tuteja N (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry 48(12): 909-930. https://doi.org/10.1016/j.plaphy.2010.08.016

Guller P, Karaman M, Guller U, Aksoy M & Kufrevioglu O I (2020). A study on the effects of inhibition mechanism of curcumin, quercetin, and resveratrol on human glutathione reductase through in vitro and in silico approaches. *Journal of Biomolecular Structure and Dynamics* 1-10. https://doi.org/10.1080/07391102.2020.1738962

Habig W H, Pabst M J & Jakoby W B (1974). The first enzymatic step in mercapturic acid formation Glutathione S-Transferases. *J. Biol. Chem* 249: 7130-7139. https://doi.org/10.1016/S0021-9258(19)42083-8

Han Q H, Huang B, Ding C B, Zhang Z W, Chen Y E, Hu C, Zhou L, Huang Y, Liao J, Yuan S & Yuan M (2017). Effects of melatonin on anti-oxidative systems and photosystem II in cold-stressed rice seedlings. Frontiers in Plant Science 8: 785. https://doi.org/10.3389/fpls.2017.00785

Heath R L & Packer L (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics 125(1): 189-198. https://doi.org/10.1016/0003-9861(68)90654-1

Hepsag F, Hayoglu I & Hepsag B (2012). Anthocyanin content of black mulberry fruit and the possibilities of using anthocyanins as colorants in the food industry. Electronic *Journal of Food Technologies* 7(1): 9-19 (In Turkish).

Huihui Z, Xin L, Yupeng G, Mabo L, Yue W, Meijun A, Yuehui Z, Guanjun L, Nan X & Guangyu S (2020). Physiological and proteomic responses of reactive oxygen species metabolism and antioxidant machinery in mulberry (*Morus alba* L.) seedling leaves to NaCl and NaHCO3 stress. Ecotoxicology and Environmental Safety 193: 110259. https://doi.org/10.1016/j.ecoenv.2020.110259

Imran M, Latif Khan A, Shahzad R, Aaqil Khan M, Bilal S, Khan A, Kang S M & Lee I J (2021). Exogenous melatonin induces drought stress tolerance by promoting plant growth and antioxidant defence system of soybean plants. AoB Plants 13(4): plab026. https://doi.org/10.1093/aobpla/plab026

Ipek M (2015). Determination of the responses of Garnem and Myrobolan 29C rootstocks to drought stress in vitro conditions. PhD Thesis, Selcuk University, Institute of Science and Technology, Konya Publication ID 409829 (In Turkish).

Jacoby R P, Millar A H & Taylor N L (2010). Wheat mitochondrial proteomes provide new links between antioxidant defense and plant salinity tolerance. *Journal of Proteome Research* 9(12): 6595-6604. https://doi.org/10.1021/pr1007834

Jahan M S, Shu S, Wang Y, Chen Z, He M, Tao M, Sun J & Guo S (2019). Melatonin alleviates heat-induced damage of tomato seedlings by balancing redox homeostasis and modulating polyamine and nitric oxide biosynthesis. BMC Plant Biology 19(1): 1-16
https://doi.org/10.1186/s12870-019-1992-7

Jain M, Mathur G, Koul S & Sarin N (2001). Ameliorative effects of proline on salt stress-induced lipid peroxidation in cell lines of groundnut (*Arachis hypogaea* L.). Plant Cell Reports 20(5): 463-468. https://doi.org/10.1007/s002990100353

Ju Y L, Yue X F, Zhao X F, Zhao H & Fang Y L (2018). Physiological, micro-morphological and metabolomic analysis of grapevine (*Vitis vinifera* L.) leaf of plants under water stress. Plant Physiology and Biochemistry 130: 501-510. https://doi.org/10.1016/j.plaphy.2018.07.036

Karimi S, Yadollahi A, Nazari-Moghadam R, Imani A & Arzani K (2012). *In vitro* screening of almond (*Prunus dulcis* (Mill.) genotypes for drought tolerance. *J Biol Environ Sci* 6(18): 263-270

Karuppanapandian T, Moon J C, Kim C, Manoharan K & Kim W (2011). Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms. *Australian Journal of Crop Science* 5(6): 709. https://doi.org/ 10.3316/informit.282079847301776

Kaya A & Doganlar Z B (2019). Melatonin improves the multiple stress tolerance in pepper (*Capsicum annuum*). Scientia Horticulturae 256: 108509. https://doi.org/10.1016/j.scienta.2019.05.036

Kaynak L & Ersoy N (1997). General properties and usage areas of plant growth regulators, *Akd. Univ. Zir. Fac. Journal* 10: 223-236 (In Turkish).

Kocacaliskan I (2008). Plant Physiology. Nobel Publications Distribution, Science and Biology Publications Series, 316 (In Turkish).

Korkmaz A, Demir O, Kocacinar F & Cuci Y (2016). Increasing tolerance to cold stress with foliar melatonin applications in pepper seedlings. *Journal of Agriculture and Nature* 19(3): 348-354 (In Turkish).

Li J, Yang Y, Sun K, Chen Y, Chen X & Li X (2019). Exogenous melatonin enhances cold, salt and drought stress tolerance by improving antioxidant defense in tea plant (*Camellia sinensis* (L.) O. Kuntze). Molecules 24(9): 1826. https://doi.org/ 10.3390/molecules24091826

Lichtenthaler H K & Wellburn A R (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Botanisches Institutder Univeristat, Kaiserstran βe 12, Postfach pp. 591-592. https://doi.org/10.1042/BST0110591

Lim S H, & Choi C I (2019). Pharmacological properties of *Morus nigra* L. (black mulberry) as a promising nutraceutical resource. Nutrients 11(2): 437. https://doi.org/ 10.3390/nu11020437

Lin Y, Fan L, Xia X, Wang Z, Yin Y, Cheng Y & Li Z (2019). Melatonin decreases resistance to postharvest green mold on citrus fruit by scavenging defense-related reactive oxygen species. Postharvest Biology and Technology 153: 21-30. https://doi.org/10.1016/j.postharvbio.2019.03.016

Liu R, Shi H, WangY, Chen S, Deng J, Liu Y, Li S & Chan Z (2014). Comparative physiological analysis of lotus (*Nelumbo nucifera*) cultivars in response to salt stress and cloning of NnCIPK genes. Scientia Horticulturae 173: 29-36. https://doi.org/ 10.1016/j.scienta.2014.04.032

Liu J, Wang W, Wang L & Sun Y (2015). Exogenous melatonin improves seedling health index and drought tolerance in tomato. Plant Growth Regulation 77: 317-326. https://doi.org/10.1007/s10725-015-0066-6

Luck H (1963). Catalase: In: Methods of Enzymatic Analysis edited by HU. https://doi.org/10.1016/B978-0-12-395630-9.50158-4

MacAdam J W, Nelson C J & Sharp R E (1992). Peroxidase activity in the leaf elongation zone of tall fescue: I. Spatial distribution of ionically bound peroxidase activity in genotypes differing in length of the elongation zone. Plant Physiology 99(3): 872-878. https://doi.org/10.1104/pp.99.3.872

Marchin R M, Ossola A, Leishman M R & Ellsworth D S (2020). A simple method for simulating drought effects on plants. Frontiers in Plant Science 10: 1715. https://doi.org/10.3389/fpls.2019.01715

Marshall J G, Rutledge R G, Blumwald E & Dumbroff E B (2000). Reduction in turgid water volume in jack pine, white spruce and black spruce in response to drought and paclobutrazol. Tree Physiology 20(10): 701-707. https://doi.org/ 10.1093/treephys/20.10.701

McCord J M & Fridovich I (1969). Superoxide dismutase: An enzymic function for erytreoeuprein (Hemoeuprein). *J. Biol. Chem* 244: 22 6049- 6055

Mehta M & Kumar A (2021). Nutrient composition, phytochemical profile and antioxidant properties of *Morus nigra*: A Review. *International Journal of Innovative Science and Research Technology* 6(2): 424-432

Michel F B (1983). Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. Plant Physiol 72: 66-70. https://doi.org/10.1104/pp.72.1.66

Moustafa-Farag M, Almoneafy A Mahmoud A, Elkelish A, Arnao MB, Li L & Ai S (2020). Melatonin and its protective role against biotic stress impacts on plants. Biomolecules 10(1): 54. https://doi.org/10.3390/biom10010054

Murashige T & Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Nakano Y & Asada K (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology 22(5): 867-880. https://doi.org/10.1093/oxfordjournals.pcp.a076232

Pandey N, Iqbal Z, Pandey BK & Sawant SV (2017). Phytohormones and drought stress: plant responses to transcriptional regulation. Mechanism of plant hormone signaling under stress. John Wiley & Sons, Hoboken, New Jersey, United States 477-504. https://doi.org/10.3389/fpls.2016.00434

Peters J L, Castillo F J & Heath R L (1989). Alteration of extracellular enzymes in pinto bean leaves upon exposure to air pollutants, ozone and sulfur dioxide. Plant Physiology 89(1): 159-164. https://doi.org/10.1104/pp.89.1.159

Poeggeler B, Thuermann S, Dose A, Schoenke M, Burkhardt S & Hardeland R (2002). Melatonin's unique radical scavenging properties–roles of its functional substituents as revealed by a comparison with its structural analogs. *Journal of Pineal Research* 33(1): 20-30. https://doi.org/10.1034/j.1600-079x.2002.01873.x

Posmyk M M & Janas K M (2009). Melatonin in plants. Acta Physiologiae Plantarum 31(1): 1. https://doi.org/10.1007/s11738-008-0213-z

Qiao Y, Yin L, Wang B, Ke Q, Deng X & Wang S (2019). Melatonin promotes plant growth by increasing nitrogen uptake and assimilation under nitrogen deficient condition in winter wheat. Plant Physiology and Biochemistry 139: 342-349. https://doi.org/10.1016/j.plaphy.2019.03.037

Reiter R J (1991). Pineal melatonin: cell biology of its synthesis and of its physiological interactions. Endocrine Reviews 12(2): 151-180. https://doi.org/10.1210/edrv-12-2-151

Rostami A A & Rahemi M (2013). Responses of caprifig genotypes to water stress and recovery. *Journal of Biological & Environmental Sciences* 7(21): 131-139

Rouhi V, Samson R, Lemeur R & Van Damme P (2006). Stomatal resistance under drought stress conditions induced by PEG 6000 on wild almond. *Communications in Agricultural and Applied Biological Sciences* 71(1): 269

Sairam R K & Saxena D C (2000). Oxidative stress and antioxidants in wheat genotypes: possible mechanism of water stress tolerance. *Journal of Agronomy and Crop Science* 184(1): 55-61. https://doi.org/10.1046/J.1439-037X.2000.00358

Sanchez F J, De Andres E F, Tenorio J L & Ayerbe L (2004). Growth of epicotyls, turgor maintenance and osmotic adjustment in pea plants (*Pisum sativum* L.) subjected to water stress. Field Crops Research 86(1): 81-90. https://doi.org/10.1016/S0378-4290(03)00121-7

Santos C V (2004). Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. Scientia Horticulturae 103(1): 93-99. https://doi.org/ 10.1016/j.scienta.2004.04.009

Sarker U & Oba S (2018). Drought stress effects on growth, ROS markers, compatible solutes, phenolics, flavonoids, and antioxidant activity in *Amaranthus tricolor*. Applied Biochemistry and Biotechnology 186(4): 999-1016. https://doi.org/ 10.1007/s12010-018-2784-5

Sekhar K M, Reddy K S & Reddy A R (2017). Amelioration of drought-induced negative responses by elevated CO_2 in field grown short rotation coppice mulberry (*Morus* spp.), a potential bio-energy tree crop. *Photosynthesis Research* 132(2): 151-164. https://doi.org/10.1007/s11120-017-0351-5

Sharma A, Wang J, Xu D, Tao S, Chong S, Yan D, Li Z, Yuan H & Zheng B (2020). Melatonin regulates the functional components of photosynthesis, antioxidant system, gene expression, and metabolic pathways to induce drought resistance in grafted *Carya cathayensis* plants. Science of the Total Environment 713: 136675. https://doi.org/10.1016/j.scitotenv.2020.136675

Shereefa L A H & Kumaraswamy M (2016). Reactive oxygen species and ascorbate-glutathione interplay in signaling and stress responses in *Sesamum orientale* L. against Alternaria sesami (Kawamura) Mohanty and Behera. *Journal of the Saudi Society of Agricultural Sciences* 15(1): 48-56. https://doi.org/10.1016/j.jssas.2014.04.007

Slinkard K & Singleton V L (1977). Total phenol analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture* 28(1): 49-55

Smirnoff N (1993). The role of active oxygen in the response of plants to water deficit and desiccation. New Phytologist 125(1): 27-58. https://doi.org/10.1111/j.1469-8137.1993.tb03863.x

Upadhyaya H, Khan M H & Panda S K (2007). Hydrogen peroxide induces oxidative stress in detached leaves of *Oryza sativa* L. Gen Appl Plant Physiol 33(1-2): 83-95

Vijayan K, Tikader A, Weiguo Z, Nair C V, Ercisli S & Tsou C H (2011). *Morus*. In Wild Crop Relatives: Genomic and Breeding Resources, pp. 75-95. Springer, Berlin, Heidelberg.

Volkert M R, Loewen P C, Switala J, Crowley D & Conley M (1994). The delta (argF-lacZ) 205 (U169) deletion greatly enhances resistance to hydrogen peroxide in stationary-phase *Escherichia coli*. *Journal of Bacteriology* 176(5): 1297-1302. https://doi.org/10.1128/jb.176.5.1297-1302.1994

Wang W, Vinocur B, Shoseyov O & Altman A (2004). Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. *Trends in Plant Science* 9(5): 244-252. https://doi.org/10.1016/j.tplants.2004.03.006

Wang Y, Reiter R J & Chan Z (2017). Phytomelatonin: a universal abiotic stress regulator. *Journal of Experimental Botany* 69(5): 963-974. https://doi.org/10.1093/jxb/erx473

Wang Z, Tang C, Xiao G, Dai F, Lin S, Li Z & Luo G (2021). Comparison of free and bound phenolic compositions and antioxidant activities of leaves from different mulberry varieties. BMC Chemistry 15(1): 1-15 https://doi.org/10.1186/s13065-021-00747-0

Xia H, Shen Y, Shen T, Wang X, Zhang X, Hu P & Deng Q (2020). Melatonin accumulation in sweet cherry and its influence on fruit quality and antioxidant properties. Molecules 25(3): 753 https://doi.org/10.3390/molecules25030753

Zhang L, Jia J, Xu Y, Wang Y, Hao J & Li T (2012). Production of transgenic *Nicotiana sylvestris* plants expressing melatonin synthetase genes and their effect on UV-B-induced DNA damage. In Vitro Cellular & Developmental Biology-Plant 48(3): 275-282 https://doi.org/10.1007/s11627-011-9413-0

Zhang N, Sun Q, Li H, Li X, Cao Y, Zhang H, Li S, Zhang L, Qi Y & Zhao B (2016). Melatonin improved anthocyanin accumulation by regulating gene expressions and resulted in high reactive oxygen species scavenging capacity in cabbage. *Frontiers in Plant Science* 7: 197 https://doi.org/10.3389/fpls.2016.00197

Zhao H J & Tan J F (2005). Role of calcium ion in protection against heat and high irradiance stress-induced oxidative damage to photosynthesis of wheat leaves. Photosynthetica 43(3): 473-476 https://doi.org/10.1007/s11099-005-0076-0

Zheng H, Zhang X, Ma W, Song J, Rahman S U, Wang J & Zhang Y (2017a). Morphological and physiological responses to cyclic drought in two contrasting genotypes of *Catalpa bungei*. Environmental and Experimental Botany 138: 77-87 https://doi.org/10.1016/j.envexpbot.2017.02.016

Zheng X, Tan D X, Allan A C, Zuo B, Zhao Y, Reiter R J, Wang L, Wang Z, Guo Y, Zhou J, Shan D, Li Q, Han Z & Zhou J (2017b). Chloroplastic biosynthesis of melatonin and its involvement in protection of plants from salt stress. Sci. Rep 7(1): 1–12 https://doi.org/10.1038/srep41236

Zhu J, Li Z, Kang H & Fan Y (2005). Effects of polyethylene glycol (PEG)-simulated drought stress on *Pinus sylvestris* var. mongolica seed germination on sandy land. Ying Yong Sheng Tai Xue Bao. *The Journal of Applied Ecology* 16(5): 801-804

Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

Pelleting with Puperabsorbent, Chitosan, and Phosphorus Fertilizer as a New Method to Improve Growth, Yield, and Physiological Attributes of Potato mini-tuber

Sevda GHASEMI GERMI[a], Morteza BARMAKI[a*], Salim FARZANEH[a], Mandana AMIRI[b]

[a]*Department of Agronomy, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, IRAN*

[b]*Department of Chemistry, Faculty of Science, University of Mohaghegh Ardabili, Ardabil, IRAN*

ARTICLE INFO

Research Article

Corresponding Author: Morteza BARMAKI, E-mail: m_barmaki@uma.ac.ir

Received: 27 July 2021 / Revised: 18 October 2021 / Accepted: 18 October 2021 / Online: 15 October 2022

Cite this article

GERMİ GHASEMİ S, BARMAKİ M, FARZANEH S, AMİRİ M (2022). Pelleting with Puperabsorbent, Chitosan, and Phosphorus Fertilizer as a New Method to Improve Growth, Yield, and Physiological Attributes of Potato mini-tuber. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 28(4):570-582. DOI: 10.15832/ankutbd.974390

ABSTRACT

Seed pelleting is a technique of covering seeds with adhesive agents to improve seed performance and plant establishment while reducing production costs. To evaluate the responses of potato superelite minitubers to different pelleting treatments both qualitatively and quantitatively, a 2-year experiment was conducted based on randomized complete block design with three replications in Mohaghegh Ardabili University, Ardabil, Iran during 2018 and 2019. Experimental treatments included 17 different combinations of zeolite (ZE) or cocopeat (CO) fillers, coating (SC) or soil application (SS) of triple superphosphate, superabsorbent polymer (SP), and chitosan (CH). The results showed a significant effect of pelleting on yield, physiological, and qualitative traits. The highest fresh and dry tuber weight belonged to SS + ZE treatment, which showed 50.6 and 49.0% increase compared to the control treatment (without pelleting), respectively. Pelleting minitubers by ZE + SS had the highest fresh tuber and biological yield (23.6 and 28.0 ton.ha^{-1}, respectively), which showed an increase of 156.5% and 145.6%, compared to the no pelleting treatment. Most pelleting treatments increased the leaf proline content and activity of the antioxidant enzymes. The highest peroxidase activity and lowest catalase activity (16.1 and 29.6 U.mg protein^{-1}.min, respectively) were observed in the CO+SC+CH treatment. Co-application of ZE, SC, and SP resulted in the highest protein, nitrate, and starch content. In addition, minituber pelleting with ZE+SS+SP increased methionine content significantly by 52.94% compared to the control. In general, the co-application of triple superphosphate, SP, and ZE increased the tuber yield and quality traits. Finally, the use of CH with these compounds resulted in improving physiological characteristics.
.

Keywords: Antioxidant activity, Methionine amino acid, Proline, Tuber yield, Zeolite

1. Introduction

Potato (*Solanum tuberosum* L.) is a non-grain crop widely cultivated worldwide due to its easy cultivation, nutrient richness, and high production. It is considered as the fourth largest crop after wheat, rice, and corn (Pawelzik & Möller 2014; Wang et al. 2020). Potato is the most crucial tuberous plant which is rich in carbohydrates, proteins, and essential amino acids for humans. Lysine and methionine are essential amino acids for humans not synthesized in the body and should be obtained from other sources. Potatoes are one of the rich sources of these amino acids (Kandi et al. 2012). The area under cultivation, production, and yield of potatoes in Iran was recorded as 148 441 ha, 5142 891 tons, and 34 646 kg/ha, respectively. Ardabil province is the second largest producer of this product after Hamadan province (Anonymous 2018). Among the superior characteristics of Ardabil province, low temperature, sufficient light, and a large difference between day and night temperatures are considered as necessary for the optimal growth in this plant (Song et al. 2014; Khan et al. 2015). The production of potatoes depends on the dynamics of nutrients in the soil and the capacity of the product to absorb nutrients in the soil (Martinset al. 2018) as well as genotype-environment interaction (Mohammadinia et al. 2021). The increasing population has led to an increase in demand for this product around the world. Thus, more attention should be paid to innovation in potato production to increase its quantitative and qualitative characteristics (Glover & Poole 2019; Hacıyusufoğlu 2020).

Seed pelleting is considered as the most useful methods used for improving seed germination and increasing seedling growth, especially under environmental stress (Rocha et al. 2019). Changing the shape of the seed or placing chemical compounds on the seed coat improves and regulates germination and growth, as well as increasing yield (Shinde et al. 2020). Seed pelleting and coating are two types of seed coatings used for commercial application (Taghizoghi et al. 2018). In the seed coating method, a thin layer of various substances such as pesticides, growth regulators, fertilizers, and nutrients and binders was added to the outer surface of the seed without improving the seed shape. Pelleting is one of the methods in which other required materials are used in addition to disinfecting the seeds such as adding nutrients to the seeds and their rounding (Mandal et al. 2015). Seed pelleting

improves the external structure of the seed and makes it uniform, which results in sowing seeds accurately. Moreover, seed priming and pelleting can increase the quality of seed, leading to an improvement in seedling growth power and reducing production costs (Szerement et al. 2014; Karagoz & Yucel 2020).

Potato minituber was produced in vitro conditions, ranging from 0.1 to 10 g or more, 4 to 7 mm diameter, and 10 to 12 mm length. Therefore, the cost of these tubers for planting is higher than that of the typical seed (Çalışkan et al. 2020). Pelleting those using nutrients, growth regulators, and inert substances is considered as a method for increasing the size of this minituber to be acceptable for cultivation in field conditions (Ravichandran et al. 2015). This method increases the weight and volume of tuber and standardizes the size of seed (Rykaczewska 2016). Some studies focused on pelleting potato seed and developed countries refused to release useful compounds or methods. In one study, two small potato tuber cultivars were pelleted using different combinations. Based on the results, the use of Acacia leaf powder increased germination, growth, and yield indices, including the number of tubers (Ravichandran et al. 2015). Pelleting with chitosan (CH) increased the chlorophyll content and catalase and peroxidase enzyme activities in peppers (Kheiri et al. 2016). Peanut seed pelleting with salicylic acid composition increased the quantitative and qualitative traits of this plant by increasing the activities of various enzymes (Dong et al. 2019). The use of salicylic acid and ethylene for pelleting and granulation improved the germination and growth indices of sugar beet (Pirasteh-Anosheh & Emam 2019).

Due to an increase in food per capita and calorie consumption, attempts were made to produce more agricultural products globally, especially in developing countries (Aksoy & Beghin 2004). Potatoes are the most critical nutrient source in nutritional importance due to the presence of essential amino acids, vitamins, and minerals in human nutrition. Due to the diverse distribution pattern of potatoes, this product has provided food security as a strategic plant in the world and Iran, especially in deprived areas. Therefore, innovations based on potato planting science leading to an increase in quantitative traits in line with qualitative characteristics are of considerable importance (Beumer et al. 2021). The present experiment aims to determine yield, physiological, and qualitative responses of potato minitubers to different pelleting treatments with zeolite (ZE), cocopeat (CO), triple superphosphate, CH, and superabsorbent (SP).

2. Material and Methods

2.1. Plant material and experimental design

To evaluate the growth, yield, physiological, and qualitative responses of potato minituber to different pelleting treatments under field conditions, an experimental randomized complete block design with three replications in the field of agricultural research and Natural resources of Mohaghegh Ardabili University located North-West of Iran (38°15′N and 48°20′E and altitude of 1350 m above sea level) were conducted during 2017-2018. Figure 1 displays monthly climatic parameters recorded at a weather station located adjacent to the experimental site for two years.

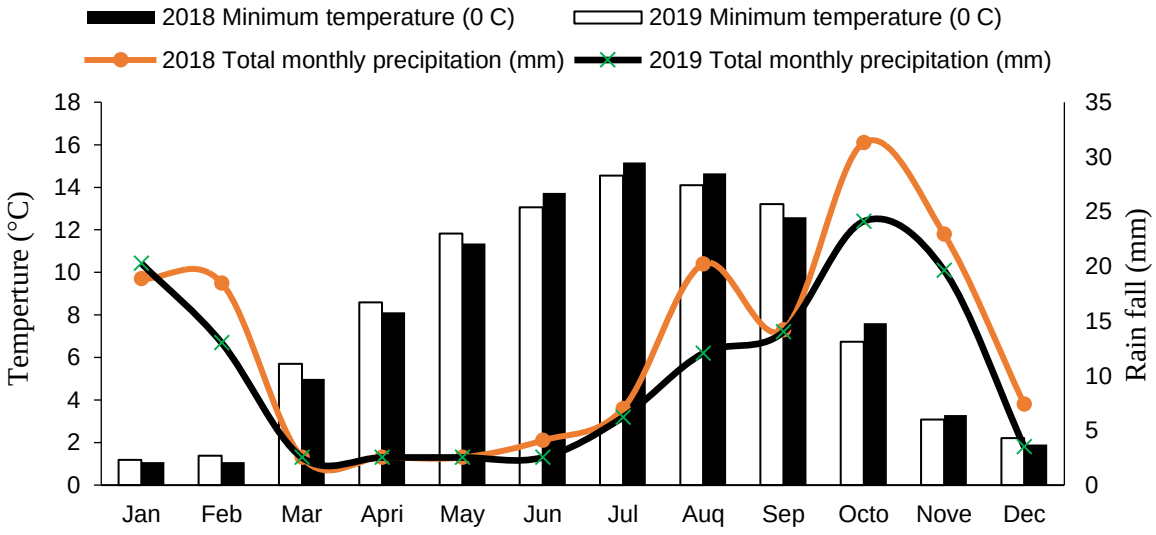


Figure 1-Climate conditions (temperature and precipitation) during the growing season 2018 and 2019

Experimental treatment included 17 treatments (using triple superphosphate fertilizer as a coating (SC) or soil application (SS, ZE, CO, SP, and CH) were tested in combination with the control treatment (without pelleting) (Table 1). In this study, Agria cultivar was used. This cultivar is obtained from a cross between Quarta × Semlo cultivars, which has some characteristics such as medium late maturity, high yield, tuber elongated, high dry matter percentage, rapid foliage development, high height, and susceptible to tuber tuberculosis and potato 'Y' virus. The growth period of the Agria cultivar is 120 to 130 days.

Table 1- Combination of experimental treatments in the present study

Treatment	*Combination*	*Treatment*	*Combination*
T1	SP + CH + SC + ZE	T10	CH + SC + CO
T2	CH + SC + ZE	T11	SP + SC + CO
T3	SP + SC + ZE	T12	SC + CO
T4	SC + ZE	T13	SP + CH + SS + CO
T5	SP + CH + SS + ZE	T14	CH + SS + CO
T6	CH + SS + ZE	T15	SP + SS + CO
T7	SP + SS + ZE	T16	SS + CO
T8	SS + ZE	T17	Control (without pelleting)
T9	SP + CH + SC + CO		

ZE: Zeolite; CO: Cocopeat; SC: Superphosphate coating; SS: Soil application of superphosphate; SP: Superabsorbent; CH: Chitosan

2.2. Minituber pelleting method

Potato minitubers were prepared from Dasht-e Zarrin Company in Ardabil province and placed in a warehouse with indirect light at 18-20 °C for one week to germinate. To prepare the CH solution (Sigma-Aldrich Company, USA), a 1% solution of acetic acid was designed and placed on a magnetic stirrer. Then, solutions of 5 g per liter of CH were prepared. In addition, chitosan powder was added slowly at 60 °C. After stirring for five to six hours when all of the CH particles were dissolved and the solution was clear, about 25% by the weight of the CH powder used glycerol (as Plasticizer) was added to the solution. In this study, Arabic gum was used as an adhesive for minitubers. To prepare a solution of Arabic gum, 1.5 l of water was exposed to a temperature of 70 °C, and then 50 g of Arabic gum was added to each liter of water was added, which was utterly powdered and sifted in a mortar to the solution. Further, it was placed on a magnetic stirrer (half an hour) (Shaddel et al. 2018). In the next stage, the pelleting material was applied. First, the tubers were impregnated with Arabic gum and then covered with ZE or CO and placed in a laboratory environment at the room temperature for about 25 °C for one day to be dried. After ensuring the tubers to dry, the tubers were covered with triple superphosphate powder in the next day using an electric mixer. Based on the experimental treatments, the tubers which were not covered with superphosphate were added to the soil. Finally, CH and SP were applied to the minitubers based on experimental treatments, and thus the pelleting process was completed. Pelleting with different compounds was done to create a layer of coating on the surface of the tuber and the whole surface was impregnated with the desired composition, which was easily done due to the use of Arabic gum as adhesive. After ensuring drying, the pelleted minitubers were transferred to the field for planting. The weight of each plate was considered to be about 0.1 to 0.2 g. Planting was conducted with a distance between plants of 25 cm, a distance between rows of 75 cm, and a planting depth of 5-10 cm in five rows of cultivation (2×3 m^2) was conducted on 30 April and 2 May for first and second years, respectively.

After planting, in addition to fertilizing based on soil test in Table 2, 180 kg nitrogen per ha in two stages from urea source, 120 kg phosphorus per ha from pentoxide phosphorus at planting, and 150 kg potassium per ha from potassium sulfate source), the operation included irrigation and weed control (manually three times), insects, and pests. Irrigation was conducted according to the needs of the plant and a total of eight irrigations. To control the deciduous pest of Colorado potato beetle, once spraying with Zolon herbicide at two liters per hectare rate was conducted 75 days after planting.

Table 2- Physico-chemical characteristics of experimental soil at depths of 0 to 30 cm (on average)

Soil texture	*Sand*	*Silt*	*Clay*	*Organic carbon (%)*	*Total Nitrogen (mg.kg^{-1})*	*Available Phosphorous (mg.kg^{-1})*	*Available Potassium (mg.kg^{-1})*	*Electrical conductivity (dS.m^{-1})*	pH
Sandy loam	52	22	26	1.17	1012	6.1	198	2.68	7.09

2.3. Yield and yield component parameters

Tuber yield and yield components such as the number of tubers, fresh and dry (70 °C for 48 h) tuber weight, and biological yield (shoots + roots + tubers weight) were calculated and measured from the middle three rows while ignoring border effects and ten plants were randomly selected. The harvest time was on 5 and 9 August in the first and second years, respectively.

2.4. Chlorophyll index and leaf area index (LAI)

After the flowering stage, the chlorophyll index was performed by manual chlorophyll meter (SPAD 502 PLUS model, England), and the LAI was measured by leaf area meter (CI202 model, USA) (Roosjen et al. 2018).

2.5. Proline content

Leaf-free proline content was determined by using Bates et al. (1973) method. First, 0.5 g of the fresh leaf was ground with quarts in porcelain pestle and mortar and treated with 10 mL sulfosalicylic acid. The homogenate was centrifuged at 13000 g for

10 min. The red surface layer and standard samples were simultaneously placed in a spectrophotometer, and the absorption of the samples was determined at the wavelength of 520 nm. The amount of proline content (µmol g^{-1} FW) was calculated using regression equations and the standard curves.

2.6. Extraction and assay of enzymatic antioxidant activities

Antioxidant enzyme extraction was determined using the method of Sunohara & Matsumoto (2004) with slight modifications. First, 200 mg of leaves were sliced and homogenized in 10 mL of 25 mM K-P buffer (pH 7.8) containing one mM AsA, 0.4 mM EDTA, and 2% PVPP at four °C for 1 min. The homogenate solution was centrifuged at 15,000 g at 4 °C for 20 min, and the supernatant was filtered through filter paper (Whatman® No.1, England). The filtrate was collected to determine the activities of catalase (EC 1.11.1.6) and peroxidase (EC 1.11.1) enzymes (Ghahremani et al. 2019).

Catalase activity was determined based on Sunohara and Matsumoto's (2004) method with some modifications. First, it was assayed in a 2 mL reaction mixture including 1.9 mL of 50 mM K-P buffer (pH 7.0) containing 25 mM H_2O_2 and 0.1 mL of enzyme extract for 1 min. Then, CAT activity was measured in terms of the decomposition of H_2O_2 at 240 nm and activity calculated per leaf protein for 1 min.

In the next procedure, peroxidase activity was assayed by the method proposed by Chance & Maehly (1955). An alcoholic liquid of the tissue extract (100 µl) was added to 3 mL of assay solution including 3 mL of reaction mixture containing 13 mM guaiacol, five mM H_2O_2, and 50 mM sodium (Na)-phosphate (pH 6.5). An increase in the optical density at 470 nm for 1 min at 25 °C was recorded using a spectrophotometer.

2.7. Tuber quality characteristics

The method of Kandi et al. (2012) was used for measuring qualitative traits including the percentage of protein, lysine, and methionine. Noda et al. (1995) method was applied for determining the tuber starch content using the phenol-sulfuric acid method. In addition, nitrate was measured by the proposed method of Errebhi et al. (1998).

2.8. Statistical analysis

After checking the normal distribution of the data by Kolmogorov-Smirnov and Shapiro-Wilk test, using Statistical Analysis System software (SAS Institute, Cary, NC, USA, *ver*. 9.2) was used for statistical analyses. When the result of ANOVA showed a significant treatment effect, the least significant difference (LSD) test was applied to compare the means at 0.05 probability level. Simple correlations between traits were performed using SAS software. Principal component analysis (PCA) and cluster analysis were performed by Ward method using Minitab statistical software (*ver*. 19).

3. Results and Discussion

3.1. Effect of minituber pelleting on yield and yield components

As shown in Table 3, mixed ANOVA of 2-year data indicated a significant effect of minituber pelleting on yield and yield components such as fresh and dry tuber weight, number of tuber per plant, tuber and biological yield ($P \leq 0.01$). The highest fresh and dry tuber weight (487.6 and 69.9 g, respectively) belonged to T8 (SS+ZE), which showed an increase of 102.6% and 96.3% compared to the control treatment (without pelleting). The lowest these traits were related to T17 (without pelleting) by 240.7 and 35.6 g, respectively (Table 3). In this regard, Bahador et al. (2015) reported that ZE application leads to an increase in the available moisture in the seeds, which results in increasing root moisture and uptake rate, and transferring nitrogenous substances. Bybordi (2016) indicated that the significant effect of ZE on plant height, leaf number, dry weight, and seed yield of rapeseed leading to an increase in the average of these traits.

Table 3- Comparison of two-year means of yield and yield components of potato minituber by different pelleting treatments

	Fresh tuber weight (g.plant^{-1})	*Dry tuber weight (g.plant^{-1})*	*Number of tuber per plant*	*Tuber yield (ton.ha^{-1})*	*Biological yield (ton.ha^{-1})*
Year					
First	366.45±91.47 a	51.96±11.52 a	7.35±1.78 b	17.21±4.52 a	20.45±5.08 a
Second	343.26±91.55 a	53.32±15.88 a	8.67±2.28 a	19.04±5.67 a	22.21±6.33 a
LSD (α=0.05)	24.17	2.77	0.51	1.95	2.46
Treatments					
T1	319.7±13.1 ef	46.1±2.5 fg	7.3±0.4 de	16.3±0.6 de	19.1±0.8 de
T2	361.6±49.7 b-f	60.7±8.2 bc	7.1±0.5 ef	17.9±2.6 cde	21.7±3.1 b-e
T3	350.2±23.2 b-f	57.6±6.1 bcd	7.7±1.2 cde	17.8±1.6 cde	21.4±1.8 cde
T4	329.4±11.5 def	48.1±2.5 efg	7.6±0.8 de	16±1.6 de	19±1.7 de
T5	296.8±16.5 fg	42.3±2.7 gh	7.3±0.9 def	15.3±1.4 e	17.9±1.5 e
T6	340±25.6 c-f	52.7±4.8 c-f	8.7±1.0 bcd	18.6±2.2 b-e	21.9±2.5 b-e
T7	333.8±16.7 c-f	49.7±3.2 d-g	8.4±0.8 b-e	18.7±1.7 b-e	21.8±1.8 b-e
T8	487.6±35.1 a	69.9±7.6 a	8.2±0.7 b-e	23.6±2.9 a	28.0±3.4 a
T9	349.4±31 b-f	51±3.5 def	11.2±1.0 a	20.5±1.9 abc	23.7±2.0 bc
T10	344.2±39.6 c-f	46.8±3.9 fg	7.8±1.1 b-e	18.5±1.7 b-e	21.4±1.8 cde
T11	415.4±37.2 b	61.3±4.2 b	7±0.6 ef	22±1.7 ab	25.8±1.9 ab
T12	375.3±25.1 b-e	53.8±5.5 b-f	9.2±1.3 b	20.1±2.8 a-d	23.4±3.1 bc
T13	344.3±37.7 c-f	51±3.9 def	7.9±0.7 b-e	20±2.2 a-d	23.2±2.2 bcd
T14	396.7±51.3 bcd	59.3±2.8 bc	9.1±0.3 bc	18±1.8 b-e	21.7±1.9 b-e
T15	344±31.1 c-f	53.9±4.6 b-f	8.4±0.6 b-e	17.2±1.6 cde	20.5±1.8 cde
T16	403.6±65.1 bc	55.2±7.0 b-e	7.8±0.4 b-e	18.8±1.9 b-e	22.2±2.3 bcd
T17 (control)	240.7±15.8 g	35.6±2.8 h	5.8±0.3 f	9.2±0.7 f	11.4±0.8 f
LSD (α=0.05)	70.4	8.07	1.49	4.09	4.25

Means followed by the same letter in each column are not significantly different according to the LSD test.

In addition, a considerable difference was observed in the number of tubers per plant under the effect of pelleting treatments, among which T9 (SP+CH+SC+CO) had the highest number of tubers per plant (11.2 number), which showed an increase of 93.1% compared to the control (Table 3). Further, most minituber pelleting treatments resulted in a significant increase in the fresh tuber yield and biological yield. The highest fresh tuber and biological yield was reported in T8 (ZE+SS) by 23.6 and 28.0 ton.ha^{-1}, respectively, which indicated an increase of 61.0 and 59.2% compared to the control treatment, respectively. The without pelleted minituber led to the lowest tuber and biological yield by 9.2 and 11.4 ton.ha^{-1}, respectively. Due to the developed root system, potatoes need a lot of nutrients to produce high yields and larger tubers, which the soil alone cannot provide the elements required by the plant (Fernandes et al. 2017). The use of organic and mineral fertilizers can have a significant impact on potato yield. Phosphorus is one of the most restricted biomass elements present in less than one percent soluble in the soils of different regions depending on climate (Alemayehu et al. 2020). Pelleting minituber with triple superphosphate increased the yield and yield components. A balanced supply of phosphorus led to an increase in yield and phosphorus uptake in the potato plant (Soratto & Fernandes 2016). Furthermore, phosphorus availability affects the uptake and concentration of other macro-and micro-elements which can affect yield (Fernandes et al. 2017). Generally, the seed pelleting by triple superphosphate has a significant advantage in yield traits and yield components compared to its soil application. It seems that more water uptake by seeds and access to elements are considered as the factors related to the superiority of pelleting compared to the soil application of superphosphate (Kataki et al. 2016).

In the present study, the application of ZE led to an increase in yield and yield components. Zeolites are among the minerals which are significantly present in Iran, the unique property is to increase cation exchange capacity and selective bonding for ammonium and potassium to improve soil structure (Alshameri et al. 2014; Ghasemi et al. 2018). Some researchers indicated that adding ZE to the soil by changing the soil permeability facilitates water movement in the soil improves vegetative growth and increases the secondary metabolite in Amaranths (Karami et al. 2020). Zeolites with a very porous structure and an extensive inner surface stabilize the nutrients in their structure, the gradual release of which provides these nutrients for the plant for a long time, which results in increasing efficiency and ability, and accordingly yield (Jakkula and Wani 2018). The application of ZE-based on the review of sources led to an increase in yields of many crops including potatoes (13%), canola (33%), rice (13%), and rapeseed (89%) (Aghaalikhani et al. 2012; Mohammadi & Rokhzadi 2012; Heydari et al. 2017). Some researchers reported that superabsorbent polymers affect the rate of water content in soil, specific gravity, and soil structure, and the evaporation rate from the soil surface leading to morpho-physiological changes in the plant (Khan et al. 2018; Bagherifard et al. 2020). It seems that the co-application of ZE and superabsorbent led to an improvement in water availability and soil physical characteristics, as well as increasing growth and yield parameters. The maximum use of resources and optimal growth conditions due to the availability of resources can be a significant factor in improving growth and yield parameters (Barnett & Morse 2013).

3.2. Effect of minituber pelleting on physiological characteristics

Physiological characteristics such as chlorophyll index, LAI, proline content, and activity of peroxidase and catalase enzymes are significantly affected by pelleting treatments. Increasing the chlorophyll content in the leaves can help researchers in the production of plants with higher photosynthetic power. The highest chlorophyll index is related to T7 (ZE+SS+SP) by 48.4, leading to an increase of 24.1% compared to the without pelleting treatment. Further, T1, T2, T3, T4, T6, T9, T10, T11, T14, and T16 treatments were in the same statistical group with treatment T7, indicating the highest means of this trait. Control treatment (without pelleting) had the lowest chlorophyll index by 39.0 (Table 4). The chlorophyll content is closely related to nitrogen availability and synthesis chlorophyll increased in the use of triple superphosphate fertilizer, ZE, and SP due to the key role of phosphorus in the structure of enzymes and the role of ZE in reducing the leaching of elements, especially nitrogen as one of the main elements in chlorophyll structure (Heydari et al. 2017).

Table 4- Comparison of two-year means of physiological traits of potato by different pelleting treatments

	Chlorophyll index	*LAI*	*Proline content (µmol g^{-1} FW)*	*Peroxidase activity (U mg $protein^{-1}$min)*	*Catalase activity (U mg $protein^{-1}$ min)*
Year					
First	44.48±3.18 b	2.79±0.21 a	237.86±20.31 b	14.48±2.26 a	37.26±10.53 a
Second	47.55±3.53 a	2.95±0.24 a	255.78±25.13 a	14.66±2.30 a	49.51±9.94 a
LSD (α=0.05)	0.95	0.54	6.88	0.66	2.05
Treatments					
T1	47.7±1.8abc	2.92±0.1 a-d	257.7±6.9 ab	12.0±0.3gh	43.7±2.9bc
T2	47.8±1.0 abc	2.93±0.11 a-d	259.3±8.1 a	16.2±0.6 a	36.4±2.0 def
T3	48.1±1.6 ab	2.90±0.09 a-d	268.0±8.0 a	14.3±0.7 a-f	33.0±2.2 e-h
T4	46.7±0.8 a-f	2.89±0.16 a-d	262.8±7.1 a	11.5±0.4 h	52.4±2.6 a
T5	45.2±0.9 c-f	2.99±0.07abc	268.6±7.8 a	13±0.6 d-h	30.3±1.0 gh
T6	46.7±1.8 a-f	2.76±0.08 d	255.4±10.1 ab	11.9±0.4gh	29.4±2.2 h
T7	48.4±1.7 a	2.96±0.12 a-d	257±7.5 ab	13.6±0.8 b-g	36.3±1.6 d-g
T8	44.1±1.4ef	3.02±0.1 ab	262.8±9.6 a	15.9±0.6 a	30.9±2.4fgh
T9	47.6±1.0 abc	2.95±0.08 a-d	267.6±9.3 a	13.2±0.4 c-h	41.2±1.9bcd
T10	46.6±1.4 a-f	2.93±0.04 a-d	254.7±6.4 ab	16.1±0.3 a	29.6±1.4 h
T11	47.4±1.5 a-d	2.81±0.08 cd	251.4±6.6 ab	14.6±0.9 a-e	42.5±1.1bc
T12	44.0±1.9 f	2.88±0.1 a-d	238.4±7.1bc	12.7±0.4 e-h	44.5±2.4 b
T13	45.4±1.3 b-f	3.06±0.1 a	223.7±4.9 cd	15.5±0.7 ab	50.9±2.8 a
T14	46.8±1.0 a-d	2.84±0.07bcd	257.3±10.3 ab	15.0±0.9abc	41.9±3.4bcd
T15	44.5±0.9 def	2.83±0.06bcd	238.1±5.9bc	12.5±0.7fgh	40.3±1.9bcd
T16	46.9±1.0 a-d	2.91±0.07 a-d	259.1±7.1 a	14.8±0.8 a-d	38.3±1.6cde
T17 (control)	39.0±0.8 g	2.38±0.14 e	210.6±4.2 d	15.3±0.5 ab	32.5±0.8 e-h
LSD (α=0.05)	2.78	0.21	20.07	1.94	6.00

Means followed by the same letter in each column are not significantly different according tothe LSD test.

As shown in Table 4, the results indicated that the pelleting treatments significantly increased LAI. The average LAI was observed from 2.38 to 3.06. The highest LAI was related to T13 (CO+SS+CH+SP) by 3.06 and the lowest mean (2.38) was related to without pelleting treatment. The results showed no significant difference between the applications of ZE and CO in terms of LAI. In addition, the presence of triple superphosphate is necessary for increasing the LAI. Further, CH plays a significant role in increasing the average physiological traits such as LAI, proline content, and antioxidant enzymes. Chitosan, as a polysaccharide-based edible coating, has been successfully used to coat seed or fresh-cut fruits (Olawuyi et al. 2018). Chitosan action mechanism is most likely done by sending signals to synthesize plant hormones such as gibberellin and auxin, leading to some changes in physiological traits (Jiao et al. 2012; Katiyar et al. 2015). In this regard, some reported that the use of CH led to an increase in growth parameters of the potato plant by strengthening the defense mechanisms of enzymatic (antioxidant enzymes) and non-enzymatic (proline, carotenoids, etc.) (Muley et al. 2019).

Based on the results, the pelleting treatments increased the leaf proline content. The average proline content was observed from 210.6 to 268.6 µmol.g^{-1} FW.As a result, the highest free proline content was obtained from T1-T11, T14, and T16. Moreover, the lowest proline content was T17 (control) by 210.6 µmol.g^{-1} FW. In addition to increasing cellular potential, proline amino acid is involved in stabilizing the intracellular structure, redox potential of the cellular buffer, and eliminating free radicals (Muley et al. 2019). Further, proline may act as a protein-compatible hydrotrope, which results in reducing cytoplasmic acidity while maintaining the $NADP^+$/NADPH ratios specified for cellular metabolic pathways (Ashraf and Foolad 2007). In another study, CH led to an increase in proline content in the potato plant (Muley et al. 2019), which is consistent with the findings of the present study.

Regarding the means, the peroxidase activity is more affected by pelleting treatments than the catalase enzyme. Further, different results were found in these two enzymes response to pelleting treatments so that the highest peroxidase activity (16.2 U.mg $protein^{-1}$.min) were observed in T2 (ZE+SC+CH) treatment and the lowest catalase activity (29.4 U.mg $protein^{-1}$.min)

were observed in T6 (ZE+SS+CH) treatment. The mean of peroxidase and catalase activities increased from 11.5 to 16.2 and 29.4 to 52.4 U.mg protein^{-1}.min, respectively. The highest activity of peroxidase and catalase enzymes showed an increase of 5.88% and 61.23% compared to the treatment without pelleting. The highest catalase activity was related to T4 (ZE+SC) and T13 (CO+SS+CH+SP) by 52.4 and 50.9 U.mg protein^{-1}.min, respectively (Table 4). The results of the present study indicated the increasing effect of CH on the activity of antioxidant enzyme peroxidase and catalase. In this regard, Loy et al. (2019) reported that CH pelleting treatment activated antioxidant enzyme activities of Thompson seedless.

3.3. Effect of minituber pelleting on tuber quality traits

Table 5 shows minituber quality characteristics such as protein, methionine, lysine, soluble carbohydrate, nitrate, and starch contents significantly affected by pelleting treatments (p<0.01). The highest and lowest protein content (5.33 and 3.18%) was observed in T3 (ZE+SC+SP) and T13 (CO+SS+CH+SP) treatments, respectively. In the T3 treatment, an increase of 50.14% protein content occurred compared to the treatment without pelleting. Regarding the mean comparison of pelleting treatments, the highest and lowest methionine amino acid content was found in T1 and T15 (0.26 and 0.16%), respectively. The application of T1 (SP+CH+SC+ZE) treatment significantly increased methionine content by 52.94% compared to the control treatment (without pelleting) (Table 5). The average of tuber lysine content ranged from 0.37 to 0.57%. The highest lysine content was related to T1 and T12 (0.56 and 0.57%). T12 treatment significantly increased lysine content by 42.5%, compared to the treatment without pelleting (Table 5). Co-application of ZE, SC, and SP (T3 treatment) resulted in the highest average nitrate and starch content (239.7 mg.kg^{-1} and 17. 2%), respectively), which showed an increase of 16.52% and 32.30% compared to the control treatment. The lowest nitrate and starch content were related to T13 (146.6 mg.kg^{-1} and 12.4%), respectively (Table 5). Any change in nitrogen uptake and metabolism directly affects the rate of nucleic acid and protein synthesis. Taheri-Soudejani et al. (2019) reported that the availability of phosphorus in the soil is adequate on nitrogen uptake and plant use and ZE can reduce nitrogen leaching and increase plant availability. This mechanism increased the minituber protein and the content of lysine and methionine amino acids using triple superphosphate and ZE. In this regard, some reported similar results for other plant species (Gholamhoseini et al. 2013).

Table 5- Comparison of two-year means of the quality of potato minituber by different pelleting treatments

	Protein content (%)	*Methionine content (%)*	*Lysine content (%)*	*Soluble carbohydrate (mg.g^{-1} FW)*	*Nitrate content (mg.kg^{-1})*	*Starch content (%)*
Year						
First	4.23±0.75 a	0.18±0.03 b	0.44±0.08 b	11.52±1.80 a	196.8±30.5 a	14.4±2.05 a
Second	4.09±1.0 a	0.25±0.04 a	0.47±0.06 a	11.81±1.37 a	201.6±39.9 a	14.3±2.82 a
LSD (α=0.05)	0.22	0.009	0.021	0.38	6.62	0.60
Treatments						
T1	4.78±0.16 ab	0.26±0.02 a	0.56±0.03 a	12.8±0.3abc	215.0±7.4 b	12.4±0.4 g
T2	4.67±0.30 b	0.2±0.02 e-h	0.43±0.02 def	10.7±0.5ef	210.2±13.9 b	16.1±0.8 ab
T3	5.33±0.45 a	0.21±0.02 c-g	0.50±0.03abc	10.4±0.4 f	239.7±15.7 a	17.2±1.8 a
T4	4.76±0.25 ab	0.23±0.02 b-e	0.48±0.03bcd	12.2±0.3bcd	214.4±6.7 b	14.9±0.8bcd
T5	4.64±0.30 b	0.23±0.02bcd	0.49±0.03bcd	12.6±0.3 a-d	208.9±7.5 b	15.2±0.7bcd
T6	4.18±0.23 b-e	0.19±0.01ghi	0.43±0.02 d-g	11.7±0.5cde	188.1±8.5cde	13.6±0.8 d-g
T7	4.14±0.17 b-e	0.21±0.02 c-g	0.47±0.02 b-e	11.6±0.5 de	186.1±5.4 de	13.1±0.6efg
T8	4.53±0.15bc	0.22±0.02 b-f	0.48±0.01bcd	10.8±0.4ef	203.8±3.9bcd	15.3±0.4bcd
T9	4.46±0.28bcd	0.21±0.02 d-g	0.44±0.02 c-f	12.6±0.6 a-d	199.0±6.4 b-e	15.5±0.7abc
T10	4.63±0.24bc	0.21±0.02 c-g	0.41±0.01efg	13.0±0.3 ab	208.1±11.2 b	15.9±1.0 abc
T11	3.88±0.24 de	0.19±0.01ghi	0.37±0.02 g	12.2±0.5bcd	182.9±9.3 e	14.1±0.5 c-g
T12	3.99±0.50cde	0.24±0.02 ab	0.57±0.01 a	13.3±0.5 ab	188.1±18.4cde	14.4±1.6 b-f
T13	3.18±0.34 f	0.19±0.02fgh	0.42±0.02efg	10.1±0.3 f	146.6±13.6 f	12.4±0.5 g
T14	4.57±0.28bc	0.24±0.02abc	0.51±0.02 ab	9.8±0.4 f	205.5±8.7bc	15.7±0.9abc
T15	4.38±0.44bcd	0.16±0.01i	0.41±0.02fg	13.7±0.5 a	197.1±19 b-e	16.0±1.2 ab
T16	4.44±0.27bcd	0.22±0.02 b-f	0.47±0.02 b-e	13±0.2 ab	201.3±9.3 b-e	14.8±0.8 b-e
T17 (control)	3.55±0.35ef	0.17±0.02 hi	0.40±0.01fg	10.4±0.4 f	205.7±8.6bc	13.0±0.6fg
LSD (α=0.05)	0.67	0.028	0.062	1.12	19.30	1.76

Means followed by the same letter in each column are not significantly different according to the LSD test.

As shown in Tables 3, 4, and 5, the number of tubers, chlorophyll index, proline content, methionine and lysine content increased significantly during the second year by 17.96%, 6.90%, 7.53%, 38.8%, and 6.82% compared to the first year. It seems

that favorable climatic conditions in the second year, especially in terms of temperature and rainfall, led to an increase in the average growth, yield, and quality traits (Porter & Semenov 2005).

3.4. Simple correlation, cluster, and principal component analysis

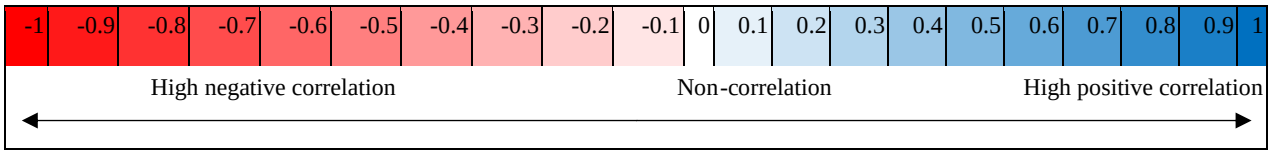
Table 6 indicates the results of simple correlations between yield, physiological, and qualitative traits. The tuber yield was positively and significantly correlated with fresh and dry tuber weight, number of tubers, biological yield, and LAI, while it was negatively correlated with methionine content. Lysine content as important qualitative traits in potatoes was positively correlated with soluble carbohydrate and starch content. In addition, positive and negative correlations were observed between the yield and morpho-physiological traits of potato and tuber yield had a positive correlation with yield components and tuber quality traits (Petros & Zelleke 2013).

Table 6- Simple correlation coefficients (Pearson) between yield, physiological, and qualitative traits of potato minituber under the influence of different seed pelleting treatments

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2	0.99**														
3	-0.67**	-0.67**													
4	0.59**	0.59**	0.31*												
5	0.59**	0.59**	0.08 ns	0.98**											
6	0.08 ns	0.09 ns	0.07 ns	0.09 ns	0.09 ns										
7	0.28*	0.28*	-0.07 ns	0.29*	0.29*	-0.03 ns									
8	0.23 ns	0.24 ns	-0.05 ns	0.25 ns	0.25 ns	0.28*	0.16 ns								
9	0.10 ns	0.10 ns	-0.01 ns	0.13 ns	0.14 ns	-0.16 ns	-0.31*	0.31*							
10	0.20 ns	0.21 ns	-0.40*	-0.10 ns	-0.10 ns	-0.22 ns	-0.11 ns	-0.20 ns	-0.34**						
11	-0.04 ns	-0.04 ns	-0.03 ns	-0.08 ns	-0.07 ns	0.29*	0.12 ns	0.03 ns	-0.09 ns	0.12 ns					
12	-0.33*	-0.33*	0.08 ns	-0.30*	-0.31*	-0.03 ns	0.02 ns	0.08 ns	0.43*	-0.30*	-0.45**				
13	-0.21 ns	-0.21 ns	-0.04 ns	-0.19 ns	-0.20 ns	-0.09 ns	0.15 ns	-0.09 ns	0.25 ns	0.13 ns	-0.25 ns	0.15 ns			
14	0.03 ns	0.03 ns	-0.02 ns	-0.08 ns	-0.09 ns	-0.04 ns	0.13 ns	0.06 ns	0.31*	-0.06 ns	-0.21 ns	0.13 ns	0.82**		
15	-0.04 ns	-0.04 ns	-0.03 ns	-0.08 ns	-0.07 ns	0.29*	0.12 ns	0.01 ns	-0.13 ns	0.01 ns	-0.32*	0.52**	-0.03 ns	-0.12 ns	
16	-0.15 ns	0.22 ns	0.33*	-0.10 ns	-0.09 ns	-0.10 ns	0.08 ns	0.07 ns	0.09 ns	0.09 ns	0.71**	0.31*	0.29*	0.86**	-0.32*

ns: non-significant; * and **: significant at the 5 and 1% probability level, respectively

1: fresh tuber weight; 2: dry tuber weight, 3: number of tuber per plant; 4: tuber yield; 5: biological yield; 6: chlorophyll index; 7: LAI; 8: proline content; 9: peroxidase activity; 10: catalase activity; 11: protein content; 12: methionine content; 13: lysine content; 14: soluble carbohydrate; 15: nitrate content; 16: starch content.



The treatment (cluster analysis) was classified for decreasing the number of experimental treatments and determining the most effective treatment. As shown in in Table 7 and Figure 2, 33 treatments were classified into three clusters, the first cluster included 11 treatments (T1, T2, T3, T4, T5, T6, T7, T9, T10, T13, and T15), the second had 5 treatments (T8, T11, T12, T14, and T16), and the control (T17) as the third cluster. The first cluster in terms of physiological characteristics such as chlorophyll index, LAI, proline, protein and soluble content, and the second cluster in terms of yield and yield components and tuber quality characteristics including fresh and dry tuber weight, tuber and biological yield, methionine and lysine content had the highest coefficients. A principal component analysis was performed to understand the correlation between characteristic and important traits (PCA) better (Figure 3). Based on the results, the first component could be explained by about 30% of the changes including proline, starch, nitrate, and protein content. Regarding the second component, 22% of the changes could be explained by the number of tubers, methionine, and lysine content. Further, the first component is called the physiological component, and the second is the qualitative component, which justifies 52% of the changes. Similar results were reported for the potato plant by other researchers (Deperi et al. 2018).

Table 7- Cluster analysis and grouping of studied variables of potato minitubers under the influence different pelleting treatments (Highest values are underlined)

Variable	*Cluster1*	*Cluster2*	*Cluster3*	*Grand centroid*
Fresh tuber weight	337.576	415.709	240.720	354.859
Dry tuber weight	50.889	59.898	35.632	52.641
Number of tubers	8.121	8.239	5.806	8.020
Tuber yield	17.875	20.480	9.213	18.132
Biological yield	21.056	24.223	11.440	21.422
Chlorophyll index	46.792	45.814	39.008	46.047
LAI	2.919	2.890	2.380	2.879
Proline	255.715	253.796	210.600	252.497
Peroxidase	13.613	14.597	15.282	14.001
Catalase	38.512	39.639	32.507	38.491
Protein	4.468	4.281	3.551	4.359
Methionine	0.210	0.222	0.173	0.211
Lysine	0.460	0.480	0.399	0.462
Soluble carbohydrate	11.941	11.836	10.420	11.821
Nitrate	201.219	196.330	205.740	200.047
Starch	14.748	14.850	12.969	14.674
Number of treatments	11	5	1	-

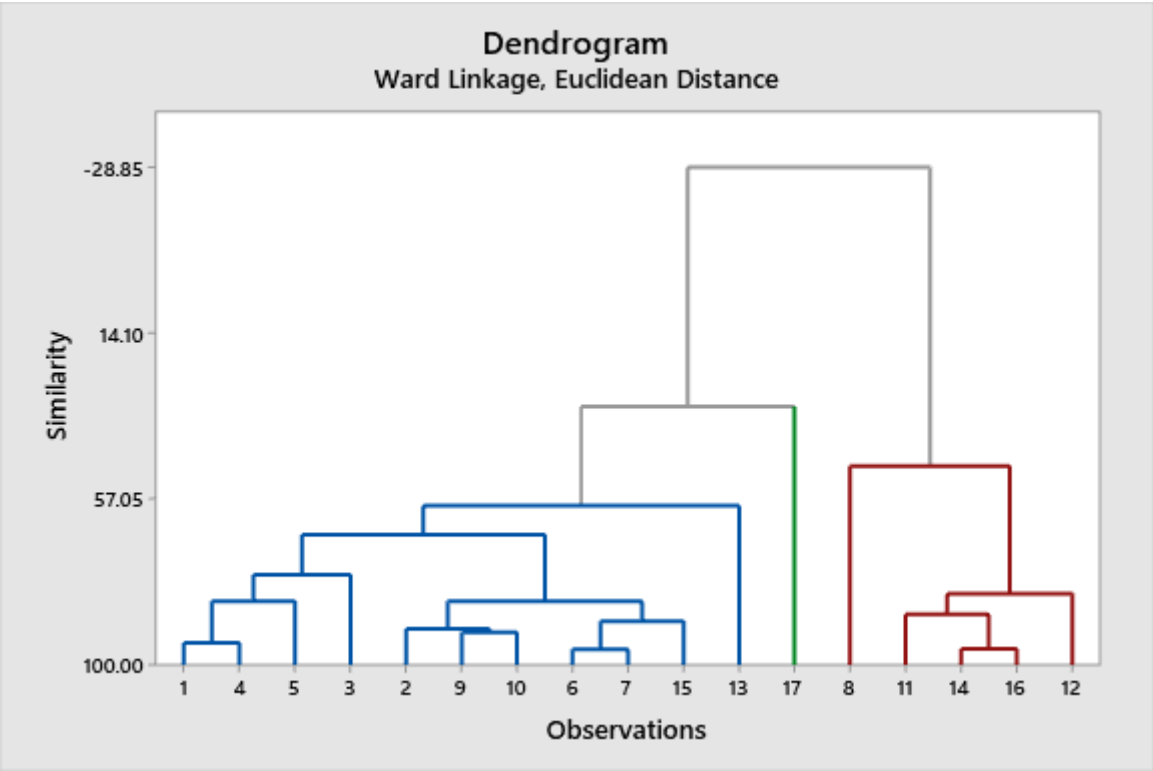


Figure 2- Grouping the combination of potato minituber pelleting treatments

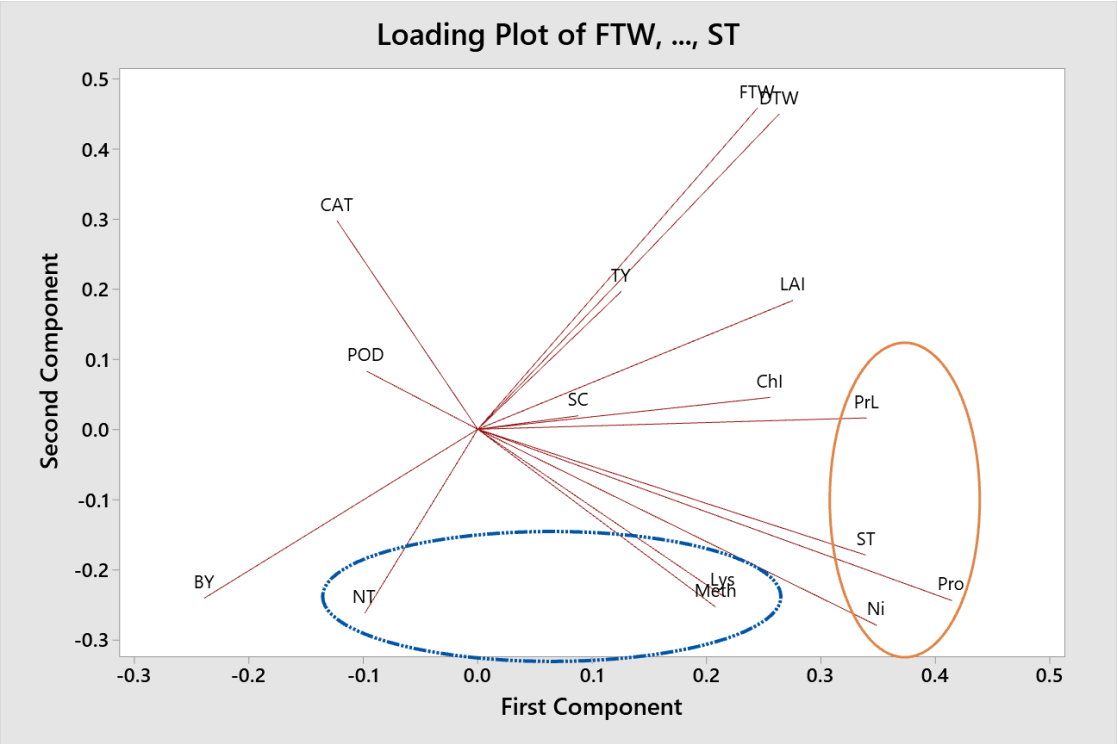


Figure 3- Principal component analysis (PCA) of yield, physiological, and qualitative traits of potato minituber

4. Conclusions

From the previous results it could be concluded that, the pelleting of potato minituber with triple superphosphate, ZE, CO, CH, and SP leads to an increase in growth, yield, and qualitative parameters of minitubers and changes in physiological traits. Therefore, the success of seed pelleting technology depends upon the selection of inexpensive and readily available pelleting agents with low cost. Collectively, cost effective, simple materials and methods are needed for use in third world countries.

Compliance with ethical standards

Conflict of interest: The authors declare that there is no conflict of interest concerning the publication of this article.

References

Aghaalikhani M, Gholamhoseini M, Dolatabadian A, Khodaei-Joghan A & Sadat Asilan K (2012). Zeolite influences on nitrate leaching, nitrogen-use efficiency, yield and yield components of canola in sandy soil. Arch. Agron. Soil Sci 58: 1149-1169. https://doi.org/10.1080/03650340.2011.572876

Aksoy M A & Beghin J C (2004). Global agricultural trade and developing countries (World Bank Publications).

Alemayehu M, Jemberie M, Yeshiwas T & Aklile M (2020). Integrated application of compound NPS fertilizer and farmyard manure for economical production of irrigated potato (Solanum tuberosum L.) in highlands of Ethiopia. Cogent Food & Agriculture 6: 1724385. https://doi.org/10.1080/23311932.2020.1724385

Alshameri A, Ibrahim A, Assabri A M, Lei X, Wang H & Yan C (2014). The investigation into the ammonium removal performance of Yemeni natural zeolite: Modification, ion exchange mechanism, and thermodynamics. Powder Technol. 258: 20-31. https://doi.org/10.1016/j.powtec.2014.02.063

Anonymous (2018). Iran Agriculture Statistics. Agricultural Publications

Ashraf M, Foolad M R (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot. 59: 206-216. https://doi.org/10.1016/j.envexpbot.2005.12.006

Bagherifard A, Hamidoghli Y, Biglouei M H & Ghaedi M (2020). Effects of drought stress and superabsorbent polymer on morpho-physiological and biochemical traits of Caper (Capparis spinosa L.). *Aust. J. Crop Sci.* 14: 13-20. https://doi.org/10.21475/ajcs.20.14.01.p1418

Bahador M, Abdali A & Lotfi A (2015). Effect of zeolite and seed priming on grain nitrogen content, leaf chlorophyll and traits dependent to grain yield of Mung bean (Vigna radiata L.). cultivars. J. Plant Proc. Func. 4: 137-147.

Barnett H J & Morse C (2013). Scarcity and growth: the economics of natural resource availability (1st ed.). RFF Press. https://doi.org/10.4324/9781315064185

Bates L S, Waldren R P & Teare I (1973). Rapid determination of free proline for water-stress studies. Plant Soil 39: 205-207. https://doi.org/10.1007/BF00018060

Beumer K, Stemerding D & Swart J A (2021). Innovation and the commons: lessons from the governance of genetic resources in potato breeding. Agric Human Values 38: 525-539. https://doi.org/10.1007/s10460-020-10169-8

Bybordi A (2016). Influence of zeolite, selenium and silicon upon some agronomic and physiologic characteristics of canola grown under salinity. Comm Soil Sci Plant Anal 47: 832-850. https://doi.org/10.1080/00103624.2016.1146898

Çalışkan M E, Yavuz C, Yağız A K, Demirel U & Çalışkan S (2020). Comparison of aeroponics and conventional potato mini tuber production systems at different plant densities. Potato Res. 64: 41-53. https://doi.org/10.1007/s11540-020-09463-z

Chance B & Maehly A C (1955). Assay of Catalase and Peroxidase. Methods in Enzymology 2: 764-775. http://dx.doi.org/10.1016/S0076-6879(55)02300-8

Deperi S I, Tagliotti M E, Bedogni M C, Manrique-Carpintero N C, Coombs J, Zhang R, Douches D & Huarte M A (2018). Discriminant analysis of principal components and pedigree assessment of genetic diversity and population structure in a tetraploid potato panel using SNPs. PloS one 13:e0194398. https://doi.org/10.1371/journal.pone.0194398

Dong Y, Wan Y, Liu F & Zhuge Y (2019). Effects of exogenous SA supplied with different approaches on growth, chlorophyll content and antioxidant enzymes of peanut growing on calcareous soil. *J. Plant Nutr.* 42: 1869-1883. https://doi.org/10.1080/01904167.2019.1648679

Errebhi M, Rosen C J, Gupta S C & Birong D E (1998). Potato yield response and nitrate leaching as influenced by nitrogen management. *Agron. J.* 90: 10-15. https://doi.org/10.2134/agronj1998.00021962009000010003x

Fernandes A M, Soratto R P, Souza Ed FC d & Job A L G (2017). Nutrient uptake and removal by potato cultivars as affected by phosphate fertilization of soils with different levels of phosphorus availability. Rev. Bras. Ciênc. Solo. 41: 1-23. https://doi.org/10.1590/18069657rbcs20160288

Ghahremani A, Moghadam E G, Tatari M & Khosroyar S (2019). Physiological responses of paneer-booti (Withania coagulans Dunal) to NaCl stress under tissue culture conditions. Not Bot Horti Agrobot Cluj Napoca 47(4): 1365-1373. https://doi.org/10.15835/nbha47411566

Ghasemi Z, Sourinejad I, Kazemian H & Rohani S (2018). Application of zeolites in aquaculture industry: a review. Rev Aquacult 10: 75-95. https://doi.org/10.1111/raq.12148

Gholamhoseini M, Ghalavand A, Khodaei-Joghan A, Dolatabadian A, Zakikhani H & Farmanbar E (2013). Zeolite-amended cattle manure effects on sunflower yield, seed quality, water use efficiency and nutrient leaching. Soil Tillage Res 126: 193-202. https://doi.org/10.1016/j.still.2012.08.002

Glover D & Poole N (2019). Principles of innovation to build nutrition-sensitive food systems in South Asia. Food Policy 82: 63-73. https://doi.org/10.1016/j.foodpol.2018.10.010

Heydari M, Mir N & Mousavi N S M (2017). The effect of phosphorus nanofertilizer modified with a novel organic compound on qualitative parameters of two types of beans. *J. Crop Improv* 19: 517-532. https://doi.org/10.22059/jci.2017.60395

Hacıyusufoğlu A F (2020). Effect of hopper diameter of seed coating machine on some coating criteria in lettuce (Lactuca sativa L.) seed. Mediterr. Agric. Sci. 33(2): 247-252

Jakkula V & Wani S (2018). Zeolites: Potential soil amendments for improving nutrient and water use efficiency and agriculture productivity. Scientific Reviews & Chemical Communications 8: 1-15

Jiao Z, Li Y, Li J, Xu X, Li H, Lu D & Wang J (2012). Effects of exogenous chitosan on physiological characteristics of potato seedlings under drought stress and rehydration. Potato Res. 55: 293-301. https://doi.org/10.1007/s11540-012-9223-8

Kandi M A S, Tobeh A, Golipouri A, Godehkahriz S J & Rastgar Z (2012). Concentration changes of lysine and methionine amino acids in potatoes varieties affected by different levels of nitrogen fertilizer. *Tech J Eng Appl Sci* 2(4): 93-96

Karagoz I & Yucel G (2020). Use of super absorbent polymers with euonymus plants (Euonymus japonicus 'Aureomarginatus') in ornamental plant cultivation. *Tar. Bil. Der*. 26: 201-211. DOI: 10.15832/ankutbd.471855

Karami S, Hadi H, Tajbaksh M & Modarres-Sanavy S A M (2020). Effect of zeolite on nitrogen use efficiency and physiological and biomass traits of amaranth (Amaranthus hypochondriacus) under water-deficit stress conditions. *J. Plant. Nutr. Soil Sci.* 20: 1427-1441. https://doi.org/10.1007/s42729-020-00223-z

Kataki S, West H, Clarke M & Baruah D C (2016). Phosphorus recovery as struvite: Recent concerns for use of seed, alternative Mg source, nitrogen conservation and fertilizer potential. Resour Conserv Recy 107: 142-156. https://doi.org/10.1016/j.resconrec.2015.12.009

Katiyar D, Hemantaranjan A & Singh B (2015). Chitosan as a promising natural compound to enhance potential physiological responses in plant: a review. *Indian J. Plant Physiol* 20: 1-9. http://dx.doi.org/10.1007/s40502-015-0139-6

Khan A, Pan X, Najeeb U, Tan D K Y, Fahad S, Zahoor R & Luo H (2018). Coping with drought: stress and adaptive mechanisms, and management through cultural and molecular alternatives in cotton as vital constituents for plant stress resilience and fitness. Bio. Res. 51: 47. http://dx.doi.org/10.1186/s40659-018-0198-z

Khan M A, Munive S & Bonierbale M (2015). Early generation in vitro assay to identify potato populations and clones tolerant to heat. Plant Cell, Tissue and Organ Culture (PCTOC) 121: 45-52. https://doi.org/10.1007/s11240-014-0677-z

Kheiri F, Barzegar T, Ghahremani Z & Rabiei V (2016). Effects of chitosan coating and hot water treatment on postharvest characteristics of fruit pepper. J. Crop Improv. 18(3): 595-608

Mandal A B, Mondal R & Dutta P M S (2015). Seed enhancement through priming, coating and pelleting for uniform crop stand and increased productivity. Journal of the Andaman Science Association 20: 26-33

Martins J D L, Soratto R P, Fernandes A & Dias P H (2018). Phosphorus fertilization and soil texture affect potato yield. Rev. Caatinga 31: 541-550. http://dx.doi.org/10.1590/1983-21252018v31n302rc

Mohammadi K & Rokhzadi A (2012). An integrated fertilization system of canola (Brassica napus L.) production under different crop rotations. Ind. Crop. Prod. 37: 264-269. https://doi.org/10.1016/j.indcrop.2011.12.023

Mohammadina S, Asghari A, Hassanpanah D, Karimizadeh R & Shokouhian A A (2021). Determining the most stable potato genotypes using ammi yield stability analysis method. *J Agr Sci-Tarim Bili* 27(2): 146-154. DOI: 10.15832/ankutbd.574082

Muley A B, Shingote P R, Patil A P, Dalvi S G &Suprasanna P (2019). Gamma radiation degradation of chitosan for application in growth promotion and induction of stress tolerance in potato (Solanum tuberosum L.). Carbohydr. Polym. 210: 289-301. https://doi.org/10.1016/j.carbpol.2019.01.056

Noda T, Takahata Y, Sato T, Hisamatsu M & Yamada T (1995). Physicochemical properties of starches extracted from sweet potato roots differing in physiological age. *J. Agric. Food Chem* 43: 3016-3020

Olawuyi I F, Park J J, Lee J J & Lee W Y (2018). Combined effect of chitosan coating and modified atmosphere packaging on fresh- cut cucumber. Food Sci. Nutr. 7: 1043-1052. https://doi.org/10.1002/fsn3.937

Pawelzik E & Möller K (2014). Sustainable potato production worldwide: the Challenge to Assess Conventional and Organic Production Systems Potato Research 57: 273-290. https://doi.org/10.1007/s11540-015-9288-2

Petros A F Y & Zelleke H (2013). Genetic variability and association between agronomic characters in some potato (Solanum tuberosum L.) genotypes in SNNPRS, *Ethiopia. Int. J. Biodivers. Conserv* 5: 523-528. https://doi.org/10.5897/IJBC2013.0548

Pirasteh-Anosheh H & Emam Y (2019). The role of plant growth regulators in enhancing crop yield under saline conditions: from theory to practice. *Iranian Journal of Crop Sciences* 21: 188-209. https://doi.org/10.29252/abj.21.3.188

Porter J R & Semenov M A (2005). Crop responses to climatic variation. Philosophical Transactions of the Royal Society B: Biological Sciences 360: 2021-2035

Roosjen P P, Brede B, Suomalainen J M, Bartholomeus H M, Kooistra L & Clevers J G (2018). Improved estimation of leaf area index and leaf chlorophyll content of a potato crop using multi-angle spectral data–potential of unmanned aerial vehicle imagery. *Int J Appl Earth Obs Geoinf* 66: 14-26

Ravichandran G, Venkatasalam E, Muthuraj R & Manorama K (2015). A method to use very small size potato (Solanum tuberosum L.) tubers as seed. *Afr. J. Plant Sci.* 9: 352-359. https://doi.org/10.5897/AJPS2014.1189

Rocha I D S, Ma Y, Souza-Alonso P, Vosátka M, Freitas H & Oliveira R S (2019). Seed coating: A tool for delivering beneficial microbes to agricultural crops. Front. Plant Sci. 10: 1357. https://doi.org/10.3389/fpls.2019.01357

Rykaczewska K (2016). The potato minituber production from microtubers in aeroponic culture. Plant Soil Environ 62: 210-214. https://doi.org/10.17221/686/2015-PSE

Shaddel R, Hesari J, Azadmard-Damirchi S, Hamishehkar H, Fathi-Achachlouei B & Huang Q (2018). Use of gelatin and gum Arabic for encapsulation of black raspberry anthocyanins by complex coacervation. *Int. J. Biol. Macromol* 107: 1800-1810

Shinde S, Paralikar P, Ingle AP & Rai M (2020). Promotion of seed germination and seedling growth of Zea mays by magnesium hydroxide nanoparticles synthesized by the filtrate from Aspergillus niger. *Arab. J. Chem* 13: 3172-3182. https://doi.org/10.1016/j.arabjc.2018.10.001

Song C, Guan Y, Wang D, Zewudie D & Li F-M (2014). Palygorskite-coated fertilizers with a timely release of nutrients increase potato productivity in rain-fed cropland. Field Crops Res 166: 10-17 https://doi.org/10.1016/j.fcr.2014.06.015

Soratto R P & Fernandes A M (2016). Phosphorus effects on biomass accumulation and nutrient uptake and removal in two potato cultivars. *Agron. J.* 108: 1225-1236. https://doi.org/10.2134/agronj2015.0333

Sunohara Y & Matsumoto H (2004). Oxidative injury induced by the herbicide quinclorac on Echinochloa oryzicola Vasing. and the involvement of antioxidative ability in its highly selective action in grass species. Plant Sci. 167: 597-606

Szerement J, Ambrożewicz-Nita A, Kędziora K & Piasek J (2014). Use of zeolite in agriculture and environmental protection. A short review Вісник Національного університету Львівська політехніка Теорія і практика будівництва pp.172-177

Taghizoghi S, Soltani E, Allahdadi I & Sadeghi R (2018). The effect of different seed coating treatments on the seed germination of canola under drought and salinity stresses: using modeling approach. *Journal of Crops Improvment* 20(3): 577-593

Taheri-Soudejani H, Heidarpour M, Shayannejad M, Shariatmadari H, Kazemian H & Afyuni M (2019). Composts containing natural and mg-modified zeolite: the effect on nitrate leaching, drainage water, and yield. Clean–Soil, Air, Water 47(8): 1800257. https://doi.org/10.1002/clen.201800257

Wang X, Guo T, Wang Y, Xing Y, Wang Y & He X (2020). Exploring the optimization of water and fertilizer management practices for potato production in the sandy loam soils of Northwest China based on PCA. Agric. Water Manag. 237: 106180 https://doi.org/10.1016/j.agwat.2020.106180

Journal of Agricultural Sciences
(TarimBilimleriDergisi)

J AgrSci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

Genoprotective Role of Purslane Methanol Extract Against Somatic Mutations Induced by Bifenthrin, a Third Generation Prethyroid Insecticide

Halit KIZILET[a], Handan UYSAL[b*]

[a]*Department of Cardiology, Erzurum Training and Research Hospital, 25100, Erzurum, TURKEY*
[b]*Department of Biology, Faculty of Science, Atatürk University, 25240, Erzurum, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Handan UYSAL, E-mail:hauysal@atauni.edu.tr
Received: 20 February 2020 / Revised: 20 October 2021 / Accepted: 20 October 2021 / Online: 15 October 2022

Cite this article
KIZILET H, UYSAL H (2022). Genoprotective Role of Purslane Methanol Extract Against Somatic Mutations Induced by Bifenthrin, a third Generation Prethyroid Insecticide. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 28(4):583-591. DOI: 10.15832/ankutbd.883842

ABSTRACT
In this study, in vitro and in vivo genotoxic effects of Bifenthrin (BIF), an important insecticide used in agricultural production, storage and processing, were investigated. The genoprotective properties of purslane (*Portulaca oleracea* L.) against the genotoxic effects of BIF were also determined by using the methanol (POmet) extract of this plant. In vivo experiments were performed with somatic mutation and recombination test (SMART) in *Drosophila melanogaster*. In in vitro studies, human peripheral blood cultures were prepared and different concentrations of BIF were applied to lymphocyte cells in accordance with the procedure of both the micronucleus (MN) and sister chromatid exchange (SCE) assay. The results obtained from all applied tests showed that BIF is genotoxic and induces chromosomal mutations. Later, another experiment was conducted and it was determined that the genotoxic effects of BIF were reduced with POmet (1:1 v/v). This result, which was observed in all in vivo and in vitro tests, shows that purslane plant is a potent radical scavenger. Due to the healing properties of POmet, gas chromatography-mass spectrometry (GS-MS) method was used to determine the components in its content. Some of the components found in the highest ratio in this extract are γ-sitosterol (21.86%), 13-docosenamide, (13.30%), palmitic acid (12.85%), stigmasteol (6.64%), campesterol (5.69%), linoleicacid (5.46%) and 2-methyl-1-hexadecanol (3.88%).

Keywords: *Drosophila melanogaster*, *Portulaca oleracea*, Household insecticides, Human peripheral blood cultures, Gas chromotography-mass spectrometry

1. Introduction

In cases where agricultural products cannot be protected from diseases and pests, it becomes difficult to obtain healthy and sufficient food. Today, about 20% of the world's grain production is lost in the pre-harvest and post-harvest stages (Durmuşoğlu et al. 2010). One of the most important causes of these losses is pests that infect agricultural products and reduce yield. Pesticides are still used as the most effective method to fight with pests (Singh et al. 2020).

Pesticides are chemicals that are used to reduce the devastating effects of live forms on human and animals and on crops such as insects, rodents, wild weeds and fungi to damage inflicted or reduced nutritional value for the food resources production, storage and consumption (Meister 1999; Pazır & Turan 2017). The pesticides group most widely used against pests are insecticides. Insecticides, which are a sub-group of pesticides used in many areas and providing control of harmful organisms, are chemical compounds used in agricultural production, in the storage of products and at homes for the purpose of killing harmful insects or preventing their reproduction. Insecticides are of great help in controlling harmful insects, which can increase product loss up to 100%. However, since insecticides are not specific, they affect not only target organisms but also non-target organisms (Sayılı & Akman 1994; Özyurt et al. 2018). The unconscious use of insecticides has been shown to cause the destruction of beneficial organisms and endanger genetic diversity (Güngör 2003).

Wild plant and animal populations are decreased as a result of intensive use of insecticides in agriculture. Therefore, it was found to be endangered and started destruction of the beneficial organisms and genetic diversity. Insecticides are poisonous compounds that are purposely released into the environment for the specific purpose of killing insects. The broad use of insecticides represents a potential risk to humans and the environment (Cantelli-Forti et al. 1993; Tiryaki et al. 2010). 30% of the world synthetic insecticides consist of pyrethroids and they are often preferred because despite being highly toxic to the target organisms, they are less toxic to birds and mammals (Mazmancı et al. 2008). Pyrethroid is an organic compound similar

to natural pyrethrins formed by pyrethrum flowers (*Chrysanthemum cinerariaefolium* and *C. coccineum*). Pyrethroids are now the bulk of commercial house hold insecticides (Robert 2002; Dev 2017).

Bifenthrin (BIF), a member of the synthetic pyrethroid family of pesticides, is a third generation insecticide used extensively in agricultural production. This group of pyrethroids is not found naturally and is more resistant to light and exhibits higher toxic activity (Mokrey & Hoagland 1989). BIF, with a half-life of approximately 7 days to 8 months, is insoluble in water or very slightly soluble, leaving a lot of residues in the soil (EXTOXNET 1996). In our country and in the world, they are used especially against aphids, fire ants, lice, fleas, spiders, ticks and flies, against ornamental plants, hops, raspberry, corn and cotton pests, as well as in homes, workplaces and schools (EPA 2010).

DNA disruption and oxidative stress play an important role in numerous cancers and pathological disorders, including carcinogenesis and aging (Soltani et al. 2009; Jacobsen-Pereira et al. 2018). Numerous experiments have shown that plant-derived natural compounds demonstrate defensive behaviors against genotoxicity caused by oxidative stress (Plazar et al. 2008; El-Nekeety et al. 2017; Rahmouni et al. 2018). Fruits and vegetables include many types of phytochemicals with antioxidant, anti-mutagenic and anti-carcinogenic properties (Arora et al. 2002; Shahidi & Ambigaipalan 2015; Janet al. 2018). Despite the development of medical science in a tremendous way in the 20th century, plants are still used in traditional medicine (Jain et al. 2007; Izquierdo-Vega et al. 2017).

Purslane (*Portulaca oleracea* L.) is a wide spread wild edible plant with green leaves and is used as a medicinal plant. It is used as salad, vegetable and medicinal plants in all parts of the world. Purslane contains more omega-3 fatty acids (α-linolenic acid in particular) than any other leafy vegetable plant. Since it is used to cure many diseases, the World Health Organisation named the plant “Global Panacea”, which means “good for every disease” (Kızılet & Uysal 2018).

In this study, the genoprotective of methanol extracts of purslane (POmet) against the possible genotoxic effects of BIF were investigated. BIF is a powerful insecticide used against insects, their eggs and larvae, especially in agricultural areas and warehouses where agricultural products are stored. However, this insecticide is also used against insects in crowded environments such as homes, schools and workplaces where people live. In this case, humans as well as insects are exposed to insecticides in both agricultural and living areas. In this study, it was aimed to determine the genotoxic effects of BIF in both *Drosophila melanogaster*, an invertebrate insect species, and humans. The genotoxic effects of BIF were determined in Drosophila by in vivo Somatic Mutation and Recombination Test (SMART). In vitro Sister Chromatid Exchange Test (SCE) and Micronucleus Test were also used to determine the genotoxic effects of BIF in humans. POmet was applied to resolve the genotoxic effects of BIF in the highest application group of all three testing techniques. Additionally, the chemical contents of the methanol extracts of *Portulaca oleracea* was also defined by gas chromatography-mass spectrometry (GS-MS) method.

2. Material and Methods

2.1. Chemicals

The Bifenthrin (CAS No:82657-04-3, state powder purity 96%), methanol (CAS No:67-56-1),dimethyl sulfoxide (CAS No:67-68-5), ethyl methane-sulfonate (CAS No:62-50-0), 5–bromo–2–deoxyuridine (CAS No: 59-14-3), potassium chloride (CAS No: 7447-40-7), giemsa (CAS No: 51811-82-6), bisbenzimide H 33342 (CAS No:23491-52-3), sodium citrate (CAS No: 6132-04-3), sodium chloride (CAS No:7647-14-5), acetic acid (CAS No: 64-19-7),chromosome medium (CAS No: F 5023), colchicine (CAS No: L 6221) and cytochalasin-B (CAS No: 14930-96-2) were purchased from the Sigma-Aldrich Company while Drosophila Instant Medium has been acquired from the Carolina Biological Supplies Company.

2.2. Preparation of the methanol extracts of Portulaca oleracea L.

Purslane plant, which was determined to be used as an antigenotoxic agent in the experiments, was collected from the vicinity of Hasancık village in Adıyaman province and from an altitude of 600-900 meters. Methanol extract (POmet) was prepared with all of the above-ground organs of the purslane plant, such as stem, leaves and flowers, which were collected in its natural environment, during the flowering period and from lands far from agriculture (Uysal et al. 2015). POmet extract was dissolved with DMSO in the course of applications.

2.3. GC-MS System and conditions

Chromatographic analyzes were conducted on the Agilent 7820 A gas chromatography system. Various temperature programs have been studied for the GC-MS process. Components determined according to POmet spectrum. GS-MS analysis of POmet according to Kızılet et al. (2019).

2.4. Experimental animals and laboratory condition

The selected two Drosophila strains (mwh and flr^3) were used in a previous study for somatic mutation and recombination test

(SMART) (Kızılet & Uysal 2019). The flies were kept according to Uysal et al. (2006) laboratory condition.

2.5. Somatic mutation and recombination test (SMART)

The method for somatic mutation and recombination test (SMART) was determined by Graf et al. (1984). According to this protocol, it was adapted to our laboratory conditions and applied. For this purpose, 4, 5, 6 and 7 ppm BIF application groups have been created and highest (7 ppm) BIF concentration was tested antigenotoxically with 1% POmet. The data is analyzed in compliance with the multiple-decision protocol of Frei &Würgler (1995).

2.6. Donors for peripheral blood assays

All donors were determined according to Kızılet et al. (2019). Permission for the study was sought from the Erzurum Provincial Training and Research Hospital Local Ethics Committee (Number: 37732058-53/2467/BEAH KAEK 2015/9-67) and the rules of the committee were observed during the inquiry. Documented informed consent was received from all patients who engaged in the study.

2.7. *Sister chromatid exchange (SCE) assay*

To determine the genotoxic effects of BIF at different concentrations (50, 100, 250 and 500 ppm) and equal concentration of 500 ppm BIF + POmet by SCE, 1–2.5 mL of human peripheral blood was added to 5 mL chromosome media. Different sets were prepared for each concentration and each donor. 5–bromo–2–deoxyuridine (BrdU) was added to all experiment sets at a final concentration of 10-4M. All substances added to the tubes were sterilized to prevent contamination during the experimental stage. The experimental sets were incubated for 72 hours at 37 °C in dark incubators. At 70 hours of the experiment, colchicine was added to the medium at a final concentration of 0.5 µg/mL to stop the mitosis at the metaphase stage. At the end of the 72 hours incubation, the tubes were centrifuged and removed the supernatants. Hypotonic solution (0.075 M KCl) was added onto pellet and incubated for 30 minutes in dark incubator at 37 °C. At the end of the time, the supernatant was removed after centrifugation. The remaining pellet was washed with a fixative consisting of a methanol/acetic acid (3:1 v/v) mixture. This procedure was repeated three times.

Peripheral blood smears were then prepared from the remaining pellet and allowed to dry in the dark for 3 days. These preparations were stained according to Rooney & Czepulkowski 1986 fluorescence plus Giemsa method. For this purpose, preparations were wetted with 0.5 µg/mL bisBenzimide (Hoechst 33342) for 20 minutes. Wet preparations were incubated for 1 hour under 366 nm UV. The preparations were then kept in a 2 X SSC (1:1 v/v 0.03 M sodium citrate/0.03 M sodium chloride) solution in a 65 °C water bath for 1 hour. Finally, the preparations were stained with 5% giemsa.

Preparations were examined at 1000 X magnification and sister chromatid changes were recorded on chromosomes in metaphases. The data obtained were analyzed with SPSS package program.

2.8. Micronucleus (MN) assay

Experimental setups for MN test were prepared by adding 5 mL chromosome medium to the test tubes containing 50, 100, 250 and 500 ppm BIF and equal concentrations of BIF+POmet (only for 500 ppm BIF). All test tubes were allowed to incubate for 72 hours. In the experimental procedure, unlike SCE, BrdU was not added to the tubes. All solutions added to the tubes were sterilized to prevent contamination. Cytochalasin-B was added to the tubes at a final concentration of 3 µg/mL at 48 hours of incubation (In order to have binucleated cells). The tubes were centrifuged at the end of the incubation and hypotonic fluid was applied to the pellet (0.075 M KCl) and incubated for 15 minutes at 37 °C. At the end of the period, the tubes were centrifuged and remaining pellets washed with fixative solution (1:3 v/v acetic acid-methanol) 3 times. Then, the supernatant was discarded and smear preparations were made from the pellet and stained with 4% giemsa. 1000 cells from each preparation were counted and investigated under a light microscope at 400 X magnification. The data obtained were analyzed with SPSS package program.

3. Results

3.1. SMART findings

The findings obtained from distilled water, dimethyl sulfoxide (DMSO), EMS, BIF and 7 ppm BIF+1% POmet application groups for the normal wings (mwh/flr^3) and serrate wings (mwh/TM3) phenotypes are shown in Table 1. As shown in Table 1, there were no significant differences between the values, which were obtained with distilled water and 1ppm DMSO applications for both normal and serrate wing phenotypes. When the BIF application groups (4, 5 and 6 ppm) were compared with the DMSO application group, inconclusive (i) results were observed for all spots, although increased mutation frequency in two phenotypes.

Table 1- Wing spot test data obtained after exposure of BIF and BIF + POmet

Compound concentration (ppm)	*Number of wings (N)*	*Small single spots (1–2 cells) (m = 2)*			*Large single spots (>2 cells) (m = 5)*			*Twin spots (m = 5)*			*Total mwh Spots (m = 2)*			*Total spots (m = 2)*			*Clone induction frequency (CIF)*
		No	*Fr.*	*D*	*No*	*Fr.*	*D*	*No*	*Fr.*	*D*	*No*	*Fr.*	*D*	*No*	*Fr.*	*D*	
Normal wings (mwh/flr³)																	
Distilled water	80	8	(0.10)		1	(0.01)		0	(0.00)		9	(0.11)		9	(0.11)		0.46
DMSO	80	9	(0.11)	i	1	(0.01)	i	0	(0.00)	i	10	(0.13)	i	10	(0.13)	i	0.51
EMS	80	29	(0.36)	+	11	(0.14)	+	3	(0.04)	i	39	(0.49)	+	43	(0.54)	+	2.00
4 BIF	80	11	(0.14)	i	0	(0.00)	-	0	(0.00)	-	11	(0.14)	i	11	(0.14)	i	0.56
5 BIF	80	12	(0.15)	i	1	(0.01)	i	0	(0.00)	-	13	(0.16)	i	13	(0.16)	i	0.66
6 BIF	80	18	(0.23)	i	0	(0.00)	-	0	(0.00)	-	18	(0.23)	i	18	(0.23)	i	0.92
7 BIF	80	21	(0.26)	+	0	(0.00)	-	0	(0.00)	-	21	(0.26)	+	21	(0.26)	+	1.07
7BIF+%1POmet	80	12	(0.15)	i	0	(0.00)	-	0	(0.00)	-	12	(0.15)	i	12	(0.15)	i	0.61
Serrate wings (mwh/TM3)																	
Distilled water	80	7	(0.09)		0	(0.00)					7	(0.09)		7	(0.09)		0.35
DMSO	80	7	(0.09)	i	0	(0.00)	i	Balancer			7	(0.09)	i	7	(0.09)	i	0.35
EMS	80	19	(0.24)	-	10	(0.13)	+	chromosome			29	(0.36)	+	29	(0.36)	+	1.49
4 BIF	80	9	(0.11)	i	0	(0.00)	-	TM3 does			9	(0.11)	i	9	(0.11)	i	0.46
5 BIF	80	9	(0.11)	i	0	(0.00)	-	not carry			9	(0.11)	i	9	(0.11)	i	0.46
6 BIF	80	10	(0.13)	i	0	(0.00)	-	the flr3			10	(0.13)	i	10	(0.13)	i	0.51
7 BIF	80	11	(0.14)	i	0	(0.00)	-	mutation.			11	(0.14)	i	11	(0.14)	i	0.56
7BIF+%1POmet	80	9	(0.11)	i	0	(0.00)	-				9	(0.11)	i	9	(0.11)	i	0.46

No: number of clones; Fr: frequency; D: statistical diagnosis +: positive; -: negative; i: inconclusive; m:multiplication factor

However, positive (+) results were observed in the highest BIF application group (7 ppm) due to the increase in the number of mutant clones for the normal wing phenotype ($P<0.05$). The clone induction frequency (CIF) was also calculated according to the values obtained in this study. While the CIF value for mwh/flr^3 genotype (normal wing phenotype) in the application of 7 ppm BIF was 1.07, this value for the distilled water and DMSO control groups was measured as 0.46 and 0.51 (Table 1).

In addition, as shown in Table 1, 1% POmet application reduced the frequency of mutations in all spots. Important variations were found between the values obtained with 7 ppm BIF and 7 ppm BIF+1% POmet applications for both normal and serrate wing phenotypes ($P<0.05$). The CIF calculated as 1.07 for mwh/flr^3 genotype (normal wing phenotype) in the 7 ppm BIF application was found to be 0.61 for 7 ppm+1% POmet application group. In the mwh/TM3 genotype (serrate wing phenotype), the CIF values were found to be 0.46 and 0.56 for the same application groups (Table 1). The decrease in CIF values in both normal and serrate wing phenotype was found statistically significant in 7 ppm+1% POmet application group.

3.2. SCE findings

The SCE values, as a result of the application to human peripheral lymphocyte cells at 50, 100, 250 and 500 ppm BIF concentrations, were detected as 4.13±0.01, 4.62±0.01, 6.50±0.03 and 7.05±0.02 respectively. The results were statistically significant ($P<0.05$). These values were determined at 3.60±0.02 for distilled water, 3.70±0.01 for negative control group DMSO and 25.96±0.02 for EMS (positive control group) (Table 2). The difference between positive control group and negative control groups are statistically significant ($P<0.05$). While the replication index (RI) values were accounted, these values decreased in all the application groups. The SCE value of BIF + POmet application at the rate of 1:1 to determine the therapeutic effect of purslane has decreased from 7.05±0.02 to 3.80±0.01 (Table 2). According to these values obtained from BIF + POmet application, the decrease observed for SCE was statistically significant ($P<0.05$).

Table 2- Statistical significance of SCE induction after exposure to four concentrations of BIF and BIF + POmet

Application Groups	*Concentration*	*RI*	*SCE/cell (Average)*	*Min.-Max. SCE*
Distilled Water	-	2.41±0.03	3.60±0.02	1-11
DMSO	%2	2.24±0.07	3.70±0.01	1-10
EMS	2mM	2.25±0.09	25.96±0.02	8-36
Bifenthrin (ppm)	50	2.33±0.08	4.13±0.01*	1-9
	100	2.01±0.07*	4.62±0.01*	1-13
	250	1.95±0.06*	6.50±0.03*	1-11
	500	1.90±0.04*	7.05±0.02*	4-13
BIF + POmet	1:1	2.25±0.11**	3.80±0.01**	1-10

*: Statistical difference is significant according to DMSO at the 0.05 level, **: Statistical difference is significant according to 500 ppm BIF at the 0.05 level.

3.3. MN findings

MN frequencies measured after exposure to varying concentrations (50, 100, 250 and 500 ppm) of BIF in human peripheral lymphocyte cells were detected as 1.100±0.73, 1.475±0.85, 1.850±0.44 and 2.050±0.68, respectively. These values were determined at 0.700±0.38 for distilled water, 0.825±0.65 for DMSO and 3.175±1.40 for EMS (Table 3). The results were statistically significant between the application and all control groups (P<0.05). In addition, as shown in Table 3 NBI decreased in all BIF application groups. NBI was found to be 1.54±0.17 in the DMSO negative control group. In the highest BIF application group, this value decreased to 1.25±0.18 (P<0.05).

In the BIF + POmet application, the MN frequency decreased from 2.050±0.68 to 0.875±0.72 (P<0.05). In this application NBI value increased from 1.25±0.18 to 1.56±0.19 (P<0.05).

Table 3- Statistical significance of MN induction after exposure to four concentrations of BIF and BIF + POmet

Application Groups	*Concentration*	*Investigated of binucleated cells*	*Number of MN within binucleat*			*MN frequency±S.E.*	*Nuclear division index (NDI)±S.E.*
			(1)	*(2)*	*(3)*		
Distilled water	-	4000	28	-	-	0.700±0.38	1.52±0.15
DMSO	%2	4000	33	-	-	0.825±0.65	1.54±0.17
EMS	2mM	4000	88	12	5	3.175±1.40	1.48±0.18
Bifenthrin (ppm)	50	4000	38	3	-	1.100±0.73	1.55±0.13
	100	4000	51	4	-	1.475±0.85*	1.46±0.21
	250	4000	66	4	-	1.850±0.44*	1.38±0.20*
	500	4000	71	4	1	2.050±0.68*	1.25±0.18*
BIF+POmet	1:1	4000	35	-	-	0.875±0.72**	1.56±0.19**

*: Statistical difference is significant according to DMSO at the 0.05 level, **: Statistical difference is significant according to 500 ppm BIF at the 0.05 level.

3.4. C-MS findings

In this study, the components found in P. oleracea were determined by GC/MS method and listed in Table 4. 23 components (99.305%) were identified for POmet. The most common compounds in POmet are γ-sitosterol (peak no. 22), 13-docosenamide (peak no. 13), (Z) and palmitic acid (peak no. 5). The total amount of these three compounds is 48.001%. Compounds such as phytol, linoleic acid, campesterol and stigmasterol were also found in different rates in the content of POmet at All of these compounds induce antigenotoxicity. Therefore, POmet can be considered as a strong radical scavenger against BIF insecticide.

Table 4- Chemical compositions of the POmet

Peak number	*Retantion time(min)*	*Component*	*Molecular formula*	*Molecular weight (g/mol)*	*% ratio in total component*
1	16.137	2,4 dihydroxy-2,4,6-trimethylcyclohexylidene-acetic acid 8-lactone	C11H16O3	196.10	2.034
2	16.727	2-cis-9-octadecenloxyethanol	C20H40O2	312.30	0.863
3	16.856	Hexahydrofamecyl acetone	C18H36O	268.27	1.067
4	18.188	Palmitic acid methyl ester	C17H34O2	270.25	0.768
5	19.104	Palmitic acid	C16H32O2	256.24	12.848
6	21.420	Phytol	C20H40O	296.30	4.101
7	22.121	Linoleic acid	C18H32O2	280.24	5.455
8	29.612	Behenicalchol	C22H46O	326.35	2.008
9	30.026	Dipalmitin	C35H63O5	568.50	1.301
10	30.481	Diisoactyl phthalate	C24H38O4	390.27	2.352
11	31.281	Docosyl acetate	C24H48O2	368.36	2.745
12	32.264	2-methyl-1-hexadecanol	C17H36O	256.27	3.880
13	33.233	13-docosenamide,(Z)=(Erucylamide)	C22H43NO	337.33	13.297
14	33.423	2-bromo octadecanol	C18H35BrO	346.18	2.177
15	33.870	Methyl epoxystearate	C19H36O3	312.26	0.917
16	34.069	Dihyaroxanthin	C17H24O5	308.16	1.303
17	35.163	14-octadecenal	C18H34O	266.26	1.247
18	36.502	α-tocopherol	C29H50O2	430.38	1.245
19	37.714	Campesterol	C28H48O	400.37	5.692
20	38.113	Stigmasterol	C29H48O	412.37	6.642
21	38.245	Ethyl iso-allocholate	C26H44O5	436.31	2.157
22	38.949	γ-sitosterol	C29H50O	414.38	21.856
23	39.467	Olean-12-en-3-one	C30H48O	424.37	3.350
Total component percentage ratio					99.305

4. Discussion

Physical factors such as solar radiation, x-rays and a wide variety of chemicals can affect cellular DNA. Oxidative stress can cause damage to lipids, proteins, and nucleic acids, resulting in improvements in signal transduction path ways, gene expression, cell mutation, and cell death (Demirci et al. 2008; Popracet et al. 2017). Pesticides are a significant group of human-made hazardous chemicals. Their probable synergistic or antagonistic side effects on humans have not yet been thoroughly studied (Demsia et al. 2007). Over the three decades since the launch of the first compounds with adequate photo-stability for agricultural use, synthetic pyrethroids have been valuable instruments for pest control in agriculture, public health and a wide range of household applications.

However, pyrethroids are not only neurotoxic to plants, but also to mammals (Soderlund et al. 2002; Costa 2015). Synthetic pyrethroids are neuropoisons that function on axons in peripheral and central nervous systems by interfering with sodium channels in mammals or insects (IPCS 1990). BIF is chemically classified as a pyrethroid. BIF interferes with the nervous system of the insect when it is eaten or touched (Miller & Salgado 1985; Yanget et al. 2018). It is more harmful to insects than it is to humans, since insects have lower body temperatures and smaller body sizes. Therefore, it can show toxic effects at high doses.

In this study, while 7 ppm BIF was showing genotoxic effects on *D. melanogaster*, upwards of 50 ppm BIF were showing genotoxic effects on human peripheral blood cells. In conclusion; the higher concentrations of BIF were caused by somatic mutation and chromosomal defects in this study. Our findings are in accordance with other research performed in a similar fashion.

BIF was determined reduce the motor activity in rats at high doses (Wolansky et al. 2007; Scollon et al. 2011). BIF alone is not harmful to rodent nerve cells at a concentration of 10-3 M. However, a house hold use substance containing BIF has been found to be neurotoxic at concentrations between 10-6 and 10-7 M. The house hold formulation of the BIF insecticide decreased the viability of rodent nerve cell cultures, while the BIF did not. Both the formulation and the active ingredient decreased the development of neuritis in vitro, although the effect of the formulation was more extreme (Tran et al. 2006). These findings indicate that inert ingredients would strengthen the developmental neurotoxic effects of BIF. According to Walker & Keith (1992) evidence of mutagenic effects from exposure to BIF are inconclusive. Studies of mouse white blood cells were positive for gene mutation. However, other tests of BIF's mutagenic effects, including the Ames test and experiments in liver rat bone marrow cells, were negative.

Sadowska-Woda (2010) has shown that BIF-induced oxidative stress induces increased lipid peroxidation and reduced antioxidant function in human peripheral blood. DNA disruption and oxidative stress play an important role in numerous cancers and pathological disorders, including carcinogenesis and aging (Soltani et al. 2009; Birch-Machin & Bowman 2016). There has lately been a great deal of interest in the anti-mutagenic and anti-carcinogenic ability of plant-derived compounds and natural food ingredients (Bhuvaneswari 2005; Xu et al. 2007; Shahidi 2009). The mechanism of defence of these structurally very diverse compounds may be multifactorial, since the anti-mutagenic behaviour of most of these chemicals is linked to their scavenging properties (Larson 1988). *P. oleracea* is a rich source of omega-3 fatty acids, gallotannins, kaempferol, quercetin, apigenin, and glutathione (Sharmaet et al. 2009; Gharneh & Hassandokht 2012; Naeem & Khan 2013). Purslane contains in large quantities l-norepinephrine (in fresh leaf 0.25%) that neurohormone of helpful to brain fatigue. It has the highest omega-3 fatty acid content of all leafy vegetables (Kumlay et al. 2010). Antioxidant and antimutagenic functions of extracts of *P. oleracea* have been previously demonstrated and are proposed to be linked to their constituents, such as A, B1, B2, C, niasinamid, nicotinic acid, α-tocopherol, β-carotene, β-alanin, β-cyanine, magnesium, calcium, potassium, iron, omega-3 fatty acids, gallotannins, kaempferol, quercetin, apigenin, flavonoids, ascorbic acid and glutathione (Simopoulos 2004).

In our study was determined that POmet, which was used in the experiment, inhibited somatic mutation in *D. melanogaster* and decreased the SCE and MN rate in human peripheral lymphocytes. Our result is supported by similar studies of purslane. Water extract of purslane significantly inhibited the DNA breakage (Behravan et al. 2011) and it's leaves stems and roots showed very strong antioxidant at in vivo and in vitro experiments on rabbits (Yu et al. 2007). Yen et al. (2001) have demonstrated that *P. oleracea* extract has antimutagenic activity against 2-amino-3-methylimidazo (4,5-f) quinoline (IQ) as a mutagenagent.

In POmet sample (Table 4), 99.305% of the total extract was identified, predominating γ-sitosterol (21.856%), 13-docosenamide, (Z) (13.297%), palmitic acid (12.848%), stigmasteol (6.642%) campesterol (5.692%), linoleic acid (5.455%) and 2-methyl-1-hexadecanol (3.880%).

β-sitosterol and γ-sitosterol are most widely found sterols in the plant. It has also been documented that the volume and function of components of the extrinsic apoptic pathway in human lung and breast adenocarcinoma cells can be impaired by γ-sitosterol (Balamurugan et al. 2011). γ-sitosterol and 13-docosenamide, (Z) also shows high antimicrobial activity (Kanimozhi & Bai 2012; Rukshana et al. 2017). Palmitic acid is the most common saturated fatty acid present in animals and plants. It is the first synthesized fatty acid in the formation of fatty acids in living things and the longer fatty acids are produced from it.

Palmitic acid, like other fatty acids, is not free in nature. Reduces hydrogen peroxide formation (Aydın 2009). Stigmasterol and campesterol are a group of phytosterols. Stigmasterol is an unsaturated plant sterol. It is also useful for the treatment of many tumours, including ovarian, lung, breast and colon cancers. It also has strong antioxidant, hypoglycaemic and thyroid inhibiting effects (Panda et al. 2009). Linoleic acid is an unsaturated essential fatty acid. Linoleic acid exhibits potent antioxidant activity as opposed to α-tocopherol (vitamin E), a known antioxidant (Ha et al. 1990).

Consequently, the higher concentration of BIF caused somatic mutation and chromosomal defects in this study. As a result of our study, the increase in mutations in SMART, and SCE and MN in in vitro tests, respectively, can be assumed as a marker of damage in genetic material. As seen above in similar studies, this genetic damage is caused by oxidative stress. Our results and previously conducted studies indicate that the effect of natural components of purslane is radically scavenging on the genotoxicagent BIF. Additionally, the repair of this damage by POmet indicates that purslane can be a strong antigenotoxic agent. This scavenger effect is due to the high concentration on the content of the compound in POmet.

References

Arora A, Sairam R K & Srivastava G C (2002). Oxidative stress and antioxidative systems in plants. *Current Science* 82: 1227-1238. https://www.jstor.org/stable/24107045

Aydın S (2009). The identification of antioxidant, antimicrobial effects and fatty acids composition of *Terfezia boudieri chatin* and *Lactarius vellereus* (Fr.). Master thesis (Published), Selçuk University, TR

Balamurugan R, Duraipandiyan V & Ignacimuthu S (2011). Antidiabetic activity of γ-sitosterol isolated from *Lippianodiflora* L. in streptozotocin induced diabetic rats. *European Journal of Pharmacology* 667(1-3): 410-418.https://doi.org/10.1016/j.ejphar.2011.05.025

Behravan J, Mosafa F, Soudmand N, Taghiabadi E, Razavi B M & Karimi G (2011). Protective effects of aqueous and ethanolic extracts of *Portulacaoleracea* L. Aerial parts on H2O2- induced DNA damage in *Lymphocytes* by comet assay. *Journal of Acupuncture and Meridian Studies* 4: 193-197.https://doi.org/10.1016/j.jams.2011.09.008

Bhuvaneswari V, Phil M, Abraham S K & Nagini S (2005). Combinatorial antigenotoxic and anticarcinogenic effects of tomato and garlic through modulation of xenobiotic-metabolizing enzymes during hamster buccal pouch carcinogenesis. Nutrition 21: 726-731.https://doi.org/10.1016/j.nut.2004.05.024

Birch-Machin M A & Bowman A (2016). Oxidative stres and ageing. *British Journal of Dermatology* 175: 26-29. https://doi.org/10.1111/bjd.14906

Cantelli-Forti G, Paolini M & Hrelial P (1993). Multiple end point procedure to evaluate risk from pesticides. *Environmental Health Perspectives* 101: 15-20.https://doi.org/10.1289/ehp.93101s315

Costa L G (2015). The neurotoxicity of organochlorine and pyrethroid pesticides. *Handbook of Clinical Neurology* 131: 135-48.https://doi.org/10.1016/B978-0-444-62627-1.00009-3

Demirci M, Hiller K A, Boslb C, Gallerb K, Schmalzb G & Schweiklb H (2008). The induction of oxidative stress, cytotoxicity, and genotoxicity by dental adhesives. *Dental Materials* 24: 362-371.https://doi.org/10.1016/j.dental.2007.06.009

Demsia G, Vlastosa D, Goumenoub M & Matthopoulos D P (2007). Assessment of the genotoxicity of imidacloprid and metalaxyl in cultured human lymphocytes and rat bone-marrow. *Mutation Research/Genetic Toxicology and Environmental* 634: 32-39.https://doi.org/10.1016/j.mrgentox.2007.05.018

Dev S (2017). Insecticides of natural origin.1st Edition. Available on Taylor & Francis eBooks, Routledge, London. ISBN 9783718659135

Durmuşoğlu E, Tiryaki O & Canhilal R (2010). Pesticide Use, Residue and Persistence Problems in Turkey, In: VII. Türkiye Ziraat Mühendisliği Teknik Kongresi, 11-15 Ocak, Ankara, Bildiriler Kitabı 2: pp. 589-607 (In Turkish)

El-Nekeety A A, Abdel-Wahhab K G, Abdel-Aziem S H, Mannaa F A, Hassan N S & Abdel-Wahhab M A (2017). Papaya fruits extracts enhance the antioxidant capacity and modulate the genotoxicity and oxidative stress in the kidney of rats fed ochratoxin A-contaminated diet. *Journal of Applied Pharmaceutical Science* 7(07): 111-121. DOI: 10.7324/JAPS.2017.70718

EPA (2010). Environmental Protection Agency. Bifenthrin Summary Document. http://www.regulations.gov/search/Regs/home.html

EXTOXNET (1996). Extension Toxicology Network. Pesticide Information Profiles - Bifenthrin. http://extoxnet.orst.edu/pips/bifenthr.htm

Frei H & Würgler F E (1995). Optimal experimental design and sample size for the statistical evaluation of data from somatic mutation and recombination test (SMART) in *Drosophila. Mutation Research* 334: 247-258.https://doi.org/10.1016/0165-1161(95)90018-7

Gharneh H A A & Hassandokht M R (2012). Chemical composition of some Iranian purslane (Portulacaoleracea) as a leafy vegetable in south parts of Iran. *Acta Horticulturae* 944: 41-44. DOI: 10.17660/ActaHortic.2012.944.4

Graf U, Würgler F E, Katz A J, Frei H, Juon H, Hall C B & Kale P G (1984). Somatic mutation test in Drosophila melanogaster. *Environmental and Molecular Mutagenesis* 6: 153-188.https://doi.org/10.1002/em.2860060206

Güngör B Ö (2003). Agricultural pollution and removal methods, In: V. Ulusal Çevre Mühendisliği Kongresi, Ankara, 1- 4 Ekim 2003 (In Turkish)

Ha Y L, Storkson J & Pariza M W (1990). Inhibition of benzo(a) pyrene-induced mouse forestomach neoplasia by conjugated dienoic derivatives of linoleic-acid. *Cancer Research* 50(4): 1097-1101

IPCS (International Programme on Chemical Safety) (1990). International Programme on Chemical Safety Environmental Health Criteria 98 Tetrametrin. World Health Organization, Geneva. https://www.who.int/ipcs/en/

Izquierdo-Vega J, Morales-González J, Sánchez Gutiérrez M, Betanzos-Cabrera G, Sosa-Delgado S, Sumaya-Martínez M, Morales-González Á, Paniagua-Pérez R, Madrigal-Bujaidar E & Madrigal-Santillán E (2017). Evidence of some natural products with antigenotoxic effects. Part 1: fruits and polysaccharides. *Nutrients* 9(2): 102.https://doi.org/10.3390/nu9020102

Jacobsen-Pereira C H, DosSantos C R, Maraslis F T, Pimentel L, Feijó A J L, Silva C I,Medeiros G S, Zeferino R C, Pedrosa R C & Maluf S W (2018). Markers of genotoxicity and oxidative stress in farmers exposed to pesticides. *Ecotoxicology and Environmental Safety* 148: 177-183.https://doi.org/10.1016/j.ecoenv.2017.10.004

Jain S, Shrivastava S, Nayak S & Sumbhate S (2007). Recent trends in *Curcuma longa* Linn. *Pharmacognosy Reviews* 1: 119-128

Jan S A, Shinwari Z K & Malik M (2018). Antioxidant and anticancer activities of *Brassica rapa*: a review. *Med Crave Online Journal of Biology and Medicine (MOJBM)* 3(5): 175-178. DOI: 10.15406/mojbm.2018.03.00094

Kanimozhi D & Bai V R (2012). Evaluation of antimicrobial activity of Cynodondactylon. *International Journal of Research in Pharmaceutical Sciences (IJRPS)* 2(2): 34-43

Kızılet H & Uysal H (2018). *Induced Genotoxicity in Human Lymphocytes by Neonicotinoids*. *Cumhuriyet Science Journal* 39(1): 201-210 (In Turkish). DOI: 10.17776/csj.406158

Kızılet H & Uysal H (2019). Induced somatic mutation during chronic exposure of chlorfenson on *Drosophila melanogaster* Oregon R (wild type). *Drosophila Information Service* 102:4-8. https://www.ou.edu/journals/dis/DIS102/DIS102.pdf

Kızılet H, Yilmaz B & Uysal H (2019). Herbal medicine against genotoxicity of dimethoate, an insecticide, in mammalian somatic cells. *Heliyon* 5(3): e01337. doi:10.1016/j.heliyon.2019. e01337

Kumlay A M, Yıldız Ö, Yurt B & Zengin H (2010). Some wild edible plants consumed traditionally in IğdırTürkiye. 1. In: 1. Traditional Foods from the Adriatic to the Caucasus Symposium 15-17 April, Tekirdağ, pp. 904

Larson R A (1988). The antioxidants of higher plants. *Phytochemistry* 27: 969-978.https://doi.org/10.1016/0031-9422(88)80254-1

Mazmancı B, Tamer L & Aşkın A (2008). Investigation of acute toxic effects of lambda-cyhalothrin in rats. *Mersin Üniversitesi Sağlık Bilimleri Dergisi* 1: 15-9 (In Turkish). https://dergipark.org.tr/tr/pub/mersinsbd/issue/19517/207886

Meister R T (1999). Farm chemicals handbook '99. Meister Publishing Company. Willoughby, Ohio ISSN: 0430-0750

Miller T A & Salgado V L (1985). The mode of action of pyrethroids on insects. In: J P Leahey (Eds), Pyrethroid Insecticides, Taylor & Francis, London pp. 43-97

Mokrey L E & Hoagland K D (1989). Acute toxicities offive synthetic pyrethroid insecticides to *daphniamagna and ceriodaphniadubia*. *Environmental Toxicology and Chemistry* 9: 1045-1051.https://doi.org/10.1002/etc.5620090811

Naeem F & Khan S H (2013). Purslane (*Portulacaoleracea* L.) as phytogenic substance - a review. *Journal of Herbs, Spices & Medicinal Plants* 19: 216-232 https://doi.org/10.1080/10496475.2013.782381

Özyurt E, Kızılet H & Uysal H (2018). Bio-*Interaction of Chlordane on Non-Target Organisms*. *Commagene Journal of Biology* 2(1): 48-54 (In Turkish).https://doi.org/10.31594/commagene.418411

Panda S, Jafri M, Kar A & Meheta B K (2009). Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma*. *Fitoterapia* 80(2): 123-126.https://doi.org/10.1016/j.fitote.2008.12.002

Pazır F & Turan F (2017). Various removal methods of some pesticide residues in fruits and vegetables. *Journal of Food and Health Science* 3(3): 109-116 (In Turkish).doi: 10.3153/JFHS17014

Plazar J, Filipic M & Groothuis G M (2008). Antigenotoxic effect of xanthohumol in rat liver slices. *Toxicology in Vitro* 22: 318-327.https://doi.org/10.1016/j.tiv.2007.09.009

Poprac P, Jomova K, Simunkova M, Kollar V, Rhodes C J & Valko M (2017). Targeting free radicals in oxidative stress-related human diseases. *Trends in pharmacological sciences* 38(7): 592-607 https://doi.org/10.1016/j.tips.2017.04.005

Rahmouni F, Saoudi M, Amri N, El-Feki A, Rebai T & Badraoui R (2018). Protective effect of *Teucriumpolium* on carbon tetrachloride induced genotoxicity and oxidative stress in rats. *Archives of Physiology and Biochemistry* 124(1): 1-9.https://doi.org/10.1080/13813455.2017.1347795

Robert L (2002). Metcalf "Insect Control" in Ullmann's Encyclopedia of Industrial Chemistry" Wiley-VCH, Weinheim. doi:10.1002/14356007.a14_263

Rooney D E & Czepulkowski B H (1986). Human Cytogenetics: a practicalapproach. First ed, IRL Press, Oxford, London

Rukshana M S, Doss A & KumariPushpa Rani T P (2017). Phytochemical screening and GC-MS analysis of leaf extract of *Pergulariadaemia (Forssk) Chiov*. *Asian Journal of Plant Science & Research* (AJPSKY) 7(1): 9-15

Sadowska-Woda I, Popowicz D & Karowicz-Bilińska A (2010). Bifenthrin-induced oxidative stress in human erythrocytes in vitro and protective effect of selected flavonols. *Toxicology in Vitro* 24: 460-464.https://doi.org/10.1016/j.tiv.2009.09.024

Scollon E J, Starr J M, Crofton K M, Wolansky M J, DeVito M J & Hughes M F (2011). Correlation of tissue concentrations of the pyrethroidbifenthrin with neurotoxicity in the rat. *Toxicology* 290: 1-6.https://doi.org/10.1016/j.tox.2011.08.002

Shahidi F & Ambigaipalan P (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. *Journal of Functional Foods* 18: 820-897.https://doi.org/10.1016/j.jff.2015.06.018

Shahidi F (2009). Nutraceutical and functional foods: whole versus processed foods. *Trends in Food Science and Technology* 20: 376-387 https://doi.org/10.1016/j.tifs.2008.08.004

Sharma A, Vijayakumar M, Rao C V, Unnikrishnan M K & Reddy G D (2009). Action of *Portulacaoleracea* against streptozotocin-induced oxidative stress in experimental diabetic rats. *Journal of Complementary and Integrative Medicine* 6: 1-12.https://doi.org/10.2202/1553-3840.1181

Simopoulos A P (2004). Omega-3 fatty acids and antioxidants in edible wild plants. *Biological Research* 37: 263-277. http://dx.doi.org/10.4067/S0716-97602004000200013

Singh R, Kumar N, Mehra R, Kumar H & Singh V P (2020). Progress and challenges in the detection of residual pesticides using nanotechnology based colorimetric techniques. *Trends in Environmental Analytical Chemistry* 26: e00086. https://doi.org/10.1016/j.teac.2020.e00086

Soderlund D M, Clarkb J M, Sheetsc L P, Mullind L S, Piccirilloe V J, Sargentf D, Stevensg J T & Weiner M L (2002). Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment. *Toxicology* 171: 3-59.https://doi.org/10.1016/S0300-483X(01)00569-8

Soltani F, Mosaffa F, Iranshahi M, Karimi G, Malekaneh M, Haghighi F & Behravan J (2009). Evaluation of antigenotoxicity effects of umbelliprenin on human peripheral lymphocytes exposed to oxidative stres. *Cell Biology and Toxicology* 25: 291-296.https://doi.org/10.1007/s10565-008-9083-9

Tiryaki O, Canhilal R & Horuz S (2010). The use of pesticides and their risks. *Erciyes University Journal of the Institute of Science and Technology* 26(2): 154-169 (In Turkish). https://dergipark.org.tr/tr/download/article-file/236259

Tran V, Hoffman N, Mofunanaya A, Pryor S C, Ojugbele O, McLaughlin A, Gibson L, Bonventre J A, Flynn K & Weeks B S (2006). Bifenthrin inhibits neurite outgrowth in differentiating PC12 cells. *Medical Science Monitor* 12: 57-62. https://www.medscimonit.com/abstract/index/idArt/445228/act/2

Uysal H, Kızılet H, Ayar A & Taheri A (2015). The use of endemic Iranian plant, *Echiumamoenum* against the ethyl methanesulfonate and the recovery of mutagenic effects. *Toxicology and Industrial Health* 31(1): 44-51.https://doi.org/10.1177/0748233712468019

Uysal H, Şişman T & Aşkın H (2006). *Drosophila* Biology and Crossover Methods (Extended 2nd Edition). Atatürk University Publications, ISBN: 975-442-111-0, No: 941, 53 s, Erzurum, Türkiye

Walker M M & Keith L H (1992). EPA's pesticide fact sheet database. CRC Press, Chelsea. ISBN 0-87371-663-9

Wolansky M J, Mc Daniel K L, Moser V C & Crofton K M (2007). Influence of dosing volume on the neurotoxicity of bifenthrin. *Neurotoxicology and Teratology* 29: 377-384.https://doi.org/10.1016/j.ntt.2007.01.007

Xu B J, Yuan S H & Chang S K C (2007). Comparative studies on the antioxidant activities of nine common food legumes against copper induced human low-density lipoprotein oxidation *in vitro*. *Journal of Food Science* 72: 522-527. DOI: 10.1111/j.1750-3841.2007.00464.x

Yang Y, Wu N & Wang C (2018). Toxicity of the pyrethroidbifenthrin insecticide. Environmental Chemistry Letters 16(4): 1377-1391.https://doi.org/10.1007/s10311-018-0765-0

Yen G C, Chen H Y & Peng H H (2001). Evaluation of the cytotoxicity, mutagenicity and antimutagenicity of emerging edible plants. *Food and Chemical Toxicology* 39: 1045-1053.https://doi.org/10.1016/S0278-6915(01)00053-9

Yu Q, Wang X & Xiong Z (2007). Comparative study of in vitro and in vivo anti-oxygen free radical effects of different parts of *Portulacaoleracea L. Practical Preventive Medicine* 2: 346-348

Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

Effects of Sequential Hydrogen Peroxide Applications on Salt Stress Tolerance in Bread Wheat Varieties

Elif Saadet ARICAN[a], Sefer DEMIRBAS[b*]

[a] *Department of Agricultural Biotechnology, Graduate School of Natural and Applied Sciences, Tekirdağ Namık Kemal University, Tekirdağ, TURKEY*
[b] *Department of Agricultural Biotechnology, Tekirdağ Namık Kemal University, Tekirdağ, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Sefer DEMIRBAS, E-mail: sdemirbas@nku.edu.tr
Received: 13 March 2021 / Revised: 26 October 2021/ Accepted: 29 October 2021 / Online: 15 October 2022

Cite this article
ARICAN E S, DEMIRBAS S (2022). Effects of Sequential Hydrogen Peroxide Applications on Salt Stress Tolerance in Bread Wheat Varieties. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 28(4):592-602. DOI: 10.15832/ankutbd.896112

ABSTRACT
Salinity is affecting plant growth and development. Low concentration of hydrogen peroxide (H_2O_2) has shown to be effective against various stress factors. In this study, effect of different H_2O_2 priming methods on growth, physiological and biochemical parameters in three wheat varieties (NKÜ Lider, Sultan-95, and Tosunbey) under salt stress were investigated. Salt stress (0 and 160 mM NaCl) was applied gradually to 100 µM H_2O_2 applied (-H_2O_2: negative control, no application; H_2O_2: positive control, 100 µM H_2O_2 applied; 1xH_2O_2: 100 µM H_2O_2 applied one year ago; 2xH_2O_2: 100 µM H_2O_2 applied second time after one year) wheat seedlings. Biochemical results showed that the lowest H_2O_2 level was in NKÜ Lider variety and in -H_2O_2 and 1xH_2O_2 groups. The lowest thiobarbituric acid reactive substances (TBARS) level was in Tosunbey variety and 2xH_2O_2 group. The highest superoxide dismutase (SOD) activity was in NKÜ Lider variety, all H_2O_2 pre-treatment caused an increase in SOD activity and 2xH_2O_2 pre-treatment caused the highest SOD activity. However, H_2O_2 and TBARS levels increased in all application groups except 2xH_2O_2 group, while the H_2O_2 amount increased and TBARS level decreased in 2xH_2O_2 group. MnSOD was not detected in any groups. CuZnSOD increased in all groups except 2xH_2O_2 groups under salt stress in Sultan-95 variety compared to FeSOD. H_2O_2 pre-treatment better tolerated salt stress, and second-applied H_2O_2 pre-treatment eliminated the stress and improved plant growth. In conclusion, it was determined that H_2O_2 re-pre-treatment to wheat seeds resulted in improvement of plant growth in tolerant varieties exposed to salt stress.

Keywords: Priming, Stress biomarkers, Wheat cultivars, Superoxide dismutase activity

1. Introduction

Many different internal mechanisms allow plants to respond to environmental changes that have occurred during their evolutionary processes. When there is a history of exposure to many types of stress, the plant changes its response in subsequent stress conditions (Bruce et al. 2007). Stress often leads to histone or DNA modifications and changes in the expression of various susceptible genes. Some of these modifications occur in a single unstable individual. Epigenetic signs preserve in the absence of stimulants, which leads to "memorization" of stresses experienced by plants in the epigenetic environmental environment (He & Li 2018).

Salt stress is one of the important abiotic stress factors that adversely affect plant growth and development (Ashraf 2009). The first response of the plant to salt stress is the decrease in leaf surface expansion rate and the halting of growth. The negative effects of salinity on plant growth are related to osmotic stress, food imbalance, specific ion effect and their combination (Ashraf & Harris 2004). Increasing salinity at the soil level limits the sustainable use of the field. High salt level cause oxidative stress because of increasing reactive oxygen species (ROS) (Rashidi et al. 2021).

Plants are developing regulatory mechanisms to adapt to various environmental stresses (Saxena et al. 2016). ROS also play a holistic role as signalling molecules in the regulation of various biological processes in plants, such as growth, development, and responses to biotic and abiotic stimuli (Baxter et al. 2014). To eliminate all the negative factors that the seed encounters, accelerate germination and seedling growth and obtain high yield, various applications called priming are applied to the seeds before sowing. Priming is defined as a controlled water intake that will initiate the necessary metabolic activity for germination, but does not allow root uptake, and is defined as a physiological process in which the plant prepares to respond more quickly to abiotic stresses (Jisha et al. 2013).

There are many used priming techniques for improving seed quality properties for example osmopriming (Farooq et al. 2017), hormopriming, hydropriming (Wani et al. 2016) and chemical priming (such as H_2O_2) (Savvides et al. 2016).

$O_2^{\cdot-}$, H_2O_2 and $OH^{\cdot}$ excessive production of ROS causes oxidative stress in cells (Parida & Das 2005). Numerous studies have shown that low concentrations of ROS, especially H_2O_2, are effective in priming against various abiotic stress factors (Savvides et al. 2016).

Triticum aestivum L. (wheat) is one of the most important global foods for human nutrition. Wheat, cultivation, and domestication as one of the main species closely related to the welfare of agriculture and settled societies; is one of the most grown products due to its high yield values, nutritional and processing properties. By 2050, the world population is estimated to reach nine billion, and the wheat yield of people's food needs to increase significantly (Jia et al. 2018). China is the largest wheat producer and consumer. China's annual wheat production is about 100 million tons (Shi & Ling 2018). In Turkey, wheat production is 20.5 million tons in 2020 (Anonymous 2021).

In this study, we investigated that the effect of different H_2O_2 priming methods on root length (RL), root dry weight (RDW), shoot length (SL), shoot dry weight (SDW), relative water content (RWC), stomatal index (SIn), stomatal conductance (SC), H_2O_2 and thiobarbituric acid reactive substances (TBARS) content, SOD activity and its isoenzyme profiling in three wheat varieties (NKÜ Lider, Sultan-95, and Tosunbey) under salt stress.

2. Material and Methods

2.1. Experimental material and design

As plant material in this study were used three wheat varieties (NKÜ Lider, Sultan-95, and Tosunbey).

The study was carried out at the laboratories of Departments of Agricultural Biotechnology, Faculty of Agriculture, Tekirdağ Namık Kemal University in 2018-2019. The experiment was arranged in a randomized split-split plot design with three replications. Each group was designed to contain at least 3 pots, and a trial was established with a total of 128 pots. The varieties, NaCl solutions and H_2O_2 pre-treatments were allotted to main plots, subplots, and sub-subplots, respectively.

2.2. H_2O_2 seed priming

The seeds of each variety were soaked in the different 100 µM H_2O_2 solutions (-H_2O_2: negative control, no application; H_2O_2: positive control, 100 µM H_2O_2 applied; 1xH_2O_2: 100 µM H_2O_2 applied one year ago; 2xH_2O_2: 100 µM H_2O_2 applied second time after one year) for 6 h under dark-room conditions for priming before sowing, and then the seeds were dried on filter paper at room temperature (Demirbas & Balkan 2020).

2.3. Plant growth condition

After priming, the 20 seeds were sown in a pot (13 cm depth; 1.5 l volume) contained perlite. The seedlings were grown in a plant growth room at 25±2/15±2 °C day/night with 16 h photoperiod, relative humidity 60% and photosynthetic flux density of approximately 250 µmol m^{-2} s^{-1}. The salt concentrations mixed in Hoagland solution weekly gradually increased, and cultivation was carried out for 5 weeks (Hoagland & Arnon 1950). On the 35th day, morphological, physiological, and biochemical measurements were made, and plant materials were packed for biochemical analysis and stored at -20 °C.

2.4. Salt stress treatment and plant harvest

Fifteen-day seedlings were watered with 0-control and 160 mM NaCl diluted in Hoagland solutions. After salt stress application, leaves were harvested on the thirty-five-days for test and analysis.

2.5. Plant growth parameters

Ten randomly selected thirty-five-days-old seedlings per replicate were divided into roots and shoots. Firstly, they were measured with a ruler for root and shoot length (cm) (RL and SL) and then, they were dried in an oven for two days at 65 °C to determine dry weights (mg) (SDW and RDW).

2.6. RWC test

The fully developed leaves of the plants were taken and weighed to determine fresh weights (FW-mg). To obtain the turgid weight (TW-mg), these leaves were waited in closed petri dishes for 24 hours between distilled water and completely wet filter paper. Turgid leaves were quickly wiped with a paper towel to remove the water accumulation on them and reweighed to

determine the TW. Then, these leaves were dried at 70 °C for 48 hours and their dry weights (DW-mg) were found. The RWC of the leaves were calculated using the following equations (Smart & Bingham 1974):

$$\text{RWC}\ (\%) = \frac{\text{FW}-\text{DW}}{\text{TW}-\text{DW}}\ \text{x}\ 100 \quad (1)$$

2.7. SIn

The Sin level was determined from the third leaves of the wheat seedlings of 35 days. Transparent nail polish was applied to the bottom surface of the leaves cut from the plant. It was expected to dry. Later, the polish was gently peeled off the sheet and adhered to the slide and kept in room conditions until the day of measurement. Under the microscope, stoma (S) and epidermis cells (E) in the unit area at 400x magnification were counted and the Sin value was calculated according to the formula below (Radoglou & Jarvis 1990). Measurements were made three times from each group.

$$\text{SIn}\ (\%) = \frac{\text{S}}{\text{S}+\text{E}}\ \text{x}\ 100 \quad (2)$$

2.8. SC

The stomatal conductivity level of plants was measured from the second leaves by DECAGON brand SC-1 leaf porometer on the 35th day of the growing period. Measurements were recorded as mmol m^{-2} s^{-1} in triplicate from each group.

2.9. Determination of H_2O_2 content

0.1 g leaf tissues were homogenized with 100 mM potassium phosphate buffer (pH 6.8). The homogenates were centrifuged at 14000 rpm for 30 min at 4 °C. After centrifugation, the supernatant reacted with peroxidase reagent. The mixture was waited at 30 °C for 10 min, and then reaction was stopped after 1 N perchloric acid was added. After 10 min, supernatant was centrifuged at 14000 rcf for 5 min at 4 °C. The H_2O_2 content was spectrophotometrically determined at 436 nm according to H_2O_2 standard (Bernt & Bergmeyer 1974).

2.10. Determination of lipid peroxidation level

The lipid peroxidation level of plants was assayed by determining the level of MDA. The content of TBARS was assayed by MDA (Madhava Rao & Sresty 2000).

2.11. Determination of total protein content and SOD activity

The total protein content of the enzyme extract was assayed by Bradford method using BSA as a standard (Bradford 1976). The used reaction mixture for measuring SOD (EC 1.15.1.1) activity contained 33 µM NBT, 10 mM L-Methionine, 0.66 mM EDTA.Na_2, and 3.3 µM riboflavin in 0.05 M Na-phosphate buffer (pH 7.8). The test tubes containing the reaction mixture (3 ml) and 200 µl enzyme extract or distilled water were shaken and left for 10 min under an illumination of 300 µmol m^{-2} s^{-1} at room temperature. Maximum reduction of NBT resulted in maximum violet color in the supernatantless reaction mixture. A non-illuminated reaction mixture served as the control. The reduction of NBT was inversely proportional to the SOD activity. One unit of SOD was defined as the amount of enzyme that inhibits 50% NBT photoreduction (Beauchamp & Fridovich 1971; Giannipolities & Ries 1977). A Mecasys Optizen POP UV/Vis spectrophotometer was used during all spectrophotometrically analyses.

2.12. Identification of SOD isoenzymes

0.2 g plant leaves were homogenised with buffer solution containing 50 mM Tris-HCl (pH 7.8), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.2% Triton X, 1 mM phenylmethanesulfonyl fluoride (PMSF) and 2% polyvinylpolypyrrolidone (PVPP). The homogenates were centrifuged at 14000 rpm at 4 °C for 10 min. The supernatants were used for electrophoretic separation of SOD isoenzymes. The isoenzymes were separated by native polyacrylamide gel electrophoresis (PAGE) using 4% stacking and 12.5% running gels with a buffer consisting of %100 at 4 °C at 80 mA for 2 h with a Junyi JY-SCZ2+ mini vertical electrophoresis. The total amount of protein applied per lane was 75 µg. After electrophoresis, the gels were stained with riboflavin, nitroblue tetrazolium (NBT) and EDTA in Na-P buffer (pH 7.8) for 45 min in the dark. The gels were washed with dIH_2O and visualized with white light (Arora & Bhatla 2017; Beauchamp & Fridovich 1973). Inhibitors of SOD before staining, such as 2 mM potassium cyanide (KCN) and 3 mM H_2O_2, were used to detect the different types of SOD (Vitória et al. 2001). After staining, the gels were visualized under UV light using a Quantum ST5 Gel Imaging System (Vilber Lourmat). SOD isoenzymes were determined densitometric using Biocapt software.

2.13. Statistical analysis

This study was carried out in 3 repetitions according to the split parcels trial pattern, which was divided in random parcels. All

physiological and biochemical data were analysed using one-way ANOVA (MSTAT) program and differences between the averages were compared with the LSD Test. Values with P≤0.05 were considered statistically significant. The results are presented in the tables as mean ± SE.

3. Results

3.1. RL and SL

RLs were increased not significantly by 1.20% under salt stress as compared to untreated plants (Figure 1). RLs in the groups which were treated by H_2O_2, $1xH_2O_2$, and $2xH_2O_2$ groups were different. RLs were decreased by 3.09% and %1.58 in H_2O_2 and $1xH_2O_2$ groups, whereas they were increased by 3.56% in the $2xH_2O_2$ group (P<0.01). We found that the Sultan-95 variety had the RL (213.74 mm) as compared to other varieties (P>0.05) (Table 1a).

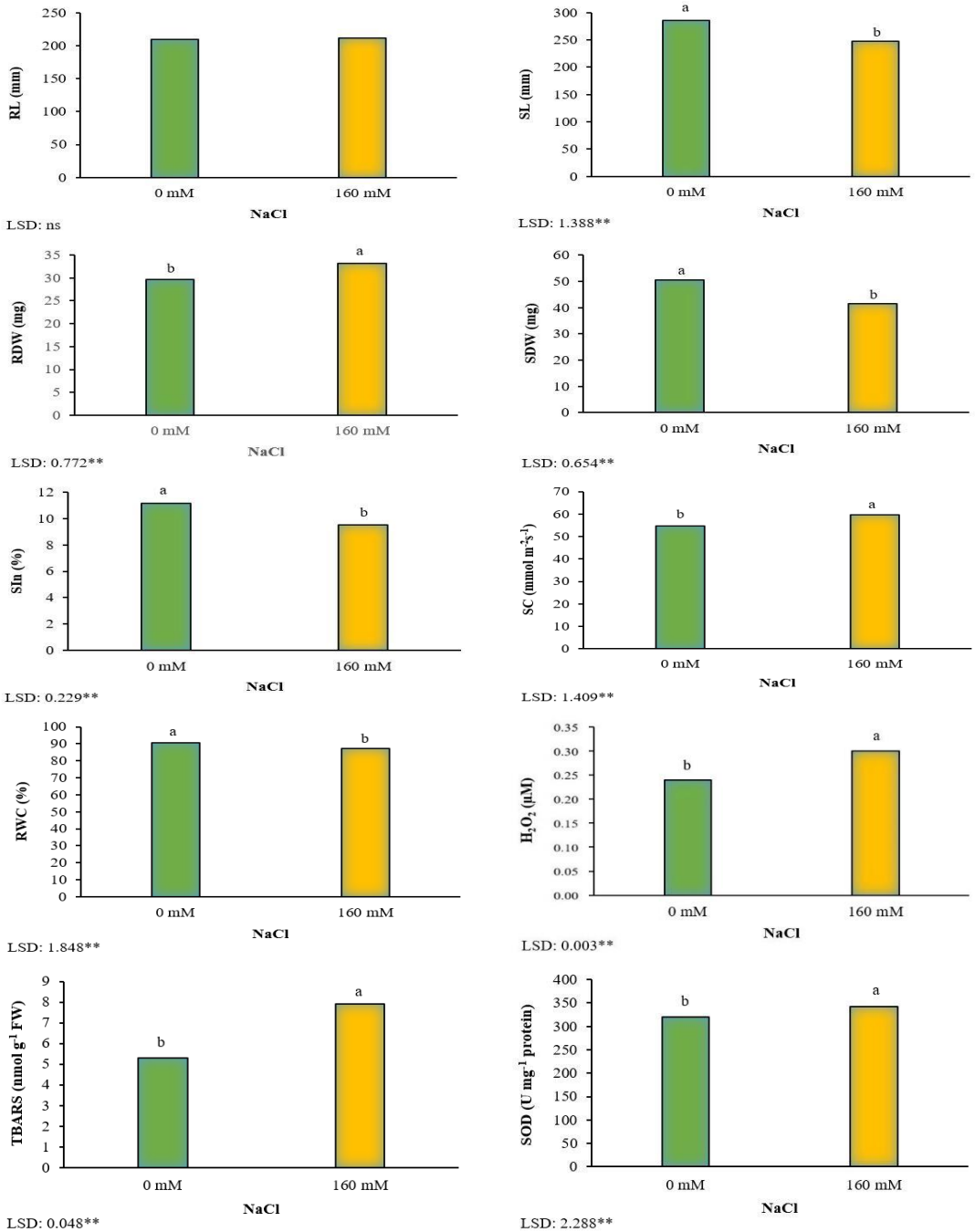


Figure 1- Effect of average NaCl on RL, RDW, SL, SDW, RWC, SIn, SC, H_2O_2, TBARS and SOD activity parameters in wheat seedlings. Means values followed by different letters are significantly different at P<0.05 (* Significant correlations at P value ≤0.05, and ** at P value ≤0.01. ns: non-significant).

Table 1- Changes in RL (a), RDW (b), SL (c), and SDW (d) parameters of seed primed wheat seedlings with H_2O_2 under salt stress

a) RL (mm)		*Groups*					
Variety	NaCl (mM)	-H_2O_2	H_2O_2	1xH_2O_2	2xH_2O_2	**Variety Average**	
NKÜ Lider	0	169.56 j	205.67 f	218.33 d	219.78 cd	**NKÜ Lider**	209.44
	160	232.61 b	196.11 ghı	203.83 f	229.67 b		
Sultan-95	0	217.00 d	189.89 ı	200.33 fgh	246.50 a	**Sultan-95**	213.74
	160	219.89 cd	226.44 bc	196.17 ghı	213.67 de		
Tosunbey	0	226.22 bc	194.33 hı	200.83 fgh	231.33 b	**Tosunbey**	209.92
	160	218.33 d	199.72 fgh	206.83 ef	201.78 fg		
H_2O_2 Average		213.94 b	202.03 c	204.39 c	223.79 a		
LSD		H_2O_2: 3.014**	VarietyxNaClxH_2O_2: 7.382**			Variety: ns	

b) RDW (mg)		*Groups*					
Variety	NaCl (mM)	-H_2O_2	H_2O_2	1xH_2O_2	2xH_2O_2	**Variety Average**	
NKÜ Lider	0	17.40 j	34.15 c-f	34.07 c-f	35.52 cde	**NKÜ Lider**	30.47 b
	160	23.63 ı	29.63 g	28.88 gh	40.50 a		
Sultan-95	0	19.89 j	26.85 h	23.35 ı	38.37 ab	**Sultan-95**	31.40 ab
	160	35.88 bc	35.80 bcd	35.35 cde	35.71 bcd		
Tosunbey	0	32.45 f	28.25 gh	32.87 ef	33.20 def	**Tosunbey**	32.34 a
	160	29.77 g	29.62 g	36.26 bc	36.27 bc		
H_2O_2 Average		20.50 c	30.72 b	31.80 b	36.59 a		
LSD		H_2O_2: 1.091**	VarietyxNaClxH_2O_2: 2.673**			Variety: 1.282*	

c) SL (mm)		*Groups*					
Variety	NaCl (mM)	-H_2O_2	H_2O_2	1xH_2O_2	2xH_2O_2	**Variety Average**	
NKÜ Lider	0	340.22 ı	282.89 f	235.28 j	236.28 ıj	**NKÜ Lider**	252.09 c
	160	292.61 e	205.89 m	183.00 n	240.56 ı		
Sultan-95	0	330.28 b	284.56 f	218.00 l	248.56 h	**Sultan-95**	259.13 b
	160	280.06 f	215.78 l	227.00 k	268.78 g		
Tosunbey	0	337.78 a	322.56 c	298.50 d	289.83 e	**Tosunbey**	288.83 a
	160	289.44 e	294.00 de	231.67 jk	246.83 h		
H_2O_2 Average		311.73 a	267.61 b	232.24 d	255.11 c		
LSD		H_2O_2: 1.963**	VarietyxNaClxH_2O_2: 4.808**			Variety: 3.061**	

d) SDW (mg)		*Groups*					
Variety	NaCl (mM)	-H_2O_2	H_2O_2	1xH_2O_2	2xH_2O_2	**Variety Average**	
NKÜ Lider	0	57.24 b	52.45 c	44.13 g	46.86 f	**NKÜ Lider**	45.41 b
	160	44.33 g	41.83 hı	34.69 j	41.71 hı		
Sultan-95	0	46.97 f	49.55 de	34.38 j	49.75 de	**Sultan-95**	42.99 c
	160	42.55 gh	41.19 hı	39.83 ı	39.67 ı		
Tosunbey	0	63.71 a	62.60 a	50.92 cd	48.61 ef	**Tosunbey**	49.94 a
	160	47.71 ef	48.88 def	44.27 g	32.83 j		
H_2O_2 Average		50.42 a	49.42 b	41.37 d	43.24 c		
LSD		H_2O_2: 0.925**	VarietyxNaClxH_2O_2: 2.266**			Variety: 0.844**	

*: Significant correlations at P value ≤ 0.05, and **: at P value ≤0.01. ns: non-significant

SLs were decreased by 13.11% (P<0.01) under salt stress as compared to untreated plants (Figure 1). SLs were decreased by 14.15%, 25.50% and %18.16 in H_2O_2 and 1xH_2O_2, and 2xH_2O_2 groups (P<0.01). We found that the Tosunbey variety had the longest shoot (288.83 mm) (P<0.01) as compared to other varieties (Table 1c).

3.2. RDW and SDW

RDW was increased significantly by 11.48% under salt stress as compared to untreated plants (Figure 1). RDW in the groups which were treated by H_2O_2, 1xH_2O_2, and 2xH_2O_2 groups were different. RDW was increased by 49.85%, 55.12% and 78.49% in H_2O_2, 1xH_2O_2 and 2xH_2O_2 groups (P<0.01). We found that the Tosunbey variety had the highest dry root weight (32.34 mg) (P<0.01) as compared to other varieties (Table 1b).

SDW was decreased by 17.75% (P<0.01) under salt stress as compared to untreated plants (Figure 1). SDW was decreased by 1.98%, 17.95% and 14.24% in H_2O_2 and 1xH_2O_2 and 2xH_2O_2 groups (P<0.01). We found that the Tosunbey variety had the highest dry shoot weight (49.94 mg) (P<0.01) as compared to other varieties (Table 1d).

3.3. RWC

RWC was decreased not significantly under salt stress as compared to untreated plants (Figure 1). RWC in the groups which were treated by H_2O_2, $1xH_2O_2$, and $2xH_2O_2$ groups were different. RWC was increased not significantly by 2.59% and 0.80% in H_2O_2 and $2xH_2O_2$ groups, whereas they were decreased by 0.20% in $1xH_2O_2$ group. We found that the Sultan-95 variety had the highest RWC (90.51%) as compared to other varieties (P>0.05) (Table 2a).

Table 2- Changes in RWC (a), SIn (b) and SC (c) parameters of seed primed wheat seedlings with H_2O_2 under salt stress

a) RWC (%)		***Groups***					
Variety	NaCl (mM)	-H_2O_2	H_2O_2	$1xH_2O_2$	$2xH_2O_2$	**Variety Average**	
NKÜ Lider	0	91.32	94.04	87.72	90.87	**NKÜ Lider**	89.13
	160	87.55	89.23	84.08	88.23		
Sultan-95	0	88.11	91.87	91.97	92.74	**Sultan-95**	90.51
	160	87.92	91.57	87.94	91.95		
Tosunbey	0	89.39	91.48	88.12	91.07	**Tosunbey**	87.46
	160	85.70	85.50	89.06	79.36		
H_2O_2 Average		88.33	90.62	88.15	89.04		
LSD		H_2O_2:^{ns}		VarietyxNaClxH_2O_2:^{ns}		Variety:^{ns}	

b) SIn (%)		***Groups***					
Variety	NaCl (mM)	-H_2O_2	H_2O_2	$1xH_2O_2$	$2xH_2O_2$	**Variety Average**	
NKÜ Lider	0	8.98 jk	18.30 a	12.29 d	6.70 m	**NKÜ Lider**	11.01 a
	160	10.06 gh	14.15 c	10.68 efg	6.87 m		
Sultan-95	0	9.85 hı	8.09 l	9.07 ıjk	17.22 b	**Sultan-95**	9.66 c
	160	6.42 mn	9.66 hıj	11.24 e	5.73 no		
Tosunbey	0	8.70 kl	10.40 fgh	6.09 mn	17.92 ab	**Tosunbey**	10.31 b
	160	12.39 d	11.01 ef	10.98 ef	4.98 o		
H_2O_2 Average		9.40 c	11.94 a	10.06 b	9.90 b		
LSD		H_2O_2: 0.324**		VarietyxNaClxH_2O_2: 0.793**		Variety: 0.321**	

c) SC (mmol $m^{-2}s^{-1}$)		***Groups***					
Variety	NaCl (mM)	-H_2O_2	H_2O_2	$1xH_2O_2$	$2xH_2O_2$	**Variety Average**	
NKÜ Lider	0	58.50 c-h	56.37 f-j	52.60 ı-l	57.80 d-h	**NKÜ Lider**	57.80
	160	54.53 h-l	59.83 b-f	62.90 abc	59.90 b-f		
Sultan-95	0	50.40 l	51.47 kl	56.67 e-ı	59.43 b-g	**Sultan-95**	55.58
	160	54.70 g-l	58.03 c-h	62.37 a-d	51.57 jkl		
Tosunbey	0	55.77 f-k	55.97 f-k	56.07 f-k	45.43 m	**Tosunbey**	58.21
	160	62.40 a-d	61.47 a-e	64.80 a	63.77 ab		
H_2O_2 Average		56.05 b	57.18 b	59.23 a	56.32 b		
LSD		H_2O_2: 1.993**		VarietyxNaClxH_2O_2: 4.880**		Variety: ^{ns}	

*: Significant correlations at P value ≤ 0.05, and **: at P value ≤ 0.01. ns: non-significant

3.4. SIn

Stomal index was decreased significantly by 14.63% under salt stress as compared to untreated plants (Figure 1). Stomal index was increased by H_2O_2 applications. Stomal index was increased by 27.02%, 7.02% and 5.32% in H_2O_2, $1xH_2O_2$ and $2xH_2O_2$ groups (P<0.01). We found that the NKÜ Lider variety had the highest stomal index (11.01%) (P<0.01) as compared to other varieties (Table 2b).

3.5. SC

SC was increased significantly by 9.10% under salt stress as compared to untreated plants (Figure 1). SC in the groups which were treated by H_2O_2, $1xH_2O_2$, and $2xH_2O_2$ groups were different. SC was increased by 2.02%, 5.67% and 0.48% in H_2O_2, $1xH_2O_2$ and $2xH_2O_2$ groups (P<0.01). We found that the Tosunbey variety had the highest SC (58.21 mmol $m^{-2}s^{-1}$) as compared to other varieties (P>0.05) (Table 2c).

3.6. H_2O_2

H_2O_2 content was increased significantly by 25.00% under salt stress as compared to untreated plants (Figure 1). H_2O_2 contents were increased by 11.54% and 3.85% in H_2O_2 and $2xH_2O_2$ groups (P<0.01). We found that the NKÜ Lider variety had the lowest H_2O_2 content (0.26 µM) (P<0.01) as compared to other varieties (Table 3a).

Table 3- Changes in H_2O_2 (a), TBARS (b) and SOD (c) parameters of seed primed wheat seedlings with H_2O_2 under salt stress

a) H_2O_2 (µM)		***Groups***					
Variety	NaCl (mM)	-H_2O_2	H_2O_2	1xH_2O_2	2xH_2O_2	**Variety Average**	
NKÜ Lider	0	0.21 lm	0.21 lm	0.20 m	0.27 fg	**NKÜ Lider**	0.26 c
	160	0.26 gh	0.25 hı	0.35 b	0.33 c		
Sultan-95	0	0.24 ıj	0.29 de	0.22 kl	0.23 jk	**Sultan-95**	0.28 a
	160	0.27 fg	0.44 a	0.26 gh	0.25 hı		
Tosunbey	0	0.30 d	0.28 ef	0.23 jk	0.20 m	**Tosunbey**	0.27 b
	160	0.27 fg	0.27 fg	0.28 ef	0.33 c		
H_2O_2 Average		0.26 c	0.29 a	0.26 c	0.27 b		
LSD		H_2O_2: 0.005**	VarietyxNaClxH_2O_2: 1.136**			Variety: 0.005**	

b) TBARS (nmol/g FW)		***Groups***					
Variety	NaCl (mM)	-H_2O_2	H_2O_2	1xH_2O_2	2xH_2O_2	**Variety Average**	
NKÜ Lider	0	6.18 j	6.42 ı	5.11 o	5.80 l	**NKÜ Lider**	7.22 a
	160	8.60 d	10.75 a	7.73 f	7.15 h		
Sultan-95	0	3.91 s	4.86 p	6.38 ı	4.52 q	**Sultan-95**	6.34 b
	160	7.50 g	9.52 b	8.39 e	5.60 m		
Tosunbey	0	6.14 jk	4.90 p	4.13 r	5.32 n	**Tosunbey**	6.29 b
	160	7.61 fg	6.99 h	9.18 c	6.01 k		
H_2O_2 Average		6.66 c	7.24 a	6.82 b	5.73 d		
LSD		H_2O_2: 0.068**	VarietyxNaClxH_2O_2: 0.164**			Variety: 0.052**	

c) SOD (U mg^{-1} protein)		**Groups**					
Variety	NaCl (mM)	-H_2O_2	H_2O_2	1xH_2O_2	2xH_2O_2	**Variety Average**	
NKÜ Lider	0	302.85 k	312.07 j	379.23 f	335.53 h	**NKÜ Lider**	400.33 a
	160	324.51 ı	315.66 j	467.94 b	764.84 a		
Sultan-95	0	194.81 q	402.08 e	418.31 d	311.76 j	**Sultan-95**	318.35 b
	160	219.84 p	235.82 o	325.07 ı	439.11 c		
Tosunbey	0	245.27 n	339.29 h	326.07 ı	269.50 m	**Tosunbey**	274.20 c
	160	199.53 q	180.96 r	354.35 g	278.63 l		
H_2O_2 Average		247.80 d	297.65 c	378.50 b	399.90 a		
LSD		H_2O_2: 3.151**	VarietyxNaClxH_2O_2: 7.719**			Variety: 3.204**	

*: Significant correlations at P value ≤ 0.05, and **: at P value ≤ 0.01. ns: non-significant

3.6. TBARS

TBARS level was increased significantly by 49.15% under salt stress as compared to untreated plants (Figure 1). TBARS levels in the groups which were treated by H_2O_2, 1xH_2O_2, and 2xH_2O_2 groups were different. TBARS level was increased by 8.71% and 2.40% in H_2O_2 and 1xH_2O_2 groups, whereas the level was decreased by 13.96% in the 2xH_2O_2 group (P<0.01). We found that Tosunbey variety had the lowest TBARS level (6.29 nmol/g FW) as compared to other varieties (P<0.01) (Table 3b).

3.7. SOD activity and isoenzyme profiling

SOD activity was increased significantly by 7.02% under salt stress as compared to untreated plants (Figure 1). SOD activity was increased by H_2O_2. SOD enzyme activity was increased by 20.12%, 52.74% and 61.38% in H_2O_2, 1xH_2O_2 and 2xH_2O_2 groups (P<0.01). We found that NKÜ Lider variety had the highest SOD activity (400.33 U mg^{-1} protein) (P<0.01) as compared to other varieties (Table 3c).

We found that two SOD isoenzymes showed the activity. MnSOD isoenzyme activity was not determined in all wheat varieties. CuZnSOD isoenzyme had higher activity than FeSOD isoenzyme except in 2xH_2O_2 group in Sultan-95 variety under salt stress (Figure 2-3).

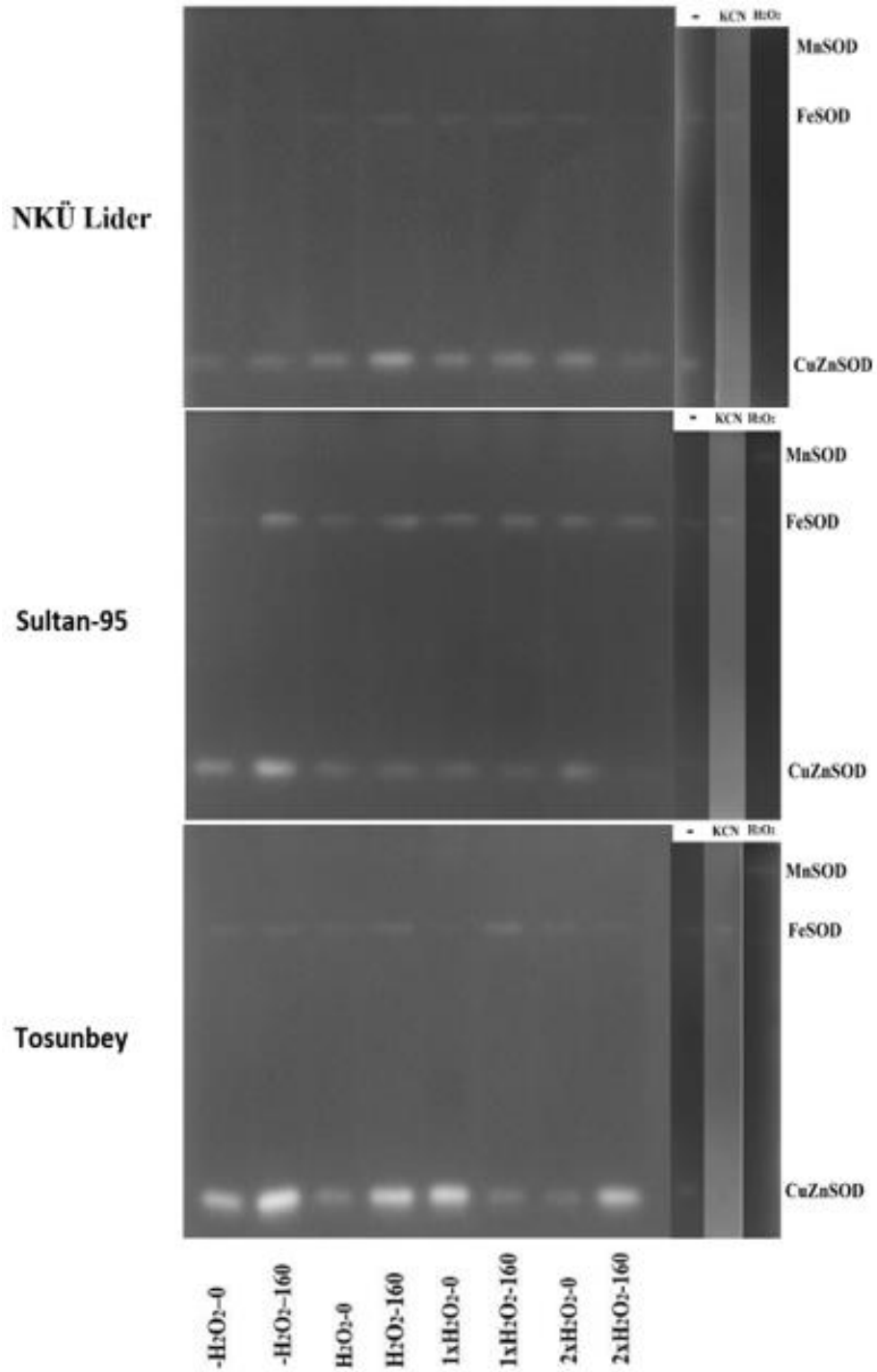


Figure 2- The effects of H_2O_2 treatments (-H_2O_2, H_2O_2 and 1xH_2O_2 and 2xH_2O_2) on native-PAGE separation of SOD isoenzymes (MnSOD, FeSOD and CuZnSOD) in wheat varieties under salt stress (0 and 160 mM NaCl)

CuZnSOD isoenzyme activity in NKÜ Lider variety, in the 0 mM NaCl groups were found to be increased by 30.36%, 18.32% and 15.84%, respectively, in the H_2O_2, 1xH_2O_2 and 2xH_2O_2 groups compared to the -H_2O_2 group. In Sultan-95 variety, CuZnSOD isoenzyme activity in the 0 mM NaCl groups increased by 9.60% and 12.42% in the H_2O_2 and 2xH_2O_2 groups, respectively, compared to the -H_2O_2 group. In Tosunbey variety, CuZnSOD isoenzyme activity in the 0 mM NaCl groups reduced by 14.44% and 23.78% in the H_2O_2 and 2xH_2O_2 groups compared to the -H_2O_2 group. It increased by 19.15% in the 1xH_2O_2 group (Figure 2-3).

CuZnSOD isoenzyme activity in NKÜ Lider variety under salt stress increased in all H_2O_2 groups compared to the control group. In Sultan-95 variety, only 2xH_2O_2 application decreased the activity by 74.53%. In Tosunbey variety, 1xH_2O_2 application decreased the activity by 11.48% (Figure 2-3).

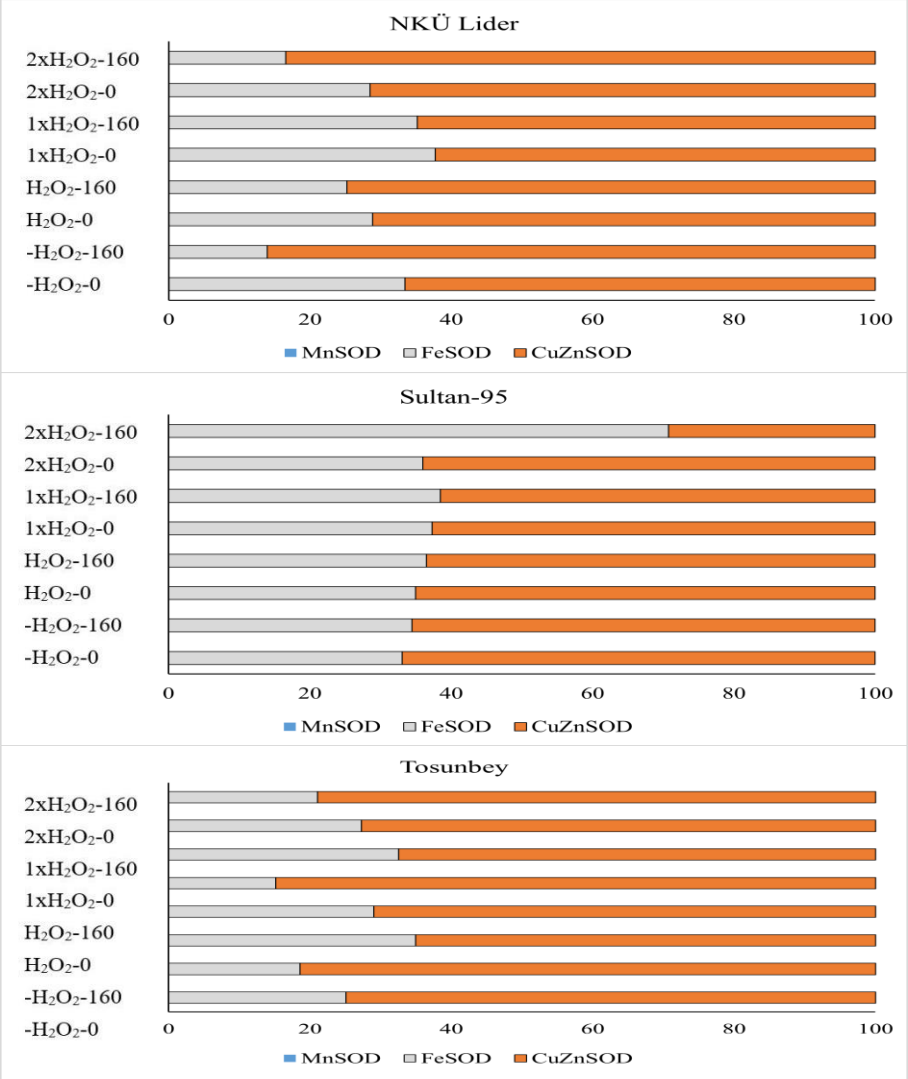


Figure 3- Effect of H_2O_2 pre-treatments (-H_2O_2, H_2O_2 and 1xH_2O_2 and 2xH_2O_2) on percentage distribution of SOD isoenzymes under salt stress in wheat varieties (a: NKÜ Lider, b: Sultan-95, c: Tosunbey)

1xH_2O_2 application caused an increase of 43.95% in FeSOD isoenzyme activity although 2xH_2O_2 application decreased the activity by 10.04% in NKÜ Lider variety. In Sultan-95 variety, all H_2O_2 applications caused an increase of 17% to 29% in the FeSOD isoenzyme activity. In Tosunbey variety, 1xH_2O_2 and 2xH_2O_2 applications caused decreases of 36.22% and 14.42%, respectively, in the activity (Figure 2-3).

1xH_2O_2 application caused an increase of 39.94% in the FeSOD isoenzyme activity in NKÜ Lider variety compared to the control group (-H_2O_2-0) under salt stress. However, -H_2O_2, H_2O_2 and 2xH_2O_2 applications decreased the activity by 60.91%, 5.55% and 56.05%, respectively. In Sultan-95 variety, all H_2O_2 applications caused increases of 26% to 68%. In Tosunbey variety, H_2O_2 and 1xH_2O_2 applications caused increases of 36.50% and 28.95% in the FeSOD isoenzyme activity under salt stress (Figure 2-3).

4. Discussion

Hydrogen peroxide causing oxidative stress in high concentrations in plants functions as a signal molecule in stimulating the plant defense system at low concentrations (Saxena et al. 2016). In previous studies, H_2O_2 has been applied by the researchers as a seed priming agent (Savvides et al. 2016) and has been reported to have a positive effect on the growth and development of wheat (Ashfaque 2014) and triticale (Demirbas & Balkan 2020) under stress conditions. Here, we focused on how H_2O_2 affects wheat seedlings under salt stress one generation after H_2O_2 treatment. Compared to the -H_2O_2 treatment group, H_2O_2 application caused an increase in RWC, SC, SIn and RDW, while the SL, RL, and SDW decreased. 1xH_2O_2 treatment caused an increase in SC, SIn, and RDW, and a decrease in RWC, SL, RL, and SDW. While 2xH_2O_2 application caused an increase in SL and RDW, and a decrease in RWC, SC, SIn, SL, and SDW (Table 1 and 2).

The highest RWC, RL, RDW and FeSOD isoenzyme band density was observed in Sultan-95 variety (Table 1a, b; Table 2a; Figure 2). The highest SC, SL, and SDW and the lowest TBARS level were observed in Tosunbey variety (Table 1c, 1d, 2c). The highest SIn, SOD activity and CuZnSOD isoenzyme band density and the lowest H_2O_2 level were observed in NKÜ Lider variety (Table 2b, 3c, Figure 2).

Triple interaction (Variety x NaCl x H_2O_2) was statistically significant for all parameters except RWC (Table 1-3). This result indicated that there was not any effect of H_2O_2 pre-treatment on leaf water status. The correlation between the parameters showed that there was a positive relationship between RWC and SDW and SIn. This result indicated that leaf water content affected stomata density and shoot development. The positive correlation between SC and H_2O_2 and TBARS indicated that stomata gas exchange arranged oxidative stress in wheat seedlings. The positive correlation between RDW and SOD and H_2O_2 showed that increasing H_2O_2 level with SOD activity increased root growth (Table 4).

Table 4- Correlation matrix of growth, physiologic and biochemical parameters in wheat seedlings

Variable	*RWC*	*SC*	*RL*	*RDW*	*SL*	*SDW*	*SIn*	*SOD*	*H_2O_2*	*TBARS*
RWC	1.000	-0.334**	0.035^{ns}	-0.048^{ns}	0.104^{ns}	0.287*	0.242*	0.061^{ns}	-0.220^{ns}	-0.122^{ns}
SC		1.000	0.203^{ns}	0.200^{ns}	-0.370**	-0.233*	-0.087^{ns}	0.065^{ns}	0.349**	0.457**
RL			1.000	0.455**	-0.101^{ns}	-0.062^{ns}	0.221^{ns}	0.027^{ns}	0.112^{ns}	0.035^{ns}
RDW				1.000	-0.419**	-0.219^{ns}	0.019^{ns}	0.253*	0.277*	0.121^{ns}
SL					1.000	0.768**	-0.058^{ns}	-0.396**	-0.352**	-0.512**
SDW						1.000	0.213^{ns}	-0.283*	-0.259*	-0.372**
SIn							1.000	-0.222^{ns}	-0.388**	0.089^{ns}
SOD								1.000	0.152^{ns}	0.008^{ns}
H_2O_2									1.000	0.421**
TBARS										1.000

*: Significant correlations at P value ≤ 0.05, and **: at P value ≤ 0.01. ns: non-significant
Hydrogen peroxide (H_2O_2), relative water content (RWC), root dry weight (RDW), root length (RL), shoot dry weight (SDW), shoot length (SL), stomatal conductance (SC), stomatal index (SIn), superoxide dismutase (SOD), thiobarbituric acid reactive substances (TBARS)

H_2O_2 level, TBARS level, SOD activity, CuZnSOD and FeSOD isoenzyme band density increased in H_2O_2 group compared to -H_2O_2 group at salt tolerance level of H_2O_2 pre-treatment. In 1xH_2O_2 group, TBARS level, SOD activity and FeSOD isoenzyme band density increased, CuZnSOD isoenzyme band density decreased and H_2O_2 level did not change. In the 2xH_2O_2 group, H_2O_2 level and SOD activity increased, TBARS level, CuZnSOD and FeSOD isoenzyme band density decreased (Table 3 and Figure 2). These results have shown the similarity to wheat plants' response to pre-treatment with AsA (Athar et al. 2008), K (Ahanger & Agarwal 2017) and SNP (Ali et al. 2017) against salt stress. And salt stress in wheat plants caused an increase in SOD activity. This result is similar to studies of (He et al. 2009) and (Ashfaque 2014). MnSOD is a constitutive antioxidant enzyme in mitochondria, and it can vary between species and varieties, but in general, it is quite stable under environmental stresses (Asensio et al. 2012). In this study, MnSOD isoenzyme activity was not determined in any groups (Figure 2). This result indicated that there was not any mitochondrial response to H_2O_2 pre-treatment.

On the other hand, the increase in the amount of H_2O_2 and TBARS in the groups other than the 2xH_2O_2 application group showed a negative relationship compared to the above studies. In the 2xH_2O_2 application group, the amount of H_2O_2 increased while the amount of TBARS decreased. This result is similar to Tabassum et al. (Tabassum et al. 2017) study which they obtained with the second application of $CaCl_2$.

5. Conclusions

Plant growth was improved by eliminating the pressure caused by salt stress in the development of wheat seedlings by removing the second H_2O_2 pre-application. As a result, this study demonstrated for the first time that the H_2O_2 application made before sowing in wheat contributes positively to the growth of the wheat plant by stimulating the SOD activity against salt stress. It can be said that the information obtained in this study will provide an important accumulation of knowledge for many scientific studies in which the pre-application increases the plant tolerance level, how the epigenetic mechanism is triggered, and all aspects of the antioxidant defense system will be examined more comprehensively.

References

Ahanger M A & Agarwal R M (2017). Salinity stress induced alterations in antioxidant metabolism and nitrogen assimilation in wheat (*Triticum aestivum* L.) as influenced by potassium supplementation. *Plant Physiology and Biochemistry* 115: 449-460. https://doi.org/10.1016/j.plaphy.2017.04.017

Ali Q, Daud M K, Haider M Z, Ali S, Rizwan M, Aslam N, Noman A, Iqbal N, Shahzad F, Deeba F, Ali I & Zhu S J (2017). Seed priming by sodium nitroprusside improves salt tolerance in wheat (*Triticum aestivum* L.) by enhancing physiological and biochemical parameters. *Plant Physiology and Biochemistry* 119: 50-58. https://doi.org/10.1016/j.plaphy.2017.08.010

Arora D & Bhatla S C (2017). Melatonin and nitric oxide regulate sunflower seedling growth under salt stress accompanying differential expression of Cu/Zn SOD and Mn SOD. *Free Radical Biology and Medicine* 106: 315-328. https://doi.org/10.1016/j.freeradbiomed.2017.02.042

Asensio A C, Gil-Monreal M, Pires L, Gogorcena Y, Aparicio-Tejo P M & Moran J F (2012). Two Fe-superoxide dismutase families respond differently to stress and senescence in legumes. *Journal of Plant Physiology* 169(13): 1253-1260. https://doi.org/10.1016/j.jplph.2012.04.019.

Ashfaque F (2014). Exogenously applied H_2O_2 promotes proline accumulation, water relations, photosynthetic efficiency and growth of wheat (*Triticum aestivum* L.) under salt stress. *Annual Research & Review in Biology* 4(1): 105-120 https://doi.org/10.9734/arrb/2014/5629

Ashraf M (2009). Biotechnological approach of improving plant salt tolerance using antioxidants as markers. In *Biotechnology Advances* 27(1): 84-93. https://doi.org/10.1016/j.biotechadv.2008.09.003

Ashraf M & Harris P J C (2004). Potential biochemical indicators of salinity tolerance in plants. *Plant Science* 166(1): 3-16. https://doi.org/10.1016/j.plantsci.2003.10.024.

Athar H ur R, Khan A & Ashraf M (2008). Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. *Environmental and Experimental Botany* 63(1–3): 224-231 https://doi.org/10.1016/j.envexpbot.2007.10.018.

Baxter A, Mittler R & Suzuki N (2014). ROS as key players in plant stress signalling. *Journal of Experimental Botany* 65(5): 1229-1240. https://doi.org/10.1093/jxb/ert375

Beauchamp C & Fridovich I (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry* 44(1): 276-287. https://doi.org/10.1016/0003-2697(71)90370-8

Beauchamp C O & Fridovich I (1973). Isozymes of superoxide dismutase from wheat germ. *BBA - Protein Structure* 317(1): 50-64. https://doi.org/10.1016/0005-2795(73)90198-0

Bradford M M (1976). A rapid and sensitive method for the quantition of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Bruce T J A, Matthes M C, Napier J A & Pickett J A (2007). Stressful "memories" of plants: Evidence and possible mechanisms. *Plant Science* 173(6): 603-608. https://doi.org/10.1016/j.plantsci.2007.09.002.

Demirbas S & Balkan A (2020). The effect of H_2O_2 pre-treatment on antioxidant enzyme activities of triticale under salt stress. *JOTAF* 8: 1169-1178. https://doi.org/10.7546/crabs.2020.08.17.

Farooq S, Hussain M, Jabran K, Hassan W, Rizwan M S & Yasir T A (2017). Osmopriming with $CaCl_2$ improves wheat (*Triticum aestivum* L.) production under water-limited environments. *Environmental Science and Pollution Research* 24(15): 13638-13649. https://doi.org/10.1007/s11356-017-8957-x.

Giannipolities N & Ries S K (1977). Superoxide dismutase occurance in higher plants. *Plant Physiology* 59(2): 309-314. https://doi.org/10.1104/pp.59.2.309

He Y & Li Z (2018). Epigenetic Environmental memories in plants: establishment, maintenance, and reprogramming. *Trends in Genetics* 34(11): 856-866. https://doi.org/10.1016/j.tig.2018.07.006

He L H, Gao Z Q & Li R Z (2009). Pretreatment of seed with H_2O_2 enhances drought tolerance of wheat (*Triticum aestivum* L.) seedlings. *African Journal of Biotechnology* 8(22): 6151-6157. https://doi.org/10.5897/ajb09.490.

Hoagland D R & Arnon D I (1950). The water-culture method for growing plants without soil. Circular. *California Agricultural Experiment Station*, 347 (2nd edit), 32

Jia M, Guan J, Zhai Z, Geng S, Zhang X, Mao L & Li A (2018). Wheat functional genomics in the era of next generation sequencing : An update. *The Crop Journal* 6(1): 7-14. https://doi.org/10.1016/j.cj.2017.09.003

Jisha K C, Vijayakumari K & Puthur J T (2013). Seed priming for abiotic stress tolerance: an overview. *Acta Physiologie Plantarum* 35: 1381-1396. https://doi.org/10.1007/s11738-012-1186-5

Madhava Rao K V & Sresty T V S (2000). Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. *Plant Science* 157(1): 113-128. https://doi.org/10.1016/s0168-9452(00)00273-9

Parida A K & Das A B (2005). Salt tolerance and salinity effects on plants: A review. *Ecotoxicology and Environmental Safety* 60(3): 324-349. https://doi.org/10.1016/j.ecoenv.2004.06.010

Radoglou K M & Jarvis P G (1990). Effects of CO_2 enrichment on four poplar clones. I. growth and leaf anatomy. *Annals of Botany* 65(6): 617–626. https://doi.org/10.1093/oxfordjournals.aob.a087978

Rashidi V, Rahimi Darabad J, Shahbazi H, Moghaddam Vahed M & Khalilvand E (2021). Heritability and genetic parameters of some antioxidant enzyme activities in barley (*Hordeum vulgare* L.) cultivars under salinity stress. *Journal of Agricultural Sciences* 27(2): 187-194. https://doi.org/10.15832/ankutbd.597545

Savvides A, Ali S, Tester M & Fotopoulos V (2016). Chemical priming of plants against multiple abiotic stresses: mission possible? *Trends in Plant Science* 21(4): 329-340. https://doi.org/10.1016/j.tplants.2015.11.003

Saxena I, Srikanth S & Chen Z (2016). Cross talk between H_2O_2 and interacting signal molecules under plant stress response. *Frontiers in Plant Science* 7: 1-16. https://doi.org/10.3389/fpls.2016.00570

Shi X & Ling H (2018). Current advances in genome sequencing of common wheat and its ancestral species. *The Crop Journal* 6(1): 15-21. https://doi.org/10.1016/j.cj.2017.11.001

Smart R E & Bingham G E (1974). Rapid estimates of relative water content. *Plant Physiology* 53: 258-260. https://doi.org/10.1104/pp.53.2.258

Tabassum T, Farooq M, Ahmad R, Zohaib A & Wahid A (2017). Seed priming and transgenerational drought memory improves tolerance against salt stress in bread wheat. *Plant Physiology and Biochemistry* 118: 362-369. https://doi.org/10.1016/j.plaphy.2017.07.007

Vitória A P, Lea P J & Azevedo R A (2001). Antioxidant enzymes responses to cadmium in radish tissues. *Phytochemistry* 57(5): 701-710 .https://doi.org/10.1016/s0031-9422(01)00130-3

Wani S H, Kumar V, Shriram V & Sah S K (2016). Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. *The Crop Journal* 4(3): 162-176. https://doi.org/10.1016/j.cj.2016.01.010

Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

Effects of Foliar Applications on Nutrient Concentrations of Kernel, Pomological Properties and Yield of 'Chandler' Walnut Variety at Different Altitudes

Nihal ACARSOY BILGIN[a*]

[a]*Ege University, Faculty of Agriculture, Department of Horticulture, İzmir, TÜRKİYE*

ARTICLE INFO
Research Article
Corresponding Author: Nihal ACARSOY BILGIN, E-mail: nihal.acarsoy@ege.edu.tr
Received: 21 April 2020 / Revised: 07 November 2021 / Accepted: 07 November 2021 / Online: 15 October 2022

Cite this article
BİLGİN A N (2022). Effects of Foliar Applications on Nutrient Concentrations of Kernel, Pomological Properties and Yield of 'Chandler' Walnut Variety at Different Altitudes. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 28(4):603-612. DOI: 10.15832/ankutbd.925268

ABSTRACT
In Turkey, the orchards are being established with the 'Chandler' walnut variety in different ecologies, nowadays. Plant nutrition applications are important for optimum yield and quality in terms of growing. In this study conducted at two different altitudes (51 and 740 m) in the orchard, foliar application as urea (5 gr/L), potassium nitrate (10 gr/L), borax (1 gr/L), manganese sulfate (2 gr/L) and zinc sulfate (1.5 gr/L) were sprayed for two years. Following the application, macro and micro nutrient content of kernel and fruit properties were determined. According to this, it was observed that foliar application of boron (B) in terms of nut weight and foliar application of potassium (K) in terms of kernel ratio ranked the first row. The shrinkage ratio, an important quality criterion for walnuts, was found to be low in the high altitude Demirci location (17.43%). However, in the low altitude Saruhanlı location, the shrinkage ratio was reduced with foliar K application. In addition, while all foliar applications had a positive effect on yield, the highest value was measured in Saruhanlı location (3.31 kg tree^{-1}). The macro and micro nutrient content of kernel evaluated, there was an increase in nutrients in the second year, except for K, calcium (Ca) and magnesium (Mg). Phosphorus (0.29 and 0.27%), iron (30.64 and 6.24 ppm), copper (7.64 and 11.35 ppm), zinc (32.42 and 27.03 ppm) and manganese (25.77 and 30.05 ppm) contents of the grain were found to be significant for Demirci and Saruhanlı locations, respectively. Values in Demirci location were higher than Saruhanli location. Additionally, it was also revealed in interaction.

Keywords: *Juglans regia*, Macro and micro nutrients, Fruit quality, Location x year x application interaction

1. Introduction

Walnut is one of the most important hard nut fruit species in the world. Yield and quality characteristics vary depending on the variety and ecological conditions at different altitudes. As it is known, high yield is the most important among production targets in terms of growing. Macro and micro nutrients have an important effect on this matter (Khayyat et al. 2007). For a long time, intensive use of soil fertilizers has caused significant soil and water-related environmental problems (Rios et al. 2020). Management of nutrients is one of the basic principles of sustainable agriculture. In this context, foliar fertilization is considered an environmentally friendly practice (Norozi et al. 2019). In plants, especially in periods when their uptake is limited and their requirements are high, nutrients must be taken from the leaves. Foliar sprays are the most effective and very rapid treatment (Fernandez et al. 2013; Ghani et al. 2021). The use of foliar fertilizer is common due to these properties (Rios et al. 2019). Nitrogen is the basic plant nutrient. When sprayed in urea form, it improves diffusion conditions by increasing the permeability of the cutin layer. In this way, it is reported to be high uptake (Kacar & Katkat 2006). Foliar N fertilizer application contributes to the rapid development of shoots in the spring period (Xu et al. 2021). At the same time, N is an important element for the synthesis of organic compounds (Carranca et al. 2018).

Another nutrient necessary for flowering and fruit formation is potassium (K). For this species with high oil content, the trees must be fertilized with K. Nut fruit species require a high amount of potassium during the fruit development period. In this case, foliar spraying is reported as an effective and rapid application in terms of potassium supply (Norozi et al. 2019; Marchand 2020).

In addition, micro-elements are effective in plant nutrition programs (Dejampour & Zeinalabedini 2006; Yıldız et al. 2007). In salty, calcareous and high pH soils, micronutrient deficiencies are eliminated by foliar application. This method is preferred because it is fast, cheap and target-oriented (Rios et al. 2019; Xie et al. 2020).

Some fruit species need high boron (B) element especially during blooming. For nut species, this element is often recommended in the fertilization program (Ellis 2016). High of pH and calcium carbonate in the experimental area soils cause low efficiency of soil-applied B fertilizers (Khoshgoftarmanesh 2012). Therefore, it can be easily transported to different organs of the tree through phloem by foliar application. Foliar B applications performed during this development period lead to fruit set and consequently increase in yield (Brown 2001; Khayyat et al. 2007). With application B, the yield of cashew nut per tree increased significantly compared to the control trees (Gavit et al. 2020). Similarly, in hazelnut, the best findings on yield and some fruit quality traits were obtained with B treatments (Horuz et al. 2021).

Zinc (Zn), which is another essential element in plant physiology, has an effect on yield and quality. Soil-applied Zn is not supplying enough Zn to the trees. This is due to the deep penetration of the roots and the difficult progression of Zn in the soil. Foliar Zn application is a common practice to compensate for Zn deficiency (Smith et al. 2021). In pistachio, it is mentioned that the application of leaf Zn spray during bud swelling and green tip period has a positive effect on fruit yield and quality, especially splitting. This application is foreseen as a solution in calcareous soils (Norozi et al. 2019).

Manganese (Mn) should be included in walnut growing programs. In cold and rainy conditions, plant root activity decreases and Mn uptake decreases. While Mn is very high in acid soils, Mn uptake is low in high pH soils. In walnuts, a significant decrease in leaf Mn concentration was observed during fruit development (Kim & Wetzstein 2005). For these reasons, similar to other elements, foliar spray treatments are more effective (Hounnou et al. 2019).

Walnut is considered as a good source of macro and micro nutrients composition. In particular, walnuts are rich in K, which consume very important for human health. On the other hand, P, Ca, Mg and Na content is also high (Yıldız & Sümbül, 2019a). Copper, Fe, and Zn contained in walnuts are necessary micronutrients for important for human metabolism (Scherz & Kirchhoff 2006). Therefore, it is present in all diet programs, including the vegetarian and vegan diet (Abdallah et al. 2015). After all, walnuts are an important food in the supply of essential elements to the human body (Simsek 2016). It was stated that nutrient content may be varied due to effects such as variety, year, ecology, maturity level and the methods of cultivation (Juranovic Cindric et al. 2018; Kabiri et al. 2019). Further, fruit quality features were improved with plant nutrition applications. In previous studies, the nutrient content of walnut kernel was revealed in different locations (Gülsoy et al. 2016; Batun et al. 2017; Kabiri et al. 2019; Ozyigit et al. 2019). In walnuts, these macro-micronutrients are extremely important in terms of fruit development, yield and quality. Additionally, it has been concluded that foliar application of K and Zn fertilizers is necessary to obtain better fruit yield and quality in pistachio (Norozi et al. 2019). In the light of the above explanations, in this study, it was aimed to determine the effects of macro and micro foliar application on nutrient content of kernel, some fruit properties and yield of 'Chandler' walnut variety in different ecological conditions with two distinct altitudes (51 and 740 m).

2. Material and Methods

Field studies were conducted in a commercial orchard located in Saruhanlı (38° 47' 31" N 27° 30' 22" E, altitude 51 m) and Demirci (39° 02' 39" N 28° 35' 56" E, altitude 740 m), Manisa province, Turkey. Drip irrigation is applied to the trees in both orchards. There is a homogeneous situation in terms of the amount of water. The maximum and minimum temperature values for the years in which the trial was conducted are presented in Table 1. Soil analysis results of the experimental orchard are given in Table 2. It was determined that the soil was loamy, slightly alkaline, moderately calcareous, poor in organic matter and salt-free. 'Chandler' walnut variety (5-years-old) was used as plant material during 2017 and 2018 years. This variety has moderately strong growth habit and winter chilling requirement is defined 700 hours. In addition, its foliation and blooming are the late period. Nuts ripening in the mid-season are large, smooth, oval shaped and fragile shell (Özçağıran et al. 2014).

Table 1- Monthly temperature values (°C)

Month	*Saruhanlı*				*Demirci*			
	2017		*2018*		*2017*		*2018*	
	Max	***Min***	***Max***	***Min***	***Max***	***Min***	***Max***	***Min***
1	9.0	-0.3	12.1	1.6	4.4	-1.8	9.3	2.9
2	14.7	2.9	15.8	5.6	9.6	2.4	11.6	5.2
3	19.6	5.6	20.3	7.9	14.3	5.4	15.0	6.9
4	23.9	6.7	28.4	8.7	17.8	8.1	23.1	12.4
5	28.7	11.7	30.8	14.8	21.7	11.9	24.7	14.3
6	34.1	16.6	33.3	16.5	27.0	16.2	27.5	16.7
7	36.6	18.6	35.6	19.1	31.7	19.9	30.3	19.2
8	35.0	19.4	36.6	20.6	30.2	19.0	31.4	20.1
9	33.6	13.3	32.1	15.9	29.1	16.9	27.2	16.8
10	25.2	8.0	25.9	9.8	19.5	10.1	22.1	12.1
11	18.8	3.2	19.2	6.7	13.9	6.1	16.1	8.6
12	15.0	5.1	11.0	2.2	10.5	4.7	8.3	2.0

Table 2- Soil analysis results at two depths (0-30 cm and 30-60 cm)

Soil Parameters	*Demirci*				*Saruhanlı*			
	0-30 cm		*30-60 cm*		*0-30 cm*		*30-60 cm*	
pH	7.77	Alkaline	7.71	Alkaline	7.74	Alkaline	7.85	Alkaline
EC (%)	0.038	Salt-free	0.048	Salt-free	0.046	Salt-free	0.047	Salt-free
$CaCO_3$ (%)	27.13	High	31.92	High	9.98	Medium	10.77	Medium
Sand (%)	42.24		46.24		44.24		44.24	
Clay (%)	28.00		24.00		30.00		30.00	
Silt (%)	29.76		29.76		25.76		25.76	
Texture		Clay loam soil		Clay loam sand		Loamy soil		Loamy soil
Organic Matter (%)	3.39	Sufficient	2.90	Sufficient	0.68	Insufficient	0.14	Insufficient
Total N (%)	0.123	Sufficient	0.106	Sufficient	0.062	Insufficient	0.056	Insufficient
Available P (ppm)	0.20	Insufficient	0.40	Insufficient	0.80	Insufficient	0.2	Insufficient
Available K (ppm)	397.70	Sufficient	329.8	Sufficient	358.9	Sufficient	310.4	Sufficient
Available Ca (ppm)	5529	High	5238	High	5238	High	5044	High
Available Mg (ppm)	351.60	High	401.70	High	632	High	649	High
Available Na (ppm)	19.80	Normal	96.10	Normal	150.4	Normal	37.6	Normal
Available Fe (ppm)	3.80	Normal	3.34	Normal	6.31	Sufficient	1.14	Insufficient
Available Zn (ppm)	0.49	Sufficient	0.77	Insufficient	0.42	Insufficient	0.54	Insufficient
Available Cu (ppm)	1.53	Sufficient	1.33	Sufficient	1.34	Sufficient	0.82	Sufficient
Available Mn (ppm)	8.12	Sufficient	6.58	Sufficient	5.8	Sufficient	3.46	Sufficient
Soluble B (ppm)	0.51	Insufficient	0.54	Insufficient	0.32	Insufficient	0.4	Insufficient

Foliar applications were sprayed as N (urea, 5 gr/L), K (potassium nitrate, 10 gr/L), B (borax, 1 gr/L), Mn (manganese sulfate, 2 gr/L), Zn (zinc sulfate, 1.5 gr/L) and control (untreated trees). As it is known, macro and micro nutrients are effective on fruit growth, yield and quality parameters of plants. In foliar fertilizers, the application dose should not exceed 0.1-0.2% in micro elements and 1-2% in macro elements on the basis of the active substance (except low biurea urea). Application doses and times vary according to the development period of the plant and the thickness of the leaf cuticle layer (Kacar 1982; Epstein & Bloom 2005; Çolakoğlu & Çiçekli 2016; Fernandez et al. 2013). Foliar application should be applied 2-3 times at 15-20 days intervals after flowering, which is the critical period for plants (Çolakoğlu & Çiçekli 2016).

It was carried out twice for each nutrient element. For B, the first application was made before the male flowers bloom, and the second application two – three weeks later. For N, K, Zn, Mn, the first application was made, after the male flowers bloomed, the second application was made two – three weeks later (Norozi et al. 2019).

The harvested fruits were separated from green peels and dried in the shade. Average nut weight was determined on precision electronic scale (0.01 g) then the kernel ratio (%) was calculated. Fruit color was measured by a CR400 model Minolta Colorimeter in CIE L* a* b*. In fruit which is accepted as 4 parts the shrinkage ratio of each part was determined as % (Şen, 1980; Beyhan, 1993; Aşkın & Gün, 1995). For yield, the total amount of nut was recorded in each tree at harvest time (kg). The kernel samples were dried at 65°C in the oven until constant weight is achieved (24-48 hours), for nutrient analysis. These samples were crushed and ground (Analytical Mill, IKA A 11 BASIC). The Kjeldahl method for N (Gerhardt Germany); the colorimetric method for P (AnalytikJena AG Germany); the flame fotometric method for Ca and K (Eppendorf Geratebaue & Netheler Hınz Gmbh Germany); atomic absorption spectrophotometer for Mg, Fe, Cu, Zn and Mn (Varian Spektr AA 220); spectrophotometic with Azomethhin-H method for B analyses were used (Varian Spektr AA 220) (Wolf 1971; Kacar & İnal 2008).

The experiment was carried out according to the design of the random blocks, with 3 replications and 3 trees per replication. 30 fruits were evaluated each year. The data were subjected to analysis of variance using SPSS 20 statistical package program. Significant differences between averages were defined by Duncan test at the $P<0.05$ significant level.

3. Results

3.1. Fruit properties and yield

In the evaluation made according to applications, years and locations, there was a statistical difference in the nut properties, in general except for the kernel color L* and a* value (Table 3). According to this, the highest nut weight was measured with B application at Saruhanlı location in 2017 and at Demirci location in both years (2017 and 2018). In contrast, the lowest value was measured in the untreated trees. Kernel ratio varied according to applications. The highest kernel ratio was obtained from B application with 54.96% at the Saruhanlı location in 2018 and K application with 57.55% at Demirci location in 2017. For b* value, a statistical difference was found at Saruhanlı in the second year and at Demirci in both years. However, applications varied on the yellow color of the kernel. The effect of applications on the shrinkage ratio of the kernel was statistically significant effective in the first year of the experiment in both locations. Accordingly, the shrinkage ratio of the kernel was found in the range of 22.50% - 56.66% in Saruhanlı and 8.33% - 30.00% in Demirci. The highest yield was achieved with control application at Saruhanlı in 2017, but at Demirci, with Zn application in both years.

According to the years; nut weight; shrinkage ratio and yield statistical difference was observed (Table 4). Thus, an increase in the features mentioned was detected in 2018. On the other hand, the statistical differences were found between locations in the majority of the properties (except nut weight and kernel ratio). It was calculated that L* value (54.07), a* value (7.22) and shrinkage ratio (17.43%) had better results in Demirci location, while b* value (27.77) and yield (3.31 kg tree^{-1}) in Saruhanlı location. On the basis of applications, it was observed that foliar application of B in terms of nut weight (13.70 g) and foliar application of K in terms of kernel ratio (52.01%) ranked the first row. For shrinkage ratio, applications were in the same statistical group, except N and Mn. All applications, except untreated trees, had an impact on yield. In addition, it was determined that foliar nutrient applications were not effective on kernel color values.

It was found out that year*location interaction for nut weight; location*application for shrinkage ratio; year*application interaction for b* value and location* year* application interaction for shrinkage ratio were significant. For the year* location interaction, changes in locations based on years were important in terms of nut weight. In terms of average value of nut weight, it varied between 12.60 g (2017) and 13.60 g (2018) in Saruhanlı location (Table 3).

To be into account year* application interaction, b* value of kernel was determined to be effective. Accordingly, the highest value was obtained from Mn application (30.05 and 29.38) with an average of 29.72 in 2018. For location* application interaction, it was found important in terms of shrinkage ratio. Regarding this feature, in the evaluation made according to the average of the data for both years in Table 3, the highest value was determined from B application (11.66% and 9.16%) with an average of 10.41% in Demirci location. However, the lowest value was stated from N application (56.66% and 36.66%) with an average of 46.66% in other location. In terms of the same feature, location* year* application interaction was important. Thus, the shrinkage ratio ranged from 5.83% (with Mn application in Demirci location in 2018) to 56.66% (with N application in Saruhanlı location in 2017).

Table 3- Location, year and application values of the nut properties

Location	*Year*	*Application*	*Nut weight (g)*	*Kernel ratio (%)*	*L* value*	*a* value*	*b* value*	*Shrinkage ratio (%)*	*Yield (kg/tree)*
Saruhanlı	2017	Control	12.27 b	50.17	51.34	8.75	29.87	29.16 ab	1.79 b
		K	12.58 ab	50.75	52.43	8.53	29.23	22.50 a	1.99 ab
		B	13.29 a	51.03	50.61	8.97	29.92	40.83 abc	3.89 a
		N	12.29 b	51.08	51.40	7.86	29.74	56.66 c	2.11 ab
		Mn	12.59 ab	52.00	51.09	8.34	28.78	34.16 ab	3.21 ab
		Zn	12.56 ab	49.22	50.25	8.05	29.48	45.00 bc	2.40 ab
	2018	Control	13.89	48.45 bc	49.65	8.54	30.14 ab	38.33	3.16
		K	12.76	54.31 ab	52.75	8.39	30.46 a	21.66	4.50
		B	13.82	54.96 a	51.28	8.42	29.22 b	22.50	4.34
		N	13.63	51.67 abc	51.90	7.95	29.92 ab	36.66	3.89
		Mn	13.81	48.51 bc	52.26	7.90	30.05 ab	38.33	4.01
		Zn	13.69	47.95 c	47.90	9.56	30.44 a	31.66	4.44
Demirci	2017	Control	11.84 d	50.59 ab	52.88	6.95	28.42 b	35.00 bc	1.11 b
		K	11.84 d	57.55 a	55.19	7.79	28.99 ab	30.00 b	1.08 b
		B	14.17 a	51.31 ab	53.87	6.67	29.12 a	11.66 a	1.42 ab
		N	12.09 cd	44.99 b	52.41	7.05	28.75 ab	16.66 a	1.24 b
		Mn	13.05 bc	49.07 b	51.26	6.77	28.44 b	41.66 c	1.24 b
		Zn	13.14 b	48.19 b	54.70	8.10	29.10 a	8.33 a	1.79 a
	2018	Control	11.97 b	49.99	53.91	7.32	28.99 ab	8.33	0.77 b
		K	12.18 ab	45.45	55.76	7.17	28.50 b	12.50	1.99 ab
		B	13.53 a	47.45	54.57	6.93	27.50 c	9.16	1.30 ab
		N	12.21 ab	47.20	54.67	7.17	28.64 ab	16.66	1.92 ab
		Mn	12.85 ab	48.30	55.05	7.20	29.38 a	5.83	1.91 ab
		Zn	13.26 ab	45.57	54.63	7.51	28.74 ab	13.33	3.05 a

The differences in the means were determined by the Duncan test according to P≤0.05

Table 4- Average values of the properties

		Nut weight (g)	*Kernel ratio (%)*	*L* value*	*a* value*	*b* value*	*Shrinkage ratio (%)*	*Yield (kg/tree)*
Year	2017	12.64 b	50.49	52.28	7.82	29.15	30.97 b	1.94 b
	2018	13.13 a	49.15	52.86	7.83	29.33	21.25 a	2.94 a
Location	Demirci	12.68	48.80	54.07 a	7.22 a	28.71 b	17.43 a	1.57 b
	Saruhanlı	13.10	50.84	51.07 b	8.43 b	29.77 a	34.79 b	3.31 a
Application	Control	12.49 d	49.80 abc	51.94	7.89	29.35	27.70 a	1.71 b
	K	12.34 d	52.01 a	54.03	7.97	29.30	21.66 ab	2.39 a
	B	13.70 a	51.19 ab	52.58	7.74	28.94	21.04 ab	2.73 a
	N	12.55 cd	48.74 bc	52.59	7.51	29.26	31.66 b	2.29 ab
	Mn	13.07 bc	49.47 abc	52.42	7.55	29.16	30.00 b	2.59 a
	Zn	13.16 b	47.73 c	51.87	8.30	29.44	24.58 ab	2.92 a
Year*Location		**	ns	ns	ns	ns	ns	ns
Location* Application		ns	ns	ns	ns	ns	**	ns
Year* Application		ns	ns	ns	ns	**	ns	ns
Location* Year* Application		ns	ns	ns	ns	ns	*	ns

*: Significant at P<0.05, **: Significant at P<0.01. ns: Non-significance.

3.2. Nutrient concentrations of kernel

As a result of foliar applications, macro and micro nutrient contents determined in 'Chandler' variety are given in Table 5. Foliar N spray was caused this element content of kernel to be much higher than others in both locations and years. In contrast, control application was observed in the last group. Considering the P contents of kernels, K and Mn (0.33%) applications for Demirci and Zn (0.31%) application for Saruhanlı took placed the first row in 2018. In the foliar K spray, this macro element was found to have the highest values as a result of kernel analysis in both locations and years. Whereas, the lowest K content was determined in the control application. All foliar applications had positive effect on this content. It was seen that Ca content of kernel is high for Saruhanlı location, K application in 2018; for Demirci location, Zn application in 2017 and Mn application in 2018. The effect of the applications on the Mg content of kernel was statistically significant. According to this, the highest Mg content of kernel was obtained from Mn application with 0.17% in 2017 and with 0.20% in 2018.

For both locations and years, micro elements such as Fe, Zn and Mn were statistically significant. Fe content of kernel was located in the first statistical group in K, B and Mn applications in Saruhanlı. Otherwise, the highest kernel Fe content was determined with the N application in Demirci. In the foliar Zn application in Saruhanlı location, this micro element was found to have the highest values, while the lowest Zn content of kernel was observed in the control group. A similar situation was determined in Mn content of kernel at the same location. Cu content of kernel was important statistically in Saruhanlı in 2018 and Demirci in both years. This content varied based on year and applications. B content, the most effective application was N foliar spray, in Saruhanlı. B content reached the highest level in the Demirci location in first year with foliar application of Zn (2.86 ppm) and second year with foliar application of B (4.53 ppm).

There was a statistical difference in macro and micro nutrients evaluated by years, except for K, Ca and Mg (Table 6). Thus, there was an increase in these nutrients in the second year. When the locations are evaluated, it was found to be an increased that P for macro and Fe, Cu, Zn and Mn for micro were significant. Nutrition contents of kernel in Demirci location were higher than the other location. Nitrogen application for N content of kernel (2.96%), K application for K content of kernel (0.55%), Mn and Zn application for Ca content of kernel (0.10%), B application for Fe content of kernel (29.76), B application for Cu content of kernel (10.55), Zn application for Zn content of kernel (31.86 ppm), Mn application for Mn content of kernel (31.13 ppm) and N application for B content of kernel (3.43 ppm) ranked the first row. Untreated trees formed the last group.

It was stated that location* year interaction for N; location* application for N, Zn, Mn and B; year* application interaction for N, Fe Mn and B and location* year* application interaction for N and Mn were significant. For year* location interaction, it was found important in terms of N amount of kernel. Considering the average value, the N content of kernel (2.905%) took the first place at the Demirci location in 2018 (Table 5). In 2017, the N content of kernel was determined as 2.44% in the same location. Considering the location* application interaction; in the evaluation made according to the average of the data for both years in Table 5, the highest amount of kernel N was obtained from Mn application (2.84 and 3.23%) with an average of 3.04% at the Demirci location. The lowest amount of kernel N was occurred from control application (2.06 and 2.21%) with an average of 2.14 at the same location. In terms of Zn amount of kernel, Mn application (34.43 and 35.31 ppm) with an average of 34.87 ppm in Demirci location was the first, and control application (17.37 and 20.66 ppm) with an average of 19.02 ppm in Saruhanlı was found in the last place. The Mn content of kernel was found in the range of 23.45 (with Zn application at Demirci location)

- 31.51 ppm (with K application at Saruhanlı location). In terms of B content of kernel, Mn application (2.97 and 4.17 ppm) with an average of 3.57 ppm in Saruhanlı location was the first, and N application (3.71 and 1.10 ppm) with an average of 2.41 ppm in the same location was found in the last place. For the year* application interaction; N, Fe, Mn and B content of kernel were determined to be effective. Accordingly, the highest value N, Fe, Mn and B content of kernel were obtained from foliar N application (3.05 and 3.26%) with an average of 3.16%, Fe application (32.28 and 35.583 ppm) with an average of 33.93 ppm, Mn application (32.97 and 35.46 ppm) with an average of 34.22 ppm, B application (4.40 and 4.53 ppm) with an average of 4.47 ppm in 2018, respectively. For the location* year* application, N and Mn contents of kernel were determined to be effective. The N content was found in the range of 3.26% (with N application in Demirci location in 2018) - 2.06% (with control application in Demirci location in 2017). The Mn of kernel was found in the range of 18.64 ppm (with K application in Demirci location in 2017) - 38.55 ppm (with Mn application in Saruhanlı location in 2018).

Table 5- Location, year and application values of the macro and micro nutrient content of kernel

Location	*Year*	*Application*	*N (%)*	*P (%)*	*K (%)*	*Ca (%)*	*Mg (%)*	*Fe (ppm)*	*Cu (ppm)*	*Zn (ppm)*	*Mn (ppm)*	*B (ppm)*
Saruhanlı	2017	Control	2.30 c	0.24	0.40 c	0.03	0.10	18.98 b	11.31	17.37 c	23.83 c	2.34 c
		K	2.78 a	0.26	0.54 a	0.06	0.10	24.85 a	11.51	26.75 b	30.05 ab	2.47 bc
		B	2.41 bc	0.27	0.46 bc	0.10	0.16	27.62 a	12.16	26.54 b	22.47 c	2.12 c
		N	2.78 a	0.29	0.46 bc	0.06	0.16	26.00 a	10.47	28.26 b	29.45 ab	3.71 a
		Mn	2.57 ab	0.26	0.43 bc	0.10	0.13	26.64 a	10.51	24.51 b	34.01 a	2.97 b
		Zn	2.63 ab	0.25	0.48 ab	0.10	0.13	25.35 a	10.43	33.40 a	28.72 b	3.62 a
	2018	Control	2.33 c	0.27 b	0.38 c	0.09 b	0.17	26.36 ab	11.36 ab	20.66 c	28.88 bc	2.84
		K	2.83 ab	0.30 ab	0.56 a	0.12 a	0.18	32.28 a	12.51 ab	28.55 ab	32.97 b	2.82
		B	2.60 bc	0.28 ab	0.50 b	0.11 a	0.18	29.41 ab	13.03 a	29.87 ab	27.92 c	4.08
		N	3.05 a	0.29 ab	0.48 b	0.09 b	0.18	25.33 b	11.13 ab	29.74 ab	32.01 bc	4.30
		Mn	2.76 ab	0.26 b	0.50 b	0.11 a	0.18	27.71 ab	10.43 b	25.82 b	38.55 a	3.14
		Zn	2.75 b	0.31 a	0.48 b	0.10 a	0.18	24.41 b	11.38 ab	32.85 a	31.77 bc	3.96
Demirci	2017	Control	2.06 d	0.31	0.37 d	0.09 b	0.14 c	28.78 a	7.59 a	33.12 ab	30.30 a	2.58 ab
		K	2.22 c	0.24	0.54 a	0.08 b	0.14 c	23.86 b	4.81 b	32.39 ab	18.64 c	2.19 b
		B	2.60 b	0.27	0.51 ab	0.10 ab	0.16 abc	29.82 a	8.14 a	30.90 ab	26.53 b	2.29 b
		N	2.75 a	0.28	0.48 abc	0.08 b	0.17 ab	30.12 a	6.51 ab	25.50 c	25.36 b	2.53 ab
		Mn	2.84 a	0.29	0.44 cd	0.10 ab	0.17 a	29.14 a	8.08 a	34.43 a	25.86 b	2.41 ab
		Zn	2.14 cd	0.31	0.44 bc	0.13 a	0.15 bc	28.04 a	6.37 ab	29.36 bc	22.97 b	2.86 a
	2018	Control	2.21 d	0.28 b	0.34 d	0.08 c	0.19 ab	30.16 d	7.65 b	36.41 a	21.86 c	3.08 b
		K	3.23 a	0.33 a	0.58 a	0.08 c	0.20 a	35.58 ab	9.83 a	34.91 ab	35.46 a	2.96 b
		B	2.96 b	0.30 ab	0.51 bc	0.10 bc	0.18 ab	32.18 cd	8.86 ab	34.63 ab	25.38 bc	4.53 a
		N	3.26 a	0.30 ab	0.50 bc	0.07 c	0.18 b	36.86 a	9.78 a	30.29 b	26.92 b	3.20 b
		Mn	3.23 a	0.33 a	0.53 ab	0.12 a	0.20 a	29.85 d	8.53 ab	35.31 ab	26.09 b	3.07 b
		Zn	2.51 c	0.30 ab	0.46 c	0.11 ab	0.18 b	33.28 bc	5.58 c	31.81 ab	23.92 bc	2.40 b

The differences in the means were determined by the Duncan test according to P≤0.05

Table 6- Average values of the macro and micro nutrient content of kernel

		N(%)	*P(%)*	*K(%)*	*Ca(%)*	*Mg (%)*	*Fe (ppm)*	*Cu (ppm)*	*Zn (ppm)*	*Mn (ppm)*	*B (ppm)*
Year	2017	2.51 b	0.27 b	0.466	0.06	0.11	26.60 b	8.99 b	28.54 b	26.51 b	2.67 b
	2018	2.81 a	0.29 a	0.489	0.06	0.12	30.28 a	10.01 a	30.90 a	29.31 a	3.36 a
Location	Demirci	2.67	0.29 a	0.479	0.05	0.11	30.64 a	7.64 b	32.42 a	25.77 b	2.84
	Saruhanlı	2.65	0.27 b	0.476	0.07	0.12	26.24 b	11.35 a	27.03 b	30.05 a	3.20
Application	Control	2.22 e	0.27	0.37 c	0.03 c	0.11	26.07 b	9.47 b	26.89 c	26.22 d	2.71 c
	K	2.76 b	0.28	0.55 a	0.05 bc	0.11	29.14 a	9.66 ab	30.65 ac	29.28 b	2.61 c
	B	2.64 c	0.28	0.49 b	0.07 ab	0.12	29.76 a	10.55 a	30.48 ac	25.57 d	3.26 ab
	N	2.96 a	0.29	0.48 b	0.04 c	0.11	29.58 a	9.48 b	28.45 bc	28.44 bc	3.43 a
	Mn	2.85 b	0.28	0.47 b	0.10 a	0.13	28.33 a	9.39 b	30.02 ac	31.13 a	2.90 bc
	Zn	2.51 d	0.29	0.46 b	0.10 a	0.11	27.77 ab	8.44 c	31.86 a	26.84 cd	3.21 ab
Year* Location		*	ns	ns	ns	ns	ns	ns	ns	ns	ns
Location* Application		**	ns	ns	ns	ns	ns	ns	*	*	**
Year* Application		*	ns	ns	ns	ns	**	ns	ns	*	*
Location* Year* Application		*	ns	ns	ns	ns	ns	ns	ns	*	ns

*: Significant at P<0.05, **: Significant at P<0.01. ns: Non-significance.

4. Discussion

4.1. Fruit properties and yield

Foliar applications of macro and micro nutrients have a positive effect on fruit quality and yield. In this study, important effects of applications were determined on fruit weight. In this regard, statistically, the highest value was obtained from the B application

for this feature. This was followed by the Zn leaf application, in general. It is also emphasized by different researchers that Zn treatments had a positive effect on nut weight in Pecan (Hounnou et al. 2019) and pistachio (Najizadeh & Khoshgoftarmanes 2019) than the untreated trees. As a result of Zn and B foliar application, nut weight and kernel ratio amount increased compared to other application for Persian walnut trees (Keshavarz et al. 2011). In the same variety, the highest value was obtained with B application in terms of nut weight (Acarsoy Bilgin et al. 2018). Similar results were found in this current study. Additionally, among the different Zn forms, zinc sulfate is the lowest-cost Zn treatment. As a matter of fact, Hounnou et al. (2019) achieved positive results with the same application. Confirming this, the zinc sulfate treatment had a positive effect on nut and kernel weight in both years. In Hazelnut B + Zn applications increased nut size and kernel weight (Horuz et al. 2021). In almond, soil and foliar N applications did not significantly affect the nut and kernel weight (Morais et al. 2020). A somewhat similar finding was obtained in this current study.

The plant nutrient deficiency has been reported to cause problems such as the shrinkage ratio of the kernel that is important for this variety (Şen 1986). In this study, the shrinkage ratio was low with the K application in Saruhanlı location and these values were determined to be 22.50% (first year) and 21.66% (second year). Besides, it was found that shrinkage ratio varied according to location and years. There are differences in the shrinkage ratio of the genotypes (Simsek 2010). In another study on the same walnut cultivar, shrinkage ratio was approximately half of the control with K application (Acarsoy Bilgin et al. 2021). In this context, it was concluded that K foliar application is important in terms of kernel quality.

One of the most important properties in commercial production is high efficiency. Keshavarz et al. (2011) reported that the foliar application of zinc dramatically increased both fruit set and yield of walnut. The yield increase was recorded with Zn foliar application in pistachio (Mohammadmehdi et al. 2019; Najizadeh & Khoshgoftarmanes 2019). There was a positive effect of B applications on yield (Acarsoy Bilgin et al. 2018). Zinc application was less effective than B application in terms of fruit yield effect by Keshavarz et al. (2011). A similar result was achieved in this study. In the research by Morais et al. (2020) investigating the effects of combined soil and foliar nitrogen fertilization applications on almond trees, it was stated that N fertilization was not effective on yield. On the contrary, in the current study, an increase in yield was recorded with foliar N application compared to the control in both locations and years. The higher yield of cashew nut was obtained with the foliar application of boron (0.25%) + zinc (0.5%) in addition to the application of N P K (1 : 0.5 : 0.5) (Gavit et al. 2020). In Tombul Hazelnut variety, the highest yield was obtained with the B + Zn applications (Horuz et al. 2021). The aforementioned fertilizers had a positive effect on Chandler walnut in this study. The effects of genotype, environment and interaction on fruit quality characteristics are mentioned (Forde, 1975). It was stated that the quality characteristics of 5 hazelnut varieties varied according to the altitudes (100, 350 and 800 m) (Gülsoy et al. 2019). Koyuncu et al. (2002) reported that the walnut grown at high altitudes are small, but the color values give better results. In addition, it was stated that the genotype selected at 710 m altitude in the Northeastern Anatolia Region increased the kernel weight and yield when grown in the Yolova ecology at sea level (Orman 2018). In Uşak ecology, at an average altitude of 650 m, 800 m, and 900 m, in the 'Chandler' variety, nut weight increased depending on the altitude (Büyüksolak et al. 2020). On the contrary, it decreased in this study. In a different study conducted our, it was emphasized that 'Chandler' variety may be suitable for Saruhanlı ecology (Bilgin et al. 2018). In support of this, in the current study of 'Chandler' walnut variety, it was observed that the properties were superior in Saruhanlı location compared to Demirci location, which has a high altitude. This situation is thought to be a result of favorable climatic conditions.

Ramos (1998) reported that walnut yield and fruit quality characteristics changed according to ecological conditions. One of the causes of the shrinkage is high temperature damage (Forde, 1975). Due to the low summer temperatures in the Demirci location, the weight that has not been filled up completely has decreased. In support of this, it was observed that the shrinkage ratio increased in Saruhanlı location, which has high summer temperatures.

4.2. Nutrient concentrations of kernel

Walnuts are a good source of macro and micro nutrients, which are very beneficial for human health (Abdallah et al. 2015; Simsek, 2016). Nutrient contents of 'Chandler' variety were revealed in the Uşak region, which is at an altitude of about 900 m in the inner western Anatolia, by Yıldız & Sümbül (2019a). According to the results of the analysis; N contents of kernels were found 3.11%, P 352.63 mg/100g, K 449.02 mg/100g, Ca 159.42 mg/100g, Mg 122.45 mg/100g, Fe 2.96 mg/100g, Zn 2.07 mg/100g, Mn 3.78 mg/100g and Cu 1.61 mg/100g. On the other hand, there are many selection studies in our country. Mineral substance contents of selected genotypes were determined (Gülsoy et al. 2016; Simsek, 2016; Yılmaz & Akça, 2017; Yıldız & Sümbül, 2019b; Acar & Kazankaya, 2020). In some research; the variation of K, P, Mg, Ca, Fe, Zn, Mn and Cu were found at 170-548, 223- 380, 81-549, 37-453, 1.20-6.90, 1.10-3.80, 1.20-18.37 and 0.50-3.22 mg/100g, respectively (Cosmulescu et al. 2010; Özcan et al. 2010; Tapia et al. 2013). According to the literature mentioned, it was determined that N, P, Ca and Mn content were low; K and Zn content were high; Mg and Fe contents were similar in this study.

In this current study, the differences in mineral content of kernel were determined in year, location and foliar nutrient application. In addition, interactions were found to be effective in some applications. The positive effects of the applications occurred compared to the control of the nutrients of the kernel. On the other hand, with the N foliar application, the N content of kernel ranked the first row in both locations and years. A similar situation was observed in the K application.

Mineral composition of kernel was varied significantly among accessions at 650-1996 m altitude in Morocco (Kabiri et al. 2019). In this study, the change depending on the locations was determined in the research conducted at different altitude. In general, mineral content was found higher in Demirci location. Differences have emerged in the studies conducted on nutrient content in walnut varieties and genotypes in our country and in the world. It is thought that there are different factors in the emergence of this condition such as genetic characteristics, climate conditions, soil type, agricultural practice and harvest dates (Caglarirmak 2003; Ozcan et al. 2010; Yılmaz & Akça 2017; Gülsoy et al. 2016; Batun et al. 2017).

No data were found on the effect of foliar nutrient applications on the kernel nutrient content of walnuts. On the other hand, applications of K_2SO_4 and $ZnSO_4$ affected concentrations of P, K, Mg, Zn, Mn and Fe in the leaves of pistachio, but nutrient treatments had no effect on leaf concentration of N (Norozi et al. 2019). In this study carried out in the Chandler variety, these applications had a positive effect on the kernel N content. In pecan trees treated with Zn, manganese was low, but Cu and Fe contents were different. However, its effects have varied over the years (Hounnou et al. 2019). In this current study, however, the positive effect of Zn application was observed. Similar to the change in leaf nutrient content by years, the same change was observed in fruit content.

5. Conclusions

Plant nutrition applications are important in terms of growing. In this context, foliar application of B and Zn was observed in terms of nut weight. The shrinkage ratio, an important quality criterion for walnuts, was found to be low in the high altitude Demirci location. However, in the low altitude Saruhanlı location, the shrinkage ratio was reduced with foliar K application. When the data were evaluated in general, walnut yield and fruit quality characteristics changed according to ecological conditions. Further, all foliar applications had positive effect on yield. The differences in mineral content of kernel were determined in year, location and application. But in general, mineral content was found higher in Demirci location. Thus, it was observed that the properties were superior in Saruhanlı location compared to Demirci location, which has a high altitude.

Acknowledgements

Thanks to Ege University Scientific Research Projects Coordination Unit for the financial support provided to the conducting of this research.

References

Acar S & Kazankaya A (2020). Some Fruit Properties and Macro-Micro Element Contents of Selected Walnuts (*Juglans regia* L.) from Batman Region. *YYU Journal of Agricultural Science* 30(2): 243-251. doi:10.29133/yyutbd.698507 (in Turkish).

Acarsoy Bilgin N, Yağız Y & Mısırlı A (2021). Effects of Some Nutrient Foliar Application on Yield and Quality in Walnut (cv. 'Chandler'). Agriculture Engineering. issue: 371: 51-58. doi:10.33724/zm.728414 (in Turkish).

Acarsoy Bilgin N, Şen F, Yağmur B, Özaktan H & Akbaba M (2018). The First Findings to Fruit Characteristics of Nutrient and PGPR Applications on Chandler Walnut Variety. International Conference on Agriculture, Forest, Food, Veterinary Sciences and Technologies. Çeşme, İzmir. 769-775

Abdallah I B, Tlili N, Martinez-Force E, Rubio A G P, Perez-Camino M C, Albouchi A & Boukhcina S (2015). Content of carotenoids, tocopherols, sterols, triterpenic and aliphatic alcohols, and volatile compounds in six walnuts (*Juglans regia* L.) varieties. Food Chem., 173: 972–978

Aşkın M A & Gün A (1995). Studies on breeding of Çameli and Bozkurt walnuts (*Juglans regia* L.) by selection. II. National Horticultural Congress in Turkey. pp. 461-463. 3-6 October Adana (in Turkish).

Batun P, Bakkalbaşı E, Kazankaya A & Cavidoğlu İ (2017). Fatty Acid Profiles and Mineral Contents of Walnuts from Different Provinces of Van Lake. GIDA. 42(2): 155-162. doi: 10.15237/gida.GD16062

Beyhan Ö (1993). Research on Selectional Breeding of Darende Walnut (*Juglans regia* L.). Doctorate thesis. Yüzüncü Yıl University. Graduate School of Natural and Applied Sciences. Van. (in Turkish).

Bilgin S, Şen F, Özeker E & Acarsoy Bilgin N (2018). Determination of Morphological and Pomological Characteristics of Some Walnut Cultivars in Menemen Conditions. *COMU J. Agric. Fac* 6(1): 31–39 (in Turkish).

Brown P (2001). Can boron correct transient nutrient deficiencies. *Fluid Journal* pp. 3

Büyüksolak Z N, Aşkın M A, Kahramanoğlu İ & Okatan V (2020). Efects of Altitude on the Pomological Characteristics and Chemical Properties of 'Chandler' Walnuts: A Case Study in Uşak Province. Acta Agrobotanica. Volume 73, Issue 3, Article 7333. doi: 10.5586/aa.7333

Caglarirmak N (2003). Biochemical and physical properties of some walnut genotypes (*Juglan regia* L.) of the East Black Sea region of Turkey. Nahrung/Food 41(1): 28-32

Carranca C, Brunetto G & Tagliavini M (2018). Nitrogen nutrition of fruit trees to reconcile productivity and environmental concerns. Plants. 7(1):4. doi:10.3390/plants7010004

Cosmulescu S, Botu M & Trandafir I (2010). Mineral composition and physical characteristics of walnut (*Juglans regia* L.) cultivars originating in Romania. *Selcuk Journal of Agriculture and Food Sciences* 24(4): 33-37. (in Turkish).

Çolakoğlu H & Çiçekli M (2016). Foliar Fertilization Guide. Toros Agriculture. (in Turkish).

Dejampour J & Zeinalabedini M (2006). Determination of some vegetative and bloom characteristics of some local apricots in Azarbaijan (Iran) ecological conditions. Acta Hort 717: 63–65. doi: 10.17660/ActaHortic.2006.717.9

Ellis Z (2016). Effect of Foliar Nutrient Combinations on Zinc Uptake in Almonds. A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant Science in the Jordan of College of Agricultural Sciences and Technology California State University, Fresno.master's thesis (unpublished)

Epstein E & Bloom AJ (2005). Mineral Nutrition of Plants: Principles and Perspectives, pp. 380

Fernandez V, Sotiropoulos T & Brown P (2013). Foliar Fertilization: Scientific Principles and Field Practices. University of California Division of Agriculture and Natural Resources. International Fertilizer Industry Association (IFA). 144 pp. Paris, France.

Forde H I (1975). Walnuts. In: Editors Janickand J, Moore J N. Advances in Fruit Breeding pp. 439-455

Gavit S, Dodak SB & Khobragade NH (2020). Application of different levels of NPK along with soil and foliar application of zinc and boron in cashew for increasing production with sustainability. IJCS 2020; 8(1): 3091-3094 doi:10.22271/chemi.2020.v8.i1au.8740

Ghani M A, Mushtaq A, Ziaf K, Ali B, Jahangir M M, Khan R W, Khan I, Azam M & Noor A (2021). Exogenously Applied GA3 Promotes Plant Growth in Onion by Reducing Oxidative Stress Under Saline Conditions. *Journal of Agricultural Sciences* 27(2): 122–128. Doi: 10.15832/ankutbd.621546

Gülsoy E, Kaya T, Pehluvan M & Şimşek M (2016). Some pomological properties and chemical contents of selected walnut (*Juglans regia* L.) genotypes from Iğdır province. *Anadolu J Agr Sci* 31: 309-314. doi: 10.7161/anajas.2016.31.3.309-314

Gülsoy E, Şimşek M & Çevik C (2019). Determination of Fruit Quality Traits in Some Hazelnut Cultivars Grown at Different Altitudes and Locations in Ordu Province. *(International Journal of Agriculture and Wildlife Science* 5(1): 25-30. doi: 10.24180/ijaws.506932 (in Turkish).

Hounnou L, Brorsen B W, Biermacher J T & Rohla C T (2019). Foliar applied zinc and the performance of pecan trees. *Journal of Plant Nutrition*, 42(5): 512-516. doi:10.1080/01904167.2019.1567771

Horuz A, Güneş A, Turan M, Demir T, Serdar Ü, Ozlu E, Karaman M R & Fırıldak G (2021). The Effects of Different Micronutrient Fertilizers on cv. Tombul Hazelnut Yield and Certain Nut Properties. Erwerbs-Obstbau. 63:107–114. doi: 10.1007/s10341-021-00546-w

Juranovic Cindric I, Zeiner M & Hlebec D (2018). Mineral Composition of Elements in Walnuts and Walnut Oils. Int. J. Environ. Res. Public Health, 15, 2674. doi:10.3390/ijerph15122674

Kabiri G, Bouda S, Elhansali M & Haddioui A (2019). Biochemical characterization and antioxidant activity of walnut kernel (*Juglans regia* L.) of accessions from Middle and High Atlas in Morocco. Acta Scientiarum. Biological Sciences, v. 41, e46411. doi: 10.4025/actascibiolsci.v41i1.46411

Kacar B & İnal A (2008). Plant Analysis, Nobel Publication, Ankara, No: 1241, 892 (in Turkish).

Kacar B & Katkat AV (2006). Plant nutrition. Nobel Publication No: 849. 593 s (in Turkish).

Kacar B (1982). Fertilizers and Fertilization Technique. 1982. Ankara, 341 s (in Turkish).

Keshavarz K, Vahdati K, Samar M, Azadegan B & Brow PH (2011). Foliar Application of Zinc and Boron Improves Walnut Vegetative and Reproductive Growth. Hort Technology. 21(2):181-186. doi: 10.21273/HORTTECH.21.2.181

Khayyat M, Tafazoli E, Eshghi S & Rajaee S (2007). Effect of nitrogen, boron, potassium and zinc sprays on yield and fruit quality of date palm. *American Eurasian J. Agric&Environ.* Sci. 3: 289-296

Khoshgoftarmanesh A H (2012). Advanced plant nutrition concepts. Isfahan, Iran: Isfahan University of Technology Publication, 538 pp.

Kim T & Wetzstein H Y (2005). Seasonal fluctuations in nutrients and carbohydrates in pecan leaves and stems. *Journal of Horticultural Science & Biotechnology* 80(6):681–688. doi:10.1080/14620316.2005.11511998

Koyuncu F, Koyuncu M A, Erdal İ & Yaviç A (2002). Chemical composition of fruits of some walnut (*Juglans regia* L.) selections. FOOD, 27(4): 247-251

Marchand M (2020). Efficiency of foliar applications of potassium sulphate on walnut production. XXX International Horticultural Congress IHC2018. doi: 10.17660/ActaHortic.2020.1280.15

Mohammadmehdi N, Valizadeh Kaji B, Karimi R & Sedghi MN (2019). Effects of Foliar Application of Potassium and Zinc on Pistachio (*Pistacia vera* L.) Fruit Yield. *International Journal of Horticultural Science and Technology* 6(1): 113-123. doi: 10.22059/ijhst.2019.278757.286

Morais M C, Aires A, Barreales D, Rodrigues M A, Ribeiro A C, Gonçalves B & Silva A P (2020). Combined Soil and Foliar Nitrogen Fertilization Effects on Rainfed Almond Tree Performance. *Journal of Soil Science and Plant Nutrition* 20: 2552–2565. doi: 10.1007/s42729-020-00321-y

Najizadeh A & Khoshgoftarmanes A H (2019). Effects of foliar applied zinc in the form of ZnSO4 and Zn-amino acid complexes on pistachio nut yield and quality. *Journal of Plant Nutrition* 42(18): 2299-2309. doi: 10.1080/01904167.2019.1655043

Norozi M, ValizadehKaji B, Karimi R & Nikoogoftar Sedghi M (2019). Effects of Foliar Application of Potassium and Zinc on Pistachio (*Pistacia vera* L.) Fruit Yield. *International Journal of Horticultural Science and Technology* 6(1): 113-123. doi: 10.22059/ijhst.2019.278757.286

Orman E (2018). Agro-Morphological and Molecular Identification of Some Local Walnut (Juglans regia L.) Genotypes. (PhD. In Horticulture). Ege University Graduate School of Natural and Applied Sciences Bornova/İzmir (in Turkish).

Ozcan MM, Iman C & Arslan D (2010). Physico-chemical properties, fatty acid and mineral content of some walnuts (*Juglans regia* L.) types. Agricultural Sciences 1: 62-67. doi:10.4236/as.2010.12009

Ozyigit İ İ, Uras M E, Yalcin İ E, Severoglu Z, Demir G, Borkoev B, Salieva K, Yucel S, Erturk Ü & Solak A O (2019). Heavy Metal Levels and Mineral Nutrient Status of Natural Walnut (*Juglans regia* L.) Populations in Kyrgyzstan: Nutritional Values of Kernels. Biological Trace Element Research. 189:277-290. doi: 10.1007/s12011-018-1461-4

Özçağıran R, Ünal A, Özeker E & İsfendiyaroğlu M (2014). Temperate Climate Fruit Species, Nuts Volume III, Ege University Faculty of Agriculture Publications No: 566 (in Turkish).

Ramos DE (1998). Walnut Production Manual. University of California. Division of Agriculture and Natural Resources. Publication, 3373. 319 pp.

Rios J J, Yepes-Molina L, Martinez-Alonso A & Carvajal M (2020). Nanobiofertilization as a novel technology for highly efficient foliar application of Fe and B in almond trees. R. Soc. Open Sci. 7: 200905. doi: 10.1098/rsos.200905

Rios J J, Garcia-Ibanez P & Carvajal M (2019). The use of biovesicles to improve the efficiency of Zn foliar fertilization. Colloids and Surfaces B: Biointerfaces. Volume 173, Pages 899-905. doi: 10.1016/j.colsurfb.2018.10.057

Scherz H & Kirchhoff E (2006). Trace elements in foods: Zinc contents of raw foods-A comparison of data originating from different geographical regions of the world. *J. Food Compos. Anal* 19: 420-433

Simsek M (2010). Selection of walnut types with high fruit bearing and quality in Sanliurfa population. *International Journal of the Physical Sciences Vol*. 5(7), pp. 992-996

Simsek M (2016). Chemical, mineral, and fatty acid compositions of various types of walnut (*Juglans regia* L.) in Turkey. *Bulgarian* Chemical Communications. 48(1): 66-70

Smith C A, Walworth J L, Richard M J, Joshua J & Sherman D (2021). Does Foliar Zinc Application Boost Leaf Photosynthesis of 'Wichita' Pecan Fertigated with Zinc-EDTA? HORTSCIENCE 56(5): 579-582. doi:10.21273/HORTSCI15692-21

Şen S M (1980). Studies on the Breeding of Walnuts (Juglans regia L.) in North East Anatolia and the Eastern Black Sea Region by Selection. Associate Professor Thesis, (unpublished) Ataturk University. Erzurum (in Turkish).

Şen S M (1986). Walnut Cultivation. Eser Printing House, Samsun (in Turkish).

Tapia M I, Sanchez-Margado J R, Garcia-Parra J, Ramirez R, Hernandez T & Gonzales-Games D (2013). Comparative study of the nutritional and bioactive compounds content of four walnut (*Juglans regia* L.) cultivars. *Journal of Food Composition and Analysis* 31: 232-237. doi: 10.1016/j.jfca.2013.06.004

Wolf B (1971). The determination of boron in soil ekstracts, plant material components, manures, waters and nutrient solutions, Soil Sci. Plant Anal 2(5): 363-374. doi: 10.1080/00103627109366326

Xie R, Zhao J, Lu L, Brown P H, Guo J & Tian S (2020). Penetration of foliar-applied Zn and its impact on apple plant nutrition status: in vivo evaluation by synchrotron-based X-ray fluorescence microscopy. Horticulture Research 7: 147. doi: 10.1038/s41438-020-00369-y

Xu C, Guo S & Wang J (2021). Exogenous nitrogen uptake and allocation in chestnut. *Can. J. Plant Sci*. 101: 307–314. doi: 10.1139/cjps-2019-0326

Yıldız A, Yıldız A, Doran İ, Aydın A & Keleş D (2007). The effects of inorganic and organic fertilizers on the growth, yield and fruit quality of apricot species Precoce de Tyrinthe. Alatarım 6(2): 1-8p (in Turkish).

Yılmaz S & Akça Y (2017). Determination of biochemical properties and fatty acid composition of new walnut (*Juglans regia*) genotypes. J. Agric. Fac. Gaziosmanpasa Univ. 34 (2): 74-80

Yıldız E & Sümbül A (2019a). Mineral content in kernel of some local and foreign walnut cultivars and genotypes. *Mustafa Kemal University Journal of Agricultural Sciences* 24 (3):174-180

Yıldız E & Sümbül A (2019b). Determination of Mineral Content in Kernel of Walnut Genotypes Selected from Uşak/Turkey. *Adnan Menderes University Journal of the Faculty of Agriculture* 16(2):179-183 doi: 10.25308/aduziraat.562837 (in Turkish).

Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

Investigation of The Roles of Hydrogen Peroxide and NADPH Oxidase in The Regulation of Polyamine Metabolism in Maize Plants under Drought Stress Conditions

Mehmet DEMİRALAY[a,d], Aykut SAĞLAM[b*], Fuat YETİŞSİN[c,d], Asım KADIOĞLU[d]

[a]*Department of Forest Engineering, Faculty of Forestry, Artvin Çoruh University, Artvin, 08100, TURKEY*

[b]*Department of Molecular Biology and Genetics, Faculty of Sciences, Karadeniz Technical University, Trabzon, 61080, TURKEY*

[c]*Department of Food Processing, Vocational High School of Technical Sciences, Mus Alparslan University, Mus, 49100, TURKEY*

[d]*Department of Biology, Faculty of Sciences, Karadeniz Technical University, Trabzon, 61080, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Aykut SAĞLAM, E-mail: saglama@ktu.edu.tr
Received: 14 January 2020 / Revised: 08 November 2021 / Accepted: 08 November 2021 / Online: 15 October 2022

Cite this article
DEMIRLIAY M, SAĞLAM A, YETIŞSIN F, KADIOĞLU A (2022). Investigation of The Roles of Hydrogen Peroxide and NADPH Oxidase in The Regulation of Polyamine Metabolism in Maize Plants under Drought Stress Conditions. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 28(4):613-625. DOI: 10.15832/ankutbd.861008

ABSTRACT
The relationship between hydrogen peroxide and the metabolism of polyamines and the role of NADPH oxidase (NOX) in that relationship under drought conditions remains unclear. To reveal the relationship, expression levels of the genes in polyamine metabolism, such as arginine decarboxylase, agmatine aminohydrolase, spermidine synthase, S-adenosyl methionine decarboxylase, diamine oxidase, and polyamine oxidase were determined by RT PCR under drought stress combined with exogenous hydrogen peroxide (H_2O_2) and diphenyleneiodonium chloride (DPI) treatments in maize seedlings. In addition, some basic stress parameters (leaf water potential, lipid peroxidation), levels of polyamines (putrescine, spermidine, and spermine), and gene expression of NOX were measured under drought stress. Exogenous H_2O_2 induced the polyamine content by up-regulating polyamine-synthesizing genes and downregulating polyamine oxidizing genes. When the NOX enzyme was inhibited by DPI, the polyamine pathway tended towards degradation instead of production. Exogenous H_2O_2 regulated the metabolism of polyamines to promote their synthesis, and NOX played a key role in that regulation.

Keywords: Antioxidant enzymes, Arginine decarboxylase, Diphenyleneiodonium chloride, Polyamine oxidation, Reactive oxygen species

1. Introduction

Polyamines, similar to hormones, have been considered to function as regulatory molecules in several basic cellular and physiological processes such as cell division, differentiation, and proliferation, cell death, membrane stabilization, DNA replication, protein synthesis, flower formation and development, leaf senescence, fruit development and maturation, biotic and abiotic stress responses (Alcazar et al. 2006a; Kusano et al. 2008). Recent studies of transgenic and mutant plants with excess or less polyamine suggest that polyamines play a protective role against abiotic stress (Alcazar et al. 2006b; Kusano et al. 2008; Gill & Tuteja 2010). For instance, the excessive level of putrescine in the Arabidopsis plant by overexpression of the Arginine decarboxylase 2 (*ADC2*) gene has probably stimulated stomal closure, and this decrease in water loss increased drought tolerance (Alcazar et al. 2010a). In a study by Kusano et al. (2007), Arabidopsis *acl5/spms* double mutant plants, which are unable to produce spermine, became hypersensitive to drought and saline stress. In the same study, however, the externally applied spermine mitigated the effects of the stresses mentioned in the plant. Besides, Yamaguchi et al. (2006; 2007) reported that the susceptibility to drought and salt stresses increased in the studies with double mutants of *spms1*/*acl5-1* of Arabidopsis to investigate the protective role of spermine against abiotic stress.

Drought stress is known to increase the generation of reactive oxygen species (ROS) in cell compartments such as chloroplasts, peroxisomes, and mitochondria. Be exposed to environmental stresses, such as drought, chilling, heat, or high light irradiation, is considered to lead to high concentrations of ROS, such as superoxide, H_2O_2, single oxygen, and hydroxyl radical (Das & Roychoudhury 2014; Akyol et al. 2020). If drought stress is prolonged, ROS production becomes dominant over the scavenging capacity of the antioxidant system, resulting in extensive cellular damage and death (Cruz de Carvalho 2008). On the other hand, ROS function as signal molecules in plants (Waszczak et al. 2018), controlling growth, development, responses to biotic and abiotic environmental stimuli, and programmed cell death (Bailey-Serres & Mittler 2006). The production of H_2O_2 is strongly associated with the catabolic processes of polyamine. Because H_2O_2 is not only produced by the of NADPH oxidases

(NOX), but also by amine oxidases (Cona et al. 2006a; 2006b). Apoplastic polyamine oxidase in tobacco and polyamine oxidase encoded by the Arabidopsis polyamine oxidase 3 (*AtPAO3*) gene have been documented to contribute to the production of H_2O_2 (Moschou et al. 2008a; Wu et al. 2010). Polyamine oxidation has been reported to be regulated by polyamine oxidases at different levels of expression in *Arabidopsis thaliana*, thus rendering ROS levels is quite complex (Fincato et al. 2011). Many studies confirm that *NOX* homologs are essential for ROS accumulation in plants (Kwak et al. 2003; Sagi et al. 2004; Torres et al. 2005). Mutants of the NADPH oxidase genes have been employed in most of these studies. ROS accumulation, especially H_2O_2, decreases in these plants. Papadakis & Roubelakis-Angelakis (2005) have suggested that the cross-linking between polyamines and NOX regulates protoplast regeneration in a tobacco plant. Andronis et al. (2014) have found that exogenously applied polyamines stimulate NADPH oxidase-dependent oxygen consumption in Arabidopsis. In the study, the amount of oxygen in the mutant plants carrying the overexpressed *AtPAO3* gene decreased compared to the mutant *Atpao3* mutant plants with incomplete function. In the *Atpao3* mutants, the amount of H_2O_2 was low. In the case of *AtPAO3* over-expression, the opposite was the case. Andronis et al. (2014) stated that there could be a very strong relationship between polyamines and H_2O_2, and this relationship could also be associated with NOX. Furthermore, Liu et al. (2019) determined that exogenously applied putrescin contributed to the development of resistance against pathogenic bacteria in *Arabidopsis thaliana*. The resistance was found to be dependent on hydrogen peroxide and NOX (*RbohD* and *RbohF*). In addition, Seo et al. (2019) found out that *NtRbohD* and *NtRbohF* genes were downregulated in *SAMDC* overexpressing *Nicotiana tabacum* under salt stress conditions. Consequently, they concluded that polyamines inhibited the production of ROS. However, the relationship between NADPH oxidase and polyamines has not been fully elucidated under drought stress conditions yet. In the current study, the hypothesis that NOX acts as a regulator in the metabolism of polyamines under drought stress conditions has been tested. For this purpose, the biosynthesis pathway of polyamines in drought-stressed maize plants was studied in detail after the application of H_2O_2 and a NOX inhibitor.

2. Material and Methods

2.1. Plant material and treatments

Maize seeds (*Zea mays* L cv Akpinar sensitive to drought, (Guven 2013) provided from Karadeniz Agricultural Research Institute, Samsun, Turkey, were sterilized with 0.1% $HgCl_2$ for 3 min and washed with sterilized distilled water (Terzi et al. 2014). Ten seeds were grown in pots containing peat and sand (5:1) in a growth chamber, which was set to 16 h light (PPFD on the leaf surface per 400 $\mu mol.m^{-2}.s^{-1}$), 8 h dark photoperiod at 25 ºC ± 2 and ambiance humidity 60% ± 5. Four-week-old seedlings were cut from near bottom then immersed distilled water for alleviating injury stress, and subsequently, plants were divided three groups as followed i) Control (3% PEG 6000 only, -0.5 MPa), ii) 3% PEG 6000 + 10 mM H_2O_2 (Terzi et al. 2014) and iii) 3% PEG 6000 + 100 µM DPI (NOX inhibitor diphenyleneiodonium chloride, Potocký et al. 2007). All treatments were done on the excised seedlings immersed in the test solutions mentioned. The seedlings were kept in the test solutions for 6 hours. At the end of this period, the third leaves from the base of the seedlings were selected and used in the experiments.

2.2. Leaf water potential

Leaf water potential (MPa) was measured by using a C52 thermocouple psychrometer (Wescor Inc.). Leaf disks approximately 6 mm in diameter were placed in the C52 sampling chamber and monitored for one hour with the PSYPRO data logger (Saglam et al. 2011).

2.3. Lipid peroxidation

The quantity of thiobarbituric acid reactive substances (TBARS) was measured to determine lipid peroxidation according to Heath & Packer (1968). Leaf samples (0.25 g) were homogenized with 20% trichloroacetic acid (TCA) including 5% thiobarbituric acid (TBA) and centrifuged at 10000 g for 10 minutes at 4 ºC. TBARS content was expressed as nmol g^{-1} dry weight.

2.4. Endogenous H_2O_2 content

The endogenous content of H_2O_2 was measured according to Velikova et al. (2000). Frozen leaf samples (0.25 g) were homogenized with 1% TCA and centrifuged at 15000 g for 15 minutes at 4 ºC. The resultant supernatant was added to the reaction mixture, including 10 Mm of potassium phosphate buffer (pH 7), and 1 M of potassium iodide (KI). Absorbance was measured at 390 nm. The H_2O_2 content was given as nmol g^{-1} dry weight.

2.5. Enzyme activities

Leaf samples (0.1 g) were pulverized in liquid nitrogen and extracted with 1.8 ml of extraction buffer (100 mM K_2HPO_4, 0.1 mM EDTA pH 7.0, 0.1% Triton). The extracts were centrifuged at 15000 g for 20 minutes at 4 ºC. The resultant supernatant was used to determine enzyme activity.

The activity of NADPH oxidase (EC 1.6.3.1) was measured by Cakmak & Marschner (1988). NADPH oxidase activity was determined based on NADPH's oxidation at 340 nm (ε= 6.2 mM cm^{-1}). Enzyme activity was determined based on the decrease in absorbance for 3 minutes in the reaction medium consisting of 100 mM of potassium phosphate buffer (pH 7.0), 0.1 mM of EDTA, and 100 µM of NADPH.

Catalase (EC 1.11.1.6) activity was determined according to Aebi (1983). Enzyme activity was determined by measuring a 1 mL reaction mixture containing 50 mM of potassium phosphate buffer (pH 7.0), 30 mM of H_2O_2, and 20 µl of enzyme extract at 240 nm for 5 minutes. The catalase activity was calculated by using the extraction coefficient ε = 39.4 mM cm^{-1} for H_2O_2.

Peroxidase (EC 1.11.1.7) activity was determined according to Urbanek et al. (1991). The enzyme activity was determined by measuring the 2 mL reaction mixture containing 100 mM of potassium phosphate buffer (pH 7.0), 0.1 mM of EDTA, 5 mM of guaiacol, 15 mM of H_2O_2, and 50 µl of enzyme extract for 1 minute at 470 nm. The peroxidase activity was calculated using the extinction coefficient (ε = 26.6 mM^{-1} cm^{-1}).

The activity of superoxide dismutase (EC 1.15.1.1) was determined according to Beauchamp & Fridovich (1971). The reaction was started by adding 2 µM of riboflavin to 1 mL reaction medium containing 50 mM of potassium phosphate buffer (pH 7.8), 0.1 mM of EDTA, 13 mM of methionine, 75 µM of nitro blue tetrazolium and 50 µl of extract. Following exposure to white light at 375 µmol m^{-2} s^{-1}, absorption values were determined at 560 nm.

2.6. Polyamine content

The determination of polyamines was performed as outlined in Terzi et al. (2014). Briefly, the fresh leaves (5g) were homogenized with 10 mL of perchloric acid 0.4 M. The homogenate was centrifuged at 3000 g for 4 minutes at 4 ºC. The supernatant was collected and perchloric acid (10 mL of 0.4M) was added to the supernatant. This mixture was then re-centrifuged. NaOH (1 M) and sodium hydrogen carbonate were added to 1 mL of the supernatant. The mixture was vortexed for 30 seconds. Subsequently, 2 ml of dansylchloride chloride was added (10 mg ml^{-1} in acetone). The reaction was stopped with 0.1 mL of 25% ammonium hydroxide. An HPLC device with UV/VIS detector (Shimadzu, LC 20 AT / Prominence, Japan) read the polyamine content. The mobile phase consisted of 0.1 M ammonium acetate and 19 M acetonitrile (65:35 v/v). The flow rate was 0.70 mL min^{-1} and the column temperature was 50 ºC. The 20 µl sample was injected into the C18 column (4.6 × 250 mm Supelco, Bellefonte, USA). Polyamines were determined at 254nm wavelength. Peak fields of the polyamines were recorded, then putrescine (put), spermine (spm), and spermidine (spd) concentrations were calculated using LabSolutions software. The concentrations were expressed in µg g^{-1} dry weight.

2.7. Protein content

Protein determination was performed spectrophotometrically according to Bradford (1976). Thirty microliters of sample were used from the extracts prepared for enzymatic activity. Bovine serum albumin (BSA) standards were prepared, and the complex of proteins with Coomassie Brillant Blue G250 was measured at 595 nm. The protein concentration was determined in mg and used for enzyme activities.

2.8. RNA isolation and cDNA synthesis

Fresh leaf samples (0.1 g) were used for total RNA isolation. Total RNA isolation was carried out using a total RNA isolation kit (RNeasy Plant Mini Kit 74904, Qiagen Netherlands). The samples frozen with liquid nitrogen were ground by tissue homogenizer (TissueLyser LT, Qiagen Germany). The amount and the purity of the RNA samples were measured using a nanodrop spectrophotometer (Thermo Scientific, Nanodrop 2000, America). A c-DNA reverse transcription kit (4368814, Applied Biosystems America) was used for the synthesis of c-DNA from the isolated total RNA. The cDNA concentration was adjusted to 2000 ng in each group. The synthesized cDNAs were stored at -20 °C until Real-Time PCR analyses were performed.

2.9. Quantitative Real-Time PCR analysis

The c-DNAs from the previous step were used in the Real-Time PCR analysis to determine gene expression. 5x HOT FIREPol Eva Green qPCR Supermix (08-36-00008, Solis Biodyne, Estonia) was used to analyze gene expression through CFX Connect Real-Time PCR system (BioRad, USA). RT PCR protocol modified according to Solis Biodyne instructions was as follows 12 minutes at 95 °C, 45 cycles at 95 °C, 30 seconds at 60 °C, 30 seconds at 72 °C. The melt curve was held in 0.5 °C increments from 60 to 95 °C. The primers were designed with Primer 3 plus (http://primer3plus.com/cgi-bin/dev/primer3plus.cgi). As a reference for gene expression, primers belonging to the Actin1 gene were used. In the study, the genes targeted for expression and primers from those genes were given in Table 1.

Table 1- The sequences of specific primers used for qRT-PCR analysis

Target gene	*NCBI accession no.*	*Primer names and their sequences*
Actin 1	NM_001155179.1	**ACT1Zm_F:** "GAAGATCACCCTGTGCTGCT" **ACT1Zm_R:** "ACCAGTTGTTCGCCCACTAG"
Arginine decarboxylase (ADC)	EU968980.1	**ADCZm_F:** "GACATCACCTGCGACAGTGA" **ADCZm_R:** "GAACAGGTTGTGCTTGCCAG"
Spermidine synthase (SPDS)	EU976300.1	**SPDSZm_F:** "TTCCCCTGTTCGATTCACCG" **SPDSZm_R:** "GCAGAACCGTTTCTTGCGTC"
Agmatine iminohydrolase (AIH)	NM_001156428.1	**AIHZm_F:** "GACGAGGATACAAACGGCCA" **AIHZm_R:** "ACGGACTGGGAGAGAACAGA"
S-Adenosylmethionine decarboxylase (SAMDC)	EU968400.1	**SAMDCZm_F:** "GGAGGCGTGAAGAAGTTCCA" **SAMDCZm_R:** "TTATCAGGAAGCAGCAGGCC"
Diamine oxidase (DAO)	NM_001152492.1	**DAOZm_F:** "ACAGCAAGTCCGAGAAGTGG" **DAOZm_R:** "TGTACCACAGCACGATGTCC"
Polyamine oxidase 1 (PAO)	NM_001111636.1	**PAOZm_F:** "CGCTACGAATACGACCAGCT" **PAOZm_R:** "TGGGCGCAGTTGATGAGAAT"
NADPH oxidase B	DQ890023.1	**NOXZm_F:** "GGGCCAGTACTTCGGTGAAA" **NOXZm_R:** "AAGCTTCACCAGGCTACGAC"

2.10. Statistical analysis

The variance analysis of means obtained as a result of the analyzes with three replications with 10 samples was performed according to the one-way ANOVA (Duncan Multiple Comparison Test) included in the package of the Statistical Package for Social Sciences (SPSS 16.0 for Windows) at $P<0.05$. The relative gene expression level was analyzed by the Bio-Rad CFX Manager 3.1. Expression levels were also assayed by SPSS software. Variance analysis of mean values was carried out by one-way ANOVA ($P<0.05$).

3. Results and Discussion

Water potential measurements were performed to determine the effect of H_2O_2 on the water content of maize plants subjected to drought stress conditions. Leaf water potential has been regarded as a good indicator of drought stress (Shaw et al. 2002). Sharma & Dubey (2005) reported in their study that the water potential of rice plants exposed to drought stress could be reduced from -0.5 MPa to -2 MPa. Similarly, drought stress in wheat reduced leaf water potential from -0.63 MPa to -2 MPa (Siddique et al. 2000). Unlike the above studies, Terzi et al. (2014) determined that the water potential of the leaf increased in drought stress conditions with the application of H_2O_2 in maize plants compared to control (PEG only). Likewise, in the current study, the leaf water status was improved by the H_2O_2 application (Figure 1a). The water potential of the H_2O_2-treated group was 1.2-fold higher than that of the control group (PEG only), and the levels of polyamine increased along with this improvement. Following the use of DPI to reduce the endogenous H_2O_2 content, there was a decrease in the leaf water potential and the polyamine content. The water potential of the DPI group was 1.3-fold lower than that of the control group. The water potential value of the DPI group was also less than that of the H_2O_2 group. The water potential of the DPI group was 1.65-fold lower than that of the H_2O_2-treated group. DPI was found to influence the water status of the plant by decreasing the water potential relative to the control group (PEG application). The reduction suggested that applying H_2O_2 could affect water potential by regulating polyamine metabolism. As is known, polyamines play a role as an osmotic regulator in drought stress (Sequera-Mutiozabal et al. 2017).

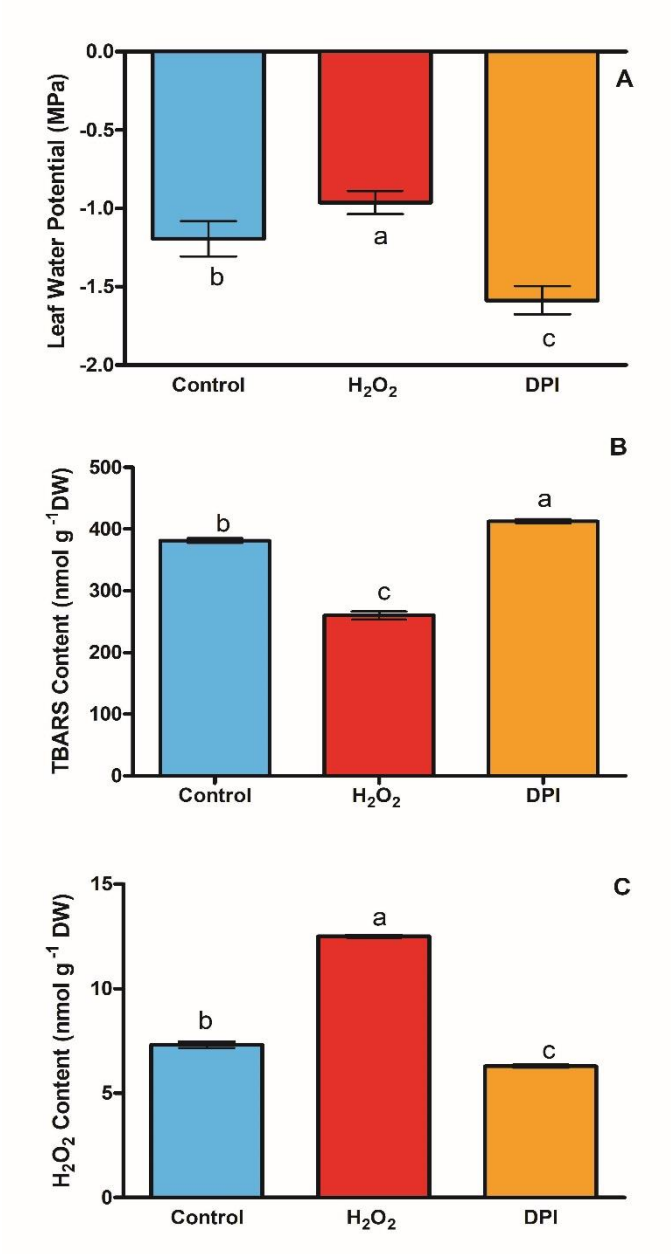


Figure 1- Effect of exogenous H_2O_2 and DPI on leaf water potential (A) TBARS (B) and endogenous H_2O_2 contents in the leaves of detached maize seedlings under PEG-induced drought stress conditions. The seedlings were submitted to three treatments: Applied with PEG (Control); applied with H_2O_2 and PEG (H_2O_2); applied with DPI and PEG (DPI). Vertical bars are standard deviations. Different letters indicate significant differences among different treatments at $P<0.05$

In the present study, lipid peroxidation was measured to determine the effects of H_2O_2 and DPI on membrane damage under drought stress. ROS is known to cause high levels of lipid peroxidation, resulting in damage to cell membranes (Munne-Bosch et al. 2001). However, in our study, the 10 mM of H_2O_2 was determined to reduce lipid peroxidation under drought stress conditions (Figure 1b). The TBARS content of the H_2O_2-treated group was 1.5-fold lower than that of the control group. The amount of TBARS measured in the DPI group was significantly increased concerning the control and the H_2O_2-treated groups. The TBARS content of the DPI group was 1.1 and 1.6-fold higher than the control and the H_2O_2-treated groups respectively. Furthermore, the application of 10 mM H_2O_2 significantly increased the endogenous level of H_2O_2 compared with the control group. The endogenous H_2O_2 content of the H_2O_2-treated group was 1.7-fold higher than that of the control group. Following DPI treatment, the level of endogenous H_2O_2 decreased significantly in comparison with the control and H_2O_2 groups (Figure 1c). The endogenous H_2O_2 content of the DPI group was 1.2 and 2.0 times lower than the control group and the H_2O_2 treated group, respectively. Here, H_2O_2 applied exogenously may have caused the endogenous hydrogen peroxide to act as a signal molecule and stimulate antioxidants. The stimulation of antioxidants in response to a non-lethal dose of hydrogen peroxide allows cells to adapt to exposure to a much larger dose and thus survive (Rodríguez-Rojas et al. 2020).

Similarly, in our study, the application of H_2O_2 increased catalase (CAT) and peroxidase (POD) activities, but the activity of superoxide dismutase (SOD) declined. The catalase activity of the H_2O_2-applied group was 1.3 and 1.4-fold higher than that of

the control and the DPI groups, respectively (Figure 2a). In the DPI group, the CAT activity was found to be lower than that of the control group. The CAT activity of the DPI group was 1.1-fold lower than that of the control group. POD activity was determined as the highest in the H_2O_2 group compared to the control and the DPI groups (Figure 2b). The peroxidase activity of the H_2O_2 applied group was 1.3 and 1.4-fold higher than the control and the DPI groups, respectively. Furthermore, because of the DPI application, the activity decreased by 1.1 times compared to the control group. In terms of superoxide dismutase, the highest activity was identified in the control group (Figure 2c). The SOD activity of the control group was 1.5 and 1.6-fold higher than that of the H_2O_2 and DPI groups, respectively. There was no difference between the H_2O_2 and DPI groups in terms of the SOD activity.

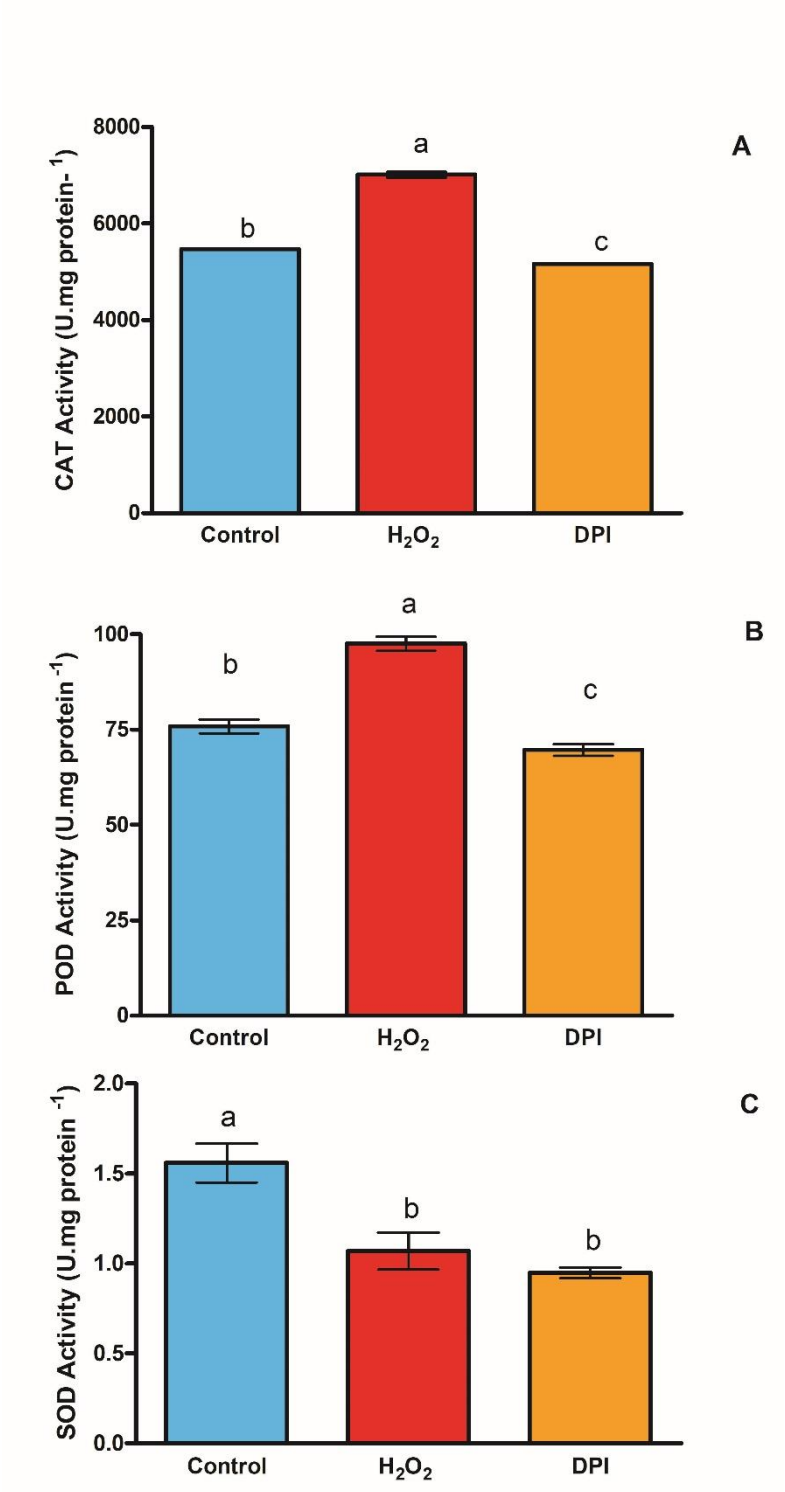


Figure 2- Effect of exogenous H_2O_2 and DPI on antioxidant enzyme activities in the leaves of detached maize seedlings under PEG-induced drought stress conditions. Catalase (A), Peroxidase (B) and Superoxide dismutase (C).

The enzyme catalase is known to scavenge excess H_2O_2 by reducing it to water and oxygen (Chaudiere & Ferrari-Iliou 1999). This enzyme has also been reported to play a role in the prevention of potential damage (such as lipid peroxidation) of H_2O_2 in membranes (Chaudiere & Ferrari-Ilioui 1999). Similarly, peroxidases have also been reported to be responsible for scavenging H_2O_2 under stress and maintaining cell membrane integrity (Parida & Das 2005). We concluded that the application of H_2O_2 protected the integrity of the cell membrane by inducing catalase and peroxidase activities and therefore reduced TBARS levels. Similar findings were reported in wheat plantings under drought conditions and in cucumber and corn plants under osmotic stress conditions (He et al. 2009; Liu et al. 2010; Terzi et al. 2014). In our study, following the application of DPI, the endogenous H_2O_2 level, NADPH oxidase activity, and *NOX* expression level decreased significantly compared to other groups. The lowest NOX activity was observed in the DPI-treated group (Figure 3a). On the other hand, exogenously applied H_2O_2 significantly increased NOX activity compared to the control. The NOX activity of the H_2O_2 treated group was 1.1 and 1.3 higher than that of the control and DPI groups, respectively. In addition to NOX activity, the DPI reduced the level of expression of the NOX gene in comparison to the control group (Figure 3b). The *NOX* expression level of the PDI applied group was 1.3 times lower than that of the control group; however, the highest *NOX* expression level was found in the H_2O_2 group (Figure 3b). The *NOX* expression of the H_2O_2 applied group was 1.4 and 1.9-fold higher than the control and the DPI groups.

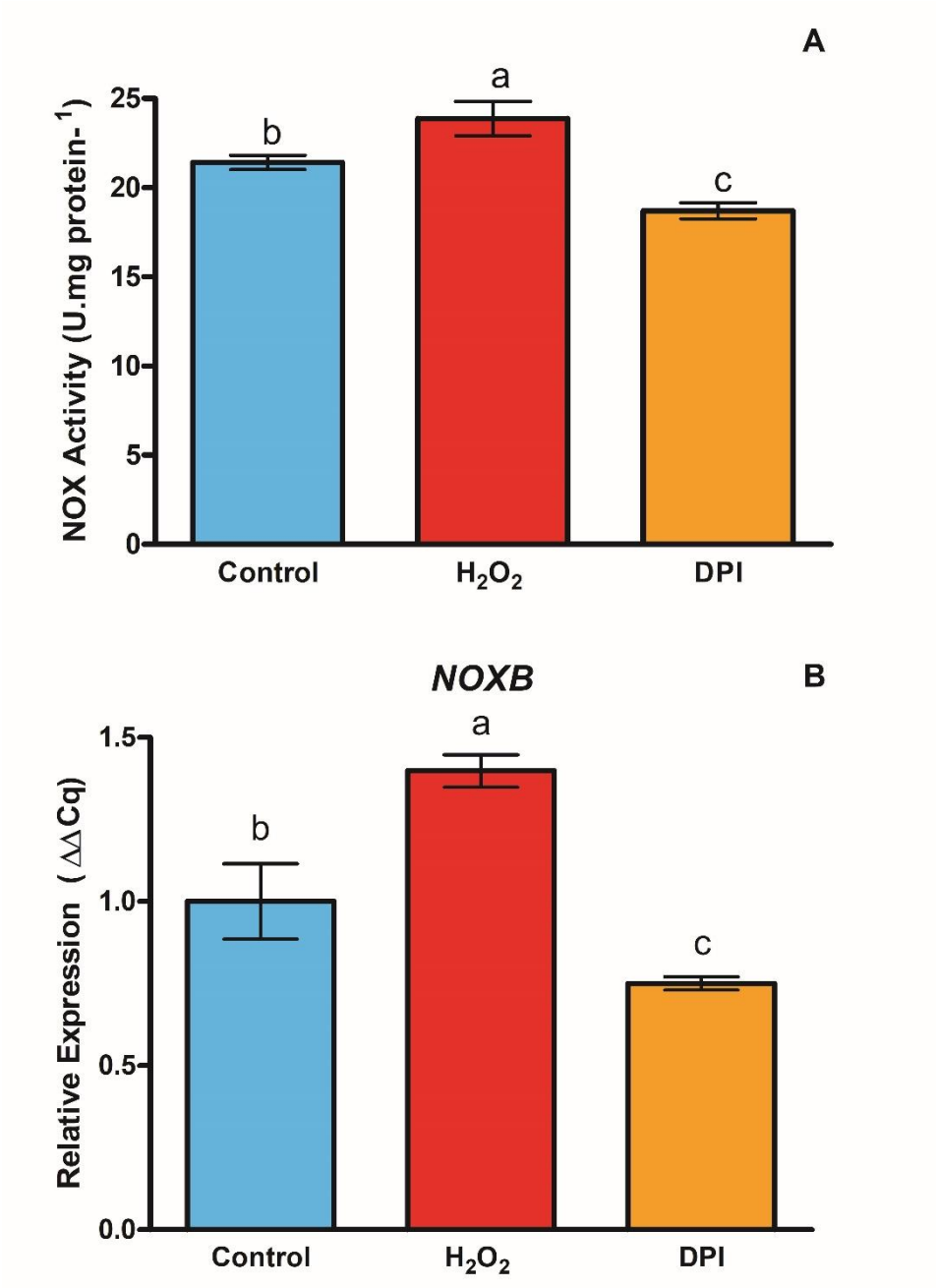


Figure 3- Effect of exogenous H_2O_2 and DPI on NADPH oxidase (NOX) activity (A) and *NOXB* gene expression (B) in the leaves of detached maize seedlings under PEG-induced drought stress conditions.

The decreases in endogenous H_2O_2 level, NADPH oxidase activity, and *NOX* expression levels and the increased amount of TBARS following the DPI application could be explained by decreasing catalase and peroxidase activities. These changes in enzyme activities showed that hydrogen peroxide acted as a signaling molecule, activated the antioxidant system and protected corn plants from the effects of stress.

Significant increases in levels of endogenous polyamines due to drought stress have been noted in some studies (Erdei et al. 1996; Galston et al. 1997; Groppa & Benavides 2008). In this study, the application of H_2O_2 under drought stress increased the levels of polyamines such as put and spm in comparison with the control group. The maximum put concentration (in all groups) was determined in group H_2O_2 (Figure 4). The putrescine level was found to be the lowest in the DPI among all groups. The put content of the H_2O_2 treated group was 1.3 and 2.1-fold higher than the control and DPI groups, respectively. The concentration of (spd) in the control group was highest among all groups. The spd content was significantly reduced by the H_2O_2 and DPI treatments relative to the control group. The spd content in the control group was 1.2 and 1.6-fold higher than the H_2O_2 and DPI groups, respectively. Furthermore, the spd content of the H_2O_2 group was found to be greater than that of the DPI group. As for the spm content, the application of H_2O_2 resulted in the accumulation of spm. The spm content of the H_2O_2-applied group was 1.4 and 2.0-fold higher than the control and DPI groups, respectively. Moreover, the lowest spm concentration was determined in the DPI group (Figure 4).

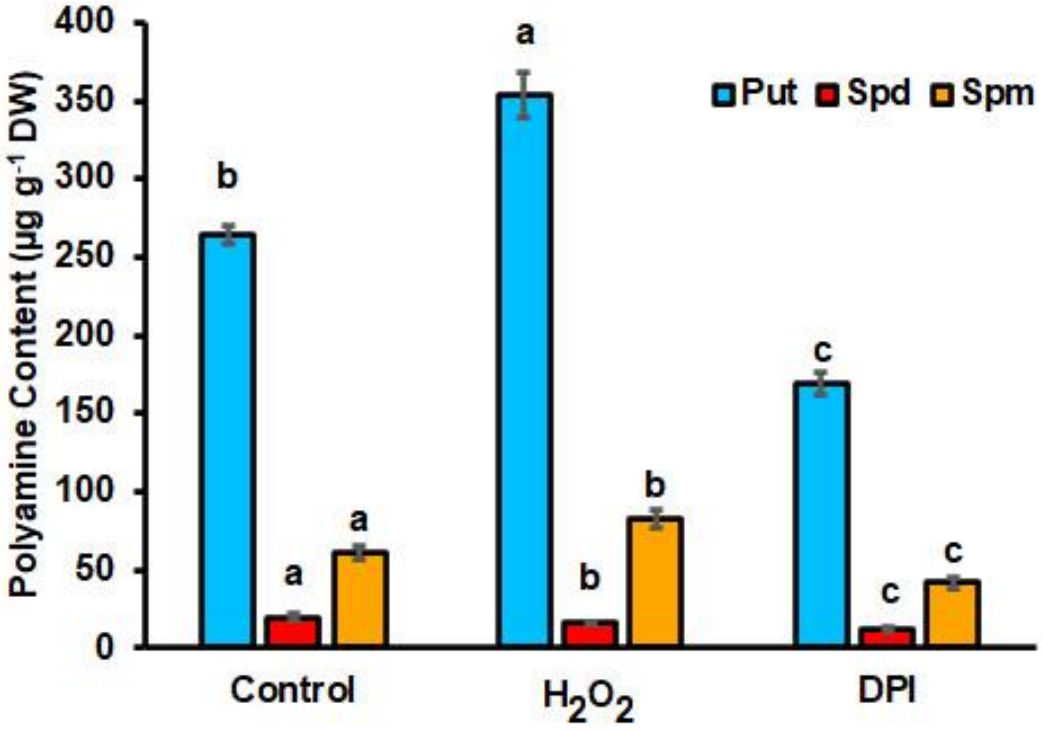


Figure 4- Effect of exogenous H_2O_2 and DPI on endogenous polyamine contents in the leaves of detached maize seedlings under PEG-induced drought stress conditions.

Abass & Mohamed (2011) suggested that the application of H_2O_2 to bean seeds stimulated polyamine content under drought conditions. Similarly, Terzi et al. (2014) exposed maize seedlings to osmotic stress after performing pre-treatment of H_2O_2 and found significant increases in the put, spm and spd contents. Besides, in our study, the decrease in polyamine level due to DPI application supported the idea that NOX-induced H_2O_2 may have a stimulating effect on the polyamine synthesis pathways.

The level of expression of the *ADC* gene, located in the first stage of the put synthesis, increased with the application of H_2O_2 in comparison to the other groups in our study. The *ADC* expression of the H_2O_2-treated group was 1.2 and 1.3-fold higher than the control and DPI groups, respectively (Figure 5a). The put pool is mainly under the control of the *ADC* gene and shows an increase in drought stress (Urano et al. 2004; Alcazar et al. 2010b). For this reason, the increase in the putrescine level in the experimental group with H_2O_2-applied may be related to the increased expression level of the *ADC* gene. Indeed, Urano et al. (2004) reported that the increase in putrescine levels in Arabidopsis plants under drought conditions was positively correlated with the stimulation of ADC transcripts. This positive relationship between ADC and putrescine, which we found in our study, was similar to the one obtained by Urano et al. (2004). Abass & Mohamed (2011) and Terzi et al. (2014), in their drought-related stress studies, determined that the application of H_2O_2 increased the polyamine content. Our findings were consistent with the

data from these researchers. In addition, the RT PCR analyses of the present study confirmed our opinion that H_2O_2 promoted the accumulation of polyamines.

In contrast to H_2O_2, DPI decreased the expression level of the *ADC* gene by 1.1-times compared to the control group (Figure 5a). The downregulation of the *ADC* gene through DPI also supported the idea that H_2O_2 derived from NOX may be necessary for the regulation of polyamine biosynthesis. The transcript level of the agmatine aminohydrolase (*AIH*) gene, which is another enzyme involved in putrescine synthesis, was enhanced with the application of H_2O_2 (Figure 5b). The *AIH* expression of H_2O_2-applied group was 2.2 and 3.3-fold higher than that of the control and the DPI groups, respectively. Because of the DPI application, the *AIH* expression level was reduced by 1.5 times compared to the control group.

The level of expression of the *AIH* gene has been reported to increase in Arabidopsis plants exposed to drought stress (Alcazar et al. 2006b). According to the results of our study, another reason for the increase in putrescine due to the application of H_2O_2 was the positive regulation of the *AIH* gene. At the same time, the decrease in the *AIH* transcript level with DPI application indicated that H_2O_2 could regulate the expression level of the gene in question, and the level of putrescine increased. Alcazar et al. (2011) and Do et al. (2014) found elevated levels of transcripts of spermidine synthase (*SPDS*) genes in Arabidopsis and rice plants, respectively, under drought stress conditions. In our study, the amount of spd was found to be decreasing in the H_2O_2 group compared to the control group. This decrease might be caused by the recovery of spermidine into the putrescine pool through PAO activity or by the reduction of transcripts of the *SPDS* gene because the *SPDS* gene in the control group was more expressed than the H_2O_2 group in our study. The expression level of *SPDS* in the control group 1.8-fold higher than H_2O_2 group (Figure 5d). Furthermore, the low level of expression of *PAO* in the H_2O_2 group compared to the control group may help explain the fluctuations in spermidine levels.

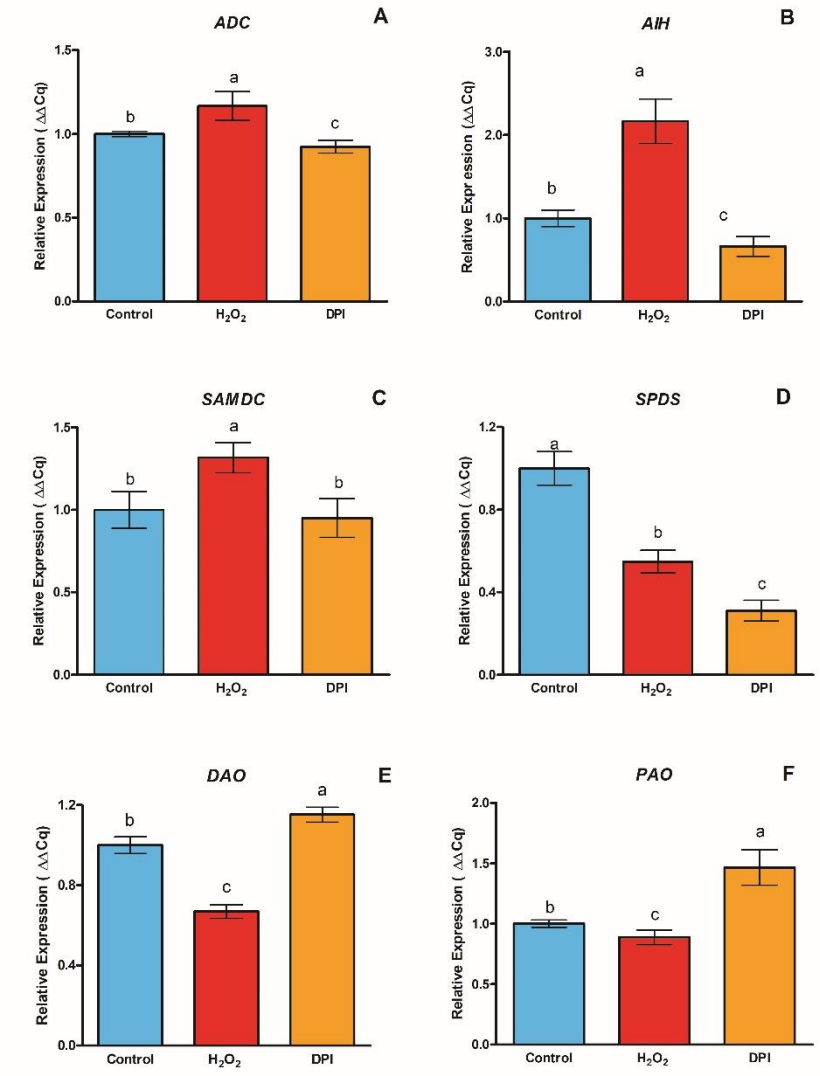


Figure 5- Effect of exogenous H_2O_2 and DPI on gene expression levels of Arginine decarboxylase (A), Agmatine iminohydrolase (B), S-adenosylmethionine decarboxylase (C), Spermidine synthase (D), Diamine oxidase (E), Polyamine oxidase (F) in the leaves of detached maize seedlings under PEG-induced drought stress conditions.

In conclusion, according to the control group, the decrease in endogenous spd level in our study due to the H_2O_2 application was consistent with the reduction in the expression level of the *SPDS* gene. In our study, the expression level of the S-adenosyl methionine decarboxylase (*SAMDC*) gene, which is involved in the synthesis of spd and spm, was determined. The level of expression of *SAMDC* was significantly enhanced by H_2O_2 treatment. The expression level of *SAMDC* in the H_2O_2-applied group was 1.3 and 1.4-fold higher than the control and the DPI groups, respectively (Figure 5c). The measurement of the degree of expression of this gene provided valuable information for the regulation of spd and spm concentrations. A limited number of studies reported increases in *SAMDC* expression levels under the stress of drought. For example, under drought stress conditions, there has been an increase in SAMDC transcripts in Arabidopsis and rice plants (Li & Chen 2000; Alcazar et al. 2011). In addition, the level of spm was reported to increase in rice plants subject to drought stress (Capell et al. 2004). In our study, under drought conditions, the *SAMDC* transcription level when H_2O_2 was applied was higher than other experimental groups. This was consistent with the increased spermine level with hydrogen peroxide application. As in our study, Terzi et al. (2014) reported increases in spm levels as a result of H_2O_2 treatment in maize seedlings subjected to osmotic stress. Furthermore, increased expression of a *SAMDC* by H_2O_2 in our study supported the idea that H_2O_2 plays an important role in the regulation of polyamine biosynthesis.

Several studies have reported that genes involved in the destruction of polyamines encode diamine oxidase (DAO) and PAO enzymes (Cohen 1998; Angelini et al. 2010; Fincato et al. 2012). In our study, the expression of the *DAO* gene was significantly decreased by H_2O_2 application in drought conditions compared to other groups. The exogenous H_2O_2 treatment reduced the expression level of the *DAO* gene as 1.5-fold than the control group (Figure 5e). This reduction may help explain the high level of put in the H_2O_2 group compared with other groups. DPI application was the group with the highest level of *DAO* transcript. The gene expression level of *DAO* in the DPI group was 1.2 and 1.7-fold higher than the control and the H_2O_2-treated groups, respectively (Figure 5e). This was confirmed by examining putrescine rates. Regulation of the *DAO* gene supported the idea that there could be a crosstalk between H_2O_2 and polyamine levels during drought stress. In our study, the levels of *PAO* transcripts in the control and DPI groups were higher than the H_2O_2 group under drought conditions. The level of expression of the *PAO* gene in the DPI group was 1.5 and 1.7 times higher than that of the control and H_2O_2 groups, respectively. On the other hand, the expression level measured in the H_2O_2 group was 1.1-fold lower than the control group (Figure 5f).

Alcazar et al. (2011) reported a rise in *PAO* expression in drought-exposed Arabidopsis plants. In another study, *PAO* transcripts were also found to increase significantly, while put and spd levels decreased in grape varieties exposed to drought stress (Hatmi et al. 2015).

On the other hand, the application of H_2O_2 which we carried out in our study resulted in a decrease in *PAO* transcripts and an increase in polyamine levels. This was evidenced by the rise in the putrescine rate in the H_2O_2 group. The increased expression level of the *PAO* gene by DPI also supported that NOX-derived H_2O_2 had a regulatory role in polyamine degradation.

Superoxide ($O_2^{\cdot -}$) and H_2O_2, the most studied ROS derivatives, have critical roles in many processes (Pitzschke et al. 2006; Dikalov et al. 2011; Suzuki et al. 2013). In our study, DPI reduced the H_2O_2 accumulation by blocking the NADPH oxidase enzyme, which resulted in significant reductions in polyamine levels. Furthermore, the levels of expression of *DAO* and *PAO* responsible for polyamine degradation increased. On the other hand, the model of polyamine oxidation initiated by the H_2O_2 signal cascade has been accepted because H_2O_2 is a direct product of polyamine oxidation (Moschou et al. 2008b). In summary, the literature indicates that polyamine metabolism can be regulated by H_2O_2, which is derived from the activities of DAO and PAO enzymes. However exogenously applied H_2O_2 in our study decreased the gene expression level of the *DAO* and *PAO*, while leading to an increase in expressions of *ADC*, *AIH*, *SPDS*, and *SAMDC*. So, according to our study, it concluded that H_2O_2 was capable of positive regulation on polyamine metabolism. Consequently, in our study, not only H_2O_2 due to polyamine oxidation, but also H_2O_2 derived from NADPH oxidase and externally applied H_2O_2 affects polyamine signaling. Because when NOX is inhibited, the metabolic pathway has shifted from synthesis to breakdown.

4. Conclusions

Based on the data obtained in this study, a model diagram showing the potential H_2O_2-polyamine relationship was established (Figure 6). The model of polyamine metabolism controlled by hydrogen peroxide can be summarized as follows. H_2O_2 which was applied externally in drought conditions, probably induced both *NOX* gene expression and enzyme activity. Then, H_2O_2 induced the expression of *ADC*, *AIH*, *SPDS*, and *SAMDC*, one of the polyamine synthesis genes, directly or indirectly, by other possible metabolic pathways. Due to the increased level of gene expression, the amount of endogenous polyamine has increased. Besides, H_2O_2 has been suggested to reduce the production of polyamine oxidation enzymes by directly or indirectly suppressing the expression of polyamine degradation genes. In conclusion, H_2O_2 plays an essential regulation role in the metabolism of polyamines by adjusting the expressions of genes for the synthesis and degradation of polyamines.

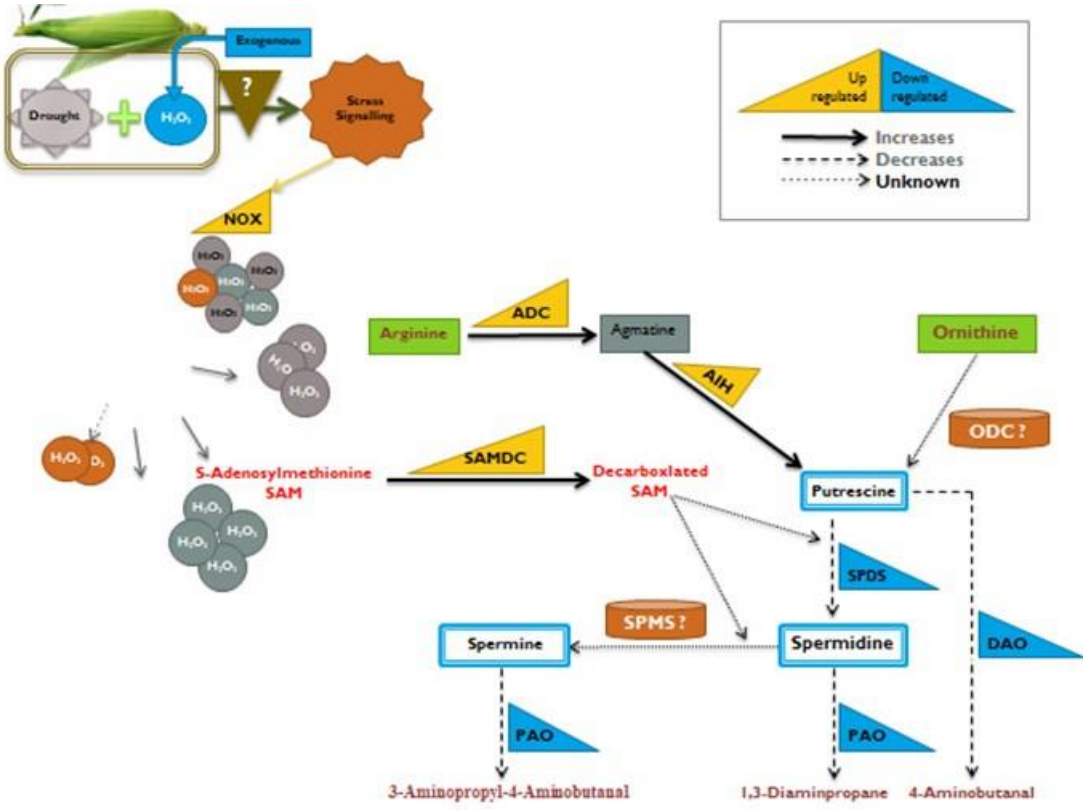


Figure 6- Putative H_2O_2-related polyamine metabolism under drought stress conditions

Acknowledgements

This study was supported by The Scientific and Technological Research Council of Turkey through the project 113Z863. Authors thanks to Prof. Dr. Ahmet YASAR for helping measurements of polyamine contents

References

Abass M S & Mohamed H I (2011). Alleviation of adverse effects of drought stress on common bean (*Phaseolus vulgaris* L.) by exogenous application of hydrogen peroxide. *Bangladesh Journal of Botany* 40: 75-83. doi: 10.3329/bjb.v40i1.8001

Aebi H E (1983). Catalase. In: Bergmeyer H U (Ed.), Methods of Enzymatic Analysis, Verlag Chemie, Germany pp. 273-286

Akyol T Y, Yılmaz O, Uzilday B, Özgür Uzilday R & Türkan I (2020). Plant response to salinity: an analysis of ROS formation, signaling, and antioxidant defense. *Turkish Journal of Botany* 44: 1-13. doi:10.3906/bot-1911-15

Alcazar R, Cuevas J C, Patron M, Altabella T & Tiburcio A F (2006a). Abscisic acid modulates polyamine metabolism under water stress in *Arabidopsis thaliana*. Physiologia Plantarum 128: 448-455. doi: 10.1111/j.1399-3054.2006.00780.x

Alcazar R, Marco F, Cuevas J C, Patron M, Ferrando A, Carrasco P, Tiburcio A F & Altabella T (2006b). Involvement of polyamines in plant response to abiotic stress. Biotechnology Letters 28: 1867-1876. doi: 10.1007/s10529-006-9179-3

Alcazar R, Planas J, Saxena T, Zarza X, Bortolotti C, Cuevas J, Bitrian M, Tiburcio A F & Altabella T (2010a). Putrescine accumulation confers drought tolerance in transgenic Arabidopsis plants overexpressing the homologous *Arginine decarboxylase 2* gene. Plant Physiology and Biochemistry 48: 547-552. doi: 10.1007/s00425-010-1130-0

Alcazar R, Altabella T, Marco F, Bortolitto C, Reymond M, Koncz C, Carasso P & Tiburcio A F (2010b). Polyamines: Molecules with regulatory functions in plant abiotic stress tolerance. Planta 231: 1237-1249. doi: 10.1016/j.plaphy.2010.02.002

Alcazar R, Bitrian M, Bartels D, Koncz C, Altabella T & Tiburcio A F (2011). Polyamine metabolic canalization in response to drought stress in Arabidopsis and the resurrection plant *Craterostigma plantagineum*. Plant Signaling and Behavior 6: 243-250. doi: 10.4161/psb.6.2.14317

Andronis A E, Moschou P N, Toumi I & Roubelakis-Angelakis K A (2014). Peroxisomal polyamine oxidase and NADPH-oxidase cross-talk for ROS homeostasis which affects respiration rate in *Arabidopsis thaliana*. Frontiers in Plant Science 5: 132. doi: 10.3389/fpls.2014.00132

Angelini R, Cona A, Federico R, Fincato P, Tavladoraki P & Tisi A (2010). Plant amine oxidases "on the move": an update. Plant Physiology and Biochemistry 48: 560-564 doi: 10.1016/j.plaphy.2010.02.001

Bailey-Serres J & Mittler R (2006). The roles of reactive oxygen species in plant cells. *Plant Physiology* 141: 311. doi: 10.1104/pp.104.900191

Beauchamp C & Fridovich I (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry 44: 276-287. doi: 10.1016/0003-2697(71)90370-8

Bradford M M (1976). Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254. doi: 10.1006/abio.1976.9999

Cakmak I & Marschner H (1988). Zinc-dependent changes in ESR signals, NADPH oxidase and plasma membrane permeability in cotton roots. Physiologia Plantarum 73: 182-186. doi: 10.1111/j.1399-3054.1988.tb09214.x

Capell T, Bassie L & Christou P (2004). Modulation of the polyamine biosynthetic pathway in transgenic rice confers tolerance to drought stress. Proceedings of the National Academy of Sciences of the United States of America 101: 9909-9914. doi: 10.1073/pnas.0306974101

Chaudiere J & Ferrari-Iliou R (1999). Intracellular antioxidants from chemical to biochemical mechanisms. Food and Chemical Toxicology 37: 949-962. doi: 10.1016/s0278-6915(99)00090-3

Cohen S S (1998). A Guide to The Polyamines. Oxford University Press, New York

Cona A, Rea G, Angelini R, Federico R & Tavladoraki P (2006a). Functions of amine oxidases in plant development and defence. Trends in Plant Science 11: 80-88. doi: 10.1016/j.tplants.2005.12.009

Cona A, Rea G, Botta M, Corelli F, Federico R & Angelini R (2006b). Flavin-containing polyamine oxidase is a hydrogen peroxide source in the oxidative response to the protein phosphatase inhibitor cantharidin in *Zea mays*. *Journal of Experimental Botany* 57: 2277-2289. doi: 10.1093/jxb/erj195

Cruz de Carvalho MH (2008). Drought stress and reactive oxygen species. *Plant Signaling and Behavior* 3: 156-165. doi: 10.4161/psb.3.3.5536

Das K & Roychoudhury A (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers in Environmental Science 2: 53. doi: 10.3389/fenvs.2014.00053

Do P T, Drechsel O, Heyer A G, Hincha D K & Zuther E (2014). Changes in free polyamine levels, expression of polyamine biosynthesis genes and performance of rice cultivars under salt stress: A comparison with responses to drought. Frontiers in Plant Science 5: 182. doi: 10.3389/fpls.2014.00182

Dikalov S, Nazarewicz R, Panov A, Harrison D G & Dikalova A (2011). Crosstalk between mitochondrial ROS and NADPH oxidases in cardiovascular and degenerative diseases: Application of mitochondria-targeted antioxidants. Free Radical Biology and Medicine 51: 85-86. doi: 10.1016/j.freeradbiomed.2011.06.033

Erdei L, Szegletes Z, Barabas K & Pestenacz A (1996). Responses in polyamine under osmotic and salt stress in sorghum and maize seedlings. *Journal of Plant Physiology* 147: 599-603. doi: 10.1016/S0176-1617(96)80052-6

Fincato P, Moschou P N, Spedaletti V, Tavazza R, Angelini R, Federico R, Roubelakis-Angelakis K A & Tavladoraki P (2011). Functional diversity inside the Arabidopsis polyamine oxidase gene family. *Journal of Experimental Botany* 62: 1155-1168. doi: 10.1093/jxb/erq341

Fincato P, Moschou P N, Ahou A, Angelini R, Roubelakis-Angelakis K A, Federico R & Tavladoraki P (2012). The members of *Arabidopsis thaliana PAO* gene family exhibit distinct tissue and organ-specific expression pattern during seedling growth and flower development. Amino Acids 42: 831-841. doi: 10.1007/s00726-011-0999-7

Galston A W, Kaur-Sawhney R, Altabella T & Tiburcio A F (1997). Plant polyamines in reproductive activity and response to abiotic stress. Botanica Acta 110: 197-207. doi: 10.1111/j.1438-8677.1997.tb00629.x

Gill S & Tuteja N (2010). Polyamines and abiotic stress tolerance in plants. Plant Signaling & Behavior 5: 26-33. doi: 10.4161/psb.5.1.10291

Groppa M D & Benavides M P (2008). Polyamines and abiotic stress: Recent advances. Amino Acids 34: 35-45. doi: 10.1007/s00726-007-0501-8

Guven F G (2013). Effect of alpha lipoic acid pre-treatment on drought tolerance in drought-sensitive and drought-resistant maize genotypes. Master thesis, Karadeniz Technical University, Turkey

Hatmi S, Gruau C, Trotel-Aziz P, Villaume S, Rabenoelina F, Baillieul F, Eullaffroy P, Clément C, Ferchichi A & Aziz A (2015). Drought stress tolerance in grapevine involves activation of polyamine oxidation contributing to improved immune response and low susceptibility to *Botrytis cinerea*. *Journal of Experimental Botany* 66: 775-787. doi: 10.1093/jxb/eru436

He L, Gao Z & Li R (2009). Pretreatment of seed with H_2O_2 enhances drought tolerance of wheat (*Triticum aestivum* L.) seedlings. *African Journal of Biotechnology* 8: 6151-6157. doi: 10.5897/AJB09.490

Heath R L & Packer L (1968). Photoperoxidation in isolated chloroplasts. I. kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics 125: 189-198. doi: 10.1016/0003-9861(68)90654-1

Kusano T, Yamaguchi K, Berberich T & Takahashi Y (2007). The polyamine spermine rescues Arabidopsis from salinity and drought stresses. Plant Signaling and Behavior 2: 251-252. doi: 10.4161/psb.2.4.3866

Kusano T, Berberich T, Tateda C & Takahashi Y (2008). Polyamines: Essential factors for growth and survival. Planta 228: 367-381. doi: 10.1007/s00425-008-0772-7

Kwak J M, Mori I C, Pei Z M, Leonhardt N, Torres M A, Dangl J L, Bloom R E, Bodde S, Jones J D G & Schroeder J I (2003). NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in Arabidopsis. *EMBO Journal* 22: 2623-2633. doi: 10.1093/emboj/cdg277

Li Z Y & Chen S Y (2000). Differential accumulation of the S-adenosylmethionine decarboxylase transcript in rice seedlings in response to salt and drought stresses. Theoretical and Applied Genetics 100: 782-788. doi: 10.1007/s001220051

Liu Z J, Guo Y K & Bai J G (2010). Exogenous hydrogen peroxide changes antioxidant enzyme activity and protects ultrastructure in leaves of two cucumber ecotypes under osmotic stress. *Journal of Plant Growth Regulation* 29: 171-183. doi: 10.1007/s00344-009-9121-8

Liu C, Atanasov K E, Tiburcio A F & Alcázar R (2019). The polyamine putrescine contributes to H_2O_2 and *RbohD*/*F*-dependent positive feedback loop in Arabidopsis PAMP-Triggered Immunity. Frontiers in Plant Science 10: 894. https://doi.org/10.3389/fpls.2019.00894

Moschou P N, Delis I D, Paschalidis K A & Roubelakis-Angelakis K A (2008a). Transgenic tobacco plants overexpressing polyamine oxidase are not able to cope with oxidative burst generated by abiotic factors. Physiologia Plantarum 133: 140-156. doi: 10.1111/j.1399-3054.2008.01049.x

Moschou P N, Paschalidis K A & Roubelakis-Angelakis K A (2008b). Plant polyamine catabolism: The state of the art. Plant Signaling and Behavior 3: 1061-1066. doi: 10.4161/psb.3.12.7172

Munne-Bosch S, Jubany-Mari T & Alegre L (2001). Drought-induced senescence is characterized by a loss of antioxidant defences in chloroplasts. Plant Cell and Environment 24: 1319-1327. doi: 10.1046/j.1365-3040.2001.00794.x

Papadakis A K & Roubelakis-Angelakis K A (2005). Polyamines inhibit NADPH oxidase-Mediated superoxide generation and putrescine prevents programmed cell death induced by polyamine oxidase-generated hydrogen peroxide. Planta 220: 826-837. doi: 10.1007/s00425-004-1400-9

Parida A K & Das A B (2005). Salt tolerance and salinity effects on plants: a review. Ecotoxicology and Environmental Safety 60: 324-349. doi: 10.1016/j.ecoenv.2004.06.010

Pitzschke A, Forzani C & Hirt H (2006). Reactive oxygen species signaling in plants. Antioxidants and Redox Signaling 8: 1757-1764. doi: 10.1089/ars.2006.8.1757

Potocký M, Jones M A, Bezvoda R, Smirnoff N & Žárský V (2007). Reactive oxygen species produced by NADPH oxidase are involved in pollen tube growth. New Phytologist 174: 742–51. doi: 10.1111/j.1469-8137.2007.02042.x

Rodríguez-Rojas A, Kim J J, Johnston P R, Makarova O, Eravci M, Weise C, Hengge R & Rolff J (2020). Non-lethal exposure to H_2O_2 boosts bacterial survival and evolvability against oxidative stress. PLoS Genetics 16(3): e1008649. doi: 10.1371/journal.pgen.1008649

Sagi M, Davydov O, Orazova S, Yesbergenova Z, Ophir R, Stratmann J W & Fluhr R (2004). Plant respiratory burst oxidase homologs impinge on wound responsiveness and development in *Lycopersicon esculentum*. Plant Cell 16: 616-628. doi: 10.1105/tpc.019398

Saglam A, Saruhan N, Terzi R & Kadioğlu A (2011). The relations between antioxidant enzymes and chlorophyll fluorescence parameters in common bean cultivars differing in sensitivity to drought stress. *Russian Journal of Plant Physiology* 58: 60-68. doi:10.1134/S102144371101016X

Seo S Y, Kim Y J & Park K Y (2019). Increasing polyamine contents enhances the stress tolerance via reinforcement of antioxidative properties. Frontiers in Plant Science 10: 1331. doi: 10.3389/fpls.2019.01331

Sequera-Mutiozabal M, Antoniou C, Tiburcio A F, Alcázar R & Fotopoulos V (2017). Polyamines: Emerging hubs promoting drought and salt stress tolerance in plants. Current Molecular Biology Reports 3: 28-36. doi: 10.1007/s40610-017-0052-z

Sharma P & Dubey R S (2005). Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. Plant Growth Regulation 46: 209-221. doi: 10.1007/s10725-005-0002-2

Shaw B, Thomas T H & Cooke D T (2002). Responses of sugar beet (*Beta vulgaris*) to drought and nutrient deficiency stress. Plant Growth Regulation 37: 77-83. doi: 10.1023/A:1020381513976

Siddique M R B, Hamid A & Islam M S (2000). Drought stress effects on water relations of wheat. Botanical Bulletin of Academia Sinica 41: 35-39

Suzuki N, Miller G, Salazar C, Mondal H A, Shulaev E, Cortes D F, Shuman J L, Luo X, Shah J, Schlauch K, Shulaev V & Mittler R (2013). Temporal-spatial interaction between reactive oxygen species and Abscisic acid regulates rapid systemic acclimation in plants. Plant Cell 25: 3553-3569. doi: 10.1105/tpc.113.114595

Terzi R, Kadioglu A, Kalaycioglu E & Saglam A (2014). Hydrogen peroxide pretreatment induces osmotic stress tolerance by influencing osmolyte and abscisic acid levels in maize leaves. *Journal of Plant Interactions* 9: 559-565. doi: 10.1080/17429145.2013.871077

Torres M A, Jones J D G & Dangl J L (2005). Pathogen-induced, NADPH oxidase-derived reactive oxygen intermediates suppress spread of cell death in *Arabidopsis thaliana*. Nature Genetics 37: 1130-1134. doi: 10.1038/ng1639

Urano K, Yoshiba Y, Nanjo T, Ito T, Yamaguchi-Shinozaki K & Shinozaki K (2004). Arabidopsis stress-inducible gene for *Arginine decarboxylase AtADC2* is required for accumulation of putrescine in salt tolerance. Biochemical and Biophysical Research Communications 313: 369-375. doi: 10.1016/j.bbrc.2003.11.119

Urbanek H, Kuzniak-Gebarowska E & Herka K (1991). Elicitation of defense responses in bean leaves by *Botrytis cinerea* polygalacturanase, Acta Physiologiae Plantarum 13: 43-50

Velikova V, Yordanov I & Edreva A (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective roles of exogenous polyamines. Plant Science 151: 59-66. doi: 10.1016/S0168-9452(99)00197-1

Waszczak C, Carmody M & Kangasjärvi J (2018). Reactive oxygen species in plant signaling. Annual Review of Plant Biology 29: 209-236. doi: 10.1146/annurev-arplant-042817-040322

Wu J, Shang Z, Jiang X, Moschou P N, Sun W, Roubelakis-Angelakis K A & Zhang S (2010). Spermidine oxidase-derived H_2O_2 regulates pollen plasma membrane hyperpolarization-activated Ca^{+2}-permeable channels and pollen tube growth. *Plant Journal* 63: 1042-1053. doi: 10.1111/j.1365-313X.2010.04301.x

Yamaguchi K, Takahashi Y, Berberich T, Imai A, Miyazaki A, Takahashi T, Michael A & Kusano T (2006). The polyamine spermine protects against high salt stress in *Arabidopsis thaliana*. FEBS Letters 580: 6783-6788. doi: 10.1016/j.febslet.2006.10.078

Yamaguchi K, Takahashi Y, Berberich T, Imai A, Takahashi, T, Michael, A J & Kusano T (2007). A protective role for the polyamine spermine against drought stress in Arabidopsis. Biochemical and Biophysical Research Communications 352: 486-490. doi: 10.1016/j.bbrc.2006.11.041

Journal of Agricultural Sciences (Tarim Bilimleri Dergisi) 2022, 28 (4) : 626 - 634 DOI: 10.15832/ankutbd.924222

Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

Microsatellite Diversity and Restriction Enzyme-based Polymorphisms of MHC Loci in Some Native Turkish Goats

Mehmet ASLAN[a], Eymen DEMİR[a], Taki KARSLI[b*]

[a]*Department of Animal Science, Faculty of Agriculture, Akdeniz University, Antalya, 07058, TURKEY*
[b]*Department of Animal Science, Faculty of Agriculture, Eskisehir Osmangazi University, Eskisehir 26160, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Taki KARSLI, E-mail: takikarsli@ogu.edu.tr
Received: 03 June 2021 / Revised: 11 November 2021 / Accepted: 13 November 2021 / Online: 15 October 2022

Cite this article
ASLAN M, DEMİR E, KARSLI T. Microsatellite Diversity and Restriction Enzyme-based Polymorphisms of MHC Loci in Some Native Turkish Goats. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 28(4):626-634. DOI: 10.15832/ankutbd.924222

ABSTRACT

Playing a key role in immunity and autoimmunity, Major Histocompatibility Complex (MHC) contains microsatellite regions and polymorphisms associated with resistance to several diseases and thermo-physiologic characteristics in farm animals. This study aims to reveal genetic diversity in four native Turkish goat populations via MHC related gene regions including MHC-linked microsatellite markers (BF1, BM1818, BM1258, SMHCC1 and DYMS1) and MHC Class II-DRB gene. A total of 120 unrelated animals belonging to Hair (HAI), Honamlı (HNM), Kabakulak (KBK) and Norduz (NRD) from different representative populations in Antalya and Van provinces were sampled and genotyped for molecular analysis. Based on MHC-linked microsatellite markers, number of alleles ranged from 8.20 (NRD) to 8.80 (HAI and KBK) across studied goat populations. Observed heterozygosity was between 0.68 (NRD) and 0.80 (KBK), whereas expected heterozygosity ranged from 0.74 (NRD) to 0.82 (KBK) in native Turkish goats. Inbreeding coefficients were 0.04, 0.13, -0.01 and 0.09 for HAI, HNM, KBK and NRD populations, respectively. A 284 bp length PCR products belonging to MHC Class II-DRB gene region were digested separately with *Pst*I, *Taq*I, *BsaH*I and *Alu*I restriction endonucleases to assess polymorphism status together with Hardy-Weinberg equilibrium in studied goat populations. P allele frequency ranged from 0.73 (KBK) to 0.95 (NRD), while p allele frequency was between 0.05 (NRD) and 0.27 (KBK) in *Pst*I polymorphism. The highest and lowest frequency of T allele were detected in HNM (0.80) and KBK (0.49), respectively, whereas frequency of t allele was between 0.20 (HNM) and 0.51 (KBK) in *Taq*I polymorphism. G and A allele frequency were between 0.16 (HNM) - 0.39 (HAI) and 0.84 (HNM) – 0.61 (HAI), respectively in *BsaH*I polymorphism, while three different genotypes and two alleles, which were different from results reported in the literature, were observed in *Alu*I polymorphism. In this study, high genetic diversity and low inbreeding were detected in native Turkish goats according to MHC-linked microsatellite markers. Similarly, native Turkish goats hold enough polymorphisms in MHC Class II-DRB gene which gives opportunity to support selection strategies against tuberculosis and heat stress in the future.

Keywords: Genetic diversity, Heat tolerance, PCR-RFLP, SSR, Tuberculosis resistance

1. Introduction

In Turkey, goat raising is practised by smallholder farmers across the country in order to obtain specific products such as milk, meat, cashmere and angora. Although, large part of goat population (more than 90%) is Hair (HAI) goat, it possesses different varieties [Kabakulak (KBK), Pavga and Çandır] adapted to different environmental conditions (Karsli et al. 2020). There are differences between HAI and its varieties in terms of body size, fertility and milk yield (Erduran & Kırbas 2010). Moreover, a recent study based on 20 microsatellite loci demonstrated that KBK is genetically different from HAI (Karsli et al. 2020). Lower jaw and convex nose are distinctive characteristics of HNM which is raised in Muğla and Antalya provinces. Norduz (NRD) possess the lowest body size and live weight among native Turkish goats and is raised in limited region of Van province and villages close to Iran border (Kirk et al. 2004).

Today, there are many factors such as climate change and infectious diseases which negatively affect sustainability of livestock sector (Radostits et al. 2007; Rovelli et al. 2020). Genetic diversity across genome and polymorphisms in related gene regions are required to face these challenges and to maintain livestock sector in the future. Indeed, today several studies have been aimed to investigate genes related to resistance to higher temperature (Basiricò et al. 2011; Liu et al. 2011) and diseases (Vaccari et al. 2006; Pisoni et al. 2010; Cecchi et al. 2017; Gowane et al. 2018; Eren et al. 2019) in livestock species. Of these genes, Major Histocompatibility Complex (MHC) consisting of MHC Class I and MHC Class II, has a key role in immunity and autoimmunity. MHC Class I molecules transporting proteins to T cells are present in almost all cells, while MHC Class II molecules known as Antigen Presenting Cells (APC) are present in certain cells such as macrophage and B cells. Previous studies

show that there are association between polymorphisms of MHC gene regions and resistance/susceptibility to several diseases (Singh et al. 2012; Shen et al. 2014; Kim et al. 2015; Kannaki et al. 2017) and thermo-physiologic traits in livestock species. Additionally, MHC-linked microsatellite markers are commonly used to reveal genetic diversity and phylogenetic relationships among different goat breeds (Salles et al. 2011; Guang-Xin et al. 2015).

Due to developing molecular techniques, animals with higher ability of heat tolerance and resistant to specific diseases can be detected easily. Moreover, by applying Marker Assisted Selection (MAS), the frequency of desired genotypes in terms of heat tolerance and resistance to disease can be increased in populations. In this regard, this paper aims i) to reveal current genetic diversity by MHC-linked microsatellite markers and ii) to obtain preliminary results of enzyme-based polymorphisms of MHC Class II-DRB gene which may be further analysed for association studies for heat tolerance and tuberculosis resistance in native Turkish goats.

2. Material and Methods

2.1 Blood collection and DNA isolation

120 unrelated blood samples were randomly chosen from HAI (n=40), HNM (n=30), KBK (n=30) and NRD (n=20) goat populations. Blood samples of NRD were obtained from Van, while the other samples were collected from representative herds raised in different districts of Antalya, Turkey. Genomic DNA was isolated from blood samples by using a salting-out method with minor modifications (Miller et al. 1988).

2.2 PCR-RFLP analysis and microsatellite genotyping

A 284 bp length of MHC Class II-DRB gene region and five MHC Class II-linked microsatellite markers were amplified with specific primer sets (Table 1).

Table 1- Characteristics of used loci in the study

	Locus	***Method***	***Primer Sequence (5'-3')***	***PCR Products (bp)***	***RE Enzyme***	***Reference***
Gene Polymorphism	MHC Class II-DRB	PCR-RFLP	F: TATCCCGTCTCTGCAGCACATTTC R: TCGCCGCTGCACACTGAAACTCTC	284	*Pst*I, *Taq*I	Singh et al. (2012)
					*Bsa*HI, *Alu*I	Yakubu et al. (2017)
Genetic Diversity	BF1	Microsatellite	F: CAACGGTCTGCAACCGAATTACC R: CAATCCGTGGGTTGGAACACAA	159-165	-	Guang-Xin et al. (2015); Salles et al. (2011)
	BM1818		F: AGCTGGGAATATAACCAAAGG R: AGTGCTTTCAAGGTCCATGC	244-266		
	BM1258		F: GTATGTATTTTTCCCACCCTGC R: GTCAGACATGACTGAGCCTG	98-126		
	DYMS1		F: TCCTGGGGATTCCCAATACC R: CATAGAAGTCTTCACTGGTG	171-199		
	SMHCC1		F: ATCTGGTGGGCTACAGTCCATG R: GCAATGCTTTCTAAATTCTGA	175-199		

PCR was performed in total of 50 µL volume containing 3.2 µL HQ buffer (GeneAll), 2.5 mM dNTPs, 10 pM of each primer, 2.5 U Taq DNA Polymerase (GeneAll), 50 ng template DNA and 30.9 µL ddH_2O. PCR amplifications were applied in initial denaturation at 94 °C for 5 mins, followed by 30 cycles at 94 °C for 45 s, at 55-58 °C (depending on locus) for 45 s and at 72 °C for 50 s. The final extension was carried out at 72 °C for 5 mins. Amplificated 284 bp length PCR products were separately digested with restriction enzymes of *Pst*I, *Taq*I, *BsaH*I and *Alu*I to assess polymorphism status of native Turkish goats. Restriction enzyme mixture containing 10 µL of amplified PCR products, 5 U restriction enzymes (Table 1), 4.5 µL enzyme buffer and 5 µL nuclease free water, were incubated according to manufacturer instructions (Thermo Scientific Inc.). PCR and RFLP products were separated on 1% and 3% agarose gel electrophoresis, respectively. On the other hand, a 96-well automatic fragment analyzer (Agilent 5200 Fragment Analyzer System, USA) was used to genotype individuals for five microsatellite markers.

2.3 Statistical analysis

Allele and genotype frequencies of PCR-RFLP data were calculated by using Popgene (Yeh et al. 1997) package software. Chi-Square ($\chi 2$) test was applied to determine deviation from Hardy-Weinberg Equilibrium (HWE). Allele size range, private allele and allele frequencies of microsatellite loci were calculated via Convert (Glaubitz 2004) program. Number of alleles (Na), number of effective alleles (Ne), observed (Ho) and expected (He) heterozygosity were calculated by Popgene (Yeh et al. 1997) software. Inbreeding coefficient (F_{IS}), the diversity between breeds (D_{ST}) and the coefficient of gene differentiation (G_{ST}) were detected by Fstat v.1.2. (Goudet 1995), whereas null allele frequency was calculated by using Ml-NullFreq (Kalinowski & Taper

2006) software, respectively. UPGMA (Unweighted Pair-Group Method with Arithmetic Mean) dendrogram was constructed by Popgene (Yeh et al. 1997), while FCA (Factorial Corresponding Analysis) and Structure analysis were done by Genetix v.4.05 (Belkhir et al. 2004) and Structure v.2.2. (Pritchard et al. 2000) programs, respectively. In order to determine the best K value, Structure Harvester (Earl & vonHoldt 2012) was utilized, while Clumpak (Kopelman et al. 2015) was used to visualise the result of structure analysis.

3. Results and Discussion

3.1 Genetic diversity among native Turkish goat populations based on MHC-linked microsatellite markers

A total of 65 alleles ranging from 11 (BM1258) to 15 (DYMS1) per marker were detected in all population (Table 2). Null allele frequency ranged from 0.00 (DYMS1) to 0.11 (BM1258) per marker with a mean of 0.05 indicating that all studied loci were highly amplificated. Mean D_{ST} and $G_{ST,}$ indicating the genetic diversity between breeds and the coefficient of gene differentiation, were 0.06 and 0.07, respectively. In this study, high number of alleles ranging from 8.20 (NRD) to 8.80 (HAI and KBK) were detected across studied goat populations by using a total of 5 different MHC-linked microsatellite markers (Table 3). Of 10 private alleles, 9 alleles were detected in NRD population, while only one private allele was observed in HNM populations (data not shown). Although, the frequency of private alleles was low (<0.3), the frequency of an allele (176 bp) at BF1 locus were at 0.60 for NRD population. Observed heterozygosity were between 0.68 (NRD) and 0.80 (KBK), whereas expected heterozygosity ranged from 0.74 (NRD) to 0.82 (KBK) across studied populations. Inbreeding coefficients were 0.04, 0.13, -0.01 and 0.09 for HAI, HNM, KBK and NRD populations, respectively (Table 3).

Table 2- Genetic diversity parameters across four native Turkish goat populations based on 5 MHC-linked loci

Loci	*AR*	*n*	*Na*	*Ne*	H_O	H_E	F_{IS}	D_{ST}	G_{ST}	*F (Null)*	*HWE*
BF1	154-180	110	13	4.52	0.82	0.78	-0.21	0.14	0.17	0.01	***
BM1818	246-270	100	12	7.58	0.68	0.87	0.18	0.02	0.03	0.09	***
BM1258	98-120	108	11	6.25	0.62	0.84	0.20	0.07	0.08	0.11	***
SMHCC1	91-199	112	14	6.20	0.69	0.84	0.15	0.01	0.01	0.06	*
DYMS1	175-205	109	15	7.53	0.89	0.87	-0.12	0.06	0.07	0.00	***
Mean	-	-	13	6.42	0.74	0.83	0.05	0.06	0.07	0.05	-

AR: Allele range, n: Number of genotyped individuals, Na: number of alleles, Ne: number of effective alleles, Ho: observed heterozygosity, He: expected heterozygosity, F_{IS}: inbreeding coefficient, D_{ST}: diversity between breeds, G_{ST}: coefficient of gene differentiation, F (Null): null allele frequency, HWE: Hardy-Weinberg equilibrium, *: P<0.05, **: P<0.01, ***: P<0.001.

Table 3- Genetic diversity parameters per studied goat populations based on 5 MHC-linked loci

Breeds	*MNA*	*MNE*	H_O	H_E	F_{IS}	*HWE*
HAI	8.80	4.83	0.74	0.80	0.04	-
HNM	8.40	5.10	0.76	0.79	0.13	1
KBK	8.80	5.36	0.80	0.82	-0.01	2
NRD	8.20	4.66	0.68	0.74	0.09	1

MNA: mean number of alleles, MNE: mean number of effective alleles, Ho: mean observed heterozygosity, He: mean expected heterozygosity, F_{IS}: mean inbreeding coefficient, HWE: number of loci deviated from Hardy-Weinberg equilibrium.

The same MHC-linked microsatellite markers were preferred by Guang-Xin et al. (2015) and Salles et al. (2011) to reveal genetic diversity in different goat breeds raised in China. Although, similar genetic diversity was reported for Dazu Black and Chuannan Black goat breeds, genetic diversity in native Turkish goat populations were higher than Chuanzhong, Enshi, Hechuan, Jining Qing, Tibetan and Yichang goat breeds raised in China (Guang-Xin et al. 2015). Additionally, mean observed heterozygosity values for native Turkish goat populations were higher than the values reported for Xuhuai, Pashmina, Changthangi, Vendi, Galla, Small East African goat breeds (Salles et al. 2011). On the other hand, higher genetic diversity was reported in previous studies conducted on native Turkish goats breeds in which Karsli et al. (2020) used 20 microsatellite markers, while Gül et al. (2020) preferred a total 22 microsatellite markers to reveal genetic structure of studied goat breeds. It is not surprising, since genetic diversity may increase by using more microsatellite markers with higher population size.

Compared to the goat breeds raised in China (Salles et al. 2011; Guang-Xin et al. 2015), lower inbreeding was detected in native Turkish goats via MHC-linked microsatellite markers. Similarly, low inbreeding was reported in previous microsatellite studies on native Turkish goats (Gül et al. 2020; Karsli et al. 2020; Tefiel et al. 2020). As highlighted by Argun Karsli et al. (2020), higher null allele frequency (>0.2) may affect estimates of observed heterozygosity and inbreeding coefficient parameters. Low null allele frequencies observed in the present study were the sign of that these parameters (observed heterozygosity and inbreeding coefficient) were calculated at high accuracy.

This study revealed that native Turkish goat populations are of high genetic diversity and low inbreeding which could be attributed to different phenomenon. As highlighted by Demir & Balcioglu (2019), Anatolia is a part of domestication centre

indicating that theoretically native Turkish goat breeds contain a large part of *C. hircus* gene pool. Additionally, sampling strategy may be another reason, since studied animals were provided from different representative herds in which animals were unrelated according to pedigree record.

Food and Agriculture Organization of the United Nations reported and recommended at least 30 microsatellite markers for molecular characterization of farm animals (FAO 2011). On the other hand, the main aim of the present study was to reveal genetic diversity based on MHC-linked microsatellite markers which are naturally less compared to other autosomal microsatellite loci. Despite of low number of used microsatellite markers, comparatively high genetic diversity (Na, Ne, Ho and He) were detected in native Turkish goat breeds. Similarly, Ceccobelli et al. (2020) reported that even decreased number of microsatellite markers from 16 to 12 was enough to distinguish a local Italian goat breed from exotic breeds.

3.2 Phylogenetic relationships among native Turkish goat populations based on MHC-linked microsatellite markers

In this study, UPGMA dendrogram (Figure 1), FCA (Figure 2) and structure (Figure 3) analysis were utilized to reveal phylogenetic relationships among native Turkish goat populations. Genetic distance and similarity values were calculated in order to construct UPGMA dendrogram. The highest genetic distance value (1.19) was observed between KBK and NRD populations, while the highest similarity value (0.87) was detected between HAI and KBK populations (Table 4).

Table 4- Nei's genetic distance (below the diagonal) and similarity (above the diagonal) values between studied populations

	HAI	***HNM***	***KBK***	***NRD***
HAI	****	0.84	0.87	0.34
HNM	0.16	****	0.86	0.33
KBK	0.13	0.13	****	0.30
NRD	1.06*	1.09*	1.19*	****

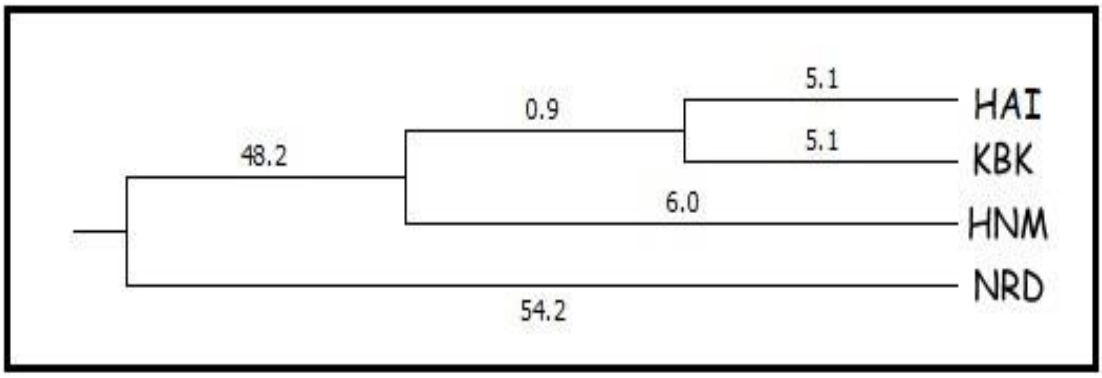


Figure 1- UPGMA denrogram among native Turkish goats based on genetic distance values (Nei 1978)

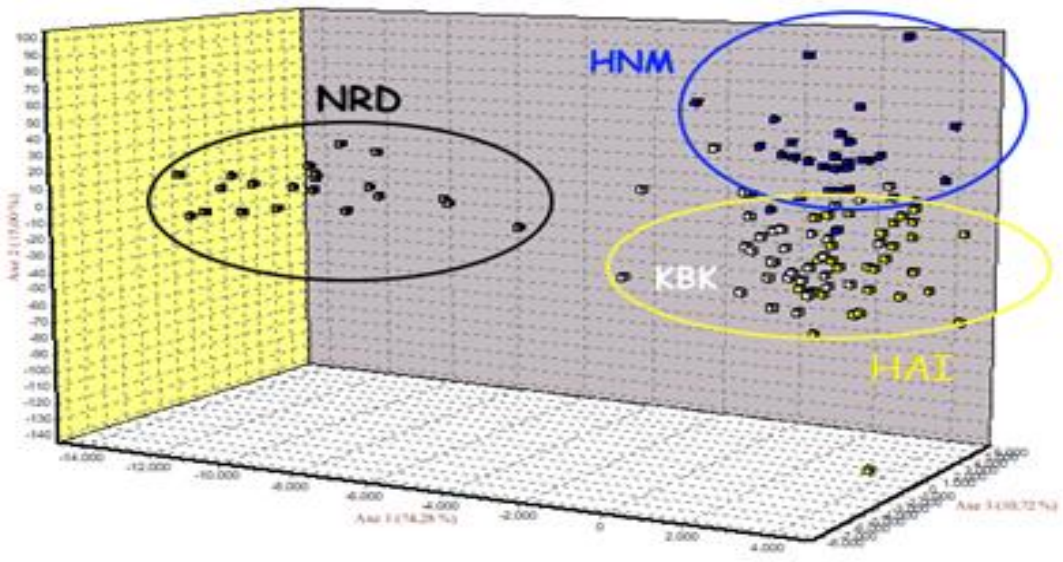


Figure 2- FCA analysis of native Turkish goats

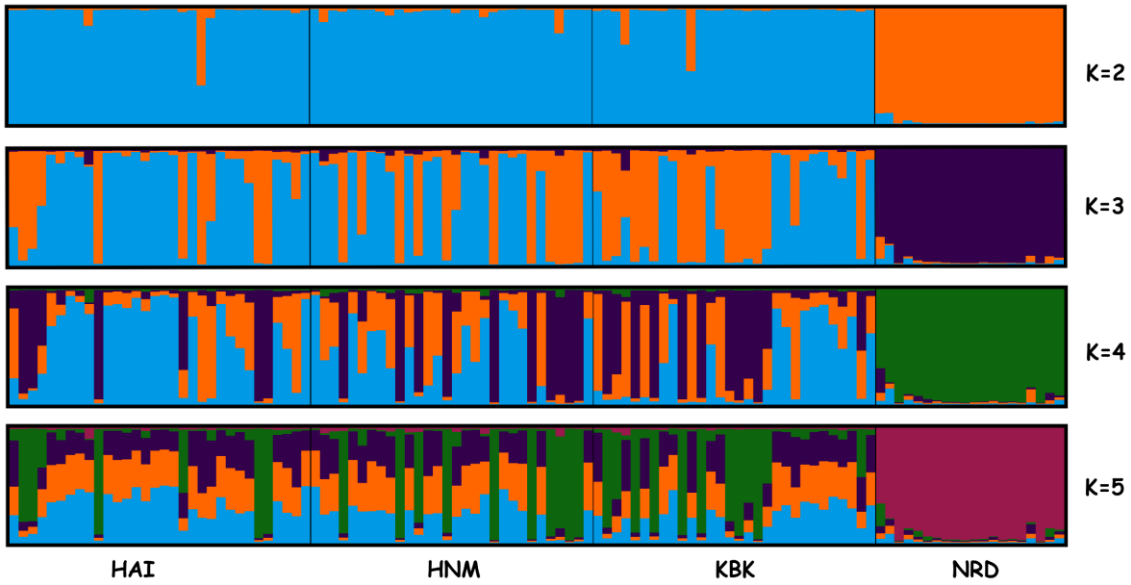


Figure 3- Population structure of native Turkish goats via STRUCTURE analysis

NRD population were clustered separately from the other populations in UPGMA dendrogram, while HAI and KBK were clustered together. HNM were found closer to HAI and KBK than NRD population. Results of UPGMA dendrogram was supported by FCA analysis in which NRD population were distinctively separate from the other populations in three-dimensional space, while KBK and HAI clustered together. Similarly, admixture was observed between HNM, HAI and KBK populations.

Although, four different Turkish goat populations were analysed in this study, the best K value was determined as 3 by Structure Harvester (Earl & vonHoldt 2012) indicating that studied individuals were assigned to three different clusters (Table 5). At K=2, NRD population were distinct clearly from the other populations, while HAI, KBK and HNM were assigned into the same clade. At K=3, similar results were obtained in which NRD was clustered separately from the other populations, while a high level of admixture was detected among HAI, KBK and HNM.

Table 5- Estimated posterior probabilities [LnPr (X|K)] and ΔK statistics for different number of clusters

Number of Cluster	*[LnPr (X|K)]*	*ΔK*
2	-2617.895	-
3	**-2530.851**	**47.860**
4	-2532.445	1.715
5	-2550.721	-

In this study, phylogenetic relationship analysis revealed that NRD population was genetically different from HAI, KBK and HNM populations in harmony with its breeding history. Indeed, NRD population is raised separately from the other goat breeds in a limited region of Turkey (Van province) and this isolation led to different genetic structure together with specific phenotypes such as lower body size. Similarly, NRD were reported to be genetically different from the other native Turkish goat breeds by Karsli et al. (2020) via 20 different microsatellite markers.

On the contrary, although they are of different morphological traits, a high level of admixture was detected among HAI, KBK and HNM populations based on phylogenetic analysis. Similarly, previous studies could not distinguish HNM and HAI breeds by microsatellite markers (Ağaoğlu & Ertuğrul 2012; Bulut et al. 2016). Conversely, a recently published study showed for the first time that KBK has become genetically different from HAI breed (Karsli et al. 2020).

3.3 Restriction enzyme-based polymorphisms of MHC loci in native Turkish goats

In order to assess the polymorphisms status of native Turkish goats, MHC Class II-DRB gene region was digested separately with *Pst*I (Figure 4a), *Taq*I (Figure 4b), *BsaH*I (Figure 4c) and *Alu*I (Figure 4d) restriction enzymes.

Two alleles (P and p) leading to three genotypes (PP, Pp and pp) were observed in native Turkish goat populations in terms of MHC Class II-DRB/*Pst*I polymorphism (Table 6). P allele frequency ranged from 0.73 (KBK) to 0.95 (NRD), while p allele frequency was between 0.05 (NRD) and 0.27 (KBK). PP was the most common genotype with ranging from 0.57 (KBK) to 0.90 (NRD), while pp was the least genotype varying between 0.00 (NRD) and 0.10 (HNM and KBK) in studied populations. The

highest and lowest frequency of Pp genotype were detected in KBK (0.33) and NRD (0.10) population, respectively. A significant deviation from HWE was detected in only HNM population. (Table 6).

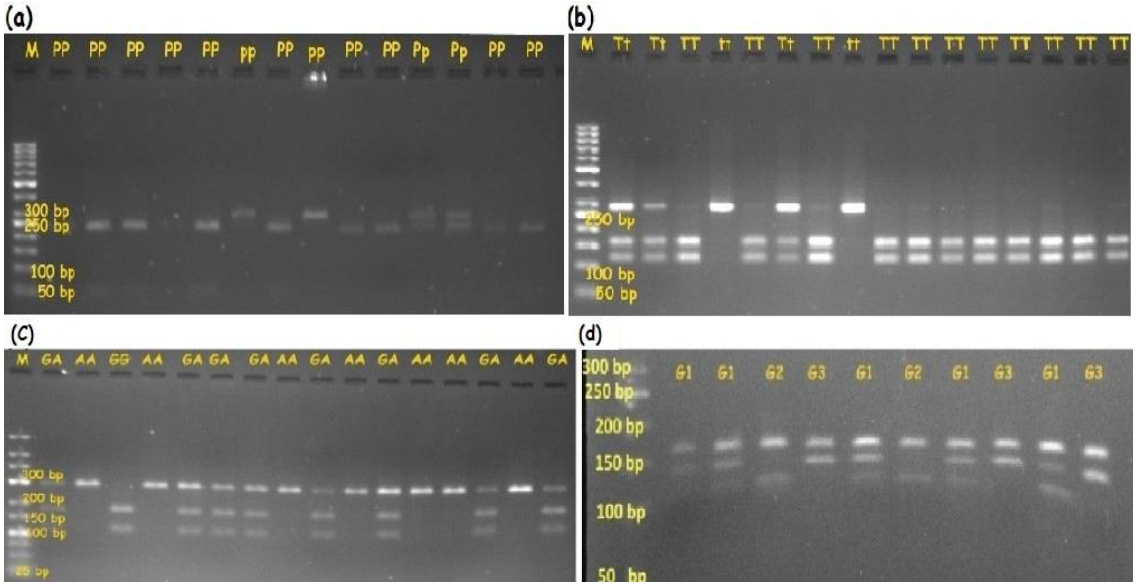


Figure 4- Digestion image of MHC Class II-DRB gene region using *Pst*I (a), *Taq*I (b), *Bsa*HI (c) and *Alu*I (d) restriction enzymes. M: Thermo 50 bp DNA ladder, Cat. No: SM0371

Table 6- Allele and genotype frequencies of *Pst*I, *Taq*I, *BsaH*I and *Alu*I, polymorphisms on MHC Class II-DRB gene region in studied populations

MHC Class II-DRB/PstI								***MHC Class II-DRB/TaqI***							
Breed	**n**	**Allele Frequency**		**Genotype Frequency**			χ^2	**Breed**	**n**	**Allele Frequency**		**Genotype Frequency**			χ^2
		P	**p**	**PP**	**Pp**	**pp**				**T**	**t**	**TT**	**Tt**	**tt**	
HAI	34	0.81	0.19	0.68	0.26	0.06	0.89[a]	**HAI**	39	0.65	0.35	0.36	0.59	0.05	3.58[a]
HNM	31	0.82	0.18	0.74	0.16	0.10	6.20*	**HNM**	30	0.80	0.20	0.67	0.27	0.06	0.83[a]
KBK	30	0.73	0.27	0.57	0.33	0.10	0.65[a]	**KBK**	30	0.49	0.51	0.20	0.57	0.23	0.54[a]
NRD	20	0.95	0.05	0.90	0.10	0.00	0.06[a]	**NRD**	20	0.60	0.40	0.45	0.30	0.25	2.82[a]
MHC Class II-DRB/BsaHI								***MHC Class II-DRB/AluI***							
Breed	**n**	**Allele Frequency**		**Genotype Frequency**			χ^2	**Breed**	**n**	**Allele Frequency**		**Genotype Frequency**			χ^2
		G	**A**	**GG**	**GA**	**AA**				**A_1**	**A_2**	**G1**	**G2**	**G3**	
HAI	40	0.39	0.61	0.00	0.78	0.22	16.01**	**HAI**	40	0.84	0.16	0.75	0.17	0.08	5.10*
HNM	31	0.16	0.84	0.00	0.32	0.68	1.14[a]	**HNM**	30	0.67	0.33	0.50	0.33	0.17	1.88[a]
KBK	30	0.28	0.72	0.03	0.50	0.47	1.60[a]	**KBK**	30	0.73	0.27	0.53	0.40	0.07	0.01[a]
NRD	20	0.30	0.70	0.05	0.50	0.45	0.73[a]	**NRD**	20	0.37	0.63	0.20	0.35	0.45	1.28[a]

$\chi^2_{0.01;1}$:3.84; $\chi^2_{0.05;1}$:6.63; * P<0.05; **P<0.01; a: Non-significant from HWE.

Similarly, presence of three different genotypes were reported in goat breeds raised in India (Jamunapari) (Singh et al. 2012) and Indonesia (Saanen, Etawah Grade and their crossbred) (Petlane et al. 2012) in MHC Class II-DRB/*Pst*I polymorphism. Moreover, Singh et al. (2012) highlighted that animals with pp genotype showed resistance to tuberculosis, while PP and Pp genotypes were commonly found in animals susceptible to tuberculosis. Frequency of pp genotype was reported as 65.04% and 90% in susceptible and resistant group for tuberculosis, respectively (Singh et al. 2012). On the other hand, pp genotype frequency was reported as 0.28, 039, and 0.35 in Saanen, Etawah Grade and their crossbred (Petlane et al. 2012). Frequency of p allele in native Turkish goat populations was lower than that of reported in goat breeds raised in India and Indonesia (Petlane et al. 2012; Singh et al. 2012). It is possible that they have developed resistance to tuberculosis during long history of breeding, since tuberculosis occurs more frequently in ruminants raised in South Asia and Africa (Humblet et al. 2009).

T and t allele together with TT, Tt and tt genotypes were detected by *Taq*I polymorphism in studied populations (Table 6). The highest and lowest frequency of T allele were detected in HNM (0.80) and KBK (0.49), respectively, whereas frequency of t allele was between 0.20 (HNM) and 0.51 (KBK) in studied populations. TT genotype frequency was between 0.20 (HNM) and 0.51 (KBK), while tt genotype frequency ranged from 0.05 (HAI) to 0.25 (NRD). The highest and lowest Tt genotype frequencies were detected in HAI (0.59) and HNM (0.27), respectively. All goat population were in HWE in terms of *Taq*I polymorphism.

Singh et al. (2012) reported that animals with Tt genotype had advantages in terms of resistance to tuberculosis than that of TT and tt genotypes in Jamunapari goat breeds in MHC Class II-DRB/*Taq*I polymorphism. Additionally, frequency of Tt genotype was reported as 0.70 in resistant group in Jamunapari (Singh et al. 2012) and 0.39, 0.15 and 0.17 in Saanen, Etawah Grade and their crossbred (Petlane et al. 2012). The lower frequency of desired genotype (Tt) in terms of tuberculosis was detected in native Turkish goat populations compared to Jumanapari (Singh et al. 2012), while higher Tt genotype frequencies were observed for HAI and KBK than Saanen goat breed (Petlane et al. 2012).

G allele frequency ranged from 0.16 (HNM) to 0.39 (HAI), while the highest and lowest A allele frequency were observed in HNM (0.84) and HAI (0.61), respectively in *BsaH*I polymorphism (Table 6). No GG genotype were detected in HAI and HNM populations, while it was present in KBK (0.03) and NRD (0.05) goats at very low levels. On the contrary, GA and AA genotypes were observed in all studied populations at variable levels ranging from 0.32 (HNM) to 0.78 (HAI) for GA genotype and ranging from 0.22 (HAI) to 0.68 (HNM) for AA genotype. Significant deviation from HWE was observed in only HAI population.

According to MHC Class II-DRB/*BsaH*I polymorphism, similar alleles and genotypes were reported by a previous study conducted on West African Dwarf, Red Sokoto and Shael goat breeds (Yakubu et al. 2017) in which animals with AA genotype had advantages in terms of heat tolerance. Yakubu et al. (2017) reported that AA allele frequencies were 0.45, 0.22 and 0.10 for West African Dwarf, Red Sokoto and Shael goat breeds, respectively. Desired genotype (AA) detected in HNM were significantly higher than the values reported by Yakubu et al. (2017) and the other native Turkish goat populations.

According to *Alu*I polymorphisms, Yakubu et al. (2017) reported three different genotypes such as GG (158, 84 and 42 bp), GC (200, 158, 84 and 42 bp) and CC (200 and 84 bp) in West African Dwarf, Red Sokoto and Shael goat breeds. Benefiting from the same primers, similar PCR fragment (284 bp) was detected in native Turkish goats. On the other hand, by using the same restriction enzyme and RFLP process, restriction fragments in native Turkish goats were different from genotype patterns reported by Yakubu et al. (2017). This variation must be further analysed by sequencing in order to obtain more information on MHC Class II-DRB/*Alu*I polymorphism.

Briefly, previous studies reported that restriction enzyme-based polymorphisms of MHC Class II-DRB gene were associated with tuberculosis resistance/susceptibility and heat tolerance in different goat breeds. Our preliminary results showed that MHC Class II-DRB gene were polymorphic in native Turkish goats. Association analysis between these polymorphisms and tuberculosis and heat tolerance should be investigated to keep sustainability of goat breeding at optimum level in Turkey.

4. Conclusions

In this study genetic diversity and phylogenetic relationships of native Turkish goat breeds were investigated via 5 different MHC-linked microsatellite markers, whereas polymorphism status of MHC Class II-DRB gene were assessed by *Pst*I, *Taq*I, *BsaH*I and *Alu*I restriction enzymes. High level of genetic diversity and low inbreeding were detected based on five MHC-linked microsatellite markers across the studied populations indicating that native Turkish goat breeds have enough genetic diversity for sustainability for the future. On the other hand, the temperature is raising globally, and diseases develop under suitable conditions in livestock species which are believed to negatively affect sustainability of livestock sector in the future. According to previous association studies on different goat breeds, native Turkish goat populations have desired genotypes for heat tolerance and resistance to tuberculosis at variable frequencies. Surprisingly, being a sub-type (variety) of HAI and raised in limited region of Southern Turkey (Elmali, Kas and Fethiye districts), KBK has the desired genotypes for tuberculosis resistance (pp and Tt) with higher frequencies than the other Turkish goat breeds. This result highlighted the importance of conserving genetic diversity not only at breeds but also sub-type level. Still, these genotypes should be investigated in terms of heat tolerance and resistance to tuberculosis and frequency of desired alleles and genotypes should be increased by applying related genes in MAS studies. Additionally, the current status of native Turkish goat breeds for the other environmental challenges and diseases should be monitored to obtain the best selection application. Additionally, a low number of MHC-linked microsatellite loci were found enough to distinguish the studied populations according to their breeding history and origins.

Acknowledgement

This work was supported by The Scientific Research Projects Coordination Unit of Akdeniz University (Project Number: FYL-2018-4301).

Ethical Statement

This research was approved by the Akdeniz University Animal Experiments Ethics Committee, Antalya, Turkey (Protocol No: B.30.2.AKD.0.05.07.00/28).

References

Ağaoğlu Ö K & Ertuğrul O (2012). Assessment of genetic diversity, genetic relationship and bottleneck using microsatellites in some native Turkish goat breeds. Small Ruminant Research 105(1-3): 53-60. https://doi.org/10.1016/j.smallrumres.2011.12.005

Argun Karsli B, Demir E, Fidan H G, & Karsli T (2020). Assessment of genetic diversity and differentiation among four indigenous Turkish sheep breeds using microsatellites. Archives Animal Breeding 63(1): 165-172. https://doi.org/10.5194/aab-63-165-2020

Basiricò L, Morera P, Primi V, Lacetera N, Nardone A & Bernabucci U (2011). Cellular thermotolerance is associated with heat shock protein 70.1 genetic polymorphisms in Holstein lactating cows. Cell Stress Chaperone 16(4): 441-448. https://doi.org/10.1007/s12192-011-0257-7

Belkhir K, Borsa P, Goudet J & Bonhomme F (2004). GENETIX 4.05, logiciel sous Windows pour la ge´ne´tique des populations. Universite´ de Montpellier II, Montpellier, France

Bulut Z, Kurar E, Ozsensoy Y, Altunok V & Nizamlioglu M (2016). Genetic diversity of eight domestic goat populations raised in Turkey. BioMed Research International 2016: 2830394. https://doi.org/10.1155/2016/2830394

Cecchi F, Russo C, Iamartino D, Galiero A, Turchi B, Fratini F, Degl'Innocenti S, Mazza R, Biffani S, Preziuso G & Cantile C (2017). Identification of candidate genes for paratuberculosis resistance in the native Italian Garfagnina goat breed. Tropical Animal Health and Production 49(6): 1135-1142. https://doi.org/10.1007/s11250-017-1306-8

Ceccobelli S, Lasagna E, Demir E, Rovelli G, Albertini E, Veronesi F, Sarti F M & Roselini D (2020). Molecular Identification of the "Facciuta Della Valnerina" Local Goat Population Reared in the Umbria Region, Italy. Animals 10: 601. https://doi.org/10.3390/ani10040601

Demir E & Balcioglu M S (2019). Genetic diversity and population structure of four cattle breeds raised in Turkey using microsatellite markers. *Czech Journal of Animal Science* 64(10): 411-419. https://doi.org/10.17221/62/2019-CJAS

Earl D A & vonHoldt B M (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4(2): 359-361. https://doi.org/10.1007/s12686-011-9548-7

Erduran H & Kırbas M (2010). Hair goat breeding and improvement studies in Konya province. In: Proceedings of the National Goat Congress, 24-26 June, Çanakkale, pp. 193-197

Eren M G, Balcioğlu M S & Demir E (2019). Detection of complex vertebral malformation in Holstein cattle reared in Antalya using allele-spesific PCR. Mediterranean Agricultural Sciences 32(3): 443-446 https://doi.org/10.29136/mediterranean.578015

FAO (2011). Molecular Genetic Characterization of Animal Genetic Resources. FAO Animal Production and Health Guidelines 9. Retrieved in August, 25, 2021 from http://www.fao.org/3/i2413e/i2413e00.htm

Glaubitz J C (2004). CONVERT: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. Molecular Ecology Notes 4(2): 309–310. https://doi.org/10.1111/j.1471-8286.2004.00597.x

Goudet J (1995). FSTAT (Version 1.2): A computer program to calculate F-Statistics. *Journal of Hereditary* 86(6): 485-486

Gowane G R, Akram N, Misra S S, Prakash V & Kumar A (2018). Genetic diversity of Cahi DRB and DQB genes of caprine MHC class II in Sirohi goat. *Journal of Genetics* 97(2): 483-492 https://doi.org/10.1007/s12041-018-0939-3

Guang-Xin E, Yong-Fu H, Yong-Ju Z, Yue-Hui M, Ri-Su N, Jia-Hua Z, Hui-Jiang G & Xin W (2015). Genetic variability of ten Chinese indigenous goats using MHC-linked microsatellite markers. Veterinary Immunology and Immunopathology 167(3-4): 196–199. https://doi.org/10.1016/j.vetimm.2015.07.013

Gül S, Yilmaz O, Gündüz Z, Keskin M, Cemal I, Ata N & Önel S E (2020). The genetic structure of the goat breeds belonging to Northwest part of Fertile Crescent. Small Ruminant Research 182: 22-28. https://doi.org/10.1016/j.smallrumres.2019.09.009

Humblet M F, Boschiroli M L & Saegerman C (2009) Classification of worldwide bovine tuberculosis risk factors in cattle: a stratified approach. Veterinary Research 40(5): 1-24 https://doi.org/10.1051/vetres/2009033

Kalinowski S T & Taper M L (2006): Maximum likelihood estimation of the frequency of null alleles at microsatellite loci. Conservation Genetics 7(6): 991-995. https://doi.org/10.1007/s10592-006-9134-9

Kannaki T R, Reddy M R, Raja Ravindra K S & Chatterjee R N (2017). Genetic diversity analysis of the major histocompatibility complex region in Indian native chicken breeds and pureline chickens using the LEI0258 microsatellite marker. *Indian Journal of Animal Research* 51(6): 998-1001

Karsli T, Demir E, Fidan H G, Aslan M, Argun Karsli B, Arik İ Z, Sahin Semerci E, Karabag K & Balcioglu M S (2020). Determination of genetic variability, population structure and genetic differentiation of indigenous Turkish goat breeds based on SSR loci. Small Ruminant Research 190: 106147 https://doi.org/10.1016/j.smallrumres.2020.106147

Kim E S, Sonstegard T S, da Silva M V G B, Gasbarre L C & Tassell C P V (2015). Genome-wide scan of gastrointestinal nematode resistance in closed Angus population selected for minimized influence of MHC. PLoS One 10(3): e0119380 https://doi.org/10.1371/journal.pone.0119380

Kirk K, Askın Y & Cengiz F (2004). Reproductive performance evaluation of artificial inseminated Norduz ewes using fresh semen. In: Proceedings of the IV National Animal Science Congress, 1-3 September, Isparta, pp. 272-279

Kopelman N M, Mayzel J, Jakobsson M, Rosenberg N A & Mayrose I (2015): CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. Molecular Ecology Resources 15(5): 1179-1191. https://doi.org/10.1111/1755-0998.12387

Liu Y, Li D, Li H, Zhou X & Wang G (2011). A novel SNP of the ATP1A1 gene is associated with heat tolerance traits in dairy cows. Molecular Biology Reports 38(1): 83-88. https://doi.org/10.1007/s11033-010-0080-8

Miller S A, Dykes D D & Polesky H F R N (1988). A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research 16(3): 1215

Nei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89(3): 583-590. https://doi.org/10.1093/genetics/89.3.583

Petlane M, Noor R R & Maheswari R R A (2012). The Genetic diversity of TLR4 MHC-DRB genes in dairy goats using PCR-RFLP technique. Media Peternakan 35(2): 91-95. https://doi.org/10.5398/medpet.2012.35.2.91

Pisoni G, Moroni P, Genini S, Stella A, Boettcher P J, Cremonesi P, Scaccabarozzi L, Giuffra E & Castiglioni B (2010). Differentially expressed genes associated with Staphylococcus mastitis in dairy goats. Veterinary Immunology and Immunopathology 135(3-4): 208-217. https://doi.org/10.1016/j.vetimm.2009.11.016

Pritchard J K, Stephens M & Donnelly P (2000). Inference of population structure using multilocus genotype data. Genetics 155(2): 945-95. https://doi.org/10.1093/genetics/155.2.945

Radostits O M, Gay C C, Hinchcliff K W & Constable P D (2007). A textbook of the diseases of cattle, horses, sheep, pigs and goats. Veterinary Medicine 10: 2045-2050

Rovelli G, Ceccobelli S, Perini F, Demir E, Mastrangelo S, Conte G, Abeni F, Marletta D, Ciampolini R, Cassandro M, Bernabucci U & Lasagna E (2020). The genetics of phenotypic plasticity in livestock in the era of climate change: A review. Italian *Journal of Animal Science*, 19(1): 997-1014. https://doi.org/10.1080/1828051X.2020.1809540

Salles P A, Santos S C, Rondina D & Weller M (2011). Genetic variability of six indigenous goat breeds using major histocompatibility complex-associated microsatellite markers. *Journal of Veterinary Science* 12(2): 127-132. https://doi.org/10.4142/jvs.2011.12.2.127

Shen H, Han G, Jia B, Jiang S & Du Y (2014). MHC-DRB1/DQB1 gene polymorphism and its association with resistance/susceptibility to cystic Echinococcosis in Chinese merino sheep. *Journal of Parasitology Research* 2014: 272601. https://doi.org/10.1155/2014/272601

Singh P K, Singh S V, Singh M K, Saxena V K, Horin P, Singh A V & Sohal J S (2012). Effect of genetic variation in the MHC Class II DRB region on resistance and susceptibility to Johne's disease in endangered Indian Jamunapari goats. *International Journal of Immunogenetics* 39(4): 314-320 https://doi.org/10.1111/j.1744-313X.2012.01092.x

Tefiel H, Ata N, Fantazi K, Yilmaz O, Cemal I, Karaca O, Chahbar M, Ameur Ameur A, & Gaouar S B S (2020). Microsatellite based genetic diversity in indigenous goat breeds reared in Algeria and Turkey. *Journal of Animal and Plant Sciences* 30(5): 1115-1122. https://doi.org/10.36899/JAPS.2020.5.0127

Vaccari G, Di Bari M A, Morelli L, Nonno R, Chiappini B, Antonucci G, Marcon S, Esposito E, Fazzi P, Palazzini N, Troiano P, Petrella A, Di Guardiola G & Agrimi U (2006). Identification of an allelic variant of the goat PrP gene associated with resistance to scrapie. *Journal of General Virology* 87(5): 1395-1402. https://doi.org/10.1099/vir.0.81485-0

Yakubu A, Salako A E, De Donato M, Peters S O, Takeet M I, Wheto M, Okpeku M & Imumorin I G (2017). Association of SNP variants of MHC Class II DRB gene with thermo-physiological traits in tropical goats. Tropical Animal Health Production 49(2): 323–336. https://doi.org/10.1007/s11250-016-1196-1

Yeh F C, Yang R C, Boyle T B J, Ye Z H & Mao J X (1997). POPGENE, The user-friendly shareware for population genetic analysis, Molecular Biology and Biotechnology Centre, University of Alberta, Canada.

Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

A Novel Machine Learning Approach: Soil Temperature Ordinal Classification (STOC)

Cansel KUCUK[a], Derya BIRANT[b], Pelin YILDIRIM TASER[c*]

[a]*Dokuz Eylul University, Graduate School of Natural and Applied Sciences, Izmir, TURKEY*
[b]*Dokuz Eylul University, Department of Computer Engineering, Izmir, TURKEY*
[c]*Izmir Bakircay University, Department of Computer Engineering, Izmir, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Pelin YILDIRIM TASER, E-mail: pelin.taser@bakircay.edu.tr
Received: 21 January 2020; Revised: 17 November 2021, Accepted: 17 November 2021 / Online: 15 October 2022

Cite this article
KUCUK C, BIRANT D, TASER YILDIRIM P (2022). A Novel Machine Learning Approach: Soil Temperature Ordinal Classification (STOC). *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 28(4):635-649. DOI: 10.15832/ankutbd.866045

ABSTRACT
Soil temperature prediction is an important task since soil temperature plays an important role in agriculture and land use. Although some progress has been made in this area, the existing methods provide a regression or nominal classification task. However, ordinal classification is yet to be explored. To bridge the gap, this paper proposes a novel approach: *Soil Temperature Ordinal Classification* (STOC), which considers the relationships between the class labels during soil temperature level prediction. To demonstrate the effectiveness of the proposed approach, the STOC method using five different traditional machine learning methods (Decision Tree, Naive Bayes, K-Nearest Neighbors, Support Vector Machines, and Random Forest) was applied on daily values of meteorological and soil data obtained from 16 stations in three states (Utah, Alabama, and New Mexico) of United States at five soil depths (2, 4, 8, 20, and 40 inches) between the years of 2011 and 2020. The experiments show that the proposed STOC approach is an efficient method for soil temperature level (very low, low, medium, high, and very high) prediction. The applied STOC models (STOC.DT, STOC.NB, STOC.KNN, STOC.SVM, and STOC.RF) showed average accuracy rates of 90.95%, 77.09%, 90.84%, 89.94%, and 90.91% on the experimental datasets, respectively. It was observed from the experimental results that the STOC.DT method achieved the best soil temperature level prediction among the others.

Keywords: Agriculture, Classification, Decision tree, Machine learning, Random forest, Soil temperature level

1. Introduction

Soil temperature greatly influences plant growth and development, soil water and salt transport, soil carbon balance, and microbial activity and chemical reactions inside the soil (Onwuka & Mang 2018). Biochemical processes, such as germination of a seed, seedling emergence, uptake operation by roots, root growth, are realized under the suitable soil temperature. The high temperature in the soil can lead to a dramatic reduction of soil resistance to physical events such as erosion and subsidence. On the other hand, some biochemical activities can stop when the temperature drops dramatically and the soil freezes. In addition, a significant decrease in temperature changes the rate of organic matter decomposition and mineralization inside the soil. If the soil temperature is suitable, chemical activities in the soil continue smoothly and regularly. Soil temperature prediction is of great importance for this purpose. If further changes in soil temperature are predicted, new strategies can be developed to prevent undesired situations.

Machine learning has recently received much attention in the soil temperature prediction field (Abyaneh et al. 2016; Li et al. 2020a; Sattari et al. 2020). It discovers significant underlying patterns in raw data by constructing a model without any human intervention. The most widely studied machine learning technique is classification. Classification is the task of categorizing an input sample data into one of the predefined classes. In the literature, there are several soil temperature prediction studies that apply different machine learning techniques, such as regression (Abyaneh et al. 2016; Alizamir et al. 2020a; Alizamir et al. 2020b; Li et al. 2020b; Sattari et al. 2020) and time series (Mehdizadeh et al. 2020; Zeynoddin et al. 2020). For example, (Alizamir et al. 2020b) proposed a deep echo state network (Deep ESN) regression model for soil temperature prediction at 10 and 20 cm depths. In another study, a new time-series model, called fractionally autoregressive integrated moving average (FARIMA), was implemented for daily soil temperature prediction at four depths (5, 10, 50, and 100 cm) (Mehdizadeh et al. 2020).

In the previous machine learning-based soil temperature prediction studies, it is assumed that there is no order between the class labels of the dataset which will be predicted. However, the class attributes of some soil data have an inherent order. Due to this situation, this study proposes the ordinal classification technique for the first time, which considers natural order between class labels to solve the problem of soil temperature level prediction.

Ordinal classification is a special type of classification which assumes the class values of the attributes in the dataset are ordered and finite (Frank & Hall 2001). The ordinal classification aims to predict the target attribute of a given new sample by considering ranking relationships between the classes. For example, the soil temperature values can be categorized as "very low", "low", "medium", "high", and "very high". The order of these values is stated as "very low" < "low" < "medium" < "high" < "very high". The prediction performance of the machine learning models can be improved regarding the ranking relationship between the class labels of the used experimental dataset. Considering this motivation, a novel ordinal classification method, named STOC, was applied to real-world soil temperature data at five different soil depths (2, 4, 8, 20, and 40 inches) in this study.

Due to the nature of the problem of soil temperature level prediction, datasets are typically ordinal. Disregarding the orders among class labels, the problem is basically treated as a multi-class classification task, and it often results in a loss of performance. A possible way out is to develop a novel approach that makes use of order relations for samples during the classification task. Exploring such ranking information can usually help to increase the effectiveness of predictive classifiers. In this study, the order information was incorporated into the classification method, thus exploiting the implicit and relative knowledge simultaneously. More specifically, this study brings together notions from the fields of agriculture and machine learning for information fusion.

Soil temperature has a great impact on the growth and development of all types of plants. Because of this reason, soil temperature prediction plays an important role in the agriculture field. The machine learning-based soil temperature prediction studies in the literature generally apply regression (Kisi et al. 2015; Abyaneh et al. 2016; Citakoglu 2017; Mehdizadeh et al. 2018; Sanikhani et al. 2018; Xing et al. 2018; Delbari et al. 2019; Feng et al. 2019; Alizamir et al. 2020a; Alizamir et al. 2020b; Hao et al. 2020; Li et al. 2020a; Li et al. 2020b; Penghui et al. 2020; Sattari et al. 2020; Shamshirband et al. 2020; Tsai et al. 2020; Abimbola et al. 2021; Bayatvarkeshi et al. 2021; Wang et al. 2021) or time series (Bonakdari et al. 2019; Li et al. 2020c; Mehdizadeh et al. 2020; Nanda et al. 2020; Zeynoddin et al. 2020) methods.

In the regression studies, DT (Sanikhani et al. 2018; Sattari et al. 2020), Support Vector Regression (SVR) (Mehdizadeh et al. 2018; Xing et al. 2018; Delbari et al. 2019; Li et al. 2020a; Li et al. 2020b; Shamshirband et al. 2020), RF (Feng et al. 2019; Alizamir et al. 2020b; Tsai et al. 2020), NN (Kisi et al. 2015; Abyaneh et al. 2016; Citakoglu 2017; Hao et al. 2020; Penghui et al. 2020; Abimbola et al. 2021; Bayatvarkeshi et al. 2021; Wang et al. 2021), ELM (Alizamir et al. 2020a) algorithms have been preferred for predicting soil temperatures. In addition, some of the time-series studies have also applied NN (Bonakdari et al. 2019; Li et al. 2020c), ELM (Mehdizadeh et al. 2020; Zeynoddin et al. 2020), SVR (Nanda et al. 2020) algorithms for the prediction performance comparison.

Most of the soil temperature prediction studies are performed at multiple depths (Kisi et al. 2015; Sanikhani et al. 2018; Shamshirband et al. 2020). Sanikhani et al. (2018) applied extreme learning machine (ELM), neural network (NN), and M5 Model Tree (M5 Tree) models on the meteorological data obtained from two stations in Turkey for predicting soil temperatures at 5, 50, and 100 cm depths. The studies also estimate the features that affect the soil temperature prediction (Feng et al. 2019; Nanda et al. 2020). Nanda et al. (2020) discovered that while rainfall data does not affect the prediction performance, the soil moisture parameter improves the accuracy of the prediction problem.

The present studies assumed that there is no order between the target attribute of the soil dataset, which will be predicted. However, the target attribute values have a natural order, such as very high, high, medium, low, very low, and it can affect the prediction performance of the soil temperature level. Because of this reason, the STOC approach, which takes into account the inherent order of class labels, is proposed to classify the soil temperature levels of the experimental dataset.

Table 1 shows the comparison of this study with the existing studies. This method differs from the existing methods in three respects. First, they performed the prediction of numeric target values, while this paper focuses on the classification of ordinal and categorical target values. Second, this study uses different methods such as KNN and NB. Third, in this study, the performance was tested with some metrics different from those used by aforementioned studies.

Table 1- Comparison of this study with the existing studies.

Reference	*Year*	*Algorithm*							*Task*			*MD*	*Country*
		DT	*SVR*	*NB*	*KNN*	*RF*	*NN*	*ELM*	*R*	*T*	*O*		
Abimbola et al. 2021	2021						√		√			√	USA
Bayatvarkeshi et al. 2021	2021						√		√			√	Iran
Wang et al. 2021	2021						√		√			√	Switzerland
Alizamir et al. 2020a	2020	√					√	√	√			√	Turkey
Alizamir et al. 2020b	2020	√				√	√		√			√	USA
Hao et al. 2020	2020						√		√			√	Switzerland
Sattari et al. 2020	2020	√							√			√	Turkey
Li et al. 2020a	2020		√			√	√		√				USA
Li et al. 2020b	2020		√				√	√	√				China
Li et al. 2020c	2020						√			√		√	Switzerland
Zeynoddin et al. 2020	2020							√		√		√	Iran
Mehdizadeh et al. 2020	2020						√			√		√	Iran
Nanda et al. 2020	2020		√			√	√			√			India
Shamshirband et al. 2020	2020		√				√		√			√	Iran
Penghui et al. 2020	2020						√		√				USA
Tsai et al. 2020	2020					√			√				Taiwan
Bonakdari et al. 2019	2019						√			√		√	USA
Delbari et al. 2019	2019		√				√		√			√	Iran
Feng et al. 2019	2019					√	√	√	√				China
Mehdizadeh et al. 2018	2018		√						√			√	Iran
Sanikhani et al. 2018	2018	√					√	√	√			√	Turkey
Xing et al. 2018	2018		√						√			√	USA
Citakoglu 2017	2017						√		√			√	Turkey
Abyaneh et al. 2016	2016						√		√			√	Iran
Kisi et al. 2015	2015						√		√			√	Turkey
Proposed approach		√	√	√	√	√	√				√	√	USA

(R=Regression, T=Time series, O=Ordinal Classification, MD=Multiple-Depth)

The novelty and main contributions of this work are as follows. (i) It is the first attempt to apply ordinal classification for soil temperature level prediction. (ii) This paper proposes a novel method, called STOC, which takes into account the relationships between the class labels during soil temperature level prediction. (iii) This study is also original in that it compares alternative base learners in conjunction with the proposed method, including decision tree (DT), Naive Bayes (NB), k-nearest neighbors (KNN), support vector machines (SVM), and random forest (RF). (iv) This is the first study using ordinal classification to predict soil temperature levels at five different soil depths. The main motivation of this study is to improve the performance of the soil temperature level prediction of the models by considering the ranking among the class labels.

2. Material and Methods

2.1. Dataset description

In this study, 16 different experimental datasets which contain daily values of meteorological and soil data obtained from 16 stations in three states (Utah, Alabama, and New Mexico) of United States at five soil depths at the interval of 01.01.2011 and 31.05.2020. Figure 1 presents the map of the stations in each state of the United States.

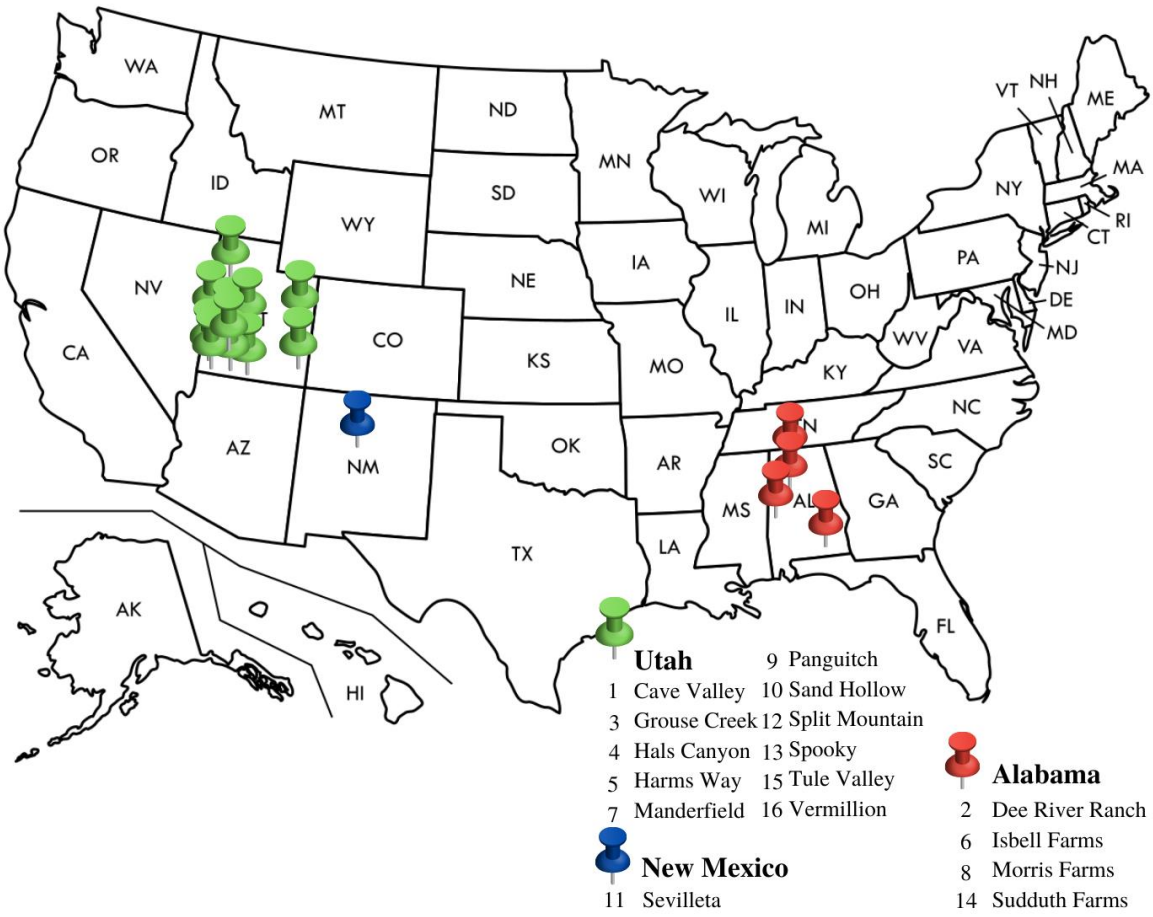


Figure 1- The map of the stations used in this study

Each data set in this study includes meteorological parameters such as air temperature, precipitation, relative humidity, solar radiation, wind speed, and vapor pressure, and soil parameters such as moisture and temperature. These real-world and publicly available datasets were gathered from the website (https://www.wcc.nrcs.usda.gov) of the National Water and Climate Center (NWCC) in the National Resources Conservation Service (NRCS) under the United States Department of Agriculture. Table 2 presents the details of meteorological and soil parameters for each dataset.

Table 2- Parameters of the datasets

Type	*Parameters*	*Unit*
Meteorological Parameters	Air Temperature Maximum	degF
	Air Temperature Minimum	degF
	Precipitation Increment	in
	Relative Humidity	pct
	Solar Radiation Average	watt/m^2
	Solar Radiation/Langley Total	langley
	Wind Speed Maximum	mph
	Wind Speed Average	mph
	Vapor Pressure – Partial	in Hg
	Vapor Pressure – Saturated	in Hg
Soil Parameters	Soil Moisture Percent -2in	pct
	Soil Moisture Percent -4in	pct
	Soil Moisture Percent -8in	pct
	Soil Moisture Percent -20in	pct
	Soil Moisture Percent -40in	pct
	Soil Temperature Observed -2in	degF
	Soil Temperature Observed -4in	degF
	Soil Temperature Observed -8in	degF
	Soil Temperature Observed -20in	degF
	Soil Temperature Observed -40in	degF

Figures 2 and 3 present the annual and seasonal variations of maximum (max) and minimum (min) soil temperature values by soil depth between 2011 and 2019, respectively. The data for 2020 are not included because they only contain soil temperature values for the first five months of the year. In Figure 2, it is clearly seen that as the soil depth increases, the minimum soil temperature increases, while the maximum soil temperature decreases for each year. Figure 3 reveals that when the air temperatures rise, especially in the spring and summer seasons, an increase in soil temperature values is also observed. In other seasons, the soil temperature decreases in direct proportion to the air temperature.

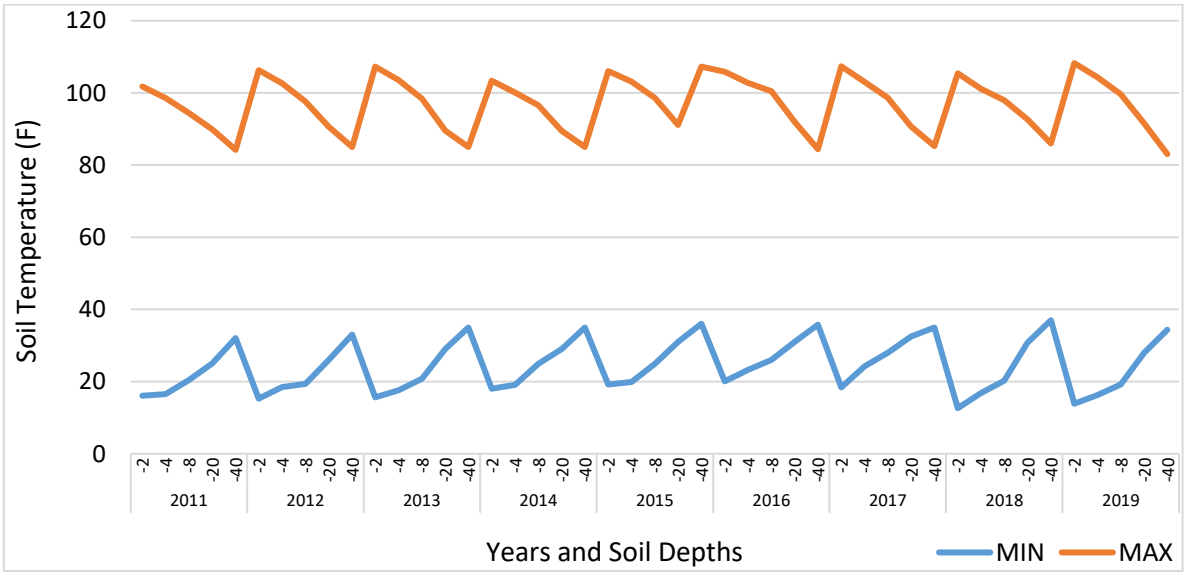


Figure 2- The annual variations of max and min soil temperature values by soil depth

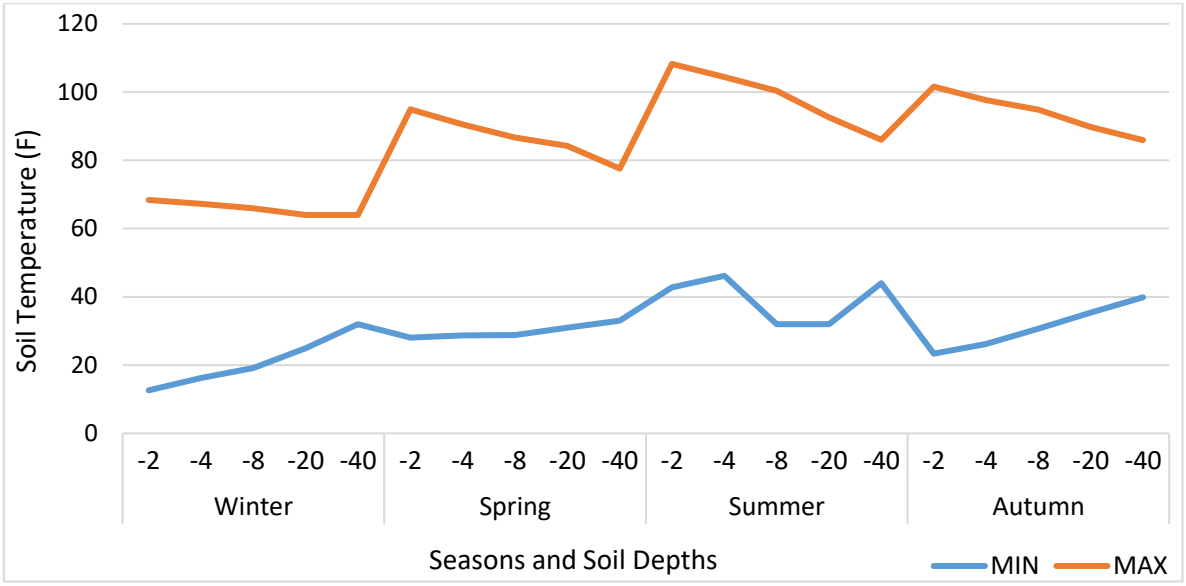


Figure 3- The seasonal variations of max and min soil temperature values by soil depth

The datasets considered in this study have passed through data preprocessing steps using the Python Scikit-learn library for the implementation of the STOC method. First, "date" and "station ID" parameters were extracted from each dataset because they are not correlated with the target parameter (soil temperature observed). Then, the hourly collected data were converted into daily data and the instances which have no soil temperature value were eliminated. Furthermore, the proposed STOC method cannot handle continuous target parameters, so, the first, soil temperature observed parameter for each soil depths in Fahrenheit degree were converted to Celsius degree, and then they were discretized into five different levels such as "very low", "low", "medium", "high", and "very high" according to specific value ranges as shown in Table 3.

Table 3- Discretization of soil temperature observed parameter

Continuous Values (Celsius)	*Continuous Values (Fahrenheit)*	*Categorical Values*
[..., 5]	[..., 41]	Very low
(5, 10]	(41, 50]	Low
(10, 15]	(50, 59]	Medium
(15, 20]	(59, 68]	High
(20, …]	(68, …]	Very high

2.2. Proposed approach

In this study, the authors propose a novel approach: STOC. This approach classifies the soil temperature levels of meteorological and soil data at different soil depths by considering the natural order of class labels. In this approach, the ordinal class classifier algorithm (Frank & Hall 2001), which converts an ordinal classification problem including multiple class labels to a binary classification problem was preferred. According to this algorithm, k different ordinal class labels are converted to binary values considering their inherent order. For example, consider that there are five different class labels (C_1, C_2, C_3, C_4, and C_5) and the order between the classes is as follows: $C_5 > C_4 > C_3 > C_2 > C_1$. In the first phase, while the class values that are higher than C_1 (C_2, C_3, C_4, and C_5) are labeled as 1, the rest of them (C_1) are labeled as 0. Then, the same processes are applied to all class labels, so the ordinal classification approach is transformed into a binary classification problem. Besides, five different traditional classification algorithms (DT, NB, KNN, SVM, and RF) were chosen as base learners for the ordinal class classifier algorithm in the STOC method.

Figure 4 shows a general overview of the proposed approach. First, in the data acquisition step, meteorological (air temperature, air pressure, precipitation, relative humidity, and solar radiation) and soil (soil temperature and soil moisture) data is obtained from the sensors (i.e. solar radiation sensor, wind speed, and direction sensor, etc.) of the Soil Climate Analysis Network (SCAN) in 16 stations of United States. These data are stored in a cloud platform. In the next step, first, the datasets are passed through a data preprocessing step (discretization and missing data elimination) and then they are converted to binary datasets to make them ready for the implementation of the STOC method. In the training phase, the STOC approach is applied to the meteorological and soil data using DT, NB, KNN, SVM, and RF as base learners. After that, the performance of the soil temperature level prediction of these models are evaluated using the n-fold cross-validation technique selecting n as 10. Then,

in the prediction phase, a new sample is predicted using the STOC method. Finally, the obtained accuracy rate and F-measure results from these models are represented in the presentation step.

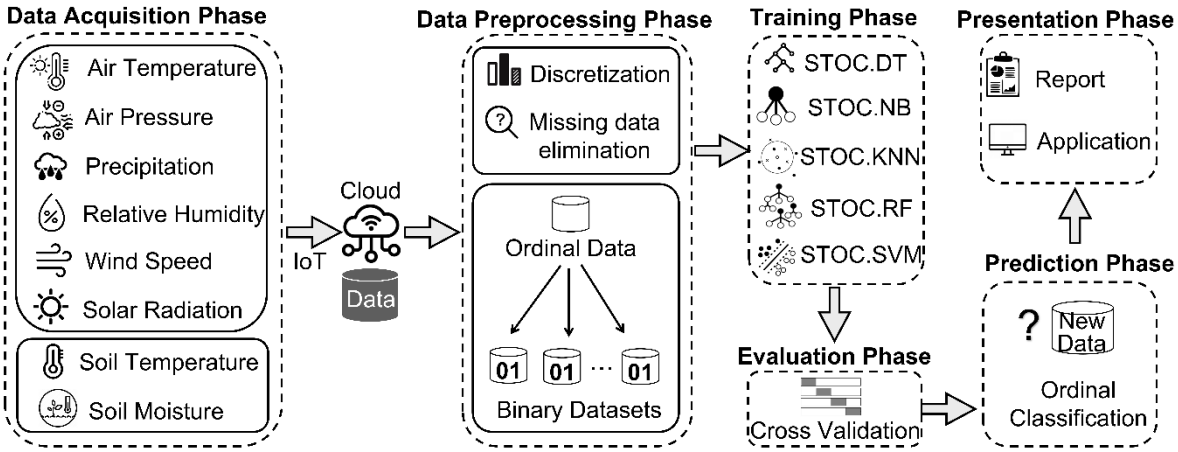


Figure 4- The general overview of the proposed STOC approach

In addition to the general overview of the proposed STOC approach, the flowchart of it is also presented in Figure 5. In the training phase, the original raw data passes through data preprocessing steps and then the ordinal values are converted to binary values using the binary decomposition method. After that, the experimental dataset is divided into training and test data. The selected ordinal classification algorithm is applied to the training data to construct the prediction model. The constructed model is then evaluated using the test data in terms of an accuracy metric. Finally, in the prediction phase, the class label of new unseen data is predicted using the constructed classification model.

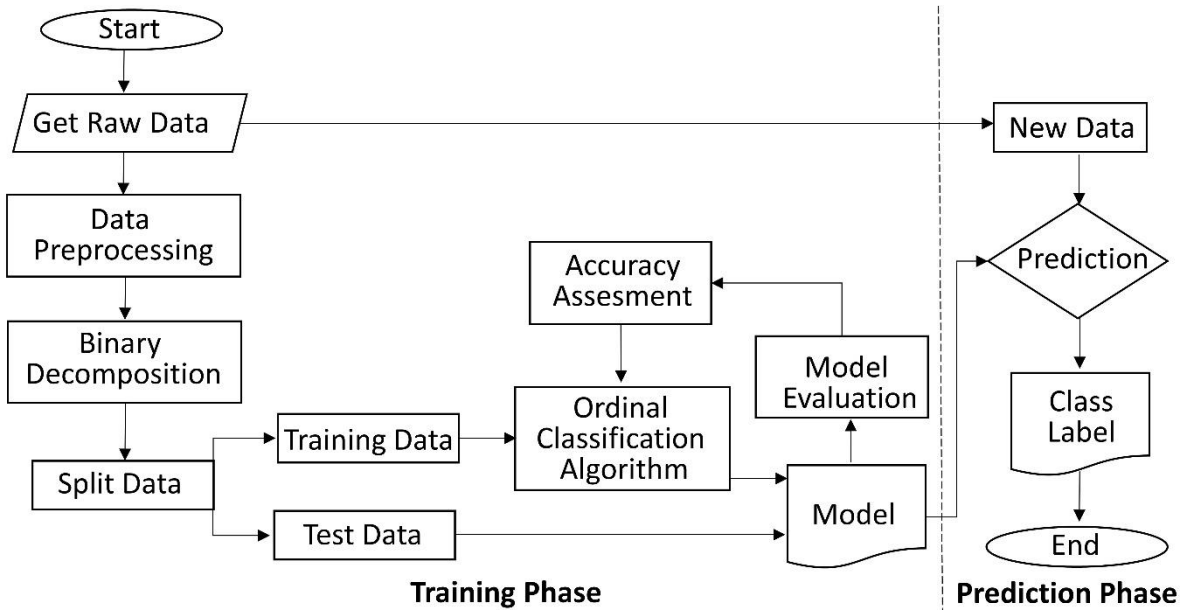


Figure 5- The flowchart of the proposed STOC approach

A tangible example of the proposed STOC approach and its differences from the traditional approach is illustrated in Figure 6. Assume that each instance in the example dataset, including daily values of meteorological and soil data, has an ordinal class attribute with five values: very low, low, medium, high, and very high. These values are on an ordinal scale, such as low < very low < medium < high < very high. In the proposed approach, first, these values are converted to binary values considering their ordinal scales. For example, the samples whose class values are higher than "very low" are labeled as 1 and the others are labeled as 0. Thus, the first dataset (D1) is obtained. In this manner, four different transformed datasets (D1, D2, D3, and D4) are constructed. Then, the base classifiers of the STOC approach (DT, NB, KNN, SVM, and RF) are applied to these datasets and multiple models are generated. The proposed application was developed by using the WEKA machine learning library (Witten et al. 2016) in the Java programming language. In the prediction step, the class label of the new data is predicted using these models by choosing the class with the highest probability. However, in the traditional approaches, the orders among class labels are disregarded. Thus, the classification algorithms are directly applied to the original dataset without any conversion process

and the model is constructed. Finally, similar to the STOC approach, new data is classified with one of the predefined classes using the model.

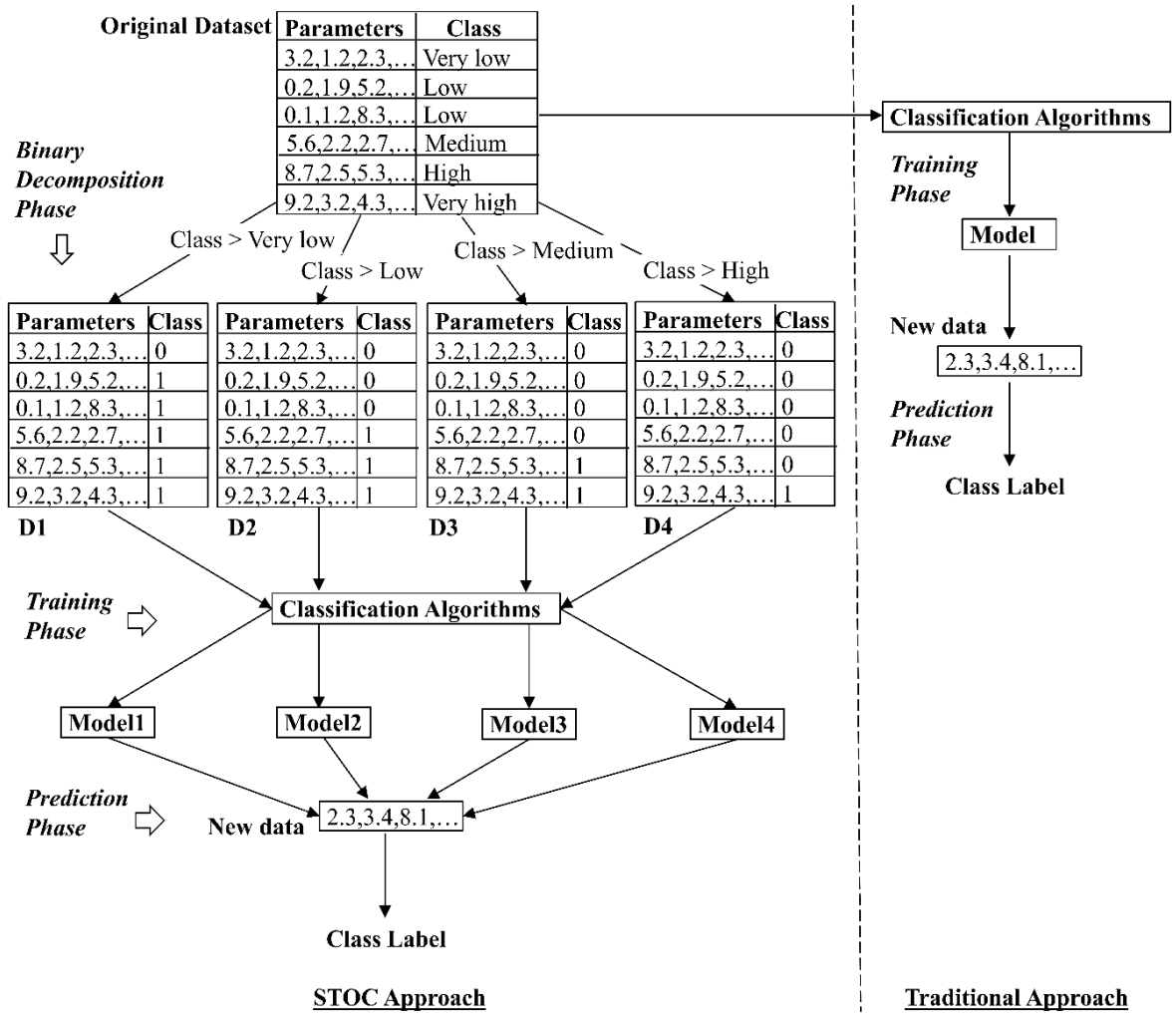


Figure 6- The comparison of the proposed STOC and traditional approaches

The main benefits of the proposed STOC approach can be summarized as follows:

- Using the meteorological data, future soil temperature levels for a region can be predicted.
- An intelligent soil management system can be designed using the predicted values.
- The crop yield in agriculture can be increased by using the predicted soil temperature levels.
- The proposed approach can play a role in reducing unnecessary resource consumption in agriculture, such as water, sensor, and pesticide.

2.3. Formal definition of the proposed method

Definition 1. STOC refers to the problem that uses ordinal data obtained from sensors and aims to construct a prediction model by considering the orders among class labels to correctly predict the level of the soil temperature according to the methodological data.

Let D be an ordinal meteorological and soil data $D=\{(x_i, y_i)_{i=1}^{N} \epsilon\ X\ x\ Y\}$, where $X \epsilon R^d$ and its related class label y_i belongs to the output space $Y=\{C_1, C_2, C_3, \ldots, C_k\}$ having k classes with a data ranking structure $C_1 < C_2 < C_3 < \ldots < C_k$, where $<$ denotes the linear order relation. In this method, five different traditional classification algorithms (DT, NB, KNN, SVM, and RF) were implemented as base learners. The aim of classification is to categorize an input vector x with one of the k class labels by selecting the maximum probability value among the evaluated probabilities for each label using a binary classification method. In this approach, the probabilities for each k class are computed as follows:

$$P(C_1|\,x) = 1 - P\,(Class > C_1|\,x) \quad (1)$$

$$P(C_j|\,x) = P(Class > C_{j-1}|x) - P\,(Class > C_j|\,x)$$
$$P(C_k|\,x) = P(Class > C_{k-1}|x)$$

Where; j={2,3,….,k}.

2.4. Proposed algorithm

Algorithm 1 presents the pseudocode of the STOC method. In the first step of this algorithm, the original dataset is passed through a binary decomposition process for converting ordinal data D to binary datasets $\{D_i\}_{i=1}^{k-1}$ to encode the ranking relationship among the classes. Here, the label y_j associated with the sample x_j is replaced with $y_j = 1$ for all $y_j > C_i$, or, $y_j = 0$ for all $y_j <= C_i$. In other words, for a particular class C_i, the values lower than or equal to C_i are labeled as 0 and the others are labeled as 1. In this way, the algorithm converts an ordinal classification problem involving k classes into k-1 binary classification problems. The dataset D^i is created for each $i \in \{1, 2, ..., k\text{-}1\}$ by using classes $\{C_1, C_2, ..., C_i\}$ against $\{C_{i+1}, C_{i+2}, ..., C_k\}$. After that, a model M_i is constructed for each binary dataset D_i using one of the classification algorithms (DT, NB, KNN, SVM, and RF) as a base learner. In other words, the first model M_1 is constructed to predict what is the probability for a given instance belonging to any class which is located higher than C_1, the second model M_2 is built to predict the probability of belonging to any class that is ordered higher than C_2, and so on. In the final step, the class label of each instance x in the test set T is predicted by choosing the maximum probability value among the evaluated probabilities for each class label using the constructed classification models M*. In this process, the probability of the first class $P(C_1)$ is computed by subtracting the probability of the upward union of classes $P(L_x > C_1)$ from 1. The probability of the last class $P(C_k)$ is computed by directly considering the probability of the upward union of classes $P(L_x\ C_{k-1})$. In other cases (for the intermediate classes, where $2 \le j \le k$-1), the probabilities are computed by considering the probabilities of both upward and downward unions of classes. In the end, the class label with the highest probability (MAX) is predicted as the final class y for the test query x.

Algorithm 1: STOC

Inputs:
D: ordinal dataset $D=\{(x_1,y_1), (x_2,y_2), ..., (x_l,y_l)\}$ with n instances
T: test set to be predicted
Y: ordinal class labels $y \in \{C_1,C_2, ...,C_k\}$ with an order $C_1 < C_2 < ... < C_k$
k: the number of classes
Output:
M:* Ordinal classifiers
$\hat{\boldsymbol{Y}}$ *:* Predicted class labels
Begin:
// Step 1 – Binary decomposition
for i = 1 **to** *k-1* **do**
foreach (x_j,y_j) **in** D
if $(y_j <= C_i)$
D_i.Add(x_j,0)
else
D_i.Add(x_j,1)
end if
end foreach
end for
// Step 2 – Model construction
for i = 1 **to** *k-1* **do**
M_i=Train(D_i)
$M^* = M^* \cup M_i$
end for
// Step 3 – Classification
foreach x **in** T
$y = M^*(x)$ = MAX (
$P(C_1) = 1 - P(L_x > C_1)$
for j = 2 **to** *k-1* **do**
$P(C_j) = P(L_x > C_{j-1}) - P(L_x > C_j)$
end for
$P(C_k) = P(L_x > C_{k-1})$)
$\hat{\boldsymbol{Y}} = \hat{\boldsymbol{Y}} \cup y$
end foreach
End Algorithm

2.5. Evaluation metrics

The proposed STOC models using five different base classifiers were compared by using the *n*-fold cross-validation technique selecting *n* as 10. The prediction performance of each model was tested in terms of accuracy rate and F-measure metric, and the obtained results were presented via tables and graphs in this study.

The accuracy rate gives a ratio of correctly classified instances to all instances in the dataset, as shown in Equation (2).

$$\text{Accuracy rate} = \frac{\#\text{ of correctly classified instances}}{\#\text{ of total instances}} \quad (2)$$

F-measure is a useful metric for presenting the classification performance of any model, which is calculated as a harmonic mean of precision and recall values as shown in Equation (3).

$$\text{F} - \text{measure} = 2 * \frac{\text{precision} * \text{recall}}{\text{precision} + \text{recall}} \quad (3)$$

3. Results

In the experimental studies, the proposed STOC method was executed on real-world meteorological and soil data from 16 stations for predicting soil temperature levels at five soil depths. In this study, five different traditional classification algorithms (DT (C4.5 algorithm), NB, KNN, SVM, and RF) were chosen as base learners for the STOC method. Except for the KNN algorithm, all other base learners were implemented with their default parameter values. In each experiment, the *k* value of the KNN algorithm was selected as $log_2(n)$(*n* is the number of instances in each dataset). To test the success of the proposed STOC method on the datasets, accuracy rate and F-measure values were evaluated.

Tables 4, 5, 6, 7, and 8 present the accuracy rate results of the applied STOC models using the traditional classification algorithms as base learners (STOC.DT, STOC.NB, STOC.KNN, STOC.SVM, and STOC.RF) on the datasets obtained from 16 stations in three states of United States at five soil depths (D). The results obtained from these tables indicate that the STOC models using five classification algorithms show different classification performances on these datasets. For example, STOC.KNN and STOC.SVM algorithms achieved the highest accuracy rate value (96.3%) on the dataset with 4 inches depth of the Sudduth Farms and Sevilleta stations, respectively. Also, when the accuracy rate results are considered in general, it is observed that STOC.NB showed a worse classification ability than the other STOC models. However, the applied STOC models, except for STOC.NB, show over 75.5% performance of the soil temperature level prediction.

Table 4- The accuracy rates (%) of the applied STOC.DT model on 16 stations at five soil depths (inch)

	Station ID															
D	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***13***	***14***	***15***	***16***
2	90.9	90.4	92.9	89	92.6	92.6	88.6	95.3	95.6	94.6	94.8	93.6	93.4	92.6	92.3	92
4	95.3	93.2	93.5	91.1	93.4	92.1	92.6	94	95.4	94.5	94.9	94	94	95.2	92.6	92.7
8	93.9	92.9	92.4	89.1	90.4	90.1	92.9	84	95.4	92.8	91.1	92.5	94.1	94.7	92	91.1
20	87.3	89.5	88.8	88.3	88.5	84.8	88.7	89.6	91.8	88.9	87.3	91	90.3	90	90.3	87.7
40	84.6	89.4	90.5	87.8	88.4	82	90.4	84.7	93.7	86.7	88.6	87.7	89.1	85.9	85.8	89.7

Table 5- The accuracy rates (%) of the applied STOC.NB model on 16 stations at five soil depths (inch)

	Station ID															
D	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***13***	***14***	***15***	***16***
2	81.1	77	84.3	84	83.2	77	80.3	81.8	85.3	79.9	83.3	89.4	87.1	79.3	85.6	81.4
4	85.1	79.7	84.5	85.3	85.5	76.3	81.5	82.8	78.5	81.7	84.9	90.7	88.3	80.2	88.1	81
8	85.9	79.5	82.5	83.2	82.6	81.2	80.8	74.4	89.1	80.8	80.8	88.1	87.5	79.3	85.9	80.9
20	75.4	78	72.6	71.2	75.3	73.4	68.4	80.8	77.3	73.8	75	76.3	75.4	76.5	78.6	66.7
40	63.5	75.9	59	55.7	51.7	63.3	65.6	72.6	69.5	70.5	58.6	58.3	64	67.1	60.5	71

Table 6- The accuracy rates (%) of the applied STOC.KNN model on 16 stations at five soil depths (inch)

	Station ID															
D	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***13***	***14***	***15***	***16***
2	91.9	91.2	92	88.6	93.2	92.1	88.8	95.1	95.7	94.3	94.9	93.4	93.6	93.4	91.2	92.7
4	95	94	94.7	90.9	93.7	93.2	92.4	95	95.7	95.5	96	93.1	94.9	96.3	92.8	93.4
8	95	93.7	92.9	89.6	91.5	90.3	93.5	83.4	95.5	93.8	91.4	93.6	94.5	95.4	92.2	92.2
20	87.4	88.7	88.3	86.8	87	87.3	86.7	90.4	88.1	88.2	87.4	89.6	89	89.7	89.9	86.3
40	83.5	87.3	86.9	82	82.9	82.4	84.5	85.8	89.6	85.9	85.8	84.3	85.7	86.1	81.6	86.4

Table 7- The accuracy rates (%) of the applied STOC.SVM model on 16 stations at five soil depths (inch)

	Station ID															
D	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***13***	***14***	***15***	***16***
2	92.1	90.4	93.1	90.2	93.6	92.3	90.4	95.4	95.8	94.9	95.6	94.1	93.9	93.5	92.2	93.3
4	95.2	93.9	94.8	92.6	94.5	92.6	92.9	95.2	95.9	95.4	96.3	93.9	95	95.9	93.6	93.8
8	95.3	93.8	93	89.8	91.5	89.3	93.2	83.4	95.6	94	91.3	94.1	94.8	95.6	93.4	92.3
20	86.6	85	87.7	86.5	87.4	86.1	84.9	89.7	87.8	87.9	85.3	90.1	89.5	88.7	90.4	86.3
40	80.2	85.7	85.3	75.5	83.1	80.8	83.4	83.9	81.8	83.9	80.8	79	81.8	85.5	77	84.7

Table 8- The accuracy rates (%) of the applied STOC.RF model on 16 stations at five soil depths (inch)

	Station ID															
D	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***13***	***14***	***15***	***16***
2	92.5	92.2	93.2	90.2	93.2	93.6	89.7	95.4	96	95.3	95.7	94.1	94.3	93.5	92.8	92.9
4	95.5	94.1	94.1	91.8	94	92.6	92.6	95.3	95.8	95.4	95.7	94.4	95	96.1	93.5	93.2
8	94.8	94	92.2	90.3	90.8	89.5	93.6	84.5	95.2	93.7	91.8	93.6	94.6	95.9	93.3	92.1
20	87.6	87	87.6	88.7	87.7	87	85.4	90.7	87.7	88.4	87.2	89.5	89.7	90.5	89.7	84.9
40	83.3	87.9	86	85.7	85.4	81.1	86.9	84.2	87.7	85.9	86.5	82.4	86.5	85.1	83.6	85.5

The average accuracy rates of the STOC models on these datasets were calculated to reveal which model is more successful in soil temperature level prediction. The obtained values are illustrated in the graph given in Figure 7. The results from this graph indicate that STOC.DT achieved the best performance of the soil temperature level prediction with an accuracy rate of 90.95%. This is probably because of the fact that the DT algorithm ignores unrelated parameters of the experimental dataset through information gain and defines logic for the split branches, and as a result, it usually provides accurate results. It is the most successful predictive model since it provides an exhaustive analysis of the consequences of each possible decision. The decision tree method divides the dataset at a much deeper level which is not as easily accomplished with other classifiers such as support of vector machines (Mim et al. 2018). According to the results given in Figure 7, it is also possible to say that STOC.RF and STOC.KNN reached higher classification abilities with quite close accuracy rates of 90.91% and 90.84%, respectively.

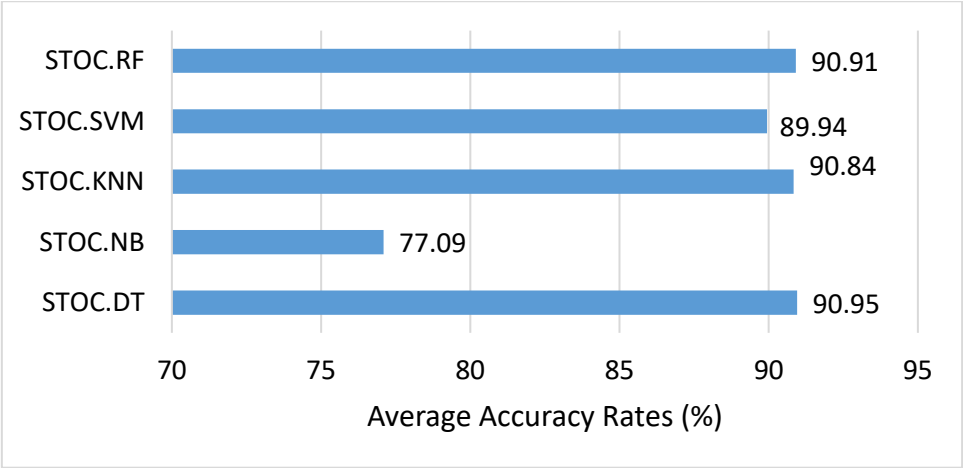


Figure 7- The average accuracy rates (%) of the applied STOC models on the 16 different stations

Furthermore, F-measure values of the applied STOC models using the traditional classification algorithms on the 16 datasets obtained in three of the United States at five soil depths were evaluated. In Figures 8, 9, and 10, the average F-measure values of the STOC models for each state were illustrated, respectively. The fact that the F-measure value is close to 1 presents that the model offers a successful classification ability. These graphs in the figures presented that the STOC.NB model gives the worst prediction performance among the others. Because the NB classifier assumes that the parameters of the dataset are independent of each other. However, some of the parameters often contain similar information and they depend on one another (Jiang et al. 2020). This may cause the NB classifier to yield inaccurate prediction results. The graphs also indicated that all the STOC models, except for STOC.NB, provide over 0.8 F-measure value. Therefore, it is possible to say that the proposed STOC approach is a successful method for soil temperature level prediction of ordinal data.

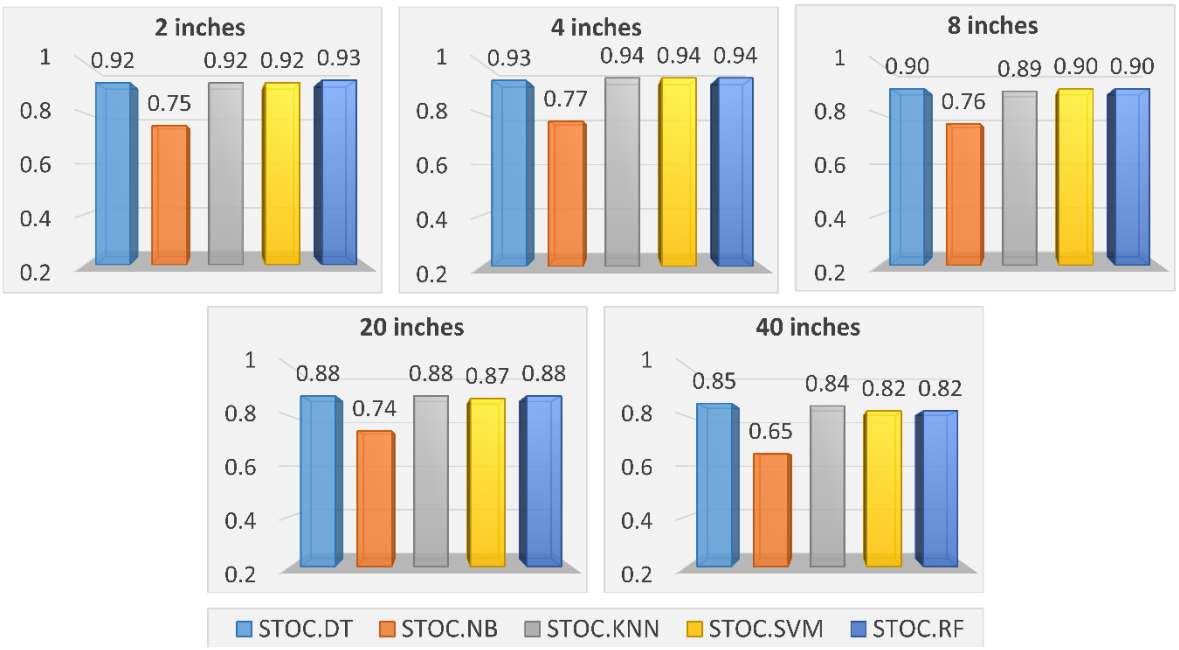


Figure 8- The average F-measure values of the applied STOC models on the Alabama state stations

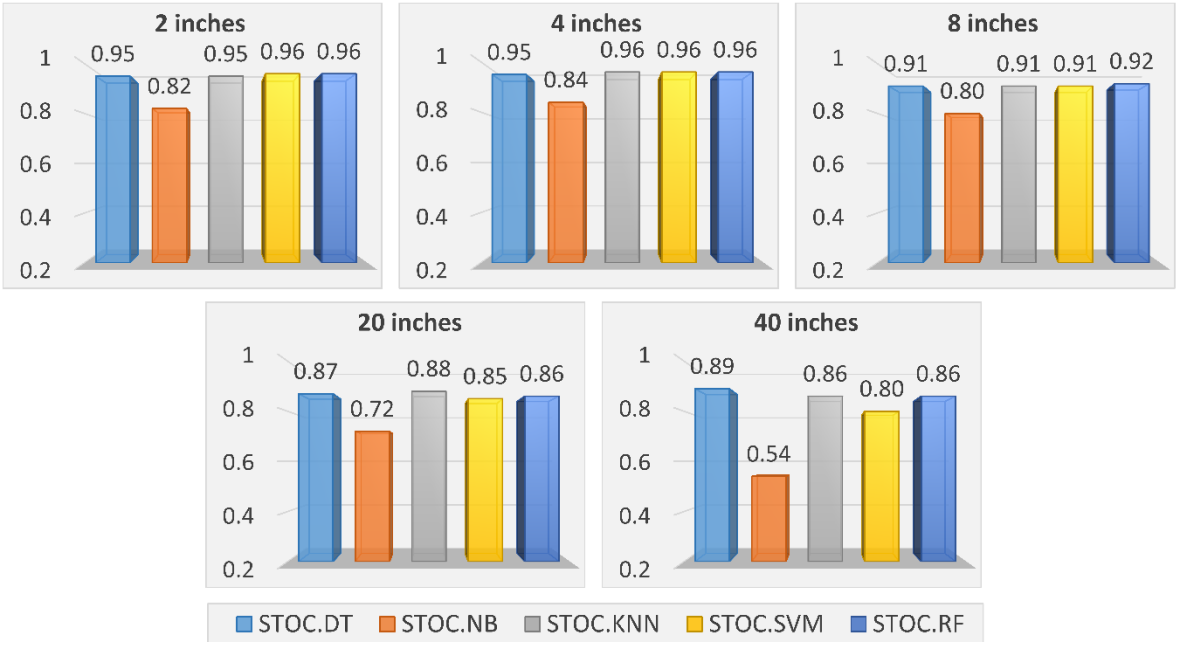


Figure 9- The average F-measure values of the applied STOC models on the Mexico state stations

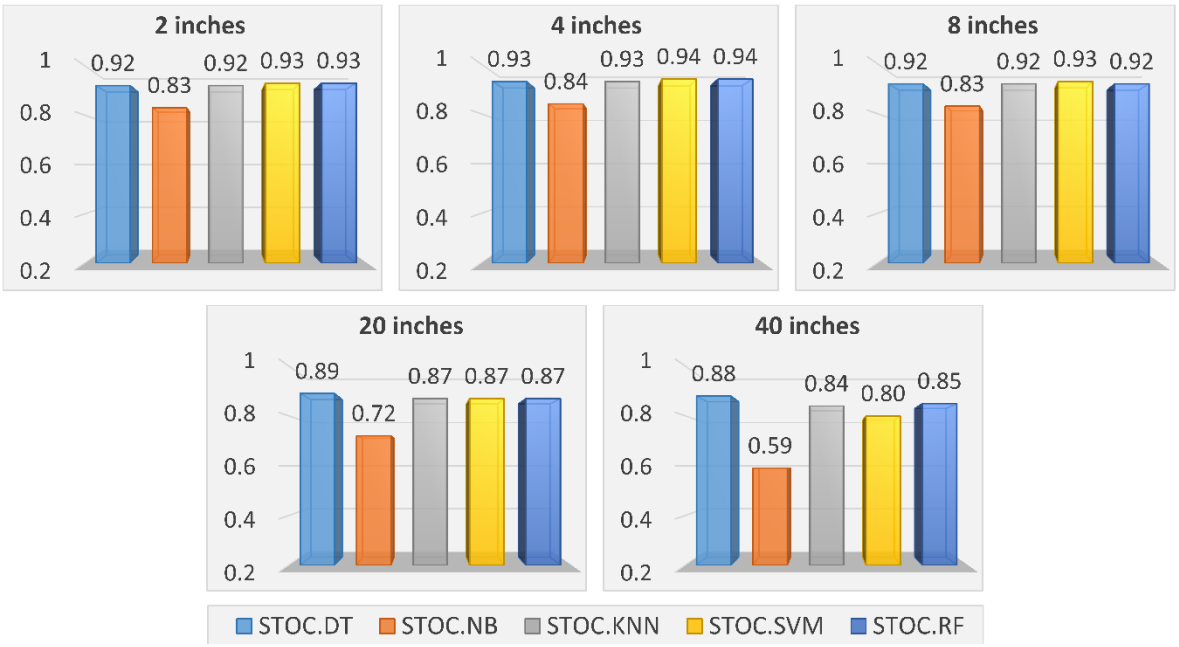


Figure 10- The average F-measure values of the applied STOC models on the Utah state stations

4. Discussion

Differently from the existing machine learning-based soil prediction studies, this study focuses on the classification of ordinal and categorical target values. It also applies different methods from the present studies, such as KNN and NB. Finally, because the target attribute value is categorical, the performances of the applied algorithms were tested with accuracy and F-measure metrics. The detailed comparison of this study with the existing studies is given in Table 1.

The main findings of this study can be summarized as follows:

- The proposed STOC method achieved high accuracy (>90%) on the prediction of soil temperature level. This means that it has a high capability of temperature prediction for new samples in the presence of ordinal historical data.

- The STOC method can be applied to any ordinal soil dataset without any prior information or specific assumptions about the given data.

- When the STOC approach was tested in combination with different classification algorithms (DT, NB, KNN, SVM, and RF), the SSOC.DT method achieved higher accuracy (90.95%) than the rest on average.

- Daily soil temperature level prediction at different depths is necessary for real-life agricultural management systems. The accuracy of prediction varies as the soil depth changes for many locations. STOC usually performed over 0.8 of F-measure value, which means that the proposed method has a good generalization ability at all soil depths. Since STOC covers multiple soil depths, it enables numerous agricultural applications, and thus it expands the use of machine learning in the field of agriculture.

- Since soil temperature is highly dependent on various parameters (i.e., meteorology, soil type, vegetation, lithology, slope angle, and background concentration), this study proposes station-based models. In other words, a separate model is built for each measurement location (monitoring node) by using the station-based soil data.

- Based on the experimental results reported in this study, it is possible to say that the proposed STOC approach is a successful method for making a prediction on ordinal soil temperature data.

- It was observed that STOC has many advantages for predicting soil temperature level, which can provide increasing crop yield, reducing unnecessary resource consumption (i.e., water, sensor, and pesticide), and providing additional information when ordinal data is available.

5. Conclusions

In this study, a novel STOC approach is proposed. The proposed approach categorizes the soil temperature levels of meteorological and soil data at different soil depths by taking into account the inherent order of class labels. While the previous soil temperature prediction studies do not consider the natural ranking between the class labels, this study considers them by using the binary decomposition technique in ordinal classification. Thus, this study is the first attempt to implement ordinal classification for soil temperature level prediction. This study is also original in that it compares alternative base learners (DT, NB, KNN, SVM, and RF) in conjunction with the proposed method.

In the experiments, the proposed STOC method with different classifiers (STOC.DT, STOC.NB, STOC.KNN, STOC.SVM, and STOC.RF) was applied on daily values of meteorological and soil data obtained from 16 stations at five soil depths and compared with each other in terms of F-measure and accuracy rate metrics. The results show that the proposed STOC method provides accurate soil temperature classification performance. The constructed STOC models, except for STOC.NB, achieved over 75.5% ability of the soil temperature level prediction. Also, it is clearly understood from the experimental result that the STOC.DT shows the best performance of the soil temperature level prediction with an average accuracy rate of 90.95%.

As a future study, a multi-output regression technique can be implemented on the same meteorological and soil data for predicting continuous soil temperature values. In addition, an improved time-series study that solves the soil temperature prediction problem using a supervised learning technique can be performed.

Acknowledgment

We thank Volkan KIRANOGLU and Goksu TUYSUZOGLU for supporting the preparation of the dataset used in this study.

References

Abimbola O P, Meyer G E, Mittlstet A R, Rudnick D R & Franz T E (2021). Knowledge-guided machine learning for improving daily soil temperature prediction across the United States. *Vadose Zone Journal* e20151: 1-18. https://doi.org/10.1002/vzj2.20151

Abyaneh H Z, Varkeshi M B, Golmohammadi G & Mohammadi K (2016). Soil temperature estimation using an artificial neural network and co-active neuro-fuzzy inference system in two different climates. *Arabian Journal of Geosciences* 9(2016): 1-9. https://doi.org/10.1007/s12517-016-2388-8

Alizamir M, Kisi O, Ahmed A N, Mert C, Fai C M, Kim S, Kim N W & Shafie A E (2020a). Advanced machine learning model for better prediction accuracy of soil temperature at different depths. PLOS ONE 15(4): 1-25. https://doi.org/10.1371/journal.pone.0231055

Alizamir M, Kim S, Kermani M Z, Heddam S, Shahrabadi A H & Gharabaghi B (2020b). Modelling daily soil temperature by hydro-meteorological data at different depths using a novel data-intelligence model: deep echo state network model. Artificial Intelligence Review 102: 1-28. https://doi.org/10.1007/s10462-020-09915-5

Bayatvarkeshi M, Bhagat S K, Mohammadi K, Kisi O, Farahani M, Hasani A, Deo R & Yaseen Z M (2021). Modeling soil temperature using air temperature features in diverse climatic conditions with complementary machine learning models. Computers and Electronics in Agriculture 185: 1-15. https://doi.org/10.1016/j.compag.2021.106158

Bonakdari H, Moeeni H, Ebtehaj I, Zeynoddin M, Mahoammadian A & Gharabaghi B (2019). New insights into soil temperature time series modeling: linear or nonlinear? Theoretical and Applied Climatology 135: 1157-1177. https://doi.org/10.1007/s00704-018-2436-2

Citakoglu H (2017). Comparison of artificial intelligence techniques for prediction of soil temperatures in Turkey. Theoretical and Applied Climatology 130: 545-556. https://doi.org/10.1007/s00704-016-1914-7

Delbari M, Sharifazari S & Mohammadi E (2019). Modeling daily soil temperature over diverse climate conditions in Iran-a comparison of multiple linear regression and support vector regression techniques. Theoretical and Applied Climatology 135: 991-1001. https://doi.org/10.1007/s00704-018-2370-3

Feng Y, Cui N, Hao W, Gao L & Gong D (2019). Estimation of soil temperature from meteorological data using different machine learning models. Geoderma 338(2019): 67-77. https://doi.org/10.1016/j.geoderma.2018.11.044

Frank E & Hall M (2001). A simple approach to ordinal classification. In: *European Conference on Machine Learning* 3-5 September, Freiburg, Germany, pp. 145-156. https://doi.org/10.1007/3-540-44795-4_13

Hao H, Yu F & Li Q (2020). Soil temperature prediction using convolutional neural network based on ensemble empirical mode decomposition. IEEE Access 9: 4084-4096. https://doi.org/10.1109/ACCESS.2020.3048028

Jiang L, Yuan P, Zhang Q & Liu Q (2020). A study of the Naive Bayes classification based on the Laplacian matrix. *International Journal of Computer Science* 47:4 713-722

Kisi O, Tombul M & Kermani M Z (2015). Modeling soil temperatures at different depths by using three different neural computing techniques. Theoretical and Applied Climatology 121: 377-387. https://doi.org/10.1007/s00704-014-1232-x

Li C, Zhang Y & Ren X (2020a). Modeling hourly soil temperature using deep BiLSTM neural network. Algorithms 13(7): 173-187. https://doi.org/10.3390/a13070173

Li Q, Hao H, Zhao Y, Ceng Q, Liu G, Zhang Y & Yu F (2020b). GANs-LSTM model for soil temperature estimation from meteorological: A new approach. IEEE Access 9: 59427-59443. https://doi.org/10.1109/ACCESS.2020.298299

Li Q, Zhao Y & Yu F (2020c). A novel multichannel long short-term memory method with time series for soil temperature modeling. IEEE Access 8: 182026-182043. https://doi.org/10.1109/ACCESS.2020.3028995

Mehdizadeh S, Behmanesh J & Khalili K (2018). Comprehensive modeling of monthly mean soil temperature using multivariate adaptive regression splines and support vector machine. Theoretical and Applied Climatology 133: 911-924. https://doi.org/10.1007/s00704-017-2227-1

Mehdizadeh S, Fathian F, Safari M J & Khosravi A (2020). Developing novel hybrid models for estimation of daily soil temperature at various depths. Soil & Tillage Research 197(2020): 1-12. https://doi.org/10.1016/j.still.2019.104513

Mim F S, Galib S M, Hasan M F & Jerin S A (2018). Automatic detection of mango ripening stages – An application of information technology to botany. Scientia Horticulturae 237: 156-163. https://doi.org/10.1016/j.scienta.2018.03.057

Nanda A, Sen S, Sharma A N & Sudheer K P (2020). Soil temperature dynamics at Hillslope scale-field observation and machine learning-based approach. Water 12(2020): 713-734. https://doi.org/10.3390/w12030713

Onwuka B & Mang B (2018). Effects of soil temperature on some soil properties and plant growth. Advances in Plants & Agriculture Research 8(1): 34-37. https://doi.org/10.15406/apar.2018.08.00288

Penghui L, Ewees A A, Beyaztas B H, Qi C, Salih S Q, Al-Ansari N, Bhagat S K, Yaseen Z M & Singh V P (2020). Metaheuristic optimization algorithms hybridized with artificial intelligence model for soil temperature prediction: Novel model. IEEE Access 8: 51884-51904. https://doi.org/10.1109/ACCESS.2020.2979822

Sanikhani H, Deo R C, Yaseen Z M, Eray O & Kisi O (2018). Non-tuned data intelligent model for soil temperature estimation: A new approach. Geoderma 330(2016): 52-64. https://doi.org/10.1016/j.geoderma.2018.05.030

Sattari M T, Avram A, Apaydin H & Matei O (2020). Soil temperature estimation with meteorological parameters by using tree-based hybrid data mining models. Mathematics 8(9): 1-21. https://doi.org/10.3390/math8091407

Shamshirband S, Esmaeilbeiki F, Zarehaghi D, Neyshabouri M, Samadianfard S, Ghorbani M A, Mosavi A, Nabipour N & Chau K W (2020). Comparative analysis of hybrid models of firefly optimization algorithm with support vector machines and multilayer perceptron for predicting soil temperature at different depths. Engineering Applications of Computational Fluid Mechanics 14(1): 939-953. https://doi.org/10.1080/19942060.2020.1788644

Tsai Y Z, Hsu K S, Wu H Y, Lin S I, Yu H L, Huang K T, Hu M C & Hsu S Y (2020). Application of random forest and ICON models combined with weather forecasts to predict soil temperature and water content in a greenhouse. Water 12(4): 1-23. https://doi.org/10.3390/w12041176

Wang X, Li W & Li Q (2021). A new embedded estimation model for soil temperature prediction. Scientific Programming 2021: 1-16. https://doi.org/10.1155/2021/5881018

Witten I H, Frank E, Hall M A & Pal C J (2016). Data Mining: Practical Machine Learning Tools and Techniques. Morgan Kaufmann Publishers, Cambridge, MA, USA https://doi.org/10.1016/C2009-0-19715-5

Xing L, Li L, Gong J, Ren C, Liu J & Chen H (2018). Daily soil temperatures predictions for various climates in United States using data-driven model. Energy 160(2018): 430-440. https://doi.org/10.1016/j.energy.2018.07.004

Zeynoddin M, Ebtehaj I & Bonakdari H (2020). Development of a linear based stochastic model for daily soil temperature prediction: One step forward to sustainable agriculture. Computers and Electronics in Agriculture 176(2020): 1-24. https://doi.org/10.1016/j.compag.2020.105636

Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

Influence of 24-Epibrassinolide on Physiological Characteristics of Tomato Seedlings Infested with Root-knot Nematode *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae)

Çiğdem GÖZEL[a*], Zeliha GÖKBAYRAK[b]

[a] *Çanakkale Onsekiz Mart University Faculty of Agriculture Department of Plant Protection 17020-Çanakkale, TÜRKİYE*
[b] *Çanakkale Onsekiz Mart University Faculty of Agriculture Department of Horticulture 17020-Çanakkale, TÜRKİYE*

ARTICLE INFO
Research Article
Corresponding Author: Çiğdem GÖZEL, E-mail: cigdemgunes@comu.edu.tr
Received: 21 June 2021 / Revised: 19 November 2021 / Accepted: 19 November 2021 / Online: 15 October 2022

Cite this article
GÖZEL Ç, GÖKBAYRAK Z (2022). Influence of 24-Epibrassinolide on Physiological Characteristics of Tomato Seedlings Infested with Root-knot Nematode *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae). *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 28(4):650-655. DOI: 10.15832/ankutbd.955398

ABSTRACT
The aim of this research is to determine the physiological responses of tomato seedlings treated with 24-epibrassinolide (EBL), given via different methods such as immersion, irrigation, and foliar spray, followed by inoculation of root-knot nematode *Meloidogyne incognita* (Kofoid & White 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae). Physiological measurements (chlorophyll, flavonols, nitrogen balance index, and anthocyanins) were carried out non-destructively with a portable chlorophyll meter at the end of the 56th days post inoculation. Results showed that chlorophyll contents of the tomato leaves were affected by both the EBL applications and the methods. Chlorophyll contents were better protected with the irrigation method. Flavonols and nitrogen balance index were inversely related with the application method. Leaf anthocyanin index was affected just by the EBL applications. Depending on the physiological aspect under observation, the method (immersion against irrigation, for instance) might present a challenging status in terms of providing protection against root-knot nematode when tomato plants are treated with EBL.

Keywords: Brassinosteroids, *Lycopersicon esculentum*, *Meloidogyne* spp., Non-destructive sampling

1. Introduction

Responses of plants to stress factors are elucidated through assessment of changes in morphology, biochemistry, and genetics. Plant stress can be caused by abiotic factors, such as drought, salinity, or heat or by biotic ones such as diseases and herbivorous pests like viruses, bacteria, or nematodes (Jagodič et al. 2017; Voglar et al. 2019). Detrimental consequences occurring after a biotic or abiotic stress can be alleviated through the utilization of synthetic elicitors (Llorens et al. 2017), chemical compounds activating plant's reaction to sustain health and productivity (Ramírez-Godoy et al. 2018). Brassinosteroids are among these elicitors, and they have been proven by various research to stimulate antioxidant defense mechanism of a plant against numerous abiotic factors (Jan et al. 2018).

Most information about protective properties of brassinosteroids in plants under stress have been provided from studies evaluating abiotic factors such as salinity, drought, or heavy-metal accumulation in the soil. In these studies, it was reported that acquisition of resistance with brassinosteroid applications derive from protection of chlorophyll content, increase in photosynthetic rate and stimulation of antioxidative system (Fariduddin et al. 2013; Yang et al. 2019; Santos de Fonseca et al. 2020). On the other hand, research pertaining to the effects of brassinosteroids in protecting plants against pathogen attacks have been quite limited. They were found to mitigate fungus-related deleterious influences in cotton (Bibi et al. 2017), cucumber (Ding et al. 2009) and barley (Ali et al. 2013).

Increases in resistance after brassinosteroid application against viruses have also been shown by Ali et al. (2014) in *Hordeum vulgare*, and by Zhang et al. (2015) in *Arabidopsis thaliana*. In recent years, responses against root-knot nematode damage in plants have also been carried out and positive results have been reported by Jasrotia & Ohri (2014; 2017), Kaur et al. (2013; 2014), Song et al. (2017) and Gözel (2021) against *Meloidogyne incognita* (Kofoid & White 1919) Chitwood 1949 (Tylenchida: Meloidogynidae).

Early detection of stress symptoms in plants presents an invaluable tool to manage and abate them before any reduction in yield and quality appears and causes an income loss. Non-destructive and in-field measurement via technological tools, thermographic or optical, have made it possible. Among these tools, leaf-clip chlorophyll-meters use chlorophyll fluorescence screening method (Agati et al. 2016), and estimate chlorophyll, flavonoids, and anthocyanins. These optical sensors have been shown to be superior to lab-based and destructive leaf chlorophyll measurement (Dong et al. 2019).

As opposed to abundant research on effects of brassinosteroids on plant growth and development as well as on response to abiotic stress, information about their role against biotic stress, specifically plant parasitic nematodes, are quite limited. Thus, aim of this study was to non-destructively evaluate whether exogenously applied 24-epibrassinolide to roots or leaves can induce protective effects on physiological aspects (chlorophyll, flavonols or antioxidants) of tomato seedlings inoculated with *M. incognita*.

2. Material and Methods

2.1. Plant material and nematode inoculum

3-4 leaved seedlings of tomato (*Lycopersicon esculentum* Mill.) cv. Heinz 2274 were obtained from a commercial nursery. These seedlings used in a study conducted at Department of Plant Protection, Faculty of Agriculture, University of Çanakkale Onsekiz Mart, Çanakkale in 2020. Second-stage juveniles (J2s) of *Meloidogyne incognita* after reproduction from a single egg mass on a cucumber plant were inoculated at the dose of 1000 per plant.

2.2. 24-epibrassinolide application and nematode inoculation

E-1641 EBL (Sigma Aldrich) was used after dilution in ethanol absolute, and working solutions of 1, 5 and 10 µM were prepared. Experimental design was shown in Table 1. Repetitive applications of irrigation and spraying were done at transplanting and on the following 7th and 14th day.

Table 1- Experimental design of the study

Methods	*EBL concentration (µM)*	*Nematode inoculation*	*Application times (x)*
Immersion (10 min.)	0 (DW)	-	1
	0 (DW+J2s)	+	1
	1	+/-	1
	5	+/-	1
	10	+/-	1
Irrigation	0 (DW)	-	3
	0 (DW+J2s)	+	3
	1	+/-	3
	5	+/-	3
	10	+/-	3
Spraying	0 (DW)	-	3
	0 (DW+J2s)	+	3
	1	+/-	3
	5	+/-	3
	10	+/-	3

2.3. Growth conditions

The pots (1.4-liter volume) into which the plants were transplanted contained 70:30 (v/v) sterilized sand and soil mixture (approx. 450 g). Later the pots were placed in a growth chamber under the conditions of 26±1 °C and 18/6 hours of photoperiod until the end of the experiment (56 days post inoculation).

2.4. Data analysis

At the end of the experiment synchronous readings using Dualex Scientific+^{TM} were made on each side (abaxial and adaxial) of the third youngest leaf and a mean value was obtained. Measured indices were chlorophyll (µg per cm^2), relative absorbance units of flavonols (0 to 3) and anthocyanins (0 to 1.5), and the nitrogen balance index (NBI), determined by the relationship between chlorophyll and flavonols.

The experiment was arranged in a completely randomized design with three replications of one plant per pot. The data for chlorophyll, flavonols, anthocyanins and NBI were analyzed using ANOVA on Minitab® (v. 17.1.0 for Windows) statistics package program. Significant differences between the means were compared using Duncan's multiple comparison test at 95% confidence level.

3. Results and Discussion

Chlorophyll contents of the tomato leaves were interactively affected by both EBL applications and the methods they were given (P= 0.001, Figure 1). Best EBL applications in the immersion group protecting the chlorophyll contents were 5 µM and 1 µM+J2s, following the distilled water (DW). Irrigated plants had better contents in all the applications except for 5 µM+J2s, 10 µM EBL+J2s and DW+J2s. Foliar spraying was generally the least supporting in all treatments, except for 5 µM EBL (Figure 1).

Effect of nematode inoculation on chlorophyll content was abated with 1 µM EBL application given via irrigation. Increasing concentration did not provide a positive response in suppressing the nematode stress comparing to control groups (Figure 1). In general, irrigating the seedlings with 1 µM EBL+J2s improved chlorophyll contents in the tomato leaves. Overall, irrigation was the best among the application methods at keeping chlorophyll at higher levels.

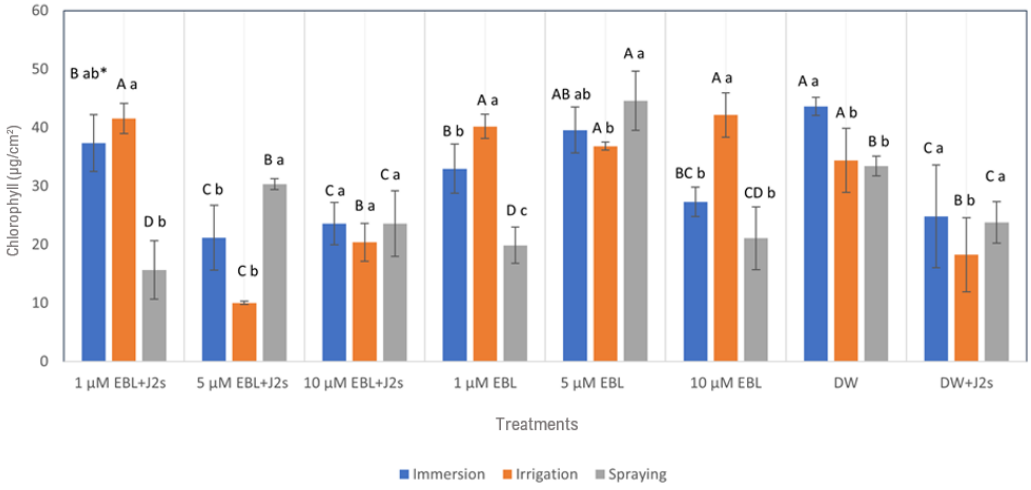


Figure 1- Effects of 24-epibrassinolide (EBL) on chlorophyll contents of tomato cv. Heinz 2274 plants given in three treatments (immersion, irrigation, and spraying) followed by *Meloidogyne incognita* inoculation at a dose of 1000 J2s. *Different capital letters denote significant differences in methods at the same concentration of EBL and different small letters indicate significance in the treatments of EBL at the same methods. P=0.001. Means±S.E.

NBI is an indicator of plant growth, using the relation between chlorophyll and flavanols contents (Figure 2). Although a significant effect of the applications was detected (P=0.019), a clear distinction was only seen between DW and 5 µM EBL+J2s. The NBI of the seedlings were in general supported by the EBL treatments in DW (nematode-free soil) more than DW+J2s, and as the concentration of EBL increased this effect was lessened. NBI was also under the influence of the methods (figure not shown, P=0.015) and it was in the decreasing order of immersion, irrigation, and foliar spraying. Significant differences were only observed between immersion and foliar spraying.

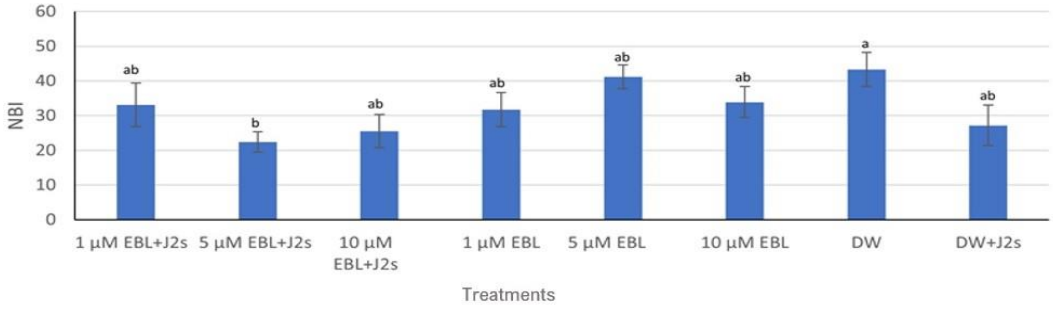


Figure 2- Effects of 24-epibrassinolide (EBL) on NBI (nitrogen balance index) of tomato cv. Heinz 2274 plants given in three treatments (immersion, irrigation, and foliar spraying) followed by *Meloidogyne incognita* inoculation at a dose of 1000 J2s. *Different letters show significant differences at P<0.05. Means±S.E.

Flavonols contents of the seedling significantly changed with the methods the EBL concentrations (p=0.021) and distinct difference was only observed between the foliar spraying and the immersion methods (Figure 3).

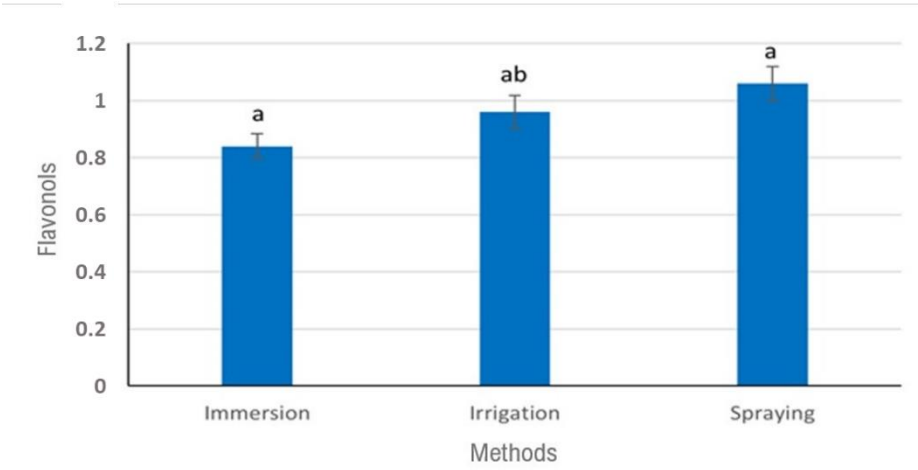


Figure 3- Effects of 24-epibrassinolide (EBL) on flavonols index (radiance absorbance unit, 0-3) of tomato cv. Heinz 2274 plants given in three treatments (immersion, irrigation, and foliar spraying) followed by *Meloidogyne incognita* inoculation at a dose of 1000 J2s. *Different letters show significant differences at P<0.05. Means±S.E.

Leaf anthocyanin contents of the seedlings at the end of the experiment was affected by the EBL applications (P= 0.036, Figure 4). The highest anthocyanin accumulation was observed in the DW+J2s application, indicating nematodes in the soil induced it as a response to the stress. However, the difference among the rest of the treatments was not clear enough to differentiate the effects of EBL. The application of 5 µM EBL provided the lowest content of anthocyanins.

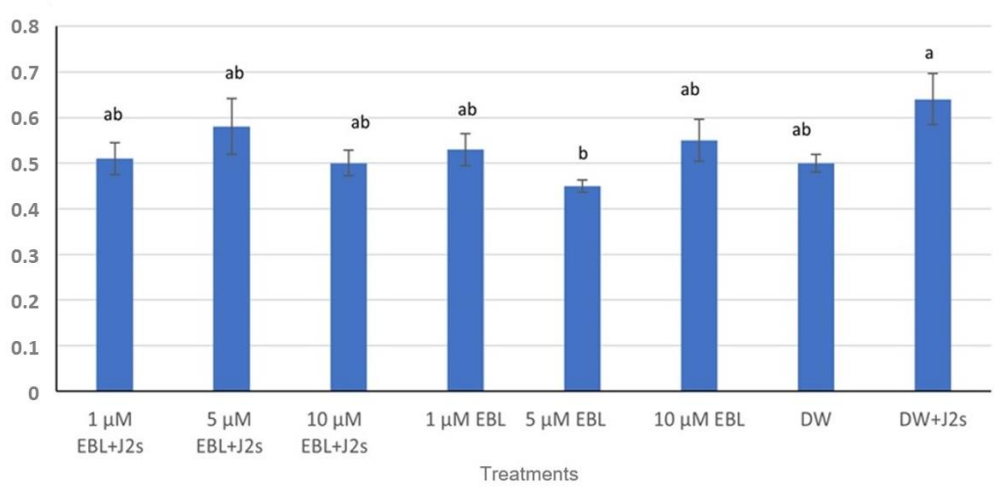


Figure 4- Effects of 24-epibrassinolide (EBL) on anthocyanins index (radiance absorbance unit, 0-1) of tomato cv. Heinz 2274 plants given in three treatments (immersion, irrigation, and foliar spraying) followed by *Meloidogyne incognita* inoculation at a dose of 1000 J2s. *Different letters show significant differences at P<0.05. Means±S.E.

Root-knot nematodes induce plants to develop galls on their roots, damaging root structure and preventing water and nutrient uptake. Most of the assimilates produced in the plant is redirected from shoot to root to support nematode's growth and reproduction (Maleita et al. 2012). Since the study of Loveys & Bird (1973), which showed that photosynthetic rate and chlorophyll content in the nematode infected plants decreased, several other reports (Melakeberhan et al. 1985; Gine et al. 2014; López-Gómez & Verdejo-Lucas (2017) also supported this observation. In the current study, a great deal of reduction in chlorophyll was also observed in the DW plants with nematode infestation.

When plants were exposed to nematode infestation, applying 1 µM EBL through immersion or irrigation resulted comparably closer chlorophyll contents to DW plants. As the concentration increased, the positive effect weakened and became negative. Similar results were obtained in rice plants to which brassinolide was given as foliar spraying (Nahar et al. 2013). Since low concentration (0.1 µM) was successful in ameliorating the effects of *M. graminicola*, but higher concentrations (1, 5, 10 µM) were not, they concluded that exogenous brassinolide created negative feedback on brassinosteroids biosynthesis. It has been also reported that brassinosteroids act as stimuli on chlorophyll through activation of chlorophyllase and Rubisco (Xia et al. 2009).

NBI, the ratio of chlorophyll to flavonols, is an index proven to be a reliable indicator of plant N content (Cartelat et al. 2005; Padilla et al. 2014). The current study found that brassinosteroids application protected the N reserve of the nematode infested plants at the lowest concentration of EBL. Schlemmer et al. (2013) stated that chlorophyll is closely related to leaf N status. One possible reason for the root treatment to cause an increased N content might be the increased root activity and increased chlorophyll concentration in the leaves. This kind of relationship between NBI and the chlorophyll content can be seen in the response of DW plants, showing a decrease in the irrigated and sprayed plants. Changes depending on the methods also revealed the inverse relationship between the flavonols and the N content, being the lowest in the sprayed plants, this relationship was also indicated in the studies of Stewart et al. (2001) and Fallovo et al. (2011).

Non-enzymatic antioxidants, such as polyphenols including flavonoids, come into play to mitigate the adverse effects of stress on plant. Although specific effects were not found significant in the current study, it was revealed that flavonols respond to method of the application. Since polyphenols are accumulated on the epidermal tissue of the leaves covering chlorophyll molecules from above, foliar spraying with epibrassinolide might have stimulated the synthesis of flavonols (Brunetti et al. 2013) with an ease of infuse on the surface or protected the integrity of the chloroplast and hence the flavonols in them. It was shown that chloroplasts have flavonoids, presenting an additional defense line against reactive oxygen species produced under stressful conditions (Triantaphylidés et al. 2008).

4. Conclusions

Studies on brassinosteroids mostly involve its effects on growth and development, and stress responses, specifically to abiotic factors. the physiological response of the tomato plant differs with the application method that EBL was given to plants. Results showed that chlorophyll contents were responsive to the EBL concentrations and the method. An inverse relationship was found between flavonols and nitrogen balance index depending on the application method. The findings of the study illustrate that possible effects of 24-epibrassinolide might be concentration-dependent and how it is administered to the plants should be taken into consideration in both scientific studies and agronomic use.

Acknowledgements

This study was financially supported by TUBİTAK with 119O660 Project number

References

Agati G, Tuccio L, Kusznierewicz B, Chmiel T, Boratoszek A, Kowalski A, Grzegorzeska M, Kosson R & Koniszewski S (2016). Nondestructive optical sensing of flavonols and chlorophyll in white head cabbage (*Brassica oleracea* L. var. *capitata* subvar. *alba*) grown under different nitrogen regimes. *Journal of Agriculture and Food Chemistry* 64: 85-94. https://doi.org/10.1021/acs.jafc.5b04962

Ali S S, Gunupuru L R, Kumar G, Khan S M, Scofield S, Nicholson P & Doohan F M (2014). Plant disease resistance is augmented in uzu barley lines modified in the brassinosteroid receptor BRI1. BMC Plant Biology 14: 227. https://doi.org/10.1186/s12870-014-0227-1

Ali S S, Kumar, G S, Khan M & Doohan F M (2013). Brassinosteroid enhances resistance to fusarium diseases of barley. Phytopathology 103: 1260-1267. http://dx.doi.org/10.1094 / PHYTO-05-13-0111-R

Bibi N, Ahmed I M, Fan K, Dawood M, Li F, Yuan S & Wang X (2017). Role of brassinosteroids in alleviating toxin-induce stress of *Verticillium dahlia* on cotton callus growth. Environmental Science and Pollution Research 24: 12281-12292. DOI: 10.1007/s11356-017-8738-6

Brunetti C, Ferdinando M D, Fini A, Pollastri S & Tattini M (2013). Flavonoids as antioxidants and developmental regulators: Relative significance in plants and humans. *International Journal of Molecular Sciences* 14(2): 3540-3555. https://doi.org/10.3390/ijms14023540

Cartelat A, Cerovic Z G, Goulas Y S, Meyer C, Lelarge J L, Prioul A, Barbottin M, Jeuffroy H, Gate P, Agati G & Moya I (2005). Optically assessed contents of leaf polyphenolics and chlorophyll as indicators of nitrogen deficiency in wheat (*Triticum aestivum* L.). Field Crops Research 91(1): 35-49. DOI: 10.1016/j.fcr.2004.05.002

Ding J, Shi K & Zhou Y H (2009). Effects of root and foliar applications of 24-epibrassinolide on fusarium wilt and antioxidant metabolism in cucumber roots. HortScience 44(5): 1340-1345. https://doi.org/10.21273/HORTSCI.44.5.1340

Dong T, Shang J, Chen J M, Liu J, Qian B, Ma B, Morrison M J, Zhang C, Liu Y, Shi Y, Pan H & Zhou G (2019). Assessment of Portable Chlorophyll Meters for Measuring Crop Leaf Chlorophyll Concentration. Remote Sensing 11(22): 2706. https://doi.org/10.3390/rs11222706

Fallovo C, Schreiner M, Schwarz D, Colla G & Krumbein A (2011). Phytochemical changes induced by different nitrogen supply forms and radiation levels in two leafy *Brassica* species. *Journal of Agriculture and Food Chemistry* 59: 4198-4207. DOI: 10.1021/jf1048904

Fariduddin Q, Khalil R R, Mir B A, Yusuf M & Ahmad A (2013). 24-Epibrassinolide regulates photosynthesis, antioxidant enzyme activities and proline content of *Cucumis sativus* under salt and/or copper stress. Environmental Monitoring and Assessment 185: 7845-7856. dx.doi.org/10.1021/jf1048904

Gine A, López-Gómez M, Vela M D, Ornat C, Talavera M, Verdejo-Lucas S & Sorribas F J (2014). Thermal requirements and population dynamics of rootknot nematodes on cucumber and yield losses under protected cultivation. Plant Pathology 63: 1446-1453. https://doi.org/10.1111/ppa.12217

Gözel Ç (2021). Effects of 24-epibrassinolide on root-knot nematode, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) in tomatoes. *Turkish Journal of Entomology* 45(1): 13-22. http://dx.doi.org/10.16970/entoted.808416

Jagodič A, Ipavec N, Trdan S & Laznik Ž (2017). Attraction behaviours: are synthetic volatiles, typically emitted by insect-damaged *Brassica nigra* roots, navigation signals for entomopathogenic nematodes (*Steinernema* and *Heterorhabditis*)? BioControl 62: 515-524. https://doi.org/10.1007/s10526-017-9796-x

Jan S, Alyemeni M N, Wijaya L, Alam P, Siddique K H & Ahmad P (2018). Interactive effect of 24-epibrassinolide and silicon alleviates cadmium stress via the modulation of antioxidant defense and glyoxalase systems and macronutrient content in *Pisum sativum* L. seedlings. BMC Plant Biology 18(1): 146. https://doi.org/10.1186/s12870-018-1359-5

Jasrotia S & Ohri P (2014). *In vitro* effect of 24-epibrassinolide on antioxidative enzymes of tomato plants during *Meloidogyne incognita* infection. *Journal of Environmental Research and Development* 9(1): 188-191

Jasrotia S & Ohri P (2017). 24-epibrassinolide reduces stress in nematode-infected tomato (*Solanum lycopersicum* L.) plants cultured *in vitro*. In Vitro Cellular & Developmental Biology-Plant 53(6): 538-545. https://doi.org/10.1007/s11627-017-9859-9

Kaur R, Ohri P & Bhardwaj R (2013). Effect of 28-homobrassinolide on susceptible and resistant cultivars of tomato after nematode inoculation. Plant Growth and Regulation 71(3): 199-205. https://doi.org/10.1007/s10725-013-9820-9

Kaur R, Ohri P & Bhardwaj R (2014). Effect of 28-homobrassinolide on non-enzymatic antioxidants of tomato plant under nematode stress. *Journal of Environmental Research and Development* 9(2): 362-367

Llorens E, García-Agustín P & Lapena L (2017). Advances in induced resistance by natural compounds: Towards new options for woody crop protection. Scientia Agricola 74(1): 90-100. https://doi.org/10.1590/1678-992X-2016-0012

López-Gómez M & Verdejo-Lucas S (2017). Penetration and post-infection development of root-knot nematodes in watermelon. *Spanish Journal of Agricultural Research* 15(4): e1010. https://doi.org/10.5424/sjar/2017154-11189

Loveys R R & Bird A F (1973). The influence of nematodes on photosynthesis in tomato plants. Physiological Plant Pathology 3: 525-529. https://doi.org/10.1016/0048-4059(73)90063-5

Maleita C M N, Curtis R H C, Powers S J & Abrantes I M (2012). Inoculum levels of *Meloidogyne hispanica* and *M. javanica* affect nematode reproduction, and growth of tomato genotypes. Phytopathologia Mediterranea 51 (3): 566-576

Melakeberhan H, Webster J M & Brooke R C (1985). Response of *Phaseolus vulgaris* to a single generation of *Meloidogyne incognita*. Nematologica 31: 191-202

Nahar K, Kyndt T, Hause B, Höfte M & Gheysen G (2013). Brassinosteroids suppress rice defense against root-knot nematodes through antagonism with the jasmonate pathway. Molecular Plant-Microbe Interactions 26: 106-115. https://doi.org/10.1094/MPMI-05-12-0108-FI

Padilla F M, Souza R, Peña-Fleitas M T, Grasso R, Gallardo M & Thompson R B (2014). Evaluation of optical sensor measurements of canopy reflectance and of leaf flavonols and chlorophyll contents to assess crop nitrogen status of muskmelon. *European Journal of Agronomy* 58: 39-52. https://doi.org/10.1016/j.eja.2014.04.006

Ramírez-Godoy A, Vera-Hoyos M P, Beltran N & Restrepo-Diaz H (2018). Application of foliar synthetic elicitors for the management of *Diaphorina citri* (Hemiptera: Liviidae) populations in Tahiti lime (*Citrus latifolia* Tanaka). HortScience 53: 1012-1020. https://doi.org/10.21273/HORTSCI13079-18

Fonseca S, Silva B R & Lobato A K (2020). 24-epibrassinolide positively modulate leaf structures, antioxidant system and photosynthetic machinery in rice under simulated acid rain. *Journal of Plant Growth Regulation* 4: 1559-1576. https://doi.org/10.1007/s00344-020-10167-4

Schlemmer M, Gitelson A A, Schepers J, Ferguson R B, Peng Y, Shanahan J & Rundquist D (2013). Remote estimation of nitrogen and chlorophyll contents in maize at leaf and canopy levels. *International Journal of Applied Earth Observation and Geoinformation* 25: 47-54. https://doi.org/10.1016/j.jag.2013.04.003

Song L X, Xu X C, Wang F N, Wang Y, Xia X J, Shi K, Zhou Y H, Zhou J & Yu J Q (2017). Brassinosteroids act as a positive regulator for resistance against root knot nematode involving respiratory burst oxidase homolog-dependent activation of MAPKs in tomato. Plant, Cell & Environment 41: 1113-1125. https://doi.org/10.1111/pce.12952

Stewart A, Chapman W, Jenkins G, Graham I, Martin T & Crozier A (2001). The effect of nitrogen and phosphorus deficiency on flavonol accumulation in plant tissues. Plant, Cell & Environment 24: 1189-1197. https://doi.org/10.1046/j.1365-3040.2001.00768.x

Triantaphylidés C, Krischke M, Hoeberichts F A, Ksas B, Gresser G, Havaux M, Breusegem F V & Mueller M J (2008). Singlet oxygen is the major reactive oxygen species involved in photooxidative damage to plants. Plant Physiology 148: 960-968. https://doi.org/10.1104/pp.108.125690

Xia X J, Huang L F, Zhou Y H, Mao, W H, Shi, K, Wu J X, Asami T, Chen Z & Yu J Q (2009). Brassinosteroids promote photosynthesis and growth by enhancing activation of Rubisco and expression of photosynthetic genes in *Cucumis sativus* L. Planta 230: 1185. https://doi.org/10.1007/s00425-009-1016-1

Voglar G E, Mrak T, Križman M, Jagodič A, Trdan S & Laznik Ž (2019). Effect of contaminated soil on multitrophic interactions in a terrestrial system. Plant and Soil 435: 337-351. DOI:10.1007/s11104-018-03903-z

Yang P, Nawaz M A, Li F, Bai L & Li J (2019). Brassinosteroids regulate antioxidant system and protect chloroplast ultrastructure of autotoxicity-stressed cucumber (*Cucumis sativus* L.) seedlings. Agronomy 9: 1-15. https://doi.org/10.3390/agronomy9050265

Zhang D W, Deng X G, Fu F Q & Lin H H (2015). Induction of plant virus defense response by brassinosteroids and brassinosteroid signaling in *Arabidopsis thaliana*. Planta 241: 875-885. DOI: 10.1007/s00425-014-2218-8

Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

Determination of Pneumatic Conveying Characteristics of Canola Seeds

Refi Ratip ÖZLÜ[a], Metin GÜNER[b*]

[a]*Republic of Turkey Ministry of Agriculture and Forestry General Directorate of Plant Production, Dumlupinar Boulevard, 06800, Çankaya, Ankara, TURKEY*
[b]*Ankara University, Faculty of Agriculture, Department of Agricultural Machinery and Technologies Engineering, Diskapi, 06110, Ankara, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Metin GÜNER, E-mail: mguner@ankara.edu.tr
Received: 12 September 2020 / Revised: 17 November 2021 / Accepted: 19 November 2021 / Online: 15 October 2022

Cite this article
ÖZLÜ R R, GÜNER M (2022). Determination of Pneumatic Conveying Characteristics of Canola Seeds. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 28(4):656-665. DOI: 10.15832/ankutbd.794097

ABSTRACT
In this study, it was aimed to determine the pneumatic conveying characteristics of canola seeds (*Brasicca napus Oleifera sp*). The physical properties, pressure drop, air velocity, power consumption characteristics of Turan variety canola seeds were investigated. Three different canola moisture (6.36%, 16.54% and 25.94%), three different pipe diameters (43.1, 54.5 and 70.3 mm), three different material feed flow rates (3.89, 5.47 and 7.49 t/h) and five different air velocities (5 compressor cycles of 850, 1150, 1450, 1750 and 2250 min^{-1}) were used and the effects of these factors on the pneumatic conveying characteristics of the canola seeds were examined. The pneumatic conveying of canola seeds was carried out at the positive pressure pneumatic conveyor. The pressure drop and power consumption have increased with the increase of air velocity. The lowest pressure drop and power consumption values were obtained in conveying with pipe diameter of 70.3 mm, canola seed moisture of 16.54%, compressor speed of 1150 rpm and conveying capacity of 3.89 t/h. The lowest power consumption was achieved with the conveying of the canola seed at 6.36% moisture, 7.49 t/h material feed flow rate and 1150 rpm compressor speed. In trials, the pressure drop decreased when the pipe diameter was increased.

Keywords: Conveying, Canola seed, Pneumatic, Pressure drop, Physical properties

1. Introduction

Canola (*Brasicca napus Oleifera sp*) forms a group of plants (formerly known as a member of the Cruciferae family) belonging to the Brasscicaceae family. Canola is a special name for a rapeseed variety that edible oil can be produced from its seeds and has been genetically modified by classical breeding methods for this purpose. Canola has high oil content and high adhesion probability. Pneumatic conveying is defined as pulling in the granular material to be transmitted along a transmission pipeline, hanging it in the air and conveying it. It is preferred for its reasons such as being flexible, no transmission loss, being suitable for automation, ensuring continuous flow.

Davison et al. (1975) stated that in case of exposure to any external load, information about the mechanical behavior of canola is required in the design of canola grinding and crushing machines, especially in the selection of grinding drum surfaces. Çalışır et al. (2005) determined some physical properties of canola seed grains such as size, geometric mean diameter, sphericity, 1000 grain weight, bulk density, terminal velocity, projection area and porosity. Razavi et al. (2009) determined the physical properties of four common Iranian varieties of canola seeds (Hyola, Okapi, Orient and SLM) such as average seed length, average diameter, geometric mean diameter, sphericity, bulk density, angles of repose, static coefficient of friction, thousand seed mass as a function of their moisture contents. İzli et al. (2009) investigated the physical and mechanical properties of three common varieties of rapeseed as a function of seed moisture content varying from 8.3 to 25.9%, from 7.7 to 27.4%, and from 7.3 to 26.4% (d.b.) for cv. Capitol, Jetneuf and Samurai, respectively. Increasing moisture content was found to increase the length, diameter, geometric mean diameter, sphericity, seed volume, surface area, thousand grain weight, porosity, angle of repose and terminal velocity, and static friction coefficient on six structural surfaces, while decreasing bulk density, true density and rupture strength. Kord et al. (2016) reported that canola stems may be an alternative source of raw materials for the particle board industry. Barekati et al. (2019) studied the effects of sowing dates and humic acid foliar application on canola cultivars seed yield and yield components.

The aim of this study is to determine the pneumatic conveying characteristics of canola seeds. Depending on the moisture change of the canola seed, physical properties such as specific mass, porosity, and conveying characteristics such as air velocity, pressure drop, and power consumption have also been determined.

2. Material and Methods

2.1. Material

In this study, Turan variety canola seeds (*Brasicca napus Oleifera sp*) were used. The pneumatic conveying of canola seeds was carried out at the positive pressure pneumatic conveyor shown in Figure 1.

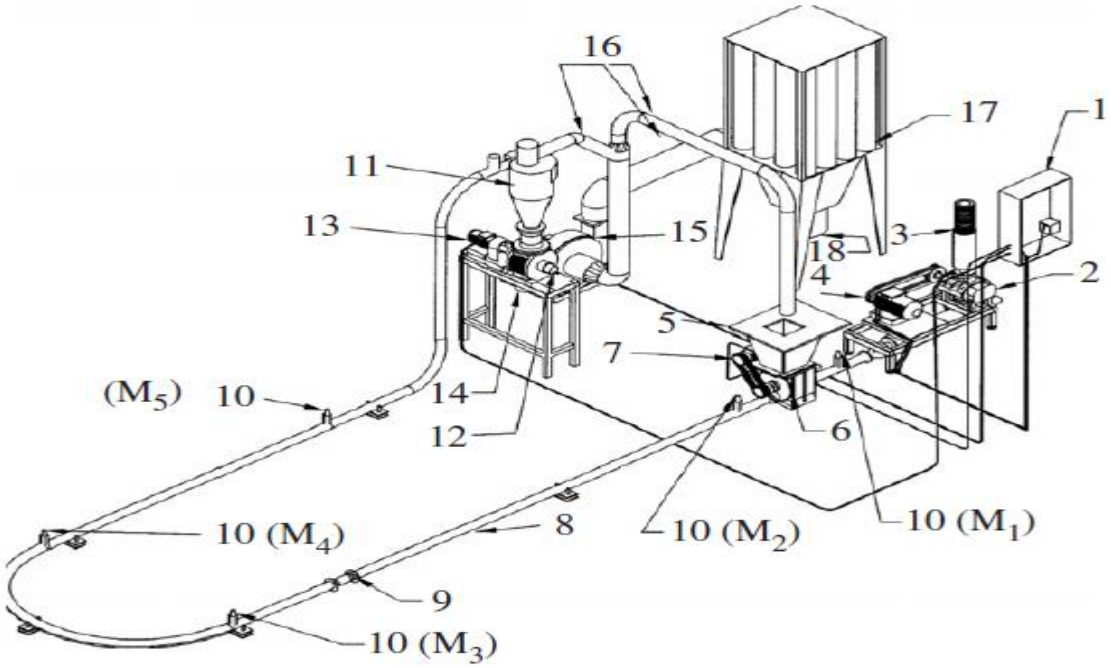


Figure 1- The general arrangement of the positive pressure pneumatic conveyor: 1: control unit; 2: roots blower; 3: air inlet filter; 4: roots blower motor; 5: seed hopper; 6: rotary feeder or hopper airlock feeder; 7: rotary feeder motor; 8: seed pipe; 9: transparent tube; 10: pressure drop measurement (M) tappings; 11: cyclone separator; 12: cyclone separator airlock; 13: cyclone separator airlock motor; 14: seed discharge; 15: vacuum blower; 16: dust pipes; 17: dust collector or exhaust air filter; 18: light foreign materials (Güner 2006).

In the measurement and analysis, 0.01 g precision electronic precision scale, 0.01 mm precision electronic digital caliper, 0.1 ml precision graduated cylinder was used to determine the volume weight and specific mass (actual density) of the material. Standard oven was used for moisture determination. Liquid toluene (C7H8) was preferred as the displacement material for volume measurement in specific mass measurements. Distilled water as conditioning and moistening material, lockable nylon bags are used to group conditioned and moistened canola seeds and keep them at desired moisture levels. During the trials and measurements, a home-type refrigerator was used to store the trial material at a constant temperature of +4 °C. Digital image processing and analysis software ImageJ / Fiji 1.47v and Myriad v8.0 softwares were used to determine projection areas. The wind tunnel device and the speed meter (anemometer) with a sensitivity of 0.1 m/s were used in the measurement of terminal velocity. The roots blower (LT–65 type, max. 80 kPa, max. 2250 min-1 produced by UKA, Konya, Turkey) was used to deliver air through the system.

2.2. Methods

In the research, 3 moisture values (6.36%, 16.54% and 25.94%) were used. The natural moisture content of canola seeds was measured as 6.36%. To increase the moisture of canola seeds to two other moisture values, water was added to the canola seeds according to the formula below.

$$Q = \frac{W_i(N_f - N_i)}{(100 - N_f)} \quad (1)$$

Where; Q, amount of water to be added (kg); Wi, the initial mass of the material (kg); Ni, the initial moisture content of the material (% w.b.); Nf, the final moisture content of the material after water addition (% w.b.).

In the moistening of canola seeds, after adding water to the seeds, seeds were mixed regularly at a constant temperature of +4 °C three times a day. This process was continued for two weeks. At the end of the second week, moisture was measured. In order to determine the physicomechanical properties of canola seeds, three replicate measurements were made at each moisture level.

The following formulas were used to determine the physical properties of canola grains (Mohsenin 1986; Sitkei 1986; Çalışır et al. 2005; İzli et al. 2009; Razavi et al. 2009).

Arithmetic mean diameter: $D_a = \frac{(L+2T)}{3}$ (2)

Geometric mean diameter: $D_g = (LT^2)^{1/3}$ (3)

Grain volume: $V_k = \frac{\pi L T^2}{6}$ (4)

Projection area: $S_a = \pi(\frac{T}{2})^2 \ [xz - plane]$ (5)

$S_a = \pi\left(\frac{L}{2}\right)(\frac{T}{2})^2 \ [xy \text{ ve } yz - planes]$

Sphericity: $\emptyset = (LT^2)^{1/3}L$ (6)

Flatness: $\varphi = 1 - \frac{T}{L}$ (7)

Where; L, length (mm); T, thickness (mm); S_a, projection area (mm^2); D_g, geometric mean diameter (mm); D_a, arithmetic mean diameter (mm); $\emptyset$, equivalent sphere diameter sphericity (%); φ, flatness; V_k, grain volume (mm^3). Since the thickness (T) of the canola in the measurements shows very close values with its width (W), the thickness dimension was accepted as equivalent to the width dimension (T = W).

The true density (ρ_t) is determined by the ratio of grain mass to the volume of the grain without voids (Mohsenin 1986; Sitkei 1986). Toluene (C_7H_8) (was used instead of water in determining the volume of the grain. Bulk density (ρ_b) was obtained by dividing the natural (void) volume of the material by the weight of the material. The following equation was used in the calculation of the equivalent sphere diameter (D_e) (Gorial & O'Callaghan 1990).

$D_e = \left[(\frac{M_d}{\rho_t})(\frac{6}{\pi})\right]^{1/3}$ (8)

Where; M_d, mass of canola seed (kg); ρ_t, true density of canola seed (kg m^{-3}), Porosity was found using the following equation.

$\varepsilon = 100(1 - \frac{\rho_b}{\rho_t})$ (9)

Where; ρ_b, bulk density of canola seed (kg m^{-3}); ρ_t, true density of canola seed (kg m^{-3})

The wind tunnel created by using the pneumatic conveyor was used to measure terminal velocities of canola grains and a digital anemometer with a speed measurement accuracy of 0.1 m s^{-1} was used. The drag coefficient of the canola seed's aerodynamic properties was calculated using the equations given below.

$C_d = \frac{2F_d}{S_a V_t^2 \rho_a} = \frac{2G_d}{S_a V_t^2 \rho_a}$ (10)

$G_d = M_d . g$ (11)

$F_d = G_d$ (12)

Where; S_a, projection area of the grain (mm^2); Vt, terminal velocity (m s^{-1}); ρa, density of air (1.28 kg m^{-3}); Gd, seed weight (N); Fd, seed drag force (N); Md, seed mass (kg); g, gravity acceleration (9.81 m s^{-2}).

Pressure transmitters (model: HUBA 0-1600 mb) were used to determine the pressure drops in the pipe. The measured values observed on DIN-Vscreen of ESM-400 48x90 device. Trials were carried out at 3 different humidity (6.36%, 16.54 and 25.94), 5 compressor cycles (850, 1150, 1450, 1750 and 2250 min^{-1}), 3 different pipe diameters (43.1, 54.5 and 70.3 mm) and 3 different material flows (3.89, 5.47 and 7.49 t h^{-1}). The moisture values of canola seed have been chosen considering the storage humidity of the canola seed. Different compressor speeds have been selected by considering the conveying velocity of the canola seed. Pipe diameters were chosen according to Güner (2006).

The trials were made with 3 replications. The difference of the pressure values before the feeder (M1) and before the cyclone (M5) at the blower outlet in the pneumatic conveyor is taken as the total pressure drop value. The air velocity in the pneumatic system was calculated from the following equation using the air flow values given in the catalogue of Longtech brand LT-65 type roots blower compressor (Güner 2006).

$$V_a = \frac{4Q_h}{60\,\pi D^2} \quad (14)$$

Where; Va , air velocity (m s^{-1}); Qh, air flow rate (m^3 min^{-1}); D, pipe diameter (m)

Seamless pipes made of St 37 steel material with 43.1 mm, 54.5 mm and 70.3 mm inner diameters were used as the conveying line in the trials. Power consumption has been found with the help of the following equation.

$$N = \sqrt{3}U.I.Cos\varphi \quad (15)$$

Where; N, power (W); U, voltage (V); I, current (A); Cos φ, power factor

In this study, two of the image processing software tools were used to determine the surface projection areas of canola seeds. The first of the computer aided image processing software tools is Myriad v8.0 digital image processing package. Along with this package, the complementary equipment specified below was also used to capture images of 30 grains randomly selected from material samples in each moisture content. Supplementary equipment used are Nikon D40X Model 10.1 megapixel SLR digital camera, appropriate size scale calibration plate, and Myriad v8.0 digital image processing software. Another computer-aided image processing software tool used in the study was ImageJ / Fiji 1.47v. Once again, numerical images of 30 canola seed grains randomly selected from each moisture content sample batch were taken using the appropriate sized scale calibration plate and digital camera. Subsequently, digital images of all seed grain taken with a digital camera; Transferred and processed for processing to a computer installed with ImageJ / Fiji 1.47v software. Minitab Statistical Software was used to evaluate the results statistically (MINITAB 2018).

3. Results and discussion

3.1. Physical properties of canola seeds

Results related to the physical properties of canola seeds in three different moisture content are given in Table 1.

Table 1- Physical properties of canola seeds

Physical properties	*Moisture contents (wet basis, %)*		
	6.36	*16.54*	*25.94*
Dimensional properties (mm)			
Length	2.14±0.011A**	2.195±0.009A	2.271±0.011B
Thickness = Width	1.83±0.0097B**	1.952±0.0079A	2.007±0.0005A
Arithmetic mean diameter	1.93±0.0086C**	2.033±0.0073B	2.095±0.0080A
Geometric mean diameter	1.928±0,0087C**	2.03±0.0074B	2.09±0.0080A
Equivalent sphere diameter	1.05	1.949	2.034
Mass properties			
Thousand grain mass (g)	3.81±0.012C**	4.195±0.038B	4.697±0.072A
Bulk density (kg/m^3)	643±0.0176A**	621.2±2.06B	597.8±0.521C
True density (kg/m^3)	1041.66±0.887C**	1064±0.263B	1075.2±0.658A
Seed (grain) volume (mm^3/seed)	3.75±0.0504C**	4.379±0.0452B	4.79±0.0531A
Sedd(grain) mass (g)	0.00381	0.004168	0.004688
Shapes properties			
Sphericity(%)	0.91±0.0034C**	0.924±0.0025A	0.921±0.0029B
Flatness (%)	0.145±0.0048A**	0.111±0.0036C	0.116±0.0042B
Porosity(%)	38.27	42.22	44.40
Projection area (mm^2)–[x–plane]-from equation	3.597±0.0375A**	3.784±0.0317A	4.05±0.0375B
Projection area (mm^2)–[y; z–planes] –from equations	3.076±0.0269C**	3.365±0.0236B	3.579±0.0269A
Projection area (mm^2)-ImageJ 1.47v from package program	3.187	3.480	3.614
Projection area (mm^2)-Myriad v8.0 from package program	2.430±0.0005 C**	3.100±0.0006 B	3.380±0.0007A
Aerodynamic properties			
Terminal velocity (m/s) (Özlü and Güner 2016)	5.8336±0.0674C**	6.8773±0.0472B	7.3064±0.0619 A
Drag coefficient	0.5418	0.4701	0.4030

**: The differences between the values given in the same lines with the same letters are statistically insignificant (P≤0.001)

From the findings in Table 1, it is seen that the length, width, arithmetic and geometric mean diameters increase with moisture content. Duc et al. (2008 determined that the geometric mean diameter in canola increases with moisture content. Ünal & Sincik (2009) found in their study with 3 different canola varieties (Capitol, Jetneuf and Samurai) that as the moisture increases, the length, thickness, geometric mean diameter and thickness values increase. They reported that longitudinal dimension, thickness (or diameter) and geometric mean diameter for 7.3%, 7.7%, and 8.3% d.b are 2.25 mm, 1.82 mm, 1.96 mm; 2.26 mm, 1.85 mm, 1.98 mm and 2.46 mm, 1.96 mm, 2.11 mm, respectively.

Işık & İzli (2016) reported that the thickness, diameter, arithmetic and geometric diameter increased in the yellow split lentil as the moisture increased at 5 different moisture values. Baran et al. (2016) found that length, width and mean geometric diameter values in canola with 8.34% moisture content as 2.35 mm, 1.93 mm and 2.07 mm, respectively. In the experiments, while the seed moisture content increased from 6.36% to 25.94%, it was observed that the weight of 1000 grains varied between 3.81 g, 4.195 g and 4.697 g, respectively, and it was determined that the weight of one thousand grain increased with moisture. As the moisture content increased, the weight of 1000 grains increased due to the canola grains absorb water and their weight increased. The following relationship was found between 1000 grain weight and moisture.

$$M_{1000d} = 0.0033 + 0.0004M_c$$

Where; M_{1000d}, thousand grain mass (g); M_c, moisture content (%, w.b.)

Similar results for thousand grain weights were recorded for canola, moringa oleifera, green wheat, soybeans, and chickpeas by other researchers (Aviara et al. 2013; Al-Manash & Rababah 2007; Duc et al. 2008; Ünal & Sincik 2009). The seed volumes of canola seeds increased with increasing moisture and similar results were found by Ünal and Sincik (2009). As the moisture content increased, the bulk volume of canola seeds decreased and their true density increased. However, if the moisture content increased to 25.94%, the increase in the true density was observed to be relatively slow.

While the moisture content of canola seed (w.b.) increased from 6.36% to 16.54%, the sphericity increased from 91.00% to 92.40%, but it was found 92.10% when the moisture content increased to 25.94%. Similar results were reported for barley by Sologubik et al. (2013) and for Okra by Sahoo & Srivastava (2002). Duc et al. (2008) reported that the sphericity decreased from 0.946 to 0.927 with the increase in moisture content. Ünal and Sıncık (2009) gave the sphericity values of canola 86.1%, 87.4% and 86.8% for 8.3%, 7.7% and 7.3% (db) of moisture content, respectively. Işık & İzli (2016) found that the sphericity increased with the increase of moisture in the yellow split lentil. Baran et al. (2016) determined the sphericity of canola as 88% for 8.34% moisture content. The sphericity of canola seeds on the contrary of flatness, firstly decreased and then increased as the moisture content increased. If the moisture content increased from 6.36% to 16.54%, the porosity increased from 38.27% to 41.62%. On the other hand, with the increase of moisture content from 16.54% to 25.94%, porosity increased to 44.40% with an increase of 2.78%. The relationship between moisture content and porosity has been determined as follows. According to this relationship, there is a polynomial relationship between moisture and porosity.

$$\varepsilon = 34.64 + 0.6437M_c - 0.0111M_c^2 \quad R^2 = 99.7$$

Where; ε, porosity (%); M_c, moisture content (%, w.b.)

Damian (2014) found that there is a polynomial relationship between the porosity and moisture contents of mustard seeds. Ünal and Sıncık (2009) found porosity values of 36.6%, 41.5% and 38.4% of canola seeds in 8.3%, 7.7% and 7.3% moisture contents, respectively. As the moisture content increased, projection areas increased in the XZ, XY and XZ-planes.

While the moisture content values were 6.36%, 16.54% and 25.94%, the terminal velocities of canola seeds were 5.8336 m s^{-1}, 6.8773 m s^{-1} and 7.3064 m s^{-1}, respectively, and terminal velocities increased with the moisture content (Özlü & Güner 2016). Duc et al. (2008) found that terminal velocities increased from 3.47 ms^{-1} to 3.91 ms^{-1} with moisture (10.03%, 14.91, 20.07, 25.06 and 30.12) in canola seeds. Kroulik et al. (2016) found the terminal velocity value for canola in 7.65 ms^{-1} at a moisture content of 8.3% (w.b.) in a study.

Bilanski et al. (1962) gave the terminal velocity of the canola as 7.62 ms^{-1}. It has been determined that terminal velocity increases as the moisture content increases in apricot kernels, chickpeas and hemp seed (Gezer et al. 2002; Konak et al. 2002; Saçılık et al. 2003). The drag coefficient (C_d) was 0.5418, 0.4701 and 0.4030 at 6.36%, 16.54% and 25.94% moisture contents, respectively. The drag coefficient decreased as the moisture content increased. The relationship between drag coefficient and moisture content was found as follows.

$$C_d = 0.58703 - 0.00709M_c \quad R^2 = 0.99$$

Where; C_d, drag coefficient, M_c, moisture content (%, d.b)

3.2. Conveying characteristics of canola seeds

The pneumatic conveying characteristics of the canola seed, which are obtained in three different moisture contents, 3 different pipe diameters, 3 different feed flow rates and 5 different air velocities, can be examined as follows.

3.2.1. The effect of pipe diameter on pressure drop

Pressure drop measurements at 16.54% and 25.9% moisture content could not be done with 43.1 mm and 54.5 mm diameter pipes due to clumping, jamming and blockages. The pressure drops of the canola with 6.36% moisture content measured in different pipe diameters at a conveying capacity of 3.89 t h^{-1} are shown in Figure 2. It is seen that the pressure drop decreases as the pipe diameter increases and air velocity decreases. Kılıçkan & Güner (2006) found that pressure drop decreased with increasing pipe diameter for cotton seed of Gülerbey and Nazilli 84 S varieties at a given conveying capacity and air velocity. Kılıçkan & Güner (2010) reported that pressure drop for pneumatic conveying of chickpea of the variety *Koçbasi* decreases as the pipe diameter increases at a constant conveying capacity and revolution of the blower.

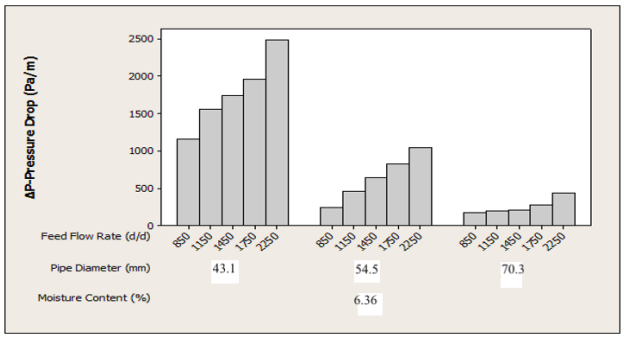


Figure 2- Effect of air velocity and pipe diameter on pressure drop at a moisture content of 6.36% and at a conveying capacity of 3.89 th^{-1}

3.2.2. Effect of conveying capacity on pressure drop

While examining the effect of conveying capacity on pressure drop; air velocity, pipe diameter and moisture content values were kept constant and pressure drops were determined due to capacity change (Figures 3-5). When the graphs are examined, it is seen that the pressure drop increases as the conveying capacity increases. Some other researcher found that the pressure drop increased as the conveying capacity increased for barley, sunflower, lentil, wheat, chickpea, and cotton seed (Güner 2006, Kılıçkan & Güner 2006; Kılıçkan & Güner 2010). When Figure 3 is examined the feed flow rate is 5.47% t/h, as the air velocity increases, the pressure drop also increases, but the tendency to increase is less than the other 2 values. This may be due to the fact that the pneumatic transmission depends on many factors such as the delivery air temperature, humidity, condition of the material, feeding of the material and the like.

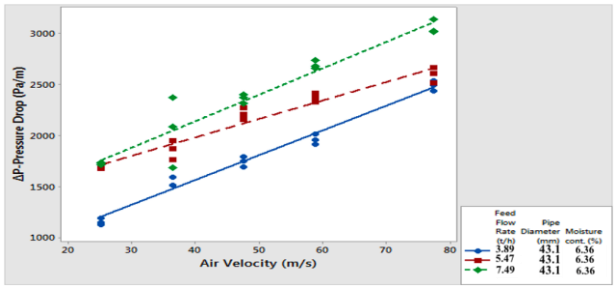


Figure 3- Pressure drop values for a pipe diameter of 43.1 mm, a moisture content of 6.36%, 5 different air velocities, and 3 different feed flow rates

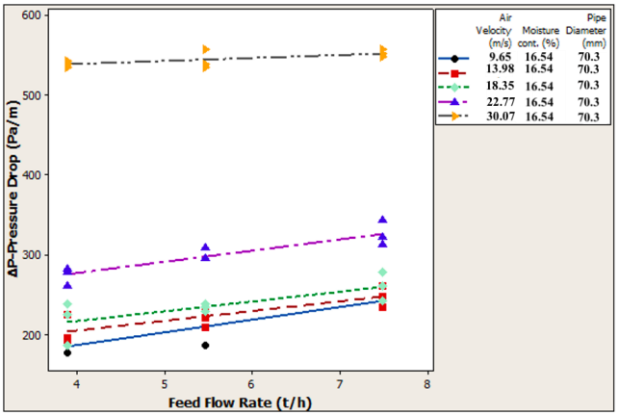


Figure 4- Pressure drop values for a pipe diameter of 70.3 mm, a moisture content of 16.54%, 5 different air velocities, and 3 different feed flow rates

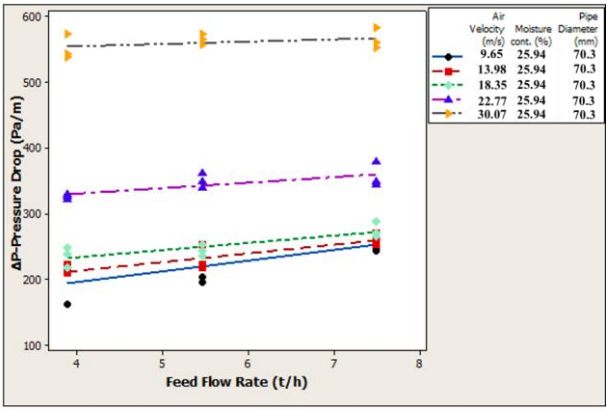
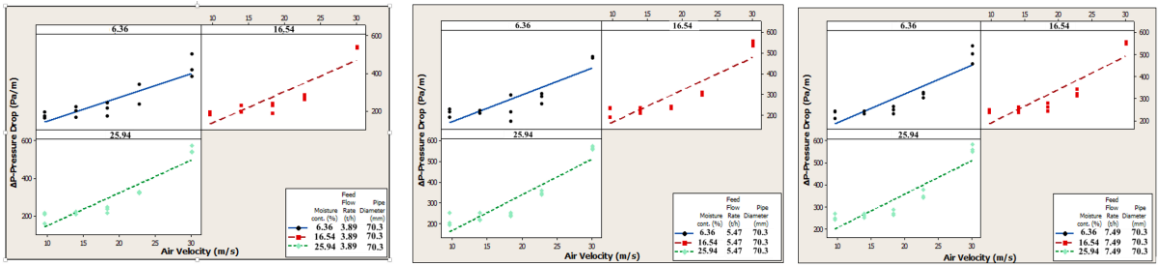


Figure 5- Pressure drop values for a pipe diameter of 70.3 mm, a moisture content of 25.94%, 5 different air velocities, and 3 different feed flow rates

3.2.3. Moisture content and pressure drop relationships

No pressure losses could be determined at 16.54% and 25.94% humidity values and 43.1 mm and 54.5 mm pipe diameters due to clumping and clogging. Therefore, trials could not be done. Pressure drops for a pipe diameter of 70.3 mm and 3 different moisture contents is given in Figure 6. Pressure drops for a pipe diameter of 70.3 mm increased as moisture content of canola seed increased.

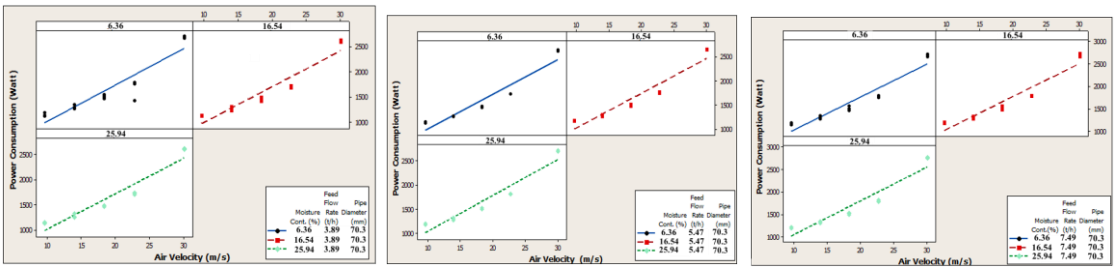


(a) (b) (c)

Figure 6- Pressure drops for a pipe diameter of 70.3 mm, 3 different moisture contents, 3 different feed flow rates, and 5 different air velocities

3.2.4. Air velocities and power consumption relationships in material conveying

Power consumption (N) of canola seeds for a diameter pipes of 70.3 mm, 3 different moisture contents, and 5 different air velocities are given in Figure 7. Power consumptions increase as air velocities increase. Güner and Kılıçkan found that the power consumption increased as the air velocities increased for barley, sunflower, lentil, wheat, chickpea, and cotton seed (Güner 2006, Kılıçkan &Güner 2006; Kılıçkan & Güner 2010).

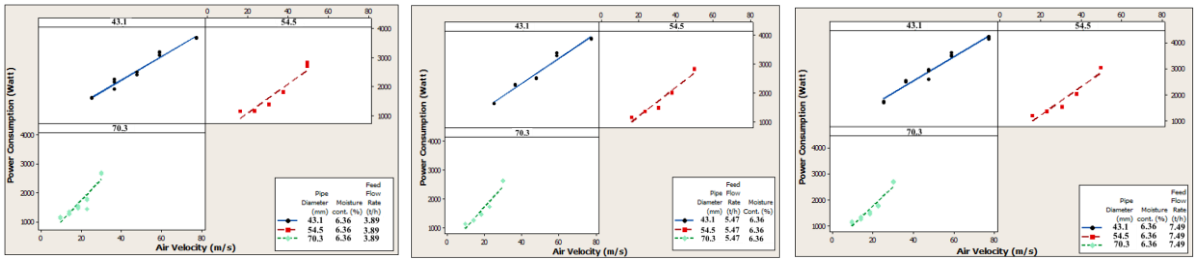


(a) (b) (c)

Figure 7- Power consumption for a diameter pipes of 70.3 mm, 3 different moisture contents,3 different feed flow rates, and 5 different air velocities [a: Q_m=3.89 t/h, b: Q_m=5.47 t/h, c: Q_m=7.49 t/h]

3.2.5. Pipe diameter, air velocity, and power consumption relationships

The relations between air velocity, feed flow rate (conveying capacity), pipe diameter and power consumption are given in Figure 8 when the moisture content is 6.36%. While the moisture content was 6.36%, the power consumption decreased as the pipe diameter increased. The reduction in power consumption was less in pipes with a diameter of 54.5 mm and pipes with a diameter of 70.3 mm. Kılıçkan and Güner (2010) found that the power decreased as the pipe diameter increased in the pneumatic conveying of chickpeas. They reported that large diameter pipes should be selected for low power consumption. The results of Kılıçkan and Güner (2010) overlap with our results. As the pipe diameter increases, the friction loss (pressure drop) decreases and this decreases the power consumption. It is an expected result.

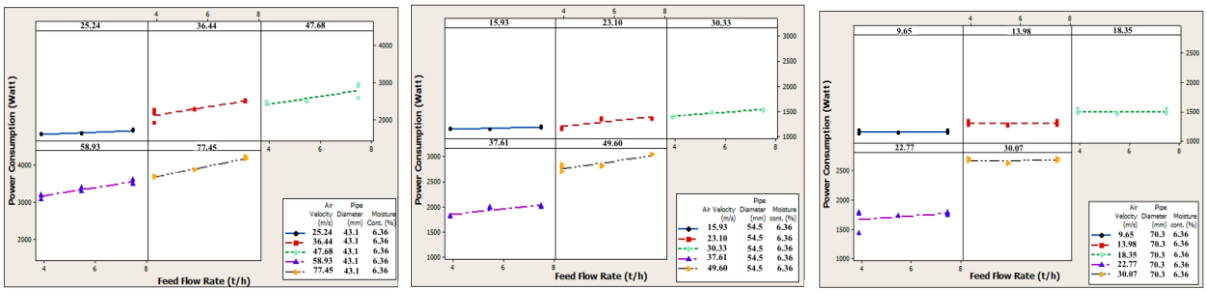


(a) (b) (c)

Figure 8- Power consumptions depending on pipe diameter, conveying capacity and air velocity under material moisture content of 6.36% [a: Q_m=3.89 t/h, b: Q_m=5.47 t/h, c: Q_m=7.49 t/h]

3.2.6. Feed flow rates (conveying capacity) and power consumption relationships

While the pipe diameters are constant, power consumption for 5 different air velocities, a moisture content of 6.36%, and feed flow rates of 3.89 th^{-1}, 5.47 th^{-1} and 7.49 th^{-1} is given in Figure 9. According to the data in the graph, the power consumption increases as feed flow rate increases. This is normal because the higher the feed flow rate, the more resistance the material applies to the airflow.



(a) (b) (c)

Figure 9- Power consumption for a moisture content of 6.36%, 3 different diameter pipes, 3 different feed flow rates, and 5 different air velocities [a: D=43.1 mm, b: D=54.5 mm, c: D=70.3 mm]

4. Conclusions

The following results can be listed on the pneumatic conveying characteristics of the Turan variety canola seeds depending on the moisture content.

1. Physico mechanical properties such as length, thickness, width, arithmetic and geometric mean diameter, projection area, volume, sphericity, flatness, equivalent sphere diameters, terminal velocity, porosity increase as the moisture content increases. The effect of moisture content on physical properties was found significant ($P \leq 0.001$).

2. The results of the projection areas found by different methods (ImageJ / Fiji 1.47v and Myriad v8.0 software) were found close to each other and this result led to the conclusion that these methods can be used in small size and soft textured materials.

3. Adhesion, clustering and jamming occurred in the conveying of the canola seed with moisture content values of 16.54% and 25.94% for pipes of 40.3 mm and 54.5 mm diameter, and therefore measurements could not be made. The reason why the canola seed cannot be transported under these conditions is the large pipe diameter and high moisture content of the canola seed. The high moisture content of the canola seed and the small diameter of the pipe led to sticking, clumping and high friction loss, which led to blockages. For this reason, material conveying trials at 16.54% and 25.94% moisture contents have been carried out with a pipe diameter of 70.3 mm and the tests have been evaluated over these outputs.

4. The increase in air velocity and feed flow rate increased pressure drop and power consumption. As the pipe diameter increased, pressure drop and power consumption decreased.

5. In order for the pressure drop to be the least, the air velocity should be chosen as small as possible and the pipe diameter should be chosen as large. In addition, care should be taken to keep the directional changes in the pneumatic system to a minimum.

6. Pneumatic conveying of materials such as canola seeds, which is high in oil content and high adhesion probability than natural storage moisture contents (such as 16.54% and 25.96%) is not efficient. It is recommended to use pipes of 70 mm and larger diameter in the transmission of the canola due to the low pressure drop and power consumption, and the absence of clogging and sticking.

Acknowledgments

This study was prepared based on the PhD thesis (Özlü 2019).

References

Al-Manash M A & Rababah T M (2007). Effect of Moisture Content on Some Physical Properties of Green Wheat. *Journal of Food Engineering* 79(4): 1467-1473. https://doi.org/10.1016/j.jfoodeng.2006.04.045

Aviara N A, Power P P & Abbas T (2013). Moisture Dependent Physical Properties of Moringa oleifera Seed Relevant in Bulk Handling and Mechanical Processing. Industrial Crops and Products 42: 96-104

Baran M F, Durgut M R, Aktaş T, Ülger P & Kayışoğlu B (2016). Determination of Some Physical Properties of Rapeseed. *International Journal of Engineering Technologies* 2(2): 49-55. https://doi.org/10.19072/ijet.65255

Barekati F, Hervan E M, Rad A H S, Mohamadi G N (2019). Effect of Sowing Date and Humic Acid Foliar Application on Yield and Yield Components of Canola Cultivars. *Tarım Bilimleri Dergisi-Journal of Agricultural Science* 25(3): 70-78. DOI: 10.15832/ankutbd.539003

Bilanski W K, Colins S H & Chiu C L (1962). The Behaviour of Seed Grains in a Vertical Wind Tunnel, Dept. of Engineering Science. Ontario Agricultural College. Guelph, Ontario. http://www.csbe-scgab.ca/docs/journal/5/5_1_29_raw.pdf/, (accessed 07 July 2017).

Çalışır S, Marakoğlu T, Öğüt T H & Öztürk Ö (2005). Physical Properties of Rapeseed (Brassica napus oleifera L.). Journal of Food Engineering 69(1): 61-66. DOI: 10.1016/j.jfoodeng.2004.07.010

Damian C (2014. Physical properties of mustard seeds (Sinapis alba L.). Lucrări Ştiinţifice - Seria Zootehnie 61: 39–44

Davison E, Middendorf F J & Bilanski W K (1975). Mechanical Properties of Rapeseed. Canadian Agricultural Engineering 17(1): 50-54

Duc L A, Han J W, Hong D J & Keum D H (2008). Physical Properties of Rapeseed (I) and (II). DOI: 10.5307/JBE.2008.33.2.101; DOI: 10.5307/JBE.2008.33.3.173.

Gezer I, Hacıseferoğulları H & Demir F (2002). Some physical properties of Hacıhaliloğlu apricot pit and its kernel. *Journal of Food Engineering* 56: 49-57. DOI: 10.1016/s0260-8774(02)00147-4

Gorial B Y & O'Callaghan J R (1990). Aerodynamic properties of grain/straw materials. *J. Agric. Eng. Res.* 46: 275-290. https://doi.org/10.1016/S0021-8634(05)80132-5

Güner M (2006). Determination of Pneumatic Conveying Characteristics of Some Agricultural Crops. TÜBİTAK Project, TOGTAG 3258

Işık E & İzli N (2016). Effects of Moisture Content on Some Physical Properties of Yellow Lentil. *Tarım Bilimleri Dergisi-Journal of Agricultural Sciences* 22: 307-316. https://doi.org/10.1501/Tarimbil_0000001389

İzli N, Ünal H & Sincik M (2009). Physical and Mechanical Properties of Rapeseed at Different Moisture Content. International Agrophysics 23: 137 -145

Kılıçkan A & Güner M (2006). Pneumatic conveying characteristics of cotton seeds. Biosystems Engineering 95(4):537-546. DOI 10.1016/j.biosystemseng.2006.08.015

Kılıçkan A & Güner M (2010). The determination of pneumatic conveying characteristics of chickpea. Turk. *J. Agric & Forestry* 34: 265-274. DOI: 10.3906/tar-0905-32

Konak M K, Çarman K & Aydın C (2002). Physical properties of chick pea seeds. Biosystems Engineering 82(1): 73-78. https://doi.org/10.1006/bioe.2002.0053

Kord B, Zare H & Hosseinzadeh A (2016).Evaluation of the mechanical and physical properties of particleboard manufactured from canola (brassica napus) straws. Maderas: Ciencia y Tecnologia 18(1): 9-18. http://dx.doi.org/10.4067/S0718-221X2016005000002

Kroulik M, Hůla J, Rybka A & Honzík I (2016). Pneumatic conveying characteristics of seeds in a vertical ascending airstream. Res. Agr. Eng. 62(2): 56-63. DOI: 10.17221/32/2014-RAE.

MINITAB 2018. https://www.minitab.com/en-us/products/minitab/free-trial/, (accessed 03 June 2018).

Özlü R R & Güner M (2016). Determination of Physical Properties of Canola Seeds at Different Moisture Levels (In Turkish). *Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi - Journal of Agricultural Faculty of Gaziosmanpasa University*, JAFAG ISSN:1300-291, E-ISSN: 2147-8848 (2016) 33 (Ek Sayı),pp.10-24

Özlü R R (2019). Determinatıon of Pneumatic Conveying Characteristics of Canola. PhD Thesis (Unpublished), Ankara University Institute of Natural and Applied Sciences Department of Agriculture Machinery and Technologies Engineering

Razavi S M A, Yeganehzad S & Sadeghi A (2009). Moisture Dependent Physical Properties of Canola Seeds. *Journal of Agricultural Science* Technology 11: 309- 322

Sacılık K, Ozturk R & Keskin R (2003). Some physical properties of hemp seed. Biosystems Engineering 86(2): 191-198. https://doi.org/10.1016/S1537-5110(03)00130-2

Sahoo P K & Srivastava A P (2002). Physical Properties of Okra Seeds. Biosystems Engineering 83: 441–448. https://doi.org/10.1006/bioe.2002.0129

Sologubik C A, Campanone L A, Pagano A M & Gely M C (2013). Effect of Moisture Content on Some Physical Properties of Barley. Industrial Crops and Products 43: 762-767. DOI:10.1016/j.indcrop.2012.08.109

Ünal H & Sıncık M (2009). Comparison of Some Engineering Properties of Rapeseed Cultivars. Industrial Crops and Products 30(1): 131-136. DOI: 10.1016/j.indcrop.2009.02.011

Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

Exploitable Potential of Biomass Energy in Electrical Energy Production in the Mediterranean Region of Turkey

Mehmet Emin BİLGİLİ [a]

[a] *Eastern Mediterranean Agricultural Research Institute, Karataş Road 17. Km Pbox: 45 01321 Adana, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Mehmet Emin BİLGİLİ, E-mail: eminbilgili@gmail.com
Received: 29 May 2021/ Revised: 23 November 2021 / Accepted: 24 November 2021 / Online: 15 October 2022

Cite this article
BİLGİLİ M E (2022). Exploitable Potential of Biomass Energy in Electrical Energy Production in the Mediterranean Region of Turkey. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 28(4):666-676. DOI: 10.15832/ankutbd.944680

ABSTRACT
Turkey is targeting to generate 30% of its total electricity production from renewable energy sources by 2023. The replacement of electrical energy in the Mediterranean Region of Turkey according to exploitable biomass data was determined in this study. Data from the Ministry of Energy and Natural Resources, the Ministry of Agriculture and Forestry, Turkish Statistical Institute, measurements and literature were used in the study. The technical potential of plant residues and animal-originated biomass in the region was analyzed. It is estimated that the existing plant and animal residues in Turkey correspond to 39% and 9%, respectively. These residues can meet 6.9% of the energy demand of the region, i.e. about 8.3 PJ/year. Employment can be established from the technologies to be used here. By generating electrical energy from biomass, 2 644 302 tons of CO_2/year can be reduced by generating electrical energy from biomass. The amount of biofertilizer that can be obtained after biogas production is 1 184 049 ton/year of biofertilizer can be obtained after biogas production. The calorific values measured to determine the energy values of agricultural residues were found to be between 13.0-20.7 MJ/kg.

Keywords: Renewable energy, Agricultural residues, Biomass potential, Energy substitution

1. Introduction

Although renewable energy sources (RES) have expanded rapidly in the last decade, it is pointed out that the share of fossil fuels in global energy demand is around 80% (GWEC 2018; IRENA 2018; Johnsson et al. 2018; REN21 2020; Afkari Sayyah et al. 2020). According to The Ministry of Energy and Natural Resources (MENR) in 2018, 85.5% of primary energy sources in Turkey were of fossil origin and 75.7% of them were imported. It is very important that to reduce foreign dependency by using domestic and renewable energy sources. Energy production technologies based on RES are environmentally friendly technologies that reduce foreign dependency.

The white document on RES prepared by the EU Commission in 1997 deems that achieving an increase of 8% between 2010-2020 and providing 20% of the electrical energy by RES as of 2020 is feasible (Brussels 2004). The main objective of the 2010-2014 strategic plan of the MENR is to achieve a level of 30% for RES energy generation in the total electricity supply as of 2023 in Turkey (MENR 2010).

Biomass is one of the promising alternatives in renewable energy sources to meet the future energy demand and reduce greenhouse gas emissions (Mao et al. 2015; Mao et al. 2018). Biomass includes all of the organic plant materials that are agricultural or forestry products and that can be used as fuel, in whole or in part, to recover energy (Atay & Ekinci 2020). Although Turkey has 78 million hectares of area, approximately 39.01 million hectares are used for agricultural applications. In addition, 5.8% of the national income is generated by agriculture (TURKSTAT 2019a). Approximately 50.01% of the total arable land in Turkey is used for agriculture, 10.7% is fallow land, fruit trees and vegetable fields cover 10.6% of the land while pastures and meadows comprise 37.9% of the total land (TURKSTAT 2019b). Turkey consists of seven geographical regions, each of which have different climate conditions as well as different agricultural activities, according to the geographical conditions and geographic area. The Mediterranean region enjoys a characteristically Mediterranean climate, with hot dry summers and moderately warm and rainy winters. Agriculture, tourism, animal husbandry, mining, industry, and forestry are the main economic activities in the Mediterranean Region. The economy of the region is looking for new energy sources to fill the energy deficit for the developing industry and expand. Biomass is the most important renewable energy source that can be used in the region. Direct burning of agricultural residues with the traditional method has negative effects on soil and air quality. In addition, it can be said that the biggest problems in agricultural residues are their high moisture content and low bulk density.

Biofuel, bioelectricity, and bioproducts can be generated from agricultural residues obtained from agricultural and livestock activities by using biomass energy technologies (NREL 2017). Biomass conversion technologies (combustion, co-firing, gasification, pyrolysis, CHP, Etherification/pressing, fermentation/hydrolysis, anaerobic digestion, etc.) were explained by Gokcol et al. (2009). Turkey meets about 37% of its total energy from primary domestic sources out of which 57% are biomass-based fuels (Demirbaş et al. 2001; Sürmen 2003; Balat 2004). The amount of agricultural residues is reported as 467-623 PJ/year and this value is equivalent to 22-27% of Turkey's energy consumption (Kar & Tekeli 2008).

A potential waste map was prepared for all provinces in Turkey (Avcıoğlu & Türker 2012; Aybek et al. 2015). The number of cattle, small ruminants, and poultry, as well as the amount of biogas production based on various criteria were calculated. According to the 2009 agricultural information, Turkey's annual biogas energy potential was determined as 2.18 billion m^3. It is estimated that the biogas energy balance based on the total biogas potential obtained from 68% cattle, 5% small ruminants and 27% poultry was approximately 49 PJ (Bilgen et al. 2015; Yelmen & Çakir 2016), recent studies have outlined a perspective for the biomass potential and technologies in Turkey. The studies by Saka & Yılmaz (2017) on the biomass potential and the situation in Turkey showed that the current annual plant residue was $142.4x10^6$ ton. According to BP (2018), Turkey's total annual energy consumption for 2017 was 6 623 PJ. It was estimated that 995 PJ of this consumption took place in the Mediterranean Region. It is estimated that Turkey's potential amount of waste biomass was approximately 8.6 Mtoe and that the amount of producible biogas was 1.5-2 Mtoe (WEB1 2019). The total dry agricultural residues potential of Turkey in 2019 was approximately $256x10^6$ ton which is equal to 8.29 Mtoe of raw fuel in value (BEPA 2020).

In terms of the importance and the future of renewable energy in Turkey, the target of MENR for 2023 is to generate 34 000 MW of hydroelectric energy, 20 000 MW of wind energy, 5 000 MW of solar energy, 1 000 MW of geothermal energy and 1 000 MW of biomass energy. In short, it is predicted that approximately one third (1/3) of the total electricity consumed in 2023 will be generated from RES (MENR 2010).

This study aims to technically find out the agricultural biomass potential in the research area of the Eastern Mediterranean Agricultural Research Institute, Adana, Turkey. Subsequently, the obtained results were used to calculate electrical energy production from these wastes in the Mediterranean. Plant and animal biomass potentials, types and quantities for the provinces in the region and their use for energy purposes were evaluated using the data for the region. In addition to the technologies to be deployed in the region, the potential contribution of biomass to the regional energy consumption was determined. These technologies are expected to contribute the creation of new employment areas, reducing CO_2 emissions, and use of biofertilizers instead of chemical fertilizers in agricultural areas.

2. Material and Methods

The study area is in the south of Turkey, situated between the Taurus Mountains and the Mediterranean Sea (Figure 1). The coordinates of the location are between 36° 32′ 33″ - 34° 34′ 11″ North latitudes and 29° 57′ 38″-36° 54′ 59″ East longitudes, and the region covers eight provinces. The area covers around 89 493 km^2 which is approximately 14% of the total area of Turkey. About 10% of the area consists of agricultural land while the region is populated by 13% of the population. The average temperature for January in the region was 6.4 °C, while the average temperature for July which was the hottest month is 26.8 °C, the annual mean temperature is 16.3 °C and the annual mean relative humidity is 63.2%. While the mean annual total precipitation was 725.9 mm, the share of the summer precipitation was 5.7%. Therefore, summer drought dominants in the region (Sensoy et al. 2008).

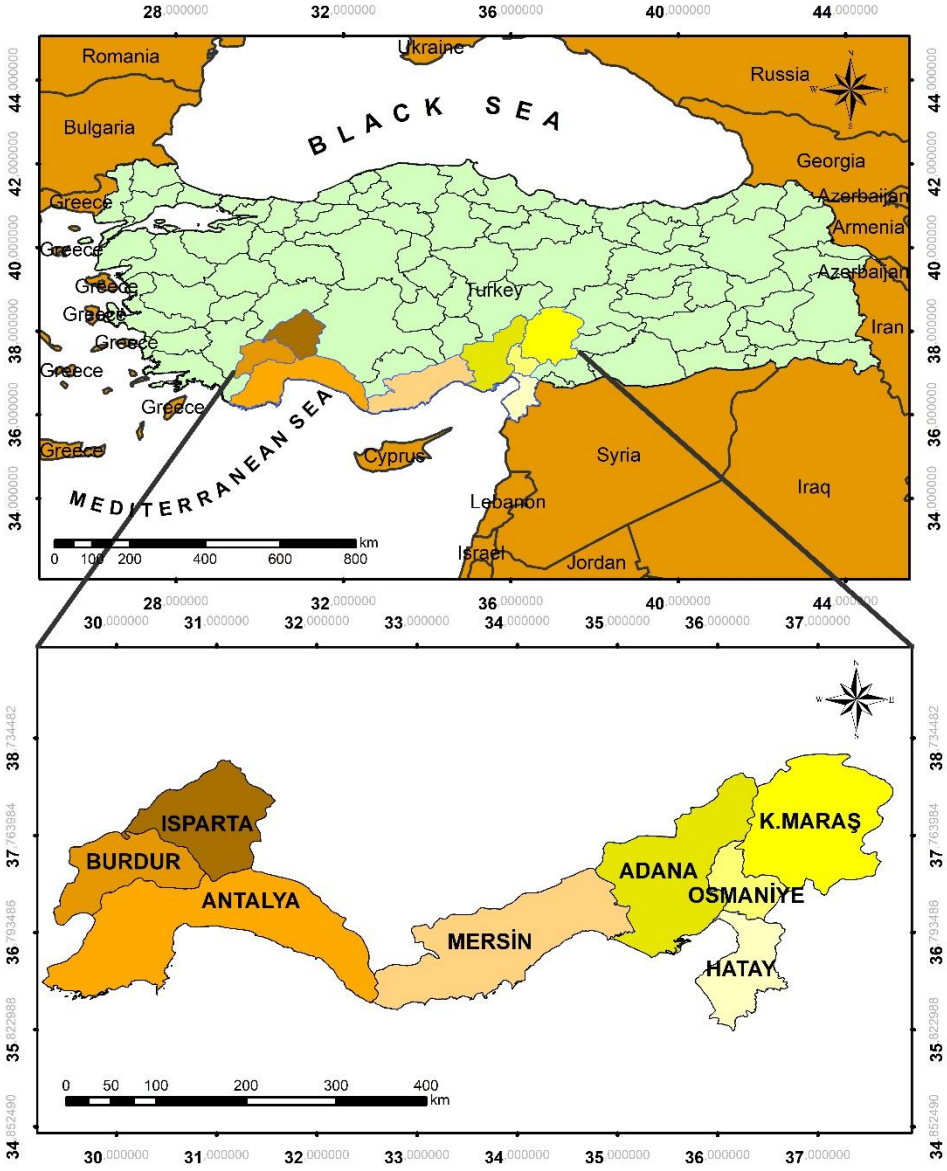


Figure 1- The Mediterranean Region

The region has an important agricultural economy and a share of more than 50% on a national basis in terms of arable land, vineyards-orchards, vegetable fields, and production. Greenhouse production is practiced the most in the Mediterranean Region in Turkey. The region also ranks first place in soybean, peanut, and corn production. In Turkey, 41% of cotton, 90% of peanut, 91% of soybean, 80% of sesame seeds, 100% of rose, 65% of anise seed, 29% of watermelon, 26% of vegetables, 22% of tomatoes, 90% of citrus fruits, 100% of banana, 18% of grapes and 16% of olives production are grown in the Mediterranean Region (TURKSTAT 2019b). A total of 34 different potential crops (trees, vegetables, and field crops) are grown in the region, but others are not grown in sufficient quantities for potential biomass energy.

The Mediterranean Region has significant potential in terms of animal husbandry. 7.9% of the country's cattle, 13.0% of sheep and goats and 9.3% of the poultry population are raised in the region (TURKSTAT 2019b).

According to TURKSTAT data for 2019, the population density of the region (person) and the amount of electrical energy consumed based on provinces (MWh/year) and agricultural areas (ha) are shown in Table 1 (TURKSTAT 2019b).

Table 1- Available biomass potential and status in the region according to 2019 data

Parameters	*Adana*	*Antalya*	*Burdur*	*Hatay*	*Isparta*	*K. Maraş*	*Mersin*	*Osmaniye*
Population (person)	2 237 940	2 511 700	270 796	1 628 894	444 914	1 154 102	1 840 425	538 759
Electricity consumption (MWh/yr)	6 754 795	8 608 939	793 877	4 390 056	1 007 211	3 902 864	4 605 510	3 709 730
Agricultural area (ha)	476 654	361 707	149 669	221 800	187 914	351 362	333 173	121 753
Number of animals								
Cattle	253 632	190 250	213 418	143 099	147 331	212 240	127 521	78 427
Small ruminant	833 027	1 273 635	410 055	414 512	537 493	925 327	1 695 174	229 174
Poultry	6 556 620	534 248	213 374	693 030	289 362	1 738 801	21 217 866	720 813
Manure amount Waste quantity (ton/yr)								
Cattle	114 562	85 934	96 398	64 636	66 548	95 866	57 600	35 424
Small ruminant	18 813	28 764	9 261	9 361	12 139	20 898	38 284	5 176
Poultry	87 051	7 093	2 833	9 201	3 842	23 086	281 707	9 570
The energy equivalent of animal waste (MWh/yr)								
Cattle	118 787	89 103	99 953	67 020	69 002	99 402	59 724	36 731
Small ruminant	3 200	4 893	1 575	1 593	2 065	3 555	6 513	880
Poultry	157 958	12 871	5 140	16 696	6 971	41 890	511 169	17 365
Annual vegetal production (ton/yr)								
Orchard (tree number)	27 782 884	25 997 154	5 425 826	34 117 471	18 577 757	27 020 858	65 438 725	5 415 963
Field crops	1 620 639	442 097	207 996	512 006	169 243	787 130	401 125	542 261
Vegetable	427 523	6 121 463	331 157	122 397	133 537	134 605	2 226 641	27 632
Biomass yield (kg/da) (kg/tree)								
Orchard (kg/tree)	3.76	3.47	2.14	3.32	2.79	2.18	2.59	3.21
Field crops (kg/da)	423.64	98.89	51.47	455.88	33.29	166.41	185.31	342.77
Vegetable (kg/da)	1124.95	2067.20	2017.38	579.26	1331.64	529.48	2218.83	600.74
Residues amount (ton/yr)								
Orchard	104 366	90 248	11 633	113 364	51 839	58 781	169 365	17 396
Field crops	1 211 740	147 948	38 024	494 768	28 712	337 498	213 901	341 562
Vegetable	193 145	26 943 421	149 109	55 087	67 820	54 619	1 328 945	12 245
The energy equivalent of vegetal residual (MWh/yr)								
Orchard	217 590	101 799	17 804	131 721	67 165	91 109	223 898	12 906
Field crops	3 620	434	110	1 463	83	999	637	1 023
Vegetable	364	48.429	268	103	122	100	2 479	23

The outputs of the study were obtained in three consecutive steps in eight provinces in the Mediterranean Region.

In the first step, statistical data published by TURKSTAT (2019a, 2019b), the Ministry for Agriculture and Forestry, and MENR as well as data regarding biomass resources in Turkey, plant, and animal production values in the "Biomass Energy Potential Atlas" (BEPA 2020) and data from the regional project executed by the Eastern Mediterranean Agricultural Research Institute and other literature (Başçetinçelik et al. 2006; Tolay et al. 2008; Bilgili et al. 2008; Kayişoğlu & Diken 2019, etc.) were utilized and are presented in Table 1. These are I) Population (person) living in the region, electricity consumption (MWh/year), agricultural lands (ha), II) In the assessment, animal species (cattle, small ruminant, and poultry) numbers (number) and plant products (9 field crops, 3 vegetable crops and 22 different types of trees). The residue amounts (ton/year) according to the plant product quantities and animal numbers were determined, no significant residue potential was obtained from other than these.

In the second step, I) Plant and animal waste potentials of the region were calculated as technical potential (ton/year). II) The lower calorific value (MJ/kg) of the plant residues in the region were analyzed in the laboratory (Table 2), and the biogas calorific value (MJ/m^3) of animal biomass was calculated according to the equation given in a literature (Samah 2016; Ozcan et al. 2015).

In the third step, I) The substitution equivalence (MWh/year) of the electrical energy consumed in the region was calculated by converting the "technical potential" of the total biomass of the region into electrical energy. II) The amount of biofertilizer that can be produced from the residue of the production facility of the calculated biogas potential (ton/year) was also determined in the study. III) The CO_2 emissions (ton of CO_2/year) for the production of both "substitution electrical energy" that can be obtained from biomass in the region as well as "substitution biofertilizer" to replace chemical fertilizer that can be obtained after biogas production were calculated.

Table 2- Average calorific values (Avg. CV) (MJ/kg) and standard deviations (Std. dev) of agricultural residues.

Crops	*Agricultural residue*	*Avg. CV (MJ/kg)*	*Std. dev. (MJ/kg)*
Orchard	Apricots	19.3	0.64
	Olives	18.1	0.48
	Figs, Almonds	18.4	0.51
	Sourcherries, Plums, Pistachios, Walnuts	19.0	0.54
	Grapefruit, Lemons, Oranges, Mandarins, Apples, Pears, Quinces, Armeniaca Vulgaris, Cherries, Peaches, Nectarines, Loquats, Pomegranates, Persimmons.	17.6	0.86
Field crops	Wheat	17.9	0.88
	Maize	18.4	0.34
	Barley, Rye	17.5	0.72
	Oats	17.4	0.67
	Soybeans	19.4	0.66
	Peanuts	20.7	0.64
	Paddy	13.0	0.07
	Cotton	18.2	0.33
Vegetable	Peppers	17.5	0.67
	Aubergines	17.4	1.09
	Tomatoes	15.4	0.93

The technical potential quantities (kg/da, kg/tree) of annual usable residues from crop production were calculated based on 4 replicate measurements and observations on a dry basis during each crop's harvest or pruning period during the study conducted in the region. Field crops and vegetable residue, annual yield, and residue rates per hectare were determined. The annual amount of residue for vineyard and orchard production was calculated as the total residue left after the pruning of each tree (Bilgili et al. 2008). The thermal production method was preferred as a suitable method for direct burning energy generation for plant residues (Tezçakar & Can 2010; Ozcan et al. 2012; Yılmaz & Saka 2017).

The total annual manure production in each province was calculated based on types and number of animals and the total manure production per one animal was calculated with the equations found in the literature (Gonzalez-Salazar et al. 2014), Equation (1-3), and in Table 1. Yılmaz & Saka (2017) proposed an anaerobic system to increase the use of energy obtained from animal manure. Biogas (methane (55-75% CH_4), carbon dioxide (25-45% CO_2), 1-10% hydrogen (H_2), 0-0.3% nitrogen (N_2) and 0-3% hydrogen sulfide (H_2S)) is formed as result of anaerobic digestion (Weiland 2010).

The calorific values were generally determined according to the methane content in biogas (Öztürk et al. 2015). The calorific value of 65% methane gas (6.1 kWh/m^3) in the biogas was converted (Samah 2016) and subsequently calculated as MWh in this study.

Daily livestock activities affect the amount of manure and the cumulative biogas potential. Animal species, diet, body weight, total solids ratio, volatile matter ratio in solids, waste availability, and biogas efficiency have a high impact on the waste potential of animals. While the availability of poultry manure is very high, bovine manure can be collected in less amount and the availability of manure is much lower for small ruminant animals due to the limited time in the shelter.

The next procedure was followed to calculate the amount of biogas production: the amount of annual manure of three animal species in 8 provinces was calculated with Equation (1, 2 and 3), and the amount of total annual manure was estimated with Eq. (4) (Gonzalez-Salazar et al. 2014). The number of each species was determined according to the data of BEPA (2020).

$$\sum_{i}^{8} AB_c = \sum NA_c.(M.(0.365)).SC.WA \quad (1)$$

$$\sum_{i}^{8} AB_s = \sum NAs.(M.(0.365)).SC.WA \quad (2)$$

$$\sum_{i}^{8} AB_p = \sum NAp.(M.(0.365)).SC.WA \quad (3)$$

$$\sum_{i,j}^{8,3} TAB = \sum ABc + \sum ABs + \sum ABp \quad (4)$$

Where TAB is total animal biomass (ton/year), j is animal species (3 species),

Where: í, Number of provinces; c, s and p: cattle, small ruminants and poultry, AB: the amount of animal manure (ton/year), NA: Number of animals (number), M: the amount of daily produced animal manure (kg/day), k: coefficient (0.365 year/ton), SC: solid content (%), WA: Waste availability (%).

The amount of manure was determined based on the daily feeding and manure accumulation habits of the animal species. In other words, the amount of manure produced (M) by cattle, small ruminants, and poultry was estimated as 15, 3, and 0.074 kg/day, respectively (Hart 1960). Table 3 shows that the characterization of waste varies.

Table 3- Physical properties of animal manure (Hart 1960)

		Wet waste amount			
Animal species	*Body weight (kg)*	*% of weight*	*(kg/day)*	*Solid content (%)*	*Waste data (kg/day)*
Poultry	1.5-2.3	3-5	0.08-1.6	10-90	0.074
Small ruminants	30-75	4-5	2-3	23-30	3
Cattle	135-800	5-6	10-30	5-25	15

Waste availability (WA) was selected as 55% for cattle, 13% for small ruminants, and 99% for poultry, taking into account the time the animals remain in shelters (Avcıoğlu & Türker 2012). Availability is a measure of a collectible manure fraction that varies with livestock conditions (Ekinci et al. 2010, Yılmaz & Saka 2017).

The total biomass obtained from animal manure was determined by multiplying the total number of animals, the total amount of dry manure, the availability value, and solids content of the manure (Equations 1-3). The solids content (SC) factors were taken as 25%, 23%, and 11.5% for poultry, small ruminants, and cattle, respectively (Ekinci et al. 2010). Annual total biomass amount according to animal species in eight provinces is given in the Eqs. (1-3):

The total annual biomass amount of 3 animal species in eight provinces was calculated with Equation 4. The total energy from animal manure (MWh/year) was determined by multiplying the total amount of animal manure (ton/year), the thermal value of biogas (MJ/m^3) and the coefficient of gas engine efficiency (%) (Özcan et al. 2012). The amount of biogas production was accepted as approximately 200 m^3 per ton of dry manure, the calorific value of biogas was taken into account as 22.7 MJ/m^3 (6.1 kWh/m^3) (Özcan et al. 2015; Samah 2016; Görmüş 2018; Yılmaz & Saka, 2017). The gas engine efficiency used in converting biogas to electrical energy was taken as 44% (Horlock 1997; Özcan et al. 2012). The total amount of electrical energy that can be obtained depending on the animal species for each province is given in Eqs. (5- 7):

$$\sum_i^8 AEc = \sum ABc.v.e \quad (5)$$

$$\sum_i^8 AEs = \sum ABs.v.e \quad (6)$$

$$\sum_i^8 AEp = \sum ABp.v.e \quad (7)$$

Where: AE is the amount of energy produced (MWh), v is Energy value of biogas (MWh/m^3), e is electrical efficiency of gas engine (%).

The amount of electrical energy from animal biomass (MWh/year) is given in Equation 8:

$$\sum_{i,j}^{8,3} AEh = \sum AEc + \sum AEs + \sum AEp \quad (8)$$

Where; AEh is the amount of electrical energy from animal biomass (MWh/year).

The amount of plant residue in eight provinces was determined by Equations 9-11:

$$\sum_{i,h}^{8,22} VOP = \sum_h^{22} NT\,.\,AA.\,WA.\,k \quad (9)$$

$$\sum_{i,f}^{8,9} FCP = \sum_f^9 FC\,.AA.\,WA.\,k \quad (10)$$

$$\sum_{i,s}^{8,3} VP = \sum_s^5 VP\,.AA.\,WA.\,k \quad (11)$$

Where; VOP is Vineyard-orchard tree potential (ton/year), NT is Number of trees (units), AA is Actual amount (kg/tree), WA is Waste availability (%), k is the residue coefficient for the plant species.

The amount of available waste was first calculated by multiplying the residual amounts with the Equation (9- 11) and some reference coefficients and plant production values for the estimation of the amount of energy that can be obtained from plant residues (Hart, 1960). Subsequently, the energy content of the residues was calculated by using Equation (12-14). The electrical energy values of plant products in the region were calculated as:

$$\sum_{i,h}^{8,22} AE_{VOP} = \sum_h^{22} VOP\,.v.e.Hu \quad (12)$$

$$\sum_{i,f}^{8,9} AE_{FCP} = \sum_{i,f}^{8,9} FCP\ .v.e.Hu \quad (13)$$

$$\sum_{i,s}^{8,3} AE_{VP} = \sum_{i,s}^{8,3} VP\ .v.e.Hu \quad (14)$$

Where; FC is Field crops (kg/da); FCP: Field crops potential (ton), f: Field crops group (9 items), VP: the amount of total vegetable (ton/year), vg: vegetable group (3 items), v: Biomass energy value (MWh/ton), e: used engine efficiency (40%). Hu: lower calorific value (MJ/kg).

The calorific values (MJ/kg) of agricultural residue types were determined with a stoichiometric analysis method **(**Bilgili et al. 2008). The calorific values of each vegetable residue were measured using a bomb calorimeter (Bilgili 2015). The calorimeter method was based on a TS ISO 1928 standard. At least 4 measurements (by calorimeter) were made to calculate the calorific values and the arithmetic average and standard deviation of the determinations were taken to obtain the calorific values of the relevant pruning/vegetable/plant residues are shown in Table 2.

Electrical energy in total crop production was calculated with Equation 15:

$$\sum_{i,b}^{8,3} AEb=\sum AEvop+\sum AEfcp+\sum AEvp \quad (15)$$

Where; b is 3 plant groups (Field crops, Vegetable and Vineyard-orchard).

The total amount of electrical energy that can be obtained from biomass in the region was calculated with Equation 16:

$$\sum_{i,x}^{8,2} AE_{total}=\sum_{i,b}^{8,3} AEb+\sum_{i,j}^{8,3} AE\,h \quad (16)$$

Where; x is the energy group that can be obtained from plant and animal biomass.

In conclusion, the analyzes carried out revealed the potential biomass for each province in the region, the supply of waste from animal and plant production, and the annual amounts of electrical energy that they corresponded to (Yılmaz & Saka 2017). Equation 17 was used to calculate the remaining solids by taking approximately 15% for separator and other losses for 3 animal species in the study to determine the amount of biofertilizer after biogas production (Bilgili et al. 2018):

$$\sum OF_{BIO}=(\sum TAB)\ 0.85 \quad (17)$$

Where; OF is the amount of biofertilizer (ton/year), TAB the amount of animal manure (ton/year). 0.85 is the availability coefficient.

CO_2 emissions were calculated according to 2019 electricity energy consumption data and its replacement in the Mediterranean Region. According to the Development Bank of Turkey (DBT) (2018), electricity generation from fossil fuels in Turkey was 512 tonCO_2/GWh, and electricity generation from biomass was 26 ton CO_2/GWh in Turkey (Bilgili 2020). The CO_2 emission was calculated with Equations 18 and 19:

Fossil: CO_2 =512 (ton CO_2/GWh). current energy consumption (GWh) (DBT 2018) (18)

Biomass: $\sum CO_2=(\sum AEh+\sum AEFC).26$ ton (ton CO_2/GWh) (Bilgili 2020) (19)

Where; AEh + AE_{FC} is energy production from Biomass (GWh).

The amount of CO_2 that can be obtained from the total replaced electrical energy is given in Equation 20.

$$\sum CO_2=(\sum AEtotal).26 \text{ ton} \quad (20)$$

The amount of CO_2 emission that can be provided by biofertilizer that can be used to replace chemical fertilizer was calculated according to Sturm (2011) and Gellings & Parmenter (2016).

3. Results and Discussion

The status of the provinces in the region and the available biomass potential in the study are shown in Table 1. According to the data obtained as a result of the calculation using TURKSTAT (2019b), While average electricity consumption in Turkey was 2 761 kWh/year/person, the electricity consumption in the Mediterranean Region was calculated as 3 178 kWh/year/person. The total number of animals in Turkey was 408 433 823, out of which 84% were poultry, 12% were small ruminants and 4% were cattle.

The Mediterranean Region's contribution to total animal production in Turkey was calculated as 10%. The total amount of animal waste in the corresponding region of Turkey was 1 184 049 ton/year which corresponds to 9%. The animal waste potential of the region, as seen in Table 1, is not directly proportional to the number of animals and the amount of waste. Table 3 shows

that the characterization of waste potential varies according to the animal species. As the size of the animal increased, the amount of waste per animal naturally increased. Yılmaz & Saka (2017), animal manure properties varied according to many factors. The physical properties of animal manure can vary depending on weight, eating habits, and the season.

Although Table 1 indicates that Mersin had a higher number of animals compared to Burdur, the proportional waste potential according to the highest animal number in the region was calculated for Burdur with approximately 91% and Mersin with 6%. This is due to the difference in animal species (Burdur has mainly cattle while poultry is dominant in Mersin). Estimates indicated that 104 238 MJ thermal energy can be produced annually from animal wastes. This is significant in determining the locations where new energy technologies will be installed in the region.

According to the results of the research, it is possible to obtain approximately 221 million m^3 of methane gas per year from animal residues and approximately $1.4x10^6$ MWh of electrical energy per year from this gas. When the results obtained are examined, it is similar to the studies performed by Aktas et al. (2015), calculated that approximately 30 million m^3 of methane gas per year and approximately 119 million kWh electrical energy can be obtained from this methane gas from the total animal fecal waste of Tekirdağ Province.

At this stage, it is important to evaluate biomass technology to be applied. Thermal or chemical processes can be used to convert biomass into a usable form. Combustion of biogas is the most common method of thermal conversion (Özcan et al. 2012). IEA (2017) data revealed that 2000 biogas plants in Turkey that can operate only with animal manure (WEB2 2017). There are 100 licensed biogas plants in Turkey, 82 biomass, waste heat, and pyrolytic oil energy power plants with a total installed capacity of 467.37 MWe were active. 19 of these plants with an installed capacity of 79.36 MWe were in the Mediterranean Region. There were 5 of them in Mersin province, 4 in Adana, 3 each in Antalya and Kahramanmaraş, 2 in Hatay, 1 each in Osmaniye and Isparta, respectively. Burdur was the only province in the region without such a plant (WEB3 2017). The amount of annual plant residues in Turkey was $163.30x10^6$ ton, 77% of this residue was from croplands, the remaining 23% was from the production of plant residues (WEB4 2019). The Mediterranean Region contributed 13% to plant production.

According to Table 1, since orchard and vegetable crops are produced on less land than field crops, their residue amounts are also less. The highest amount of residue in the region was obtained from field crops. Yields are shown in Table 1. Adana had the highest yield of $1.9x10^6$ ton/year and 95.9% of the waste and the most residues were collected from cultivated plants. The percentages for residue amount generated by wheat, corn, sunflower, cotton, and soybean were 32.1%, 47.3%, 8.9%, 7.7%, and 4.0%, respectively. According to estimates, 5.97 Mtoe can be used every year if plant residues are used as specified. Bilgili (2020), despite its high potential availability, agricultural residues in the region have generally been directly incinerated and used for thermal purposes for the last 20 years, biomass thermal conversion facilities were not available. However, a few businesses were working with new technologies intending to generate electrical energy. Furthermore, many new facilities were in the design or commissioning phase.

Although the existing energy potential for the provinces in the region seems high, the supply chain (logistics) problem limits the capacities of potential power plants in the absence of large volumes in the field crops. Similar results were observed in several other studies (Yılmaz & Saka 2017; Bilgili 2020). However, the most suitable conditions for thermal conversion-based energy generation are to establish a medium or large-scale plant to achieve an efficient cycle (WEB5 2017). Investment and operating costs for small-scale plants are high compared to large-scale power generation (Yılmaz & Saka 2017). The central facility concept, which is as economical as the transport distance between stations (Salleh et al. 2019) and the amount of dry matter in the manure requires a solution. For example, dry matter content should be 70%, the economic distance should be around 40 km. If the dry matter content drops to 10%, the distance should be 10 km (Tafdrup 1994).

While the yield status of potential plant residue is 14.63 ton/ha-year depending on the agricultural areas in the region, the overall potential in Turkey has been calculated as 2.40 ton/ha-year (Table 1). Similar results were observed in several other studies, Bilgili et al. (2008); Yılmaz & Saka (2017); Bilgili (2020), the lowest and highest calorific values of plant residues varied between 10.3 and 21.8 MJ/kg.

Biomass waste potential in the Mediterranean Region was estimated to be around 8.3 PJ. According to the results, 6.9% of the region's energy demand can be countered by this potential. The inclusion of biomass in energy technologies investments in the region is highly recommended. These are in agreement with the results were observed in several other studies (Yılmaz & Saka 2017; BP 2018; WEB1 2019).

According to the results of this study, 1 184 049 tons/year of biofertilizer, which can be obtained after biogas production in agricultural areas can be used in the region. This is in agreement with the results of a research involving biomass-energy performed by Bilgili (2020). The literature has reported that the pure nitrogen ratio of the biogas slurry to the chemical fertilizer best improves the soil nitrogen and organic matter content. The liquid-solid part remaining after biogas production is used as fertilizer (Tolay et al. 1999; Türker 2008). For the use of this biofertilizer, the optimum digestion time is recommended as 21 days in agricultural applications (Okoroigwe 2007). If 40 t/ha biofertilizer can be applied instead of 0.5-5 t/ha conventional compost application, the need for mineral fertilizers can be reduced by 20-75% (Sidorchuk 2017).

If the animal waste in the region is used for energy replacement, it is estimated that a total of 1 461 116 ton of CO_2 in emissions will be reduced per year with the production of biofertilizers. This is in agreement with the results of an experiment involving biofertilizers application in agriculture performed by Havukainen et al. (2018), carbon footprints are 0.8 $kgCO_2/kg$ for N and 1.8 $kgCO_2/kg$ for P, whereas carbon footprints for mineral fertilizers are 1.9–7.8 $kgCO_2/kg$ for N. and 2.3–4.5 $kgCO_2/kg$ for P. Compared to the emission from mineral fertilizer production, the reduction of GHG emission in organic fertilizer production is 78% for N and 41% for P on average.

4. Conclusions

The usable biomass potential of the provinces in the Mediterranean Region was emphasized in this study. The amounts of waste of animal and plant biomass and the energy equivalency of potential of biomass were analyzed according to the statistical data of the Mediterranean Region for 2019 and the project was carried out to represent the region. A comparison of the biomass potential with literature studies showed that the estimate seems reasonable. The current biomass potential was determined and the amount of energy to be generated with biogas from animal waste and burning plant residues were calculated basis on waste. The electrical energy value of the total biomass waste in the region was calculated as 2 313 965 MWh/year in 2019. The following results can be evaluated in this context:

- ✓ The provinces with the highest plant and animal biomass potential in the region were Mersin and Adana.
- ✓ The region's total biomass potential was calculated as approximately 8.3 PJ/year and this energy can meet 6.9% of the regional demand.
- ✓ The animal waste potential was lower than plant residue potential, therefore it is likely that animal energy production-based biomass can be generated with relatively small-scale energy technologies, hybrid applications are needed.
- ✓ 1 184 049 ton/year of biofertilizer that can be obtained after biogas production can be used in agricultural areas in the region. Accordingly, approximately 537 kg of biofertilizer per hectare can be applied to all agricultural land in the region. This can benefit areas that are poor in organic matter.
- ✓ If the agricultural residues in the region are used for energy replacement, it is estimated that a total of 2 644 302 ton of CO_2 emissions are reduced with the production of approximately 1 183 186 ton of CO_2 and biofertilizers per year.
- ✓ The fact that the region's electricity consumption per capita (3 178 kWh/year/per person) is above Turkey's average (2 761 kWh/year/per person) can be expressed as an indicator of development. In this study, the per capita electricity requirement of the region (218 kWh/year/per person) may be beneficial. Alternative energy sources are important at this point.
- ✓ The approach to making useful biomass without damaging the environment and reducing CO_2 emissions on a global scale realized with this study will also contribute to the energy targets of Turkey's "Vision 2023".

In conclusion, some of the expected differences in energy production from biomass compared to other energy sources in this study can be listed as follows: 1) Strategically important energy can provide economic and political advantages to the state. 2) It is considered a reliable potential in soil protection, water, creating landscape value, energy and food production. 3) It can provide permanent job opportunities by creating new technologies and employment. 4) It is an energy source that slows down climate change by reducing greenhouse gas emissions.

References

Afkari Sayyah A H, Mohammadi S, Nikbakht A M & Khalife E (2020). Modeling and Design a Special Type of Passive Solar Greenhouse in Cold Climate by TRNSYS. *Journal of Agricultural Sciences* 26(4): 488-498. DOI: 10.15832/ankutbd.502073

Aktas T, Özer B, Soyak G & Ertürk M C (2015). Determination of the Electricity Generation Potential from Animal Biogas in Tekirdag City. *Journal of Agricultural Machinery Science* 11(1): 69-74

Atay O A & Ekinci K (2020). Characterization of pellets made from rose oil processing solid wastes/coal powder/pine bark. Renewable Energy 149: 933-939. DOI: 10.1016/j.rser.2011.11.006

Avcıoğlu A O & Türker U (2012). Status and potential of biogas energy from animal wastes in Turkey. Renewable and Sustainable Energy Reviews 16(3):1557–1561. DOI: 10.1016/j.rser.2011.11.006

Aybek A, Üçok S, İspir M A & Bilgili M E (2015). Digital Mapping and Determination of Biogas Energy Potential of Usable Animal Manure and Cereal Straw Wastes in Turkey. (In Turkish). *Journal of Tekirdag Agricultural Faculty* 12(3): 109-120

Balat M (2004). The Use of Renewable Energy Sources for Energy in Turkey and Potential Trends. Energy Exploratıon & Exploıtatıon. Volume 22. Number 4, pp.235–251. DOI: 10.1260/0144598042886317

Başçetinçelik A, Öztürk H H, Karaca C, Kaçıra M, Ekinci K, Kaya D, Baban A, Güneş K, Komitti N, Barnes I & Nieminen M (2006). A Guide on Exploitation of Agricultural Residues in Turkey, Final Report Annex XIV, Life 03 Tcy/ Tr /000061, Adana, Türkiye.

BEPA (2020). General Directorate of Renewable Energy, BEPA Graphs 2020. Retrieved in October, 10, 2020 from, http://www.yegm.gov.tr

Bilgen S, Keleş S, Sarıkaya İ & Kaygusuz K (2015). A perspective for potential and technology of bioenergy in Turkey: present case and future view. Renewable and Sustainable Energy Reviews 48:228-39. DOI: 10.1016/j.rser.2015.03.096

Bilgili M E, Başçetinçelik A ve Karaca C (2008). Determination of Some Agricultural Waste (Biomass) Potential in Çukurova Region and Comparison with Traditional Energy Sources. (In Turkish). Project number: TAGEM-TOPRAKSU- 05440C01. Publication No: TAGEM-BB-TOPRAKSU-2008/66. Tarsus.

Bilgili M E (2015). Biomass Chemical Composition Induced Air/Fuel Ratio and Determination of Some Theoretical Parameters on Thermal Efficiency. (In Turkish). TARMEK 2015 "29th National Agricultural Mechanization and Energy Congress". 2-5 September, 2015. Diyarbakır-Turkey

Bilgili M E, Akyüz A & Aybek A (2018). Electricity Consumption in Fertilizer Management of Modern Dairy Cattle Farms and Providing with Photovoltaic. (In Turkish). IGAP2018 - 1st Internatıonal GAP Agrıculture and Lıvestock Congress. April 25 - 27, 2018. Sanliurfa-Turkey

Bilgili M E (2020). "Usage of Agricultural Biomass Potential in Electricity Substitution in Adana Province". (In Turkish). *Osmaniye Korkut Ata University Journal of Natural and Applied Sciences*. Volume 3, Issue 1, 41-47

BP Statistical (2018). Review of World Energy. Retrieved in April, 10, 2020 from, https://www.bp.com/content/dam/bp/pdf/energy-economics/statistical-review-2016/bp-statistical-review-of-world-energy-2018-full-report.pdf.

Brussels (2004). European Renewable Energy Council, "Renewable Energy Targets for Europe", EREC,

Demirbaş A, Akdeniz F, Güllü D & Cağlar A (2001). Energy policies and sustainable energy options of Turkey up to 2010. Energy. Educ. Sci. Technol 7(1):19–36

DBT (2018). Development Bank of Turkey. Development Bank Environmental Management Committee. R.V: 1.03, V. Date: 20.06.2018. Ankara

Ekinci K, Külcü R, Kaya D, Yaldız O, Ertekin C & Öztürk H H (2010). The prospective of potential biogas plants that can utilize animal manure in Turkey. Energy Exploration & Exploitation 28(3): 187-205. DOI: 10.1260/0144-5987.28.3.187

Gellings C W & Parmenter K E (2016). Energy efficiency in fertilizer production and use. Efficient Use and Conservation of Energy; Gellings, CW, Ed.; Encyclopedia of Life Support Systems pp. 123-136

Gokcol C, Dursun B, Alboyaci B & Sunan E (2009). Importance of biomass energy as alternative to other sources in Turkey. Energy Policy 37(2):424-43. DOI: 10.1016/j.enpol.2008.09.057

Gonzalez-Salazar M A, Morini M, Pinelli M, Spina P R, Venturini M, Finkenrath M & Poganietz W R (2014). Methodology for estimating biomass energy potential and its application to Colombia. Applied Energy 136: 781-796. DOI: 10.1016/j.apenergy.2014.07.004

Görmüş C (2018). Determination of Biogas Energy Potential of Animal Manure in Turkey. (In Turkish). (Master's thesis, Namık Kemal University, Institute of Science). (Unpublished), Tekirdag.

GWEC (2018). Global Wind Energy Council, Global wind statistics. Retrieved in April, 18, 2019 from, http://gwec. net/global-figures/graphs

Hart S A (1960). The Management of Livestock Wastes, *Journal of the Water Pollution Control Federation* 3(2): 78-80

Havukainen J, Uusitalo V, Koistinen K, Liikanen M & Horttanainen M (2018). Carbon footprint evaluation of biofertilizers. *International Journal of Sustainable Development and Planning* 13(8): 1050-1060

Horlock J H (1997). Cogeneration-Combined Heat and Power (CHP): Thermodynamics and Economics. Krieger publishing company, Florida.

IRENA (2018). Global Energy Transformation: A Roadmap to 2050. Retrieved in May, 05, 2018 from https://www.irena.org/-/media/Files/IRENA /Agency/Publication/2018/Apr/IRENA_Report_GET_2018.pdf

Johnsson F, Kjärstad J & Rootzén J (2018). The threat to climate change mitigation posed by the abundance of fossil fuels. Climate Policy 19(2): 258-274. DOI: 10.1080/14693062.2018.1483885

Kar Y & Tekeli Y (2008). The potential of biomass residues in Turkey and their importance as energy resources. *Energy Sources. Part A*. Recovery, Utilization, and Environmental Effects 30(6):483–93. DOI: 10.1080/15567030600828974

Kayişoğlu B & Diken B (2019). The Current Situation of Renewable Energy Use in Turkey and Problems. *Journal of Agricultural Machinery Science* 15(2): 61-65

Mao C, Yongzhong F, Xiaojiao W & Guangxin R (2015). Review on research achievements of biogas from anaerobic digestion. Renewable and Sustainable Energy Reviews 45: 540-555. DOI: 10.1016/j.rser.2015.02.032.

Mao G, Huang N, Chen L & Wang H (2018). Research on biomass energy and environment from the past to the future: A bibliometric analysis. Science of the Total Environment 635: 1081-1090. DOI: 10.1016/j.scitotenv.2018.04.173.

MENR (2010). Ministry of Energy and Natural Resources, "Ministry of Energy and Natural Resources 2010-2014 Strategic Plan", (In Turkish). Retrieved in April, 10, 2018 from: http://www.enerji.gov.tr/yayinlar_raporlar/ETKB_2010_2 014_Stratejik_Plani.pdf

MENR (2018). Ministry of Energy and Natural Resources. (In Turkish). Retrived in April, 15, 2019 from, www.enerji.gov.tr

NREL (2017). Biomass Energy Basics. Retrieved in April, 25, 2020 from http://www.nrel.gov/workingwithus/re-biomass.html.

Okoroigwe E C (2007). Application of biomass technology in sustainable agriculture. Trends in Applied Sciences Research 2(6): 549-553

Ozcan M, Öztürk S, Yıldırım M & Kılıç L (2012). Electricity Energy Potential of Different Biomass Sources Based on Different Production Technologies. ELECO'2012 Electrical - Electronics and Computer Engineering Symposium pp. 162-166. Bursa- TURKEY.

Ozcan M, Öztürk S, & Oguz Y (2015). Potential evaluation of biomass-based energy sources for Turkey. *Engineering Science and Technology, an International Journal* 18(2): 178-184. DOI: 10.1016/j.jestch.2014.10.003

REN21 (2020). Renewables (Global status report). Retrieved in July, 11, 2020 from https://www.ren21.net/reports/global-status-report/

Saka K & Yılmaz İ H (2017). Agricultural biomass potential in Turkey. *International Journal of Management and Applied Science* 3(2):79-81

Salleh S F, Gunawan M F, Zulkarnain M F B & Halim A (2019). Modelling and optimization of biomass supply chain for bioenergy production. *Journal of Environmental Treatment Techniques* 7(4): 689-695

Samah E (2016). Measuring small-scale biogas capacity and production. International Renewable Energy Agency (IRENA), Abu Dhabi.

Sensoy S, Demircan M, Ulupınar U & Balta I (2008). Turkey's climate. Turkish State Meteorological Service (DMİ), (In Turkish). Ankara.

Sidorchuk A (2017). Application of Biofertilizers after Biogas Production. Retrieved in April, 10, 2020 from, https://ecodevelop.ua/en/zastosuvannya-biodobriv-020617/

Sturm V (2011). Taking into account the emissions from the production and use of mineral fertilizers by imposing a 'carbon tax'. 14th Annual Conference on Global Economic Analysis, Venice, Italy

Sürmen Y (2003). "The necessity of biomass energy for Turkish economy". Energy Sources 25(2): 83–92. DOI: 10.1080/00908310390142145.

Tafdrup S (1994). Centralized biogas plants combine agricultural and environmental benefits with energy production. Water Science and Technology 30(12): 133-141

Tezçakar M & Can O (2010). "Thermal Disposal Technologies from Waste to Energy", *2nd Waste Technologies Symposium and Exhibition*, IWES'10, 4-5November 2010, Istanbul, Turkey.

Tolay M, Söğüt N & Öztürk İ (1999). Commissioning, Operation and Maintenance of Biological Wastewater Treatment Plants, "Environmental Pollution Priorities in Turkey. Symposium III", 18-19 November 1999, Gebze Institute of Technology, Gebze-Kocaeli.

Tolay M, Yamankaradeniz H, Yardımcı S, Reiter R (2008) Biogas Production from Animal Waste, VII. National Clear Energy Symposium, UTES'2008, 17-19 December 2008, İstanbul. (In Turkish).

TURKSTAT (State Institute of Statistics) (2019a). Annual Gross Domestic Product, (In Turkish). Retrieved in April, 10, 2018 from: http://www.turkstat.gov.tr.

TURKSTAT (State Institute of Statistics). (2019b). Agricultural Structure Statistics, Retrieved in April, 16, 2020 from: http://www.turkstat.gov.tr.

Türker M (2008). Anaerobic Biotechnology and Bioenergy Production: Trends in the World and Turkey. VII. National Clear Energy Symposium, UTES'2008, 17-19 December 2008, İstanbul. (In Turkish).

WEB1 (2019). Retrieved in June, 10, 2020 from: https://www.enerji.gov.tr/tr-TR/Sayfalar/Biyokutle (In Turkish).

WEB2 (2017). IEA Bioenergy Task 37: Anaerobic Digestion Report Turkey's Situation in Biogas. Retrieved in June, 11, 2019 from: http://task37.ieabioenergy.com/country-reports.html.

WEB3 (2017). Retrieved in April, 25, 2019 from: https://www.enerjiatlasi.com/biyogaz/

WEB4 (2019). Agricultural biomass potential of Turkey. (In Turkish). Retrieved in April, 25, 2020 from: http://www.yegm.gov.tr/yenilenebilir/biyogaz.aspx

WEB5 (2017). Turkey's Biomass Power Plants. (In Turkish). Retrieved in May, 10, 2019 from: http://enerjienstitusu.com/santraller/biyokutle-santralleri

Weiland P (2010). Biogas Production: Current State and Perspectives. Applied Microbiology and Biotechnology 85(4): 849-860. DOİ: 10.1007/s00253-009-2246-7

Yelmen B & Çakir M T (2016). Biomass potential of Turkey and energy production applications. Energy Sources. *Part B.* 11 (5):428–35. DOI: 10.1080/15567249.2011.613443

Yılmaz İ H, & Saka K (2017). Exploitable biomass status and potential of the Southeastern Anatolia Region, Turkey. Energy Sources, Part B: Economics, Planning, and Policy 2018 13(1): 46–52. DOI: 10.1080/15567249.2017.1403498

Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

Risk Analysis Using Geographic Information Systems by Determining the Factors Affecting Yield in Plant Production: A case study from Ankara, Turkey

Emre YENIAY[a*], Aydın ŞIK[b]

[a]*Ministry of Agriculture and Forestry, General Directorate of Plant Production, Coordinator, Ankara, TURKEY*
[b]*Gazi University Faculty of Architecture, Department of Industrial Design, Ankara, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Emre YENİAY, E-mail: eyeniay@yahoo.com
Received: 22 March 2020 / Revised: 24 November 2021 / Accepted: 26 November 2021 / Online: 15 October 2022

Cite this article
YENIAY E, ŞIK A (2022). Risk Analysis Using Geographic Information Systems by Determining the Factors Affecting Yield in Plant Production: A case study from Ankara, Turkey. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 28(4):677-690. DOI: 10.15832/ankutbd.900997

ABSTRACT
Performing agricultural analysis is becoming much more effortless due to the rapid improvements in information technologies. Geographic Information Systems (GIS) provide more detailed data about climate, soil, topography, and irrigation values regarding agriculture; thus, allowing for performing detailed location analyses. These analyses cover agricultural investment maps, agricultural propriety areas, and plant pattern detections. The purpose of this study is to develop product-based agricultural risk analysis maps. Climate, soil, topography, and irrigation data are essential in the cultivation of agricultural products. With risk analysis, the risk values are determined for each risk factor. Applying the Analytical Hierarchy Process (AHP), which is one of the multi-criteria decision-making methods, the total risk value is calculated by prioritizing the risk factors. AHP is an efficient methodology developed to calculate scenario-based risk values by considering various possibilities.

In this study, a model is generated by studying apricot, sour cherry, and almond farming in Ankara. As a result of the development of a GIS model for Ankara, the total risk values were mapped as "high-risk areas", "medium-risk areas", "low-risk areas" and "strongly not recommended areas" according to the points they received spatially. When the maps were examined in detail; it was determined that apricot crops in Ankara province are more sensitive to climate, soil, and topography conditions than other products. Since apricot is affected by late spring frosts, it is recommended that risk factors can be reduced by taking climatic measures in areas where soil structure is suitable. It has been determined that the sour cherry crop is less sensitive to climatic and topographic conditions and is more affected by the risk factors from the soil layers; while the almond crop is more affected by the climatic conditions, though it is more tolerant to soil conditions. According to these results, apricot can be grown in large areas with medium and high-risk levels, and in limited areas with low-risk levels. Almond with a very high-risk level can be grown in large areas compared to apricot, and sour cherry can be grown in similar-sized areas with apricot, but with a lower risk level than apricot.

Keywords: Risk maps, Multi-Criteria decision Model (MCDM), Analytical hierarchy process (AHP), Fault tree analyses (FTA), Agricultural product risk maps

1. Introduction

The current world population of 7.6 billion is expected to reach 8.6 billion in 2030, 9.8 billion in 2050 and 11.2 billion in 2100, according to a United Nations last report. Numerous challenges linked to agricultural supply chains, as well as decreasing farmland, environmental problems, and the insufficient protection of natural resources have made it necessary to take urgent measures to meet the food needs of the increasing world population. Farming systems require an extensive and profound transformation from traditional practices to precision farming and to intelligent farming practices to cope with these challenges (FAO/WHO, 2018).

Approximately 13 billion hectares of the earth's surface is covered with land and 37% of this land (approximately 5 billion hectares) consists of agricultural land. Considering the distribution of the agricultural land according to its use; It is seen that field crops are grown in approximately 1.5 billion hectares of land, while perennial plants are also planted in 1.5 billion hectares. The remaining 2 billion hectares are utilized as meadows and pastures (Ministry Development of Turkey 2013).

The strategic importance of the protection and development of agricultural production, the continuity of nutrition, which is the basic need of human beings, the supply of food raw materials, and the sustainability of the agricultural sector has become even more evident during the COVID-19 process. The existing agricultural areas in the world are decreasing by 0.1% - 0.2% every 5 years. On the other hand, the world population has increased by 6.2% in the last 5 years (UN, 2013). Between 2001 and 2020, the total amount of land planted in Turkey decreased by 3 million 205 thousand hectares from 26 million 350 thousand

hectares to 23 million 145 thousand hectares. The drop rate was 12 percent. In the same period, our population increased by 18 million 219 thousand and reached 83 million 385 thousand. The rate of increase was 28% (Turkish Statistical Institute 2021). It is estimated that the population of Turkey will reach 100 million in 2040. This shows that it will be more difficult to feed the increasing population with the decreasing agricultural lands (Kritikos 2017; Pablo et al. 2014).

In the current study, the reason why apricot, sour cherry, and almond were selected for creating a risk analysis map in Ankara was due to the fact that these crops are affected the most during early spring and late autumn frosts, and also that the damage caused by moisture and sunburn reduces the economic value of these products. These factors arising from the climate can be minimized via performing site selection analysis with GIS methods.

1.1. Risk analysis

In the current situation, the existence of a potential source that may cause deficiency or loss induces a risk. On the other hand, the presence of loss is not satisfactory in defining the risk situation encountered. At this point, there is vagueness as the loss has converted from potential to tangible loss. Therefore, the risk can be defined as a combination of any loss or omissions that may occur. Moreover, the vagueness of this possibility turns into an actual omission or loss. In brief, the risk is the probability of loss or deficiency and the degree of this deficiency. Risk is equal to the sum of incompleteness and uncertainty (Benner 1978). Another equation in this regard is the following formula, which includes the “result” and the "probability of occurrence"; Risk (R) is the multiplying of “product of severity” (S) and “probability” (P). As the formula indicates, the degree of risk revealed through result and probability values can have the equivalent value for various situations. Although the risk levels are similar; there are several techniques to reduce risk. In the first case, systems that may cause minor damage should be highlighted, considering the situations that will occur when the event occurs. Concerning the second case, the main objects generating the events should be ascertained and eliminated efficiently (Şenel & Şenel 2013; Çetinyokuş & Yeniay 2019).

1.2. Geographic information systems (GIS)

GIS has many different definitions due to its use in numerous disciplines. For instance, Geographic Information Systems (GIS) can be used as a landscape planning instrument for the landscape architect, a reserve calculation instrument for a mining engineer, or an instrument for calculating recall values for an agricultural engineer. GIS is defined as a system that is “designed to solve complex planning and management problems, which consists of hardware, software, and methods that cover the containment, management, processing, analysis, modeling, and display of data for a particular location” (Research In Urbanism Series Vol. 3, 2015). In addition, the agricultural meaning of GIS is a technology that facilitates the transition from existing methods to precision agriculture (Sharma et al. 2018).

In the current study, land propriety, land survey, selection, and risk assessment for agricultural products are discussed through GIS applications. GIS and Risk analysis are combined in the same model to determine location-based agricultural risks. This study has established a framework for crop harvesting in plant production where ecological risks can be predicted and preventive measures can be taken to minimize risks. That framework identifies extensive data analysis that plays an essential role in improving the quality of GIS implementation in agriculture and provides guidelines for researchers, practitioners, and policymakers to manage massive GIS data to successfully maintain enhanced agricultural productivity (Peggion et al. 2008).

In similar studies, it has been observed that a limited number of agricultural risks are focused on a single GIS model. In addition, each of the risks creates the result with the same degree of importance. For example, it was seen that the frost risk, which significantly affects the yield, and the humidity risk, which affects the product quality, are calculated with the same degree of importance. In this study, due to Multi-Criteria Decision Model (MCDM) in Ankara, many agricultural risk layers and the sub-layers that make up these layers are scored according to the degree of importance with AHP and create a risk score from 0 to 25 (Nyeko 2012).

2. Material and Methods

2.1. Study area

In this study, Ankara is selected, since it is the capital city of Turkey and in the middle of it. Ankara has an area of 26,897 km² and its elevation above sea level is about 890 meters. Approximately 50% of the province's surface area formed in the plains is agricultural land, 28% is forest and heathland, 12% is meadow and pasture, and 10% is non-agricultural land (Ankara Governorship 2020; Turkish Statistical Institute 2020).

Kızılırmak River, one of the longest rivers in Turkey with a length of 1 355 kilometers, irrigates the east of the province, while the longest river with a length of 824 kilometers, irrigates the west of the Ankara province. Ankara stream, a branch of the Sakarya River, flows through the city center. Salt Lake is the second-largest lake in the country with 1 300 km², and with a salt rate of 32.4% it is the second saltiest lake in the world. In addition, the basin in which Salt Lake is located, is the largest closed basin in Turkey (Ankara Governorship 2020).

Ankara province has a variable geographical structure with Black Sea climate in the north, continental climate in the south and east, and temperate climate in the west. Due to these wide characteristics, the province of Ankara has been preferred in order to spread the test applicability of the current study throughout the country.

2.2. Data supply

Climate, soil, topography, and streambed maps provided by the General Directorate of State Hydraulic Works, General Directorate of Meteorology, as well as from the General Directorate of Abolished Land and Water datas (Table 1) were standardized. These data were transferred to the geographical database built-in with the ArcGIS 10.5 application and adapted to a single projection system.

The last published 30 years (1980-2010) of climatic data, collected from 228 stations in Turkey by the General Directorate of Meteorology, have been used. These data were classified monthly, and subsequently, the monthly mean values, minimum, as well as maximum values, were calculated. Consequently, data were converted to GIS format via co-kriging and inverse distance weighting (IDW) methods with the ArcGIS software, and surface spread was applied. In the process of spreading over the surface, considering the topography, values were divided into compartments with 20x20 meters size, and climate maps were generated in raster format.

Table 1- GIS Datasets

Climate Data	***Soil Data***

• Temperature
  ○ Average Temperature on a Monthly
  ○ Maximum Temperature on a Monthly
  ○ Minimum Temperature on a Monthly
  ○ Extreme Maximum Temperature on a Monthly
  ○ Extreme Minimum Temperature on a Monthly
• Precipitation
  ○ Total Precipitation on a Monthly
  ○ Summer Months Total Precipitation
  ○ Total Annual Precipitation
• Sunbathing
  ○ Total Sunbathing Times on a Monthly
  ○ Annual Total Sunbathing Time
• Evaporation
  ○ Evaporation Values on a Monthly
  ○ Average Annual Evaporation Amount
• Humidity
  ○ Average Humidity value on a Monthly
  ○ Spring Months Average Humidity value
  ○ Summer Months Average Humidity Value
• Wind
  ○ Average Wind Speed on a Monthly
• Soil Temperature,
	• Soil Depth
	  ○ Lithosolic
	  ○ Very Shallow (0-30cm)
	  ○ Shallow (30-50cm)
	  ○ Medium Deep (50-90cm)
	  ○ Deep (90-150cm)
	  ○ Very Deep (>150cm)
	• Soil Erosion
	  ○ Wind Erosion
	  ○ Rain Erosion
	• Land Use Capability
	  ○ 1-8th Class Land
	• Available Land Use
	  ○ Absolute Irrigated Farmland
	  ○ Marginal Irrigated Agricultural Land
	  ○ Absolute Dry Farmland
	  ○ Marginal Dry Farmland
	  ○ Planted Agricultural Land
	  ○ Meadow and Pasture Areas
	  ○ Wetlands
	  ○ Forest Areas
	  ○ More Fields
	• Drainage

Water Data • Areas Irrigated by Surface and Groundwater Resources • Streams • Dams and Lakes • Sub-Basins Where Groundwater Is Insufficient	***Topography*** Slope and Aspect maps are produced using the Turkish Digital Height Model (DHM) data at a resolution of 10 meters. • Height • Slope • Aspect

2.3. Statistical data

The province and district-based annual values of field, production, and yield where agricultural products are grown, offered by the Turkish Statistical Institute, were used. The average values of the last five years were calculated, and also the product-based county yield amount was calculated. Since it takes 3-5 years for fruit trees to reach economic value in perennial garden facilities, statistical data from the past 5 years have been used.

2.4. Method

Factors described graphically in the tree diagram and errors related to the used material can be attributed to human mistakes or different events that generate unintended outcomes. The Failed Tree Analyses (FTA) method can be qualitatively used to identify the reasons and events that lead to an error and can be used quantitatively, to calculate the probability of recurrence of the main

reason as well. In the design stage of a system, The FTA method is also used to calculate potential losses and collect from different design options and predict the significance of potential losses during the operation phase (Limnios 1993).

Based on deductive logic, the factors that may cause an undesirable situation are determined and analyzed. The severity of the risk arising from undesirable conditions is calculated by the FTA method. Risk factors are organized, defined, and presented on a tree diagram with a logical system. Undesirable peak events should be detected and any factor considered in this event should be analyzed. The model setup is started by using the total risk value of a selected fruit product. Consequently, sub-risks such as climate, soil, topography, and water restrictions that lead to total risk are determined. Factors that lead to sub-risks are selected based on a product and inscribed as layers. The FTA method used in complex systems focuses only on the risk of a single product (Çınar & Karacabey 2004).

2.5. Agricultural risk analysis

Various risks that farmers experience in developing countries are listed under two main headings; ecological risks which arise from the uncertainty of production amount related to climatic factors in current agricultural activities; and economic risks which arise from potential fluctuations in cost and sales. In recent years, risks such as increasingly large disasters, droughts, floods, frost, hail damages, shifting of seasons, erosion, new types of diseases, and pests are collected under the heading of ecological risks. In a recent study, researchers estimate that 23% of the field products have been lost due to adverse weather conditions. This percentage is remarkable in garden plants (Islam et al. 2018).

2.6. Step model establishment

Analytical Hierarchy Process (AHP) model is developed in the GIS environment under the capabilities of the ArcGIS software. The "weighted overlay analysis" tool, the equivalent of the AHP method in ArcGIS software, is used. During the calculation of the risk factors of the substrates constituting the distinctive climate risk factor, it was accepted that the main climate risk factor is the highest risk factor, since it would contain the highest risk in terms of plant growth. At this stage, the "fuzzy overlay analysis" tool in the ArcGIS software was used.

In that model, with the road map in figure 1, values lead to risk during the plant's growth in the climate, soil, topography, and water presence layers, and the risk probabilities are inscribed in the Figure 2. Consequently, optimum values, i.e., the lowest risk values, promote selected plant growth according to the risk matrix that has scored the lowest. In contrast, high-risk values for the plant's growing conditions are scored as the highest according to the risk matrix.

In addition, thanks to the "weighted overlap analysis tool", the risk scores from the layers can be re-scored hierarchically by assigning values between climate, soil, hill, and irrigation layers according to their importance (Tuncay & Demirel 2017).

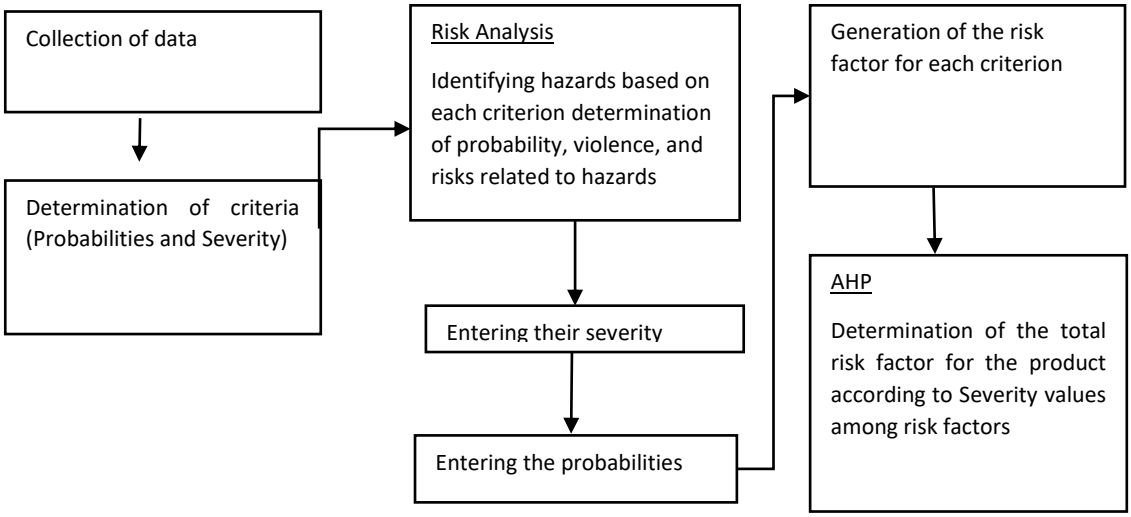


Figure 1- GIS/AHP Model Flow Chart

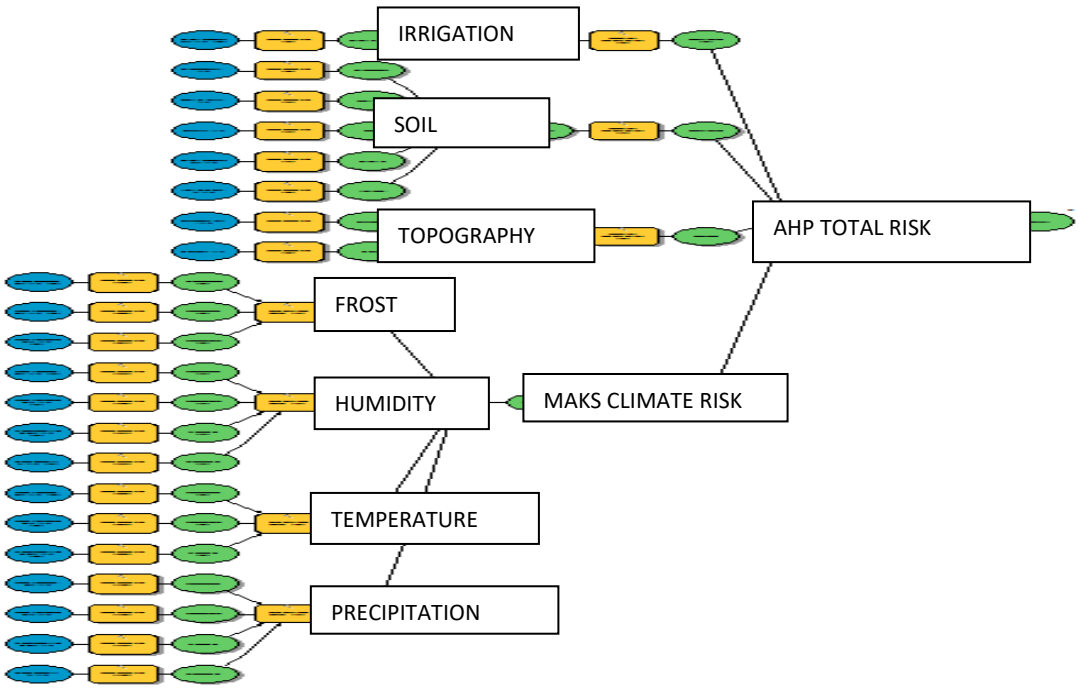


Figure 2- GIS Model (Error Tree, FMEA System hierarchical structure)

2.7. Step; GIS/AHP scoring

All of the data required on the cultivation of agricultural products were collected from crop specialists (Researchers working on crop improvement in Agricultural Research Institutes) and from published scientific studies. Data have been entered into the GIS/AHP Model by following the steps below (Çobanoğlu et al. 2007; Öztekin et al. 2008; Gür et al. 2011; Eroğlu & Mısırlı 2012; Göçmez & Seferoğlu 2014).

1. Entering the probabilities
2. Entering its severity
3. Generation of risk matrix
4. Determining risks
5. Prioritization of risks with AHP
6. Determination of total risk

In the light of this information, risk severity for each layer that is essential for the selected product's growth is filled in Table 2 with the "reclassification" tool on GIS.

Table 2- Severity Rating of AHP

Risk Matrix Severity	*Degree*	*Description*
Very light	1	Product/yield loss less than 10%
Light	2	Up to 10% product/yield loss
Moderate	3	10%-30% product/yield loss
Serious	4	30%-50% product/yield loss
Very serious	5	Product yield loss more than 50%

For instance, for the apricot crop, when the April minimum temperature value is at -10 ºC, frostbite intensity is "5" as "very high", while at 1 ºC, the frostbite intensity is "3" as "moderate", and at +6 ºC, frostbite intensity is "1" as "very low."

For example, for Apricot, the minimum temperature value for April is “5” at -10 °C, while the severity of frost is “very severe”, and at -3 °C the severity of frost is “3”, and finally at +3 °C, the severity of frost is considered as light at “3”. In addition, apricot cultivation is definitely not recommended at locations where the average temperature layer shows temperatures below -10 °C. Considering this situation, when the risk severity exceeds the "irrecoverable" level, with the "no data" command entered into the model, the information that an apricot crop is not recommended for this region is processed regardless of other conditions (Chen et al. 2009).

Table 3- Severity Rating (Example: Average Temperature Layer)

Average Temperature Layer	*State of Violence*	*Severity ating*	*Description*
+6,+10 C	Very light	1	Product/yield loss less than 10%
+3,+5C	Light	2	Up to 10% product/yield loss
-2,+2 C	Moderate	3	10%-30% product/yield loss
-5,-3 C	Serious	4	30%-50% product/yield loss
-10,-6 C	Very serious	5	Product yield loss more than 50%
> -10 C	Definitely	No data	No products can be grown

Each layer essential for the selected plant growth has been inputted with probability values such as the values given above.

Risk probability values such as the risk severity values given in Table 3 were entered for all layers that are important for the growth of the selected plant. After the risk severity values were entered into the GIS model, the "probability" values were filled as shown in Table 4. Here, the aim is to enter the information about how likely the risk severity layer is to realize the severity. This process is formed with the "Weighted Overlay Analyses" tool in GIS (Öztekin et al. 2008; Mokarram & Aminzadeh 2010; Mokarram & Hojati 2016)

Table 4- Degree of Probability

Risk Matrix Probability	*Degree*	*Description*
Very small	1	No more than once in 30 years
Small	2	2-5 times in 30 years
Moderate	3	Seen 6-10 times in 30 years
High	4	11-15 sightings in 30 years
Too high	5	Seen more than 15 times in 30 years

For instance, in Table 3, April frostbite severity values are noted for the apricot crop. Supposing that value for February, March, and May is "5" as "too high." Hence, in April, frost probability is higher than the rest of the year due to the flowering of apricot trees. The average temperature layer data of March, May, and February can be entered into the model as "3" "moderate", "2" "small", and "1" "very small" respectively (İbrahim & Pırlak 2011).

Table 5- Degree of Probability

Mounts	*Probability*	*Degree of Probability*	*Description*
February	Very small	1	No more than once in 30 years
Mart	Moderate	3	Seen 6-10 times in 30 years
April	Too high	5	Seen more than 15 times in 30 years
May	Small	2	2-5 times in 30 years

2.8. Risk Matrix

Risk= Severity x Probability; Using the probability values in Tables 5 - 7 risk matrix was created with this formula.

According to the scores after the values entered;

- Green Areas: Low-risk from 1 to 6 (inclusive) (1 pointless risk)
- Yellow Areas: Medium risk from 6 to 12 (inclusive)
- Red Areas: High risk from 12 and 25 (inclusive) (25 non-tolerable risks)

Table 6- Risk Matrix

RISK MATRIX	***Violence***				
Probability	***1 (Very Light)***	***2 (Light)***	***3 (Moderate)***	***4 (Serious)***	***5 (Very Serious)***
1 (Very small)	**1** Meanless	**2** Low	**3** Low	**4** Low	**5** Low
2 (Small)	**2** Low	**4** Low	**6** Low	**8** Medium	**10** Medium
3 (Moderate)	**3** Low	**6** Low	**9** Medium	**12** Medium	**15** High
4 (High)	**4** Low	**8** Medium	**12** Medium	**16** High	**20** High
5 (Too high)	**5** Low	**10** Medium	**15** High	**20** High	**25** Irrecoverable

The GIS model places the probability values entered for layers locational into each grid as values. A selected point in Ankara is the average temperature layer (-1 °C) in April. Hence, the severity of apricot trees damaged by frostbite is a moderate "3". The average temperature value of the same point is (-5 °C) in March, and the average temperature value is (+3 °C) in May. Therefore, the average temperature value, in other words, the frostbite severity of March and May, is 5 and 1 °C, respectively. However, considering the periods when apricot trees bloom at the selected region, in April when flowering is the highest, the probability of frostbiting is serious "5", and the probability of frostbiting in March and May is "3" and "1", respectively, since the flowering of trees is low in the months of March and May (Öztekin et al. 2008; İbrahim & Pırlak 2011).

Table 7- Risk Factor

For a selected point	*Temperature*	*State of Severity*	*Severity Rating*	*Probabilities*	*Degree of Probability*	*RISK Status*	*RISK Severity*	*Frost Risk Factor*
Average temperature value for March	-5 C	Moderate	4	High	3	Moderate	4x3 **12**	**Maximum risk severity = 15**
Average temperature value for April	-1 C	Very serious	3	High	5	High	3x5 **15**	
Average temperature value for May	+6 C	Light	1	Very Small	2	Low	1x2 **2**	

Table 7 has the frostbite risk factor for the region selected for the apricot crop. Factors essential in agricultural productivity which may include risks for products in their lack or excess can be calculated separately for the following factors; risk factors; climate (frost, humidity, temperature, precipitation, sunshine, wind), soil, topography, and water presence (drought).

Therefore, Table 8 is filled for each risk factor, and "importance assessment" is carried out using AHP through those risk factors. Scoring is determined among the risk factors with the support of Table 8, which was generated during the AHP. This is conducted with the "Weighted Overlay" analysis tool on GIS (Ünüvar & Pırlak 2016).

Table 8- AHP Severity Values

Value	*Definition*	*Description*
1	Equally important	Equally important in two options
3	Little important.	Where one criterion is slightly superior to another
5	Too important.	One criterion is considered superior to another
7	Too important.	One criterion is considered quite superior to another
9	Extremely important	One criterion is superior to another.
2, 4, 6, 8	Intermediate values	Specify intermediate values between two consecutive evaluations

After determining criteria and sub-criteria, using the Super Decision software, the interactions between the criteria can be analyzed, and the criteria affecting each other can be determined. The network structure in Figure 3 is established by generating inter-criteria connections, internal and external dependencies, and feedback with the program's help (Rabia & Terribile 2013).

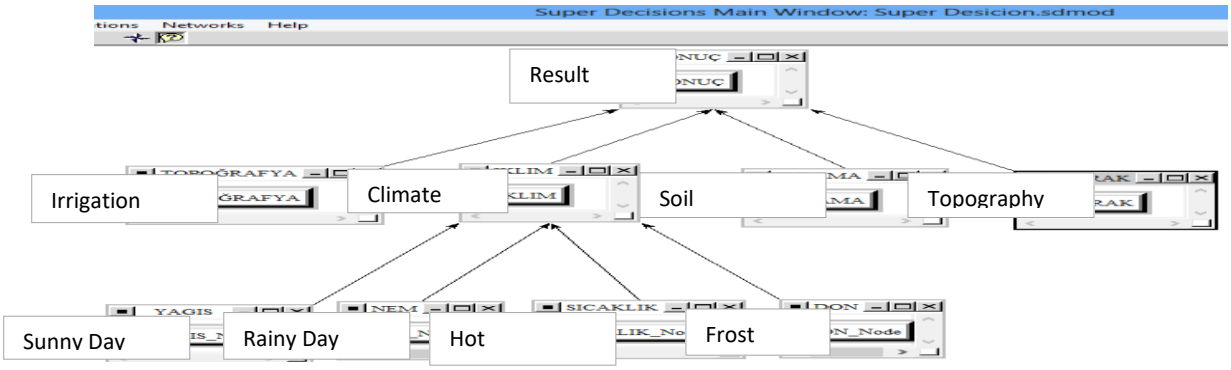


Figure 3- Super Decision Program Interface

Cross-layer "severity scores" (Figure 4) were calculated by entering the critical values of the AHP method to the Super Decision software (Figure 3). Score values can be entered directly into the GIS Model (Altay & Keskin 2018; Paulraj & Easwaran 2008).

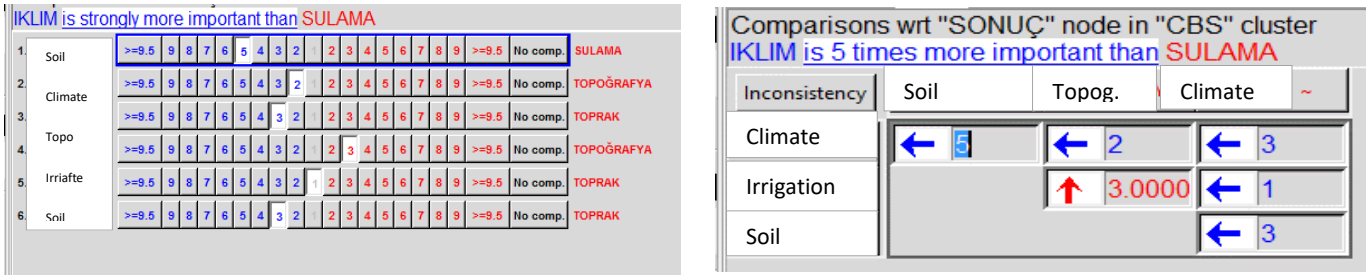


Figure 4- Entering data into the Super Decision Program

For the apricot crop selected as a model, the "supremacy" importance scores of the risk factors that were formed after all the layers under the climate, soil, topography, and irrigation layer groups were entered using Table 9. The sum of the importance values to be given to the risk factor groups should be equal to “1” (Van Chuong 2008).

Table 9- Total Risk Prioritization

Risk Factors			*Severity Weight*	*Total Risk*
Irrigation			0.10303	**Climate + Soil + Topography + Irrigation**
Soil			0.11887	
Topography			0.29271	
	Layers	**Climate Max Risk**		
Climate	Frost Humidity Temperature Precipitation	Max (Frost, Humidity, Temperature, Precipitation)	0.48090	

2.9. Step; running the model

All the steps and formulas described above to calculate total risk are processed into the GIS model developed on ArcGIS Desktop software and the values entered are run on the tools shown in Figure 2 and rendered into the pop-up windows. The blue oval shapes shown in figure 2 represent the layers entered into the model. Checkboxes colored yellow are the toolboxes that are processed. Green oval shapes, on the other hand, express the newly formed layers after the procedure. In the model, the layers that are decisive in the growing of apricot crops are primarily chosen. These layers are divided into groups as climate, soil, topography, and irrigation. The climate is divided into subgroups as frost, temperature, precipitation, and humidity. Risk probabilities and risk severity values in each sub-group are entered as described in this article's setting-up-the-model section. Scores from sub-groups are calculated with the formula “X=Max ∑Probability x Severity”. Since there are four subgroups of this factor in calculating the risk factor value of the climate group, each risk factor is calculated separately, and the maximum climate risk layer is created by selecting the maximum risk that matches with each point with the “fuzzy overlay analysis” tool.

In calculating soil, topography, and irrigation risk factors, probability and severity values in the sub-layers are entered. Risk factors for groups are calculated by multiplying the probability and severity values, which have been entered. The maximum climate risk layer described in the previous step is used for the climate.

After this stage, risk factors ranging from zero to 25 are obtained in each group. To prioritize climate, soil, topography, and irrigation with AHP, the "AHP Importance Values" shown in Table 9 are entered into the “weighted overlay analysis” tool. Since the sum of the values entered in AHP is equal to 100% of the values entered, the final values can be the “total risk” values between 0 and 25.

An example of manual calculation for a selected point is made below. in the apricot example described above, "frost risk factor" scored "15" at a chosen point. again, the calculation process is continued assuming its score is "10" from the risk factors "humidity," "temperature," and "precipitation." When calculating the climate risk factor, prioritization scores are given among the sub-groups as shown in Table 9 total risk calculation.

Climate Risk Factor= Maksimum[Frost Risk, Humidity Risk, Precipitation Risk, Temperature Risk]
Climate Risk Factor (Calculation with Virtual Values)= Maksimum[15, 10, 10, 10] = 15

When the treating was performed, the "climate risk factor" scored 15. Considering the risk matrix, the climate risk factor of the chosen point is the "high risk" level for the apricot crop.

Once again, the process was continued assuming that the soil risk factor from the same point scored "8" and the topography risk factor scored as "12" and the irrigation risk factor scored as "18". Table 9: In calculating the total risk, AHP and "importance values" scores for climate, soil, topography, and irrigation are multiplied.

Total risk = Climate x 0.48090 + Soil x 0.11887 + Topography x 0.29271 + Irrigation x 0.10303
Total risk = 15 x 0.48 + 8 x 0.12 + 12 x 0.30 + 18 x 0.10 = 12.54

The total risk value calculated for apricot farming at the chosen point after the previously explained procedure is "moderate," according to Table 6 risk matrix. Assume that the total risk score calculated for the chosen point is decision 1. Since AHP allows calculations exerted in consideration of several possibilities, a new total risk score has been calculated by assuming that the importance of the climate risk factor weight is reduced at the same point. This reduction is due to the conservative effect of measures such as a temporary greenhouse plant, running a fan on icy cold days, creating smoke by burning hay, and tying mesh against hail risk.

In order to check the model result, calculations will also be made manually for 3 different points, which are currently apricot, cherry and almond orchards. In this way, it will be possible to compare the score status of the risk maps in the lands that can grow high-yield crops.

2.10. Creating risk maps

The climate (Figure 5), soil (Figure 6), topography (Figure 7) and irrigation (Figure 8) sub-risk layers created as a result of running the GIS model are shown on the maps. While the risk value is low in the north of Ankara in the climate risk map, the situation is calculated as the opposite in the topography risk map. The soil risk map shows a complex distribution. Fixed risk has been determined in non-irrigated lands. (El-Sheikh et al. 2010)

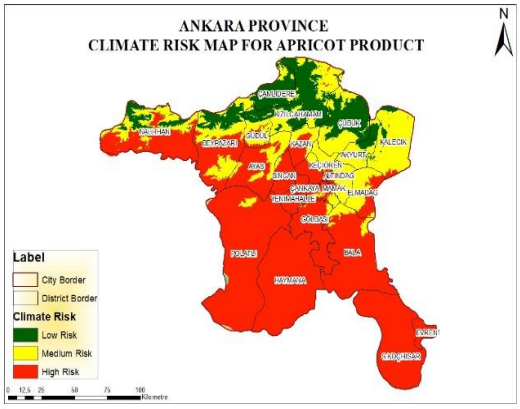


Figure 5- Climate Risk Map

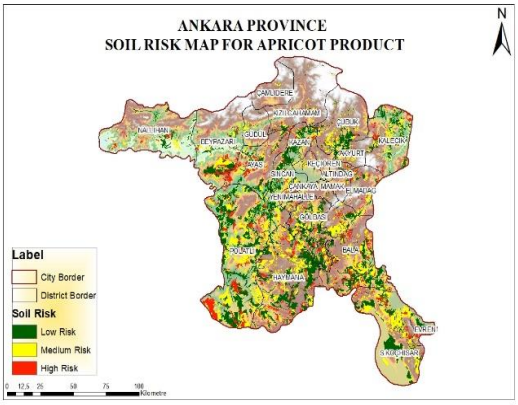


Figure 6- Soil Risk Map

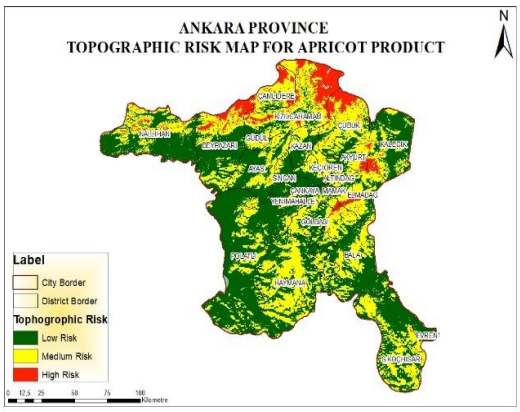


Figure 7- Topography Risk Map

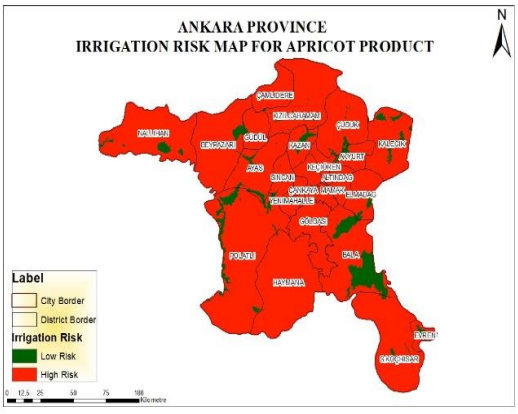


Figure 8- Irrigation Risk Map

Total Risk Map

The total risk map of apricot created by the combination of the sub-risk layers, are shown Figure 9.

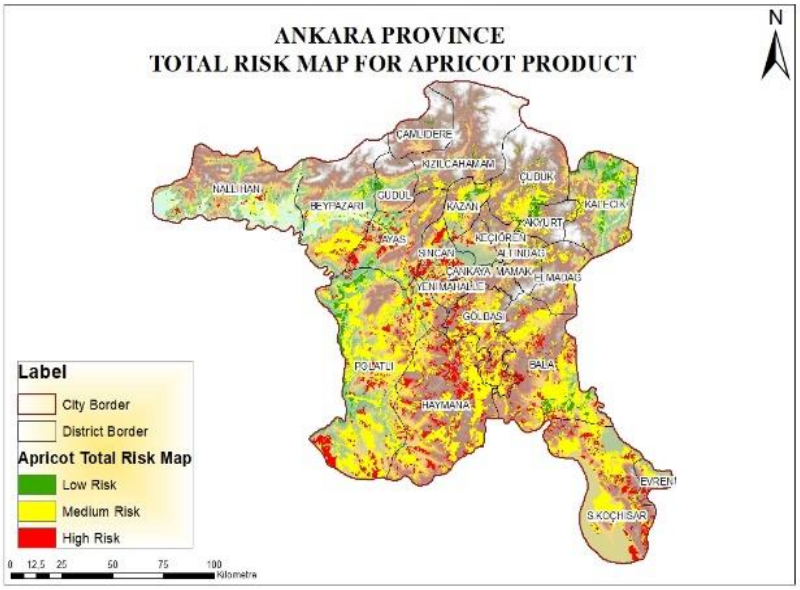


Figure 9- Apricot Risk Map in Ankara Province

Table 10- Turkish Statistical Institute 2020 Plant Production Statistics

Provincial Name	*County Name*	*Product Name*	*Planted Area (da)*	*Number of Trees*	*Production (Tons)*	*Average Yield Per Tree (kg)*
Ankara	Evren	Apricot	0	1140	68	60
Ankara	Kalecik	Apricot	499	21550	690	32
Ankara	Beypazarı	Apricot	0	1000	30	30
Ankara	Güdül	Apricot	170	6500	195	30
Ankara	Kahramankazan	Apricot	23	1800	54	30
Ankara	Keçiören	Apricot	7	535	16	30
Ankara	Yenimahalle	Apricot	0	250	7	28
Ankara	Çankaya	Apricot	15	2700	70	26
Ankara	Polatlı	Apricot	878	24400	561	23
Ankara	Çubuk	Apricot	340	5435	125	23
Ankara	Şereflikoçhisar	Apricot	0	2000	40	20
Ankara	Etimesgut	Apricot	18	215	4	19
Ankara	Haymana	Apricot	295	12550	213	17
Ankara	Ayaş	Apricot	65	9400	141	15
Ankara	Nallıhan	Apricot	5	6550	98	15
Ankara	Pursaklar	Apricot	20	400	6	15
Ankara	Sincan	Apricot	122	5270	79	15
Ankara	Mamak	Apricot	40	1290	18	14
Ankara	Gölbaşı	Apricot	1550	21792	283	13
Ankara	Elmadağ	Apricot	43	5250	63	12
Ankara	Altındağ	Apricot	24	469	4	9
Ankara	Akyurt	Apricot	49	2120	11	5
Ankara	Bala	Apricot	27	750	1	1
Total/Average			**4 190**	**133 366**	**2 777**	**21**

According to the Turkish Statistical Institute apricot maximum yield in Turkey is 95 kg, the average yield is 47 kg in 2020. When Table 10 is examined, it is understood that the average yield for the apricot crop is below the country average in all except Evren district of Ankara province. There is no closed garden in Evren district either. It is seen that the yield is quite low in the high-risk red areas on the map, while the yield is relatively high in the low-risk green areas. High and medium risk areas make up the bulk of the map.

Since it is possible to develop risk maps of different products with the same model, risk maps are generated for the sour cherry and almond crops. Probability and severity values entered into the model are predetermined for the product, and two new maps are generated.

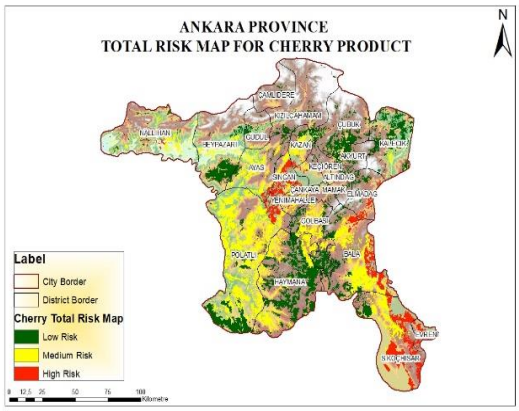


Figure 10- Sour Cherry Risk Map in Ankara Province

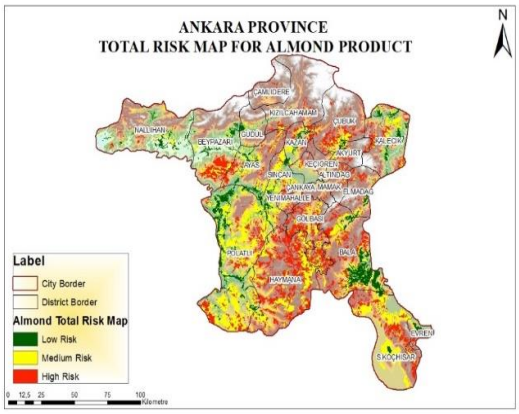


Figure 11- Almond Risk Map in Ankara Province

Table 11- Turkish Statistical Institute 2020 Plant Production Statistics

Provincial Name	*County Name*	*Product Name*	*Planted Area (da)*	*Number of Trees*	*Production (Tons)*	*Average Yield Per Tree (kg)*
Ankara	Evren	S. Cherry	20	1.270	76	60
Ankara	Akyurt	S. Cherry	88	2.720	136	50
Ankara	Güdül	S. Cherry	800	14.500	725	50
Ankara	Sincan	S. Cherry	265	6.530	327	50
Ankara	Şereflikoçhisar	S. Cherry	0	1.500	75	50
Ankara	Polatlı	S. Cherry	276	10.240	410	40
Ankara	Çankaya	S. Cherry	3	2.050	72	35
Ankara	Yenimahalle	S. Cherry	0	150	5	33
Ankara	Ayaş	S. Cherry	1.900	55.200	1.766	32
Ankara	Kalecik	S. Cherry	300	13.400	415	31
Ankara	Keçiören	S. Cherry	18	258	8	31
Ankara	Elmadağ	S. Cherry	136	7.350	221	30
Ankara	Kahramankazan	S. Cherry	67	6.040	181	30
Ankara	Çubuk	S. Cherry	1.230	19.661	590	30
Ankara	Etimesgut	S. Cherry	14	750	22	29
Ankara	Çamlıdere	S. Cherry	58	1.100	26	24
Ankara	Kızılcahamam	S. Cherry	40	6.500	143	22
Ankara	Beypazarı	S. Cherry	203	14.020	280	20
Ankara	Haymana	S. Cherry	76	2.730	54	20
Ankara	Pursaklar	S. Cherry	45	1.300	26	20
Ankara	Altındağ	S. Cherry	18	429	8	19
Ankara	Mamak	S. Cherry	8	440	8	18
Ankara	Gölbaşı	S. Cherry	520	10.824	162	15
Ankara	Nallıhan	S. Cherry	74	11.600	174	15
Ankara	Bala	S. Cherry	28	825	8	10
Total/Average			**6 187**	**191 387**	**5 918**	**31**

According to the Turkish Statistical Institute, sour cherry maximum yield in Turkey is 83 kg, the average yield is 33 kg in 2020. The current product statistics of the sour cherry crop in Table 11 are examined, it is understood that it is similar to the map created as a result of the model in Figure 10. It is seen that the risk is low in the flat areas located in the north and west of Ankara. It is observed that the risk increases in mountainous areas.

Table 12- Turkish Statistical Institute 2020 Plant Production Statistics

Provincial Name	*County Name*	*Product Name*	*Planted Area (da)*	*Number of Trees*	*Production (Tons)*	*Average Yield Per Tree (kg)*
Ankara	Evren	Almond	585	16000	560	35
Ankara	Akyurt	Almond	150	1550	47	30
Ankara	Polatlı	Almond	1.438	35800	1074	30
Ankara	Beypazarı	Almond	66	4188	105	25
Ankara	Sincan	Almond	200	1880	47	25
Ankara	Çankaya	Almond	3	925	23	25
Ankara	Çubuk	Almond	350	5595	140	25
Ankara	Etimesgut	Almond	8	200	4	20
Ankara	Güdül	Almond	70	4250	85	20
Ankara	Haymana	Almond	488	3200	65	20
Ankara	Kahramankazan	Almond	58	1959	39	20
Ankara	Kalecik	Almond	6003	16459	331	20
Ankara	Pursaklar	Almond	45	1300	25	19
Ankara	Ayaş	Almond	130	4400	79	18
Ankara	Nallıhan	Almond	330	6500	98	15
Ankara	Keçiören	Almond	2	70	1	14
Ankara	Şereflikoçhisar	Almond	600	11100	144	13
Ankara	Gölbaşı	Almond	955	18984	190	10
Ankara	Mamak	Almond	150	5874	47	8
Ankara	Altındağ	Almond	6	560	4	7
Ankara	Bala	Almond	1840	21275	106	5
Ankara	Elmadağ	Almond	590	15600	78	5
Ankara	Yenimahalle	Almond	42	1500	8	5
Total/Average			**14 109**	**179 169**	**3 300**	**18**

According to the Turkish Statistical Institute, almond maximum yield in Turkey is 70 kg, the average yield is 15 kg for 2020. When Table 12 and Figure 11 are interpreted together, it is considered that almond crops settle with less risk in mountainous areas. From the risk maps obtained as a result of the analysis made on the GIS, the areas where the crop can be grown are calculated in table 13.

Table 13- Crops area calculation

Product Name	***Low Risk Area (Da)***	***Medium Risk Area (Da)***	***High Risk Area (Da)***	***Total Areas (Da)***
Apricot	572 290	5 496 557	1 574 379	7 643 227
Sour Cherry	3 031 271	3 498 374	1 083 955	7 613 601
Almond	1 210 614	3 447 835	2 946 215	7 604 215

According to these results, all crops have a similar total growing area even if they have different risk factors. However, the sour cherry crop has more low-risk growing areas than the others, and the apricot crop has more medium risk growing areas, while the almond crop has few ‘low risk’ growing areas, with mostly 'moderate risk' and 'high risk' growing areas.

3. Conclusions

The fact that agriculture is indispensable not only for Turkey, but also for all the countries in the world during the pandemic, has made it necessary to continue with agriculture more consciously after scientific analysis. The study aims to develop a methodology to provide farmers with the highest profit from their fields with the lowest risk. As a result of entering the correct data, crop risk analysis can be performed with the developed method in this study, for millions of geographic points within a few minutes.

The risks that led to yield loss in fruit growing are pretty high. The probability of those risks remains seasonal and decreases and increases in severity as the plants grow, bloom, and bear fruits. In addition, the effect of each risk factor on yield/crop loss is different from each other. A model is developed to generate product-based risk analysis maps with the analytical hierarchy method, a multi-criteria decision-making method, risk matrix, and fault tree analysis over GIS, by considering the cumulative information specific to agriculture.

In this study, apricot, sour cherry, and almond farming in Ankara were chosen as a model. Risk maps were prepared separately for the climate, soil, topography, and irrigation factors. Total risk maps for apricot, sour cherry, and almond crops were created in general, with all the layers taken together. The reason for those ascertained risks can be easily accessed, retrospectively, with the fault tree analysis. Risk values of 3 different points chosen in Ankara, which were entered into the GIS model were compared,

and the accuracy was confirmed with manual calculations. In this manner, this analysis can be performed for millions of points on the GIS, in the time allotted for manual risk analysis calculation for each point.

When the risk maps and sub-layers are examined in detail, it was determined that the apricot tree is more susceptible to soil and topography conditions and is highly affected by risk factors from soil and topography layers. Hence, it was concluded that the total risk factors for apricot can be mitigated with improvements that can be performed in terms of soil and topography. It was also determined that the almond crop is more susceptible to climatic conditions and is highly affected by the risk factors that arise from the climatic layers. Thus, finally it was concluded that the sour cherry crop is less affected by the climate, soil, and the topography risk layers as compared to apricot and almond, and it has a high yield with less risk in irrigated areas.

Considering the climate, soil, topography, and irrigation data throughout Turkey, this study can be implemented for all plant products with known ecological demands using this developed model. After the products have been examined, a methodology study can be conducted to find answers to the questions as to which crops can grow more riskily in various regions, and which of these risks are caused by climate, soil, topography, and irrigation resources, and finally what precautions can be taken against them.

In this study, it was concluded that with the risk analysis methodology developed on GIS, the sour cherry crop can be grown in Ankara with lesser risk as compared to the other products examined.

References

Altay Y & Keskin İ (2018). Determination of Factors Affecting Wheat Production in Altınekin District by Risk Analysis. *Selcuk Journal of Agriculture and Food Sciences* 32(3): 496-501. https://doi.org/10.15316/SJAFS.2018.128 (In Turkish)

Ankara Governorship (2020). Ankara Geography http://www.ankara.gov.tr/genel-cografya-ve-yeryuzu-sekilleri

Chen Y, Yu J, Shahbaz K & Xevi E (2009). A GIS-based sensitivity analysis of multi-criteria weights. *In proceedings of the 18th world IMACS/MODSIM congress, Cairns, Australia* (pp. 13-17). https://mssanz.org.au/modsim09/

Climate change and food security: risks and responses, 2015 Food and Agriculture Organization of the United Nations, Publish Date: 01.04.2022 Web: Http://Www.Fao.Org/3/I5188e/I5188e.Pdf

Çınar Y Y & Karacabey A A T D (2004) *Example of Multi-Attribute Decision Making and "Evaluation of Financial Performance of Banks"* (Doctoral Dissertation, Department of Business Administration) (In Turkish)

Çetinyokuş S & Yeniay E (2019) Risk Analysis on Occupational Health and Safety in Carrot Production Facility. *Erciyes Journal of Agriculture and Animal Sciences* 2(3): 5-10. (In Turkish)

Çobanoğlu F, Kocataş H, Mesut Ö Z E N, Tutmuş E & Konak R (2007). An Evaluation of Determining the Effect of Climate Factors on Turkish Dried Fig Export. *Anatolian Journal of Agricultural Sciences* 22(1), 11-19. (In Turkish)

El-Sheikh R F A, Ahmad N, Shariff A R M, Balasundram S K & Yahaya S (2010). An agricultural investment map based on geographic information system and multi-criteria method. *Journal of Applied Sciences* 10(15): 1596-1602. https://scialert.net/abstract/?doi=jas.2010.1596.1602

Eroğlu Z Ö & Mısırlı A (2012). Breeding and Development of Peach. Bahçe 41(2): 37-46. (In Turkish)

Göçmez A & Seferoğlu H G (2014). Table and Dry Fig Quality Criteria and Factors Affecting Quality. *Turkish Journal of Agricultural Research* 1(1): 98-108. https://doi.org/10.19159/Tutad.29893 (In Turkish)

Islam M M, Ahamed T & Noguchi R (2018). Land suitability and insurance premiums: A GIS-based multicriteria analysis approach for sustainable rice production. Sustainability 10(6): 1759. https://doi.org/10.3390/Su10061759

İbrahim G & Pırlak L (2011). Determination of phenological and pomological characteristics of some peach cultivars grown in Eğirdir ecological conditions. Derim 28(2): 27-41. https://doi.org/10.17100/nevbiltek.210971 (In Turkish)

Kritikos M (2017). Precision agriculture in Europe: Legal, social and ethical considerations. *Europäische Kommission,, Brüssel*. https://www.Europarl.Europa.Eu/Regdata/Etudes/Stud/2017/603207/Eprs Stu(2017)603207_En.Pdf Belgium

Mokarram M & Aminzadeh F (2010). GIS-based multicriteria land suitability evaluation using ordered weight averaging with fuzzy quantifier: a case study in Shavur Plain, Iran. The International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences 38(2): 508-512

Mokarram M & Hojati M (2016). Using ordered weight averaging (OWA) for multicriteria soil fertility evaluation by GIS *(case study: Southeast Iran).* Solid Earth Discussions 1-28. https://doi.org/10.5194/se-2016-112

Nyeko M (2012). GIS and multi-criteria decision analysis for land use resource planning. *Journal of Geographic Information System Vol. 4, No. 4, page 8.* https://doi.org/10.4236/jgis.2012.44039

Pablo J, Hubbard N& Loudjani P (2014) Precision Agriculture: An Opportunıty For Eu Farmers - Potential Support With The Cap 2014-2020, *Directorate-General For Internal Policies,* Agriculture And Rural Development, European Parlement https://www.europarl.europa.eu/regdata/etudes/note/join/2014/529049/ipol-agri_nt(2014)529049_en.pdf

Paulraj A P & Easwaran N (2008) Evaluation of 'Revamped'Crop Insurance Pradhan Mantri Fasal Bima Yojana (PMFBY) among Paddy Farmers in Tamil Nadu, India. https://doi.org/10.9734/cjast/2020/v39i3431037

Peggion M, Bernardini A & Masera M (2008). Geographic information systems and risk assessment. *Scientific and Technical Research series EUR, Office for Official Publications of the European Communities*, Luxembourg.

Rabia A H & Terribile F (2013). Introducing a new parametric concept for land suitability assessment. *International Journal of Environmental Science and Development* 4(1): 15. https://doi.org/10.7763/IJESD.2013.V4.295

Sharma R, Kamble S S & Gunasekaran A (2018). Big GIS analytics framework for agriculture supply chains: A literature review identifying the current trends and future perspectives. Computers and Electronics in Agriculture 155: 103-120. https://doi.org/10.1016/j.compag.2018.10.001

Şenel B & Şenel M (2013). Risk Analysis: Fault Tree Analysis Application on Traffic Accidents in Turkey. *Anadolu University Journal of Social Sciences* 13(3). (In Turkish)

Tuncay D & Demirel N (2017). Reliability analysis of a dragline using fault tree analysis, *Scientific Journal of Mining* 56(2): 55-64. https://doi.org/10.30797/madencilik.388169 (In Turkish)

TUIK (Turkish Statistical Institute, Statistical Data Portal). Agricultural Statistics, Population Statistics (2021). https://data.tuik.gov.tr/ (In Turkish)

TSMS (Turkish State Meteorological Service, Republic of Turkey Ministry of Agriculture and Forestry). 2018. Meteorological data information sales and presentation system (In Turkish)

Ünüvar G & Pırlak L (2016). Phenological and pomological properties of some apple cultivars grafted on M9 rootstock in Karaman ecological conditions. *Nevşehir Science and Technology Journal* 5: 96-106. https://doi.org/10.17100/Nevbiltek.210971 (In Turkish)

Van Chuong H (2008). Multicriteria land suitability evaluation for crops using GIS at community level in central Vietnam. *In International Symposium on geoinformatics for spatial-infrastructure development in earth and allied sciences.*

FAO/WHO (2011). *FAO/WHO guide for application of risk analysis principles and procedures during food safety emergencies*. Rome: Food and Agriculture Organization of the United Nations.

Journal of Agricultural Sciences (Tarim Bilimleri Dergisi) 2022, 28 (4) : 691 - 703 DOI: 10.15832/ankutbd.959820

Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

Effects of Moisture Contents and Storage Temperatures on the Physical, Chemical and Microbiological Qualities of Non-Sulfitted Dried Apricots

Nihal GÜZEL[a*], Şeref TAĞI[b], Mehmet ÖZKAN[b]

[a]*Department of Food Engineering Faculty of Engineering, Hitit University Çorum, 19030, TURKEY*
[b]*Department of Food Engineering Faculty of Engineering, Ankara University Gölbaşı, Ankara 06830, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Nihal GÜZEL, E-mail: nihalguzel@hitit.edu.tr
Received: 30 June 2021/ Revised: 30 November 2021 / Accepted: 30 November 2021 / Online: 15 October 2022

Cite this article
GÜZEL N, TAĞI Ş, ÖZKAN M (2022). Effects of Moisture Contents and Storage Temperatures on the Physical, Chemical and Microbiological Qualities of Non-Sulfitted Dried Apricots. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 28(4):691-703. DOI: 10.15832/ankutbd.959820

ABSTRACT
This study was conducted to determine the changes in the physicochemical and microbiological qualities of the non-sulfited sun dried apricots (NSDAs) at three different moisture contents (MCs, 13.7, 23.5 and 27.0%) and at four different storage temperatures (4, 10, 20, and 30 °C) for 12 months in bulk and packaged samples. NSDAs were subjected to physical (moisture, water activity, pH and reflectance colour values), chemical (browning formation, β-carotene, and acidity), and microbial (counts for total aerobic mesophilic and psycrofilic bacteria, yeast and mould, xerofilic mould and yeast, osmophilic yeast, lactic acid bacteria, *Enterobacteriaceae*, *Staphylococcus-Micrococcus* spp.) analyses at one-month time intervals. Results indicated that while the fastest brown colour formation occurred in the samples containing 23.5% MC and stored at 30 °C, the browning decelerated below 10 °C. No significant change was observed in β-carotene contents of NSDAs during storage ($P>0.05$). After rehydration, osmophilic yeast count of NSDAs increased by 2.5 log colony-forming units (cfu/g). Significant reductions were observed in the microbial loads of the samples at 23.5 and 27.0% MCs with the decreasing water activity ($P<0.05$) during storage and increasing storage temperatures. However, yeast and mould counts exceeded 5.0 log cfu/g of the samples containing 27.0% MC after 2-months storage at 20 °C. Overall, we suggest that MC and storage temperature for NSDAs should be below 23.5% and 20 °C to achieve high microbial and physicochemical qualities for a year, respectively.

Keywords: Dried apricots, Colour, Water activity, Browning index, β-carotene content, Yeast and mould counts

1. Introduction

Sulfur dioxide (SO_2) treatment of apricots before drying not only prevents enzymatic and non-enzymatic browning, but also prevents microbial spoilage during drying and storage. Aside from these valuable benefits, the adverse health effects of SO_2 have been well-documented for asthmatic patients (Rose 2007). For the last decade, because of increasing consumer demand towards minimally processed and additive free products, NSDAs (Non-sulfited dried apricots) have become more prominent. However, compared to sulfited apricots, NSDAs are more susceptible to chemical and microbial deteriorations during storage at very high MC (over 34%) (Alagöz et al. 2015).

The Codex Alimentarius (Codex Alimentarius Commission 2019) requires that maximum MC of untreated sun-dried apricot should not exceed 20%, while this limit is 22% for Turkish standard (TS 485 2019). Although the NSDAs are sun-dried until their MCs are generally reduced to 10.0–13.0% for long-term storage, dried apricots with high MC (over 25%) are more desirable because of their soft mouth-feel (Davis et al. 1973; Asma 2007). Dried apricots with 25% MC have been preferred due to their softer and tender texture (Davis et al. 1973). NSDAs are rehydrated before marketing because of consumer demand for higher moisture containing dried products (Davis et al. 1973). However, NSDAs at high MCs are susceptible to microbial deteriorations especially by yeasts and molds. Also, unlike golden yellow coloured-sulfited apricots, NSDAs colour become more brown due to the browning reactions take place during sun drying, and at high storage temperatures and/or for prolonged storages (Özkan et al. 2016).

The effects of factors such as MC (Sağırlı et al. 2008), drying temperatures (Ihns et al. 2011), sulfitting methods (Coşkun et al. 2013) and SO_2 content (Türkyılmaz et al. 2012) on the various quality parameters have been studied. On the contrary, there are only a few studies on NSDAs. Alagöz et al. (2015) investigated the effects of various sorbic acid levels (488–1087 mg/kg) on the chemical and microbial qualities of NSDAs (27.2–34.3% MCs) stored at 5–30 °C. Elmacı et al. (2008) studied the chemical and sensory quality changes of modified atmosphere packaged NSDAs (17.5% MC) during storage at 5–25 °C.

However, no information is available concerning the effect of low and intermediate MCs (13.7–27.0%) on the physicochemical and microbial properties of NSDAs stored in bulk and packaged. Therefore, considering both Codex Alimentarius and TS 485 limit for MC, the aim of the present study was to investigate the changes in MCs, water activity (a_w), brown color, reflectance surface color, β-carotene content, total mesophilic aerobic, psychrophilic aerobic bacteria, total yeast and molds, xerophilic yeast-mould, osmophilic yeast, lactic acid bacteria, *Staphylococcus-Micrococcus* spp and *Enterobacteriaceae* NSDAs with various MCs (13.7, 23.5 and 27.0%) during 12 months-storage at various temperatures (4, 10, 20 and 30 °C).

2. Material and Methods

2.1. Materials

Apricots (*Prunus armeniaca* L., var. *Hacıhaliloğlu*) that contained no preservatives were supplied by Malatya Apricot Research Foundation.

Chemical and Reagents. β-carotene was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and HPLC grade liquid chromatography reagents, culture media, and all other analytical grade chemicals and reagents were obtained from Merck Co. (Darmstad, Germany). Aluminum phosphide was obtained from Platin Kimya, Inc (Istanbul, Turkey). Fresh apricots (*Prunus armeniaca* L., var. *Hacıhaliloğlu*) were supplied by Malatya Apricot Research Foundation.

2.2. Drying and fumigation

Fresh apricots (*Prunus armeniaca* L., var. *Hacıhaliloğlu*) were sun-dried for 6 days in Malatya. At the 3rd day of drying, semi-dried apricots were hand-squeezed to remove the pits and then continued for sun-drying for three 3 more days. NSDAs samples (120 kg) were put in a closable container and then left in a temperature-controlled room at 20 °C for one month so that they could equilibrate for MC. Then, the damaged ones, excessively soft and hard, and light and dark coloured dried apricots were removed. NSDAs were loosely put in crates and then fumigated to eliminate the insects and larvae present, using 2 tablets (6 g/each) containing aluminium phosphide (57%, w/w each) (Tamtoxin, Platin Kimya, İstanbul, Turkey) for each m^3 space in a confined room at room temperature for 3 days. A flow diagram of processing and storage of NSDAs is shown in Figure 1.

2.3. Rehydration

The rehydration of dried apricots was carried out on a conveyor system equipped with spray washing system using tap water at 20 °C. After rehydration, NSDAs were held in plastic storage crates at ambient temperature for 4 days for the absorption of moisture on the surface of dried apricots. To increase the MC from 13.7% to the targeted MCs (23.5 and 27.0%), rehydration was carried out in two and three steps respectively, to avoid skin peeling of the apricots. Finally, rehydrated apricots were transferred into closable plastic containers to equilibrate the respective MCs at 20 °C for 2 weeks. Following the rehydration, moisture analysis was carried out to monitor the final MC. As stated before, a part of NSDAs was kept at 13.7% to mimic the MC (10.0–13.0%) of NSDAs for long-term commercial bulk storage (Asma 2007), while MCs of 23.5 and 27.0% were mimic the commercially marketed packaged NSDAs, although these two MCs were above the both Codex Alimentarius and TS 485 limits.

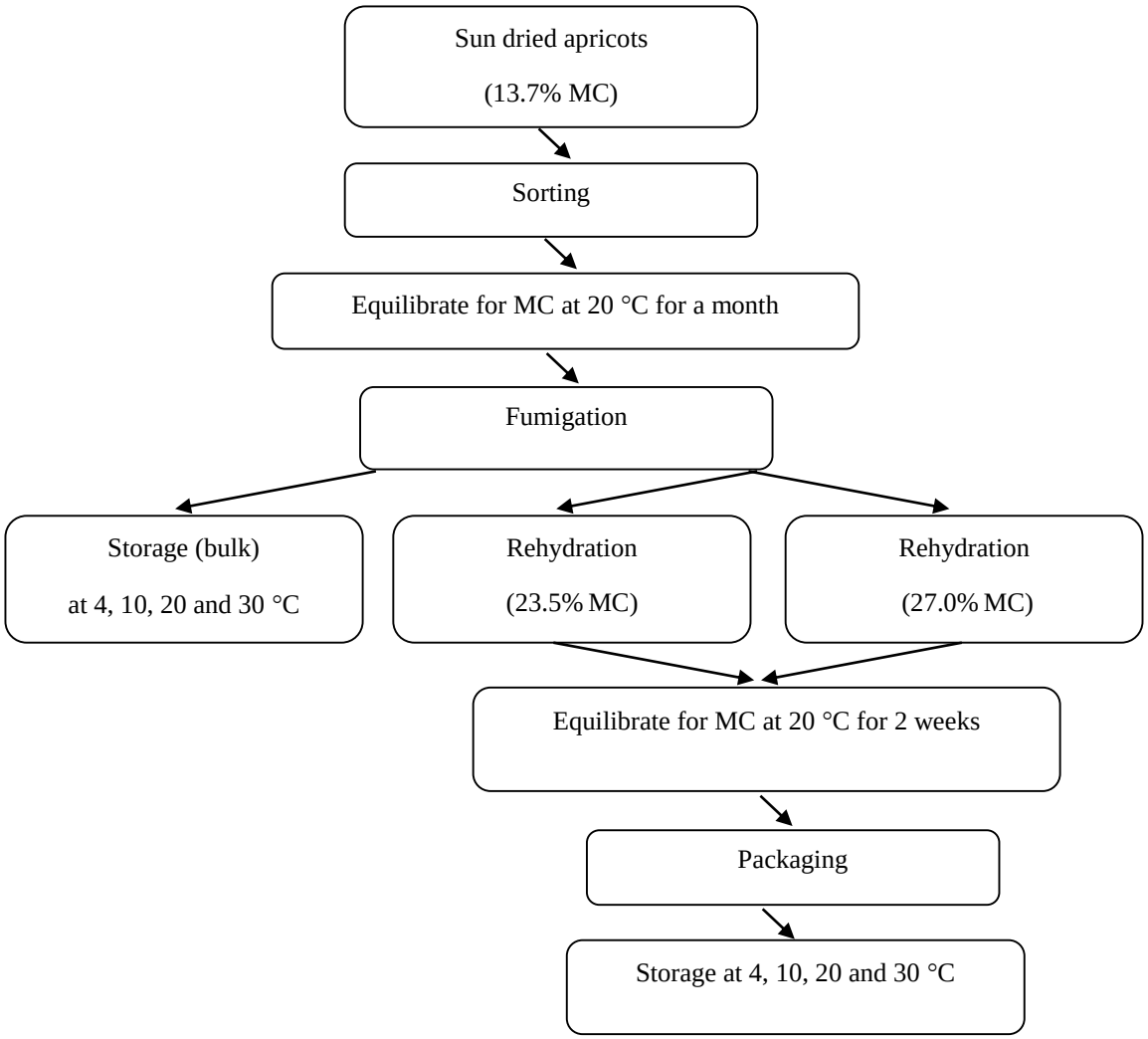


Figure 1- Flow diagram of the processing and storage of non-sulfitted dried apricots

2.4. Packaging and storage

For the packaged storage, 400-g samples at 23.5 and 27.0% MCs were placed in polystyrene trays (14 x 22 x 2.5 cm) which was then covered with PVC film (moisture permeability: 500 cm^3/cm^2.day and O_2 permeability: 20 000 dm^3 O_2/cm^2.day) to mimic consumer packaging. The PVC film was applied one layer and stretched moderately to minimize permeability change. For the bulk storage, a 10-kg sample at 13.7% MC was transferred into temperature controlled incubators (Sanyo MIR 153 and 253, Gunma, Japan) at 4, 10, 20 and 30 ºC ± 0.5 °C to mimic the commercial bulk storage conditions for a period of 12 months. For each sampling time, samples (2 packages) were randomly taken out of the storage for the various physical, chemical and microbiological analyses given below. To obtain a homogeneous sample, a-400 g sample was passed twice through a meat grinder (Tefal Maxi Power 1800 W, France) with 4 mm orifices.

2.5. Moisture Analyses, Water Activity, pH and Titratable Acidity

The MCs of NSDAs were determined using 5 g homogenized sample which was dried in a vacuum oven (Heraeus VT 6025, Hanau, Germany) at 70 °C ± 0.5 °C for 14 h with the AOAC 934.06 method (AOAC 2000). Moisture measurements were replicated four times. The MCs of the samples throughout storage was determined based on the weight changes of NSDAs due to moisture loss. Each time, 20 pieces of NSDAs for each storage temperature from the packaged and bulk stored samples were randomly picked and their weights were recorded. After weight measurements, the same 20-piece samples were immediately placed in the plastic bags which were then hot-sealed and returned to the respective incubators for the future moisture determinations. For the bulk-stored samples, these sub-samples were directly transferred in the incubators without any plastic bags.

The water activities of the homogenized NSDA samples were measured with a hygrometer (AquaLab 3, Decagon Devices, Pullman, USA) with an accuracy ± 0.003 at 25 °C. The pH of the samples was measured using a pH meter (WTW Inolab, Weilheim, Germany). The titratable acidity was measured by the method (942.15) given by AOAC (2000), and the results were expressed as "g anhydrous citric acid/100 g sample".

2.6. Browning measurement

Brown colour formation was determined by the method outlined by Baloch et al. (1973). Details of this analysis were given in Coşkun et al. (2013). Briefly, water soluble brown pigments were extracted with acetic acid containing formaldehyde and then the absorbances of supernatants were determined at 420 nm for brown colour and 600 nm for turbidity with a UV-VIS spectrophotometer (Thermo Spectronic Helios-α, Cambridge, England). Calculation of the browning was based on the difference between the absorbances at 600 and 420 nm. The browning value was expressed as "absorbance at 420 nm/g sample dried weight (DW)".

2.7. β-carotene analyses

2.7.1. Extraction

β-carotene was extracted by the method outlined in Sadler et al. (1990). A 10 g (±0.01 g) homogenized sample in 20 mL distilled water was rehydrated at +4 °C overnight and then homogenized at 13 500 rpm using a benchtop homogenizer (Heidolph SilentCrusher M, Schwabach, Germany) for 2 min. Calcium carbonate (0.2 g) as a neutralizing agent was added onto a-2 g homogenized sample in polypropylene centrifuge tube containing 25 mL extraction solvent (hexane:acetone:ethanol; 25:50:25, v/v/v). The tube was then agitated on an orbital shaker (Heidolph Unimax 2010, Schwabach, Germany) at 220 rpm until the residue became completely colourless (ca. 15 min). 5 mL distilled water was added onto the yellowish-orange extract which was then centrifuged at 9400 *g* at +4 °C for 15 min to separate the polar and nonpolar layers.

2.7.2. Preparation of sample for HPLC

The upper non-polar hexane layer (5 mL) containing β-carotene was pipetted to an amber coloured vial. The hexane was evaporated under a stream of nitrogen at 40 °C (Caliper TurboVap LV, Hopkinton, MA, U.S.A.). The resulting residue was dissolved in 200 µL tetrahydrofuran (THF) with 0.1 g/L buthylated hydroxytoluene (BHT) and then diluted with 1800 µL methanol. After filtration through a 0.22-µm polytetrafluoroethylene (PTFE) filter (Sartorious AG, Goettingen, Germany), the extract was immediately injected to HPLC.

2.7.3. Instrumentation and chromatography

For the separation and quantification of β-carotene, high performance liquid chromatography (HPLC, Agilent 1200 series, Waldbronn, Germany) with the following equipments were used: A binary pump, a photo diode array (PDA) detector, a thermostatted auto-sampler, a degasser and a thermostatted column compartment. Agilent 1200 series ChemStation rev.B.02.01 software was used to process the chromatographic data. Isocratic separation was carried out on a C_{30} (5 µm) column (250 x 4.6 mm) (Phenomenex, Inc, Los Angeles, CA, U.S.A.) with a C_{30} (5 µm) guard column (10 x 4.0 mm) (Phenomenex, Inc). Methanol:tert-buthylmethylether (65:35, v/v) solution containing 0.1 g/L BHT was the mobile phase. The flow rate was 1.0 mL/min, sample injection volume was 50 µl and column temperature was set at 30 °C. The detector was set at 450 nm. The other details are given in Türkyılmaz et al. (2013). The amount of β-carotene was calculated taking into account of recovery values (90–96%).

2.8. Surface Colour Measurement

The surface colour of NSDA samples were measured using a reflectance spectrophotometer (Minolta CM-3600d, Osaka, Japan). The measurements were recorded L* (Lightness), +a* (redness), +b* (yellowness), C* (Chroma), and h° (hue angle) colour coordinates. Browning Index (BI) and total colour changes (ΔE) were calculated using L*, a* and b* coordinates according to the following equations (Ihns et al. 2011):

$$BI = [100\ (x - 0.31)] / 0.17 \quad (1)$$

$$x = [a^* + 1.75L^*] / [5.645L^* + a^* - 3.012b^*] \quad (2)$$

$$\Delta E = [(\Delta a)^2 + (\Delta b)^2 + (\Delta L)^2]^{0.5} \quad (3)$$

2.9. Microbial analyses

The microbial analyses were carried out to evaluate the microbial contamination before and after fumigation, and after rehydration and packaging, and during storage of the NSDA samples. At each sampling day, three packages of samples were removed from the incubators and a 400-g sample was removed from the bulk stored NSDA samples. The NSDA samples were aseptically cut into halves to obtain a uniform subsample. Then, a-30 g subsample was aseptically weighed in a screw cap flask (500 mL). A 20 mL portion of 90 mL aliquot of sterile 0.1% (w/v) peptone water (PW) was added to the flask, which was left at room temperature for 15 min to prevent osmotic shock and revive the stressed microorganisms (Mackey 2000). Then, the

remaining 70 mL PW was added into the flask, which was agitated with an oscillating flask shaker (Griffin and George Ltd., UK) at half speed of the shaker's maximum speed for 1.5 min. Appropriate dilutions in PW were transferred to appropriate media for each microorganism according to APHA-directives (APHA 2002).

The microbial loads of for total mesophilic aerobic (TMAB) and psychrophilic aerobic bacteria (TPAB) were determined on plate count agar (PCA) plates by pour culture method, total yeast and molds (YM) on yeast extract glucose chloramphenicol (YGC) agar, xerophilic yeast-mould (XYM) on dichloran glycerol (DG-18) agar, osmophilic yeast (OSY) on malt yeast extract (MY-40G, osmofilic 40% glucose) agar, lactic acid bacteria (LAB) by spread culture method on de man, rogosa and sharpe (MRS) agar containing cycloheximide (100 μg/mL), *Staphylococcus-Micrococcus* spp (SM) on Baird-Parker (BP) agar (fortified with egg yolk tellurite) and *Enterobacteriaceae* (ENT) on violet red bile dextrose (VRBD) agar by pour plate method. The plates were then incubated at 28 °C for 48 h and 4 °C for 10 days for the detection of TMAB and TPAB, respectively, at 28 °C for 5–6 days for YM, XYM and OSY, at 30 °C for up to 3 days for LAB, at 37 °C for 48 h for SM and at 37 °C for 24 h for ENT. Suspected colonies for LAB, SM and ENT were confirmed with appropriate biochemical tests. All microbiological incubations were carried out in temperature-controlled incubators (Sanyo MIR 253).

2.10. Statistical analysis

Browning, β-carotene content, moisture, titration acidity, pH and microbial counts were the main variables. These variables were subjected to one-way analysis of variance (ANOVA) using the SPSS Statistics software, version 20 (IBM Statistics for Windows, Armonk, NY, USA). Statistical differences among means were determined using Duncan's multiple range tests at 5% significance level. Chemical analyses and microbiological counts were carried out twice analytical and biological triplicates, respectively.

3. Results and Discussion

3.1. Moisture content and a_w

Although the MC of NSDAs should not exceed to 20% for Codex Alimentarius and 22% for Turkish standard (TS 485), the MCs of the packaged NSDA samples were over the standards' limits. The reason for chosing higher MCs for NSDAs was due to mimic the MC of commercially marketed NSDAs. The MCs of the NSDA samples during storage at 4–30 ºC are presented in Table 1. The MCs of the NSDA samples stored at 20 and 30 ºC lost most of their MCs after 1-month storage. The decrease in MC of the NSDA samples during storage was parabolic at 20 and 30 ºC at all MCs (data not shown). For example, the samples packed in PVC film (containing 23.5 and 27.0% MC) lost 44 and 57% MCs after 1-month storage at 20 and 30 ºC, respectively. After 3-months storage, the MCs at all samples were similar and did not change much between 3- and 12-months storage at both temperatures. At the end of 1-year storage, MCs of the samples at 13.7, 23.5 and 27.0% MCs decreased by 47, 67 and 77% at 30 ºC, respectively. These observations clearly showed that most of the MCs were lost for the first 3-months storage at high temperatures (20 and 30 ºC). Moreover, the moisture loss was also higher at higher MCs, compared to lower MCs. Furthermore, as expected, storage at 30 ºC caused more moisture loss than that of 20 ºC.

Table 1-Moisture contents (%) of NSDAs stored at various temperatures for 1 year

		MCs after 1 year of storage			
MCs prior to storage (%)	*Time (months)*	*4 ºC*	*10 ºC*	*20 ºC*	*30 ºC*
13.7	1	17.7	16.1	12.2	10.6
	2	20.7	16.8	11.1	09.0
	3	21.5	18.4	10.2	08.2
	4	18.9	20.6	10.0	07.6
	6	14.0	21.9	11.7	07.3
	9	11.5	12.3	12.3	06.7
	12	11.3	25.4	09.7	07.0
23.5	1	23.1	22.3	16.5	13.2
	2	22.8	21.2	13.9	10.8
	3	22.5	20.7	12.4	09.7
	4	20.6	21.0	11.9	09.0
	6	13.5	21.9	12.8	08.5
	9	10.6	12.9	13.1	07.8
	12	10.2	18.0	11.0	07.7
27.5	1	26.5	25.1	18.1	11.7
	2	26.2	23.3	14.0	09.1
	3	26.0	22.1	12.1	08.0
	4	24.1	21.7	11.4	07.4
	6	16.5	22.1	12.2	06.9
	9	13.5	12.0	12.5	06.2
	12	13.1	17.5	10.3	06.3

The a_w values of the samples at 23.5 and 27.0% MCs were initially 0.675 and 0.696, respectively. These a_w values are in the range of a_w optimums (aw=0.60–0.70) for Maillard reactions. This will be discussed in detail in "3.2. Brown colour formation" section. The a_w values of the samples could not have been measured at 30 ºC because the samples lost most of their moisture after 1-month storage and this made impossible the samples to pass through the grinder. Moreover, a_w values of the sample stored in bulk (13.7% MC) could also have not been measured at 20 ºC due to the very low MC.

Similar to MCs, the a_w of NSDAs also decreased sharply after 1-month storage and this high decrease also continued up to 3-months storage at 20 and 30 °C. This showed that most of the moisture loss occurred during the first 3-months storage at these temperatures as a result of the loss of free water. The highest reductions in both MC and a_w values were observed in the samples stored at 30°C. The samples packaged in PVC films (at 23.5 and 27.0% MCs) could not have kept their initial MC even after 1-month storage at 30 °C. The results indicated that PVC packaging material, which is widely used for dried apricot packaging in Turkey, is not suitable for the rehydrated dried apricots stored at 30 °C.

3.2. Brown colour formation

At the beginning of storage, the absorbance values measured for brown colour at 420 nm in NSDAs ranged from 3.52 to 4.99 A_{420}/g DW, depending on MCs. The acceptable colour for sulfite-treated dried apricots was defined by Nury et al. (1960) as the time where the absorbance values at 440 nm reached to 0.3 (Davis et al. 1973). However, there has been no such absorbance value defined for NSDAs. Therefore, in the present study, only the changes in in the brown colour measured at 420 nm of the NSDA samples were compared throughout the storage. Below 10°C, no significant changes were detected for the brown colour values of the samples throughout the storage period ($P>0.05$); therefore, no kinetic parameter was calculated below 10 ºC. However, above 10 °C, significant increase in brown colour formation was observed as the storage temperature increased from 10 to 20 and 30 °C at all MCs ($P<0.05$) (Table 2). For instance, the brown colour formation for the sample containing 23.5% moisture after 12-months storage at 20 and 30 °C increased by 51 and 80%, respectively.

Table 2- *k* values for the formation of brown colour, ΔE, pH and titratable acidity in NSDA samples during storage at 20 and 30 °C

Temperature (°C)	*Initial MC (%)*	*Formation of brown colour (1/month)*	*ΔE (colour units/month)*	*pH (pH units/month)*	*Titratable acidity (g/[100g DW month])*
20	13.7	0.0598 (0.8820)[a]	0.2902 (0.9233)	–0.0140 (0.8922)	0.0276 (0.8642)
30		0.1483 (0.9533)	0.3694 (0.9310)	–0.0220 (0.8272)	0.0562 (0.9126)
20	23.5	0.0643 (0.9219)	0.1928 (0.9502)	–0.0135 (0.7316)	0.0320 (0.9480)
30		0.1568 (0.9446)	0.3402 (0.8060)	–0.0179 (0.9534)	0.0412 (0.9075)
20	27.0	0.0332 (0.8929)	0.2204 (0.7500)	–0.0098 (0.8803)	0.0477 (0.8309)
30		0.1338 (0.9025)	0.3091 (0.7978)	–0.0137 (0.8399)	0.0558 (0.8555)

[a]: Numbers in parentheses are the coefficient of determination (n=2).

The brown colour formation in NSDA samples at 13.7–27.0% MCs at 20 and 30 °C was fitted to a first-order reaction kinetic model. Investigation of determination coefficients (R^2) values at these temperatures revealed a little higher value for zero-order than for the first-order reaction rate constants. However, zero-order reaction model for brown colour formation in NSDAs would not be possible because MCs in the NSDAs changed not in a constant amount during the 12-months storage period. Therefore, brown-colour formation in NSDAs would not fit zero-order reaction kinetic model. Most studies in sulfite-dried apricots during storage showed that brown colour formation was fitted to a first-order reaction model (Sadler et al. 1990; Sağırlı et al. 2008; Türkyılmaz et al. 2012).

The increase in *k* value for NSDAs stored at 20 °C (0.0643/month) and 30 °C (0.1568 /month) at 23.5% MC clearly showed that brown colour formation was highly dependent on storage temperature. Similarly, Sağırlı et al. (2008) found *k* values as 0.0488 and 0.2411/month for brown colour formation in sulfitted (1458 mg/kg) dried apricots stored at 20 and 30 °C at 36.6% MC, respectively. In another study with lower MC (18.9–24.5%), Türkyılmaz et al. (2012) reported *k* values for sulfitted (188–3864 mg/kg) dried apricots stored at 20 and 30 °C as 0.0193 and 0.0553/month, respectively. The *k* values for brown colour formation increased by 1.5 times increasing the storage temperature from 10 to 20 °C than from 20 to 30 °C. Moreover, increasing the temperature form 20 to 30 °C caused the rate of brown colour formation to increase 4.81 and 7.53 times at 13.7 and 27% MCs, respectively. Studies on sulfited and non-sulfitted dried apricots showed that storage temperature over 20 °C caused significant increase in brown colour formation (Türkyılmaz et al. 2012; Coşkun et al. 2013; Alagöz et al. 2015). The results from the present study and above studies clearly indicated that the storage temperature should be lower than 20 °C in order to control the brown colour formation in dried apricots. To show the effects of the temperature change on brown colour formation, Q_{10} values were calculated, which were 2.36 at 10–20 °C and 3.49 at 20–30 °C. Higher Q_{10} value showed that increase in temperature at 20–30 °C had more effect on brown colour formation than at 10–20 °C.

The MCs also had significant effects on brown colour formation in NSDAs. As expected, the highest brown colour formation was observed in the NSDA samples containing 23.5% MC (a_w=0.675) at both 20 and 30 °C, which corresponds to intermediate MC. As known, the non-enzymatic Maillard browning reactions occur fastest at the intermediate MC, which corresponds to a_w=0.6–0.7 (Beveridge & Harrison 1984). The samples containing 13.7% MC had higher brown colour compared to those containing 27% MC at especially 20 °C. Increasing MC from 13.7 to 23.5% caused 10% decrease in the *k* value for brown colour formation in NSDAs stored at 30 °C, while 7.7% decrease increasing MC from 23.5 to 27%.

3.3. Changes in β-carotene content

The sample chromatogram for carotenoid composition of NSDA sample is presented in Figure 2. β-carotene was the predominant carotenoid in all NSDA samples. Similar composition was also found for the same cultivar (Hacihaliloğlu) in the studies carried out in our laboratory (Sağırlı et al. 2008; Türkyılmaz et al. 2012; Coşkun et al. 2013; Alagöz et al. 2015). β-carotene content in NSDA samples at the beginning of the storage-ranged from 6.5 to 12.8 mg/100 g DW, while 4.7–12.4 mg/100 g DW after 12-months storage. The storage time and temperature had no significant effect on the β-carotene contents of the samples at 13.7, 23.5 and 27.0% MCs during storage at 4–30 °C (P>0.05). Similarly, Alagöz et al. (2015) found that storage at 30 °C for 10 months did not affect the β-carotene content of sorbic acid treated dried apricots (P>0.05). On the contrary, Elmacı et al. (2008) reported that increasing storage time and temperature decreased the β-carotene content of NSDAs during 12-months storage. The decrease observed in β-carotene content might have been due to the higher a_w of the samples during storage. As known, one of the degradation patterns of β-carotene is the enzymatic oxidation which is catalyzed by the enzyme lipoxygenase, showing the optimum activity a_w>0.85 (Berry et al. 1996).

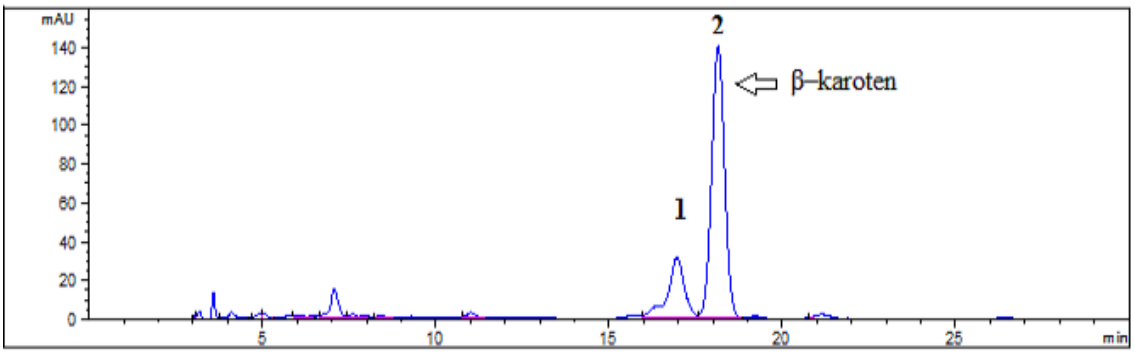


Figure 2- Carotenoid profile of NSDA sample containing 13.7% moisture at the beginning of storage

3.4. Changes in surface colour

For the dried fruits whose MC is not very low, reflectance color measurements, along with browning and carotenoid measurements, can be used to monitor the changes in the color of NSDAs throughout the storage. Results from surface color measurements are presented in Table 2. Both MC and storage temperature affected the reflectance colour values of NSDA samples. Changes in reflectance colour values were found insignificant ($P>0.05$) in the samples stored at low temperatures (4 and 10 °C). On the contrary, L* values decreased by 2.5–2.7 units in the samples at 13.7% MC, 1.8–2.0 units at 23.5% MC and 0.9–1.7 units at 27.0% MC after 12-months storage at 20 and 30 °C, respectively. Much higher decreases (3.7 and 7.4 units) were reported for L* values of the NSDA samples containing 34.0% MC and 488 mg/kg sorbic acid after 10-months storage at 20 and 30 °C, respectively (Alagöz et al. 2015). Studies showed that MCs of dried fuits had tremendeous effects on the reflectance color measurements. For example, Özkan et al. (2003) showed that increasing MC from 15.0 to 30.0% caused significant changes in L*, a*, b*, C* and h^o color values ($P<0.05$). L* value was used as a browning index in many dried fruits, such as raisins (Aguilera et al. 1987), dried figs (Piga et al. 2004) and dried pears (Joubert et al. 2001). In addition to L* value, Browning Index (BI), which was calculated from L*, a* and b* values, has also been used to evaluate brown colour formation (Castañón et al. 1999; Chutintrasri & Noomhorm 2007). Strong negative correlations between BI and browning values (A_{420}/g DW) were found in in the samples containing at 23.5% MC ($r= –0.8575$) and 27.0% MC ($r= –0.9258$) at 30 °C during 12-month storage. This result was in an agreement with the finding of Alagöz et al. (2015) who found a strong negative logarithmic correlation ($r=0.896–0.996$) between BI and browning values of sorbic acid treated dried apricots at 20 and 30 °C. These high correlations indicated that measuring the reflectance colour values of dried apricots may be a good alternative to the spectrophotometric lenghty brown color measurements. As expected, increasing the storage temperature decreased the BI values of the samples, indicating the increase in brown color formation (Table 3).

Table 3- Changes in the reflectance colour values[a] of NSDAs during storage at 20 and 30°C

Temperature (°C)	*Initial MC (%)*	*Time (month)*	*L**	*a**	*b**	*C**	*h°*	*ΔE*	*BI*
20	13.7	0	25.76±2.35 [a]	4.82±1.59 [a]	9.12±2.07 [a]	10.37±2.35	62.30±6.54	–	56.08
		12	23.19±2.17 [b]	4.65±1.65 [a]	6.85±1.63 [b]	8.36±1.98	56.28±8.39	3.43	48.59
30		0	27.30±2.29 [a]	5.40±1.67 [a]	10.42±2.41 [a]	11.79±2.68	62.59±6.08	–	61.06
		12	24.55±1.90 [b]	4.10±1.81 [a]	6.96±2.16 [a]	8.16±2.51	59.85±6.49	4.61	44.56
20	23.5	0	26.26±2.06 [a]	5.84±2.25 [a]	8.83±3.07 [a]	10.65±3.61	56.62±6.87	–	55.98
		12	24.98±2.54 [b]	5.77±2.12 [b]	8.16±2.50 [a]	10.35±3.07	56.90±7.61	1.45	55.20
30		0	25.66±2.55 [a]	6.00±2.25 [a]	9.10±2.71 [a]	10.97±3.29	56.82±7.03	–	59.52
		12	23.66±1.90 [b]	2.95±0.92 [b]	6.26±2.15 [b]	6.96±2.20	64.18±6.95	4.62	38.95
20	27.0	0	26.43±2.34 [a]	7.08±2.45 [a]	9.58±3.17 [a]	11.98±3.79	53.20±6.38	–	63.11
		12	25.54±1.83 [b]	4.86±1.70 [b]	7.34±2.64 [b]	8.87±2.96	56.25±6.91	3.28	46.76
30		0	24.32±1.66 [a]	5.58±1.28 [a]	7.98±2.14 [a]	9.77±2.35	54.62±5.13	–	55.30
		12	22.63±1.37 [b]	2.87±0.92 [b]	5.73±1.93 [b]	6.45±1.99	62.54±7.11	3.91	37.60

[a]: Colour values are expressed as mean ± standard deviation (n=30). The letters indicated for the means which are at the begining and end of the storage in the same column with different lower case superscripts are significantly different (P<0.05).

ΔE (total change in colour) value is another useful indicator which is used to show the colour change in dried fruits. ΔE of the NSDA samples increased with increasing storage temperature, which indicated that higher colour deterioration occurred at high storage temperatures (Table 2). The change in colour might be due to pigment degradation, browning reaction or both during drying and storage. The changes in ΔE values were fitted to zero-order kinetic model, which was in agreement with the former studies (Barreiro et al. 1997; Chutintrasri & Noomhorm 2007). The highest *k* values for the change in ΔE (0.3091–0.3694 colour units/month) of all NSDA samples were determined at 30 °C. Furthermore, the colour deterioration occurred faster in bulk samples than the samples stored in PVC packages at all temperatures. The results showed that the colour properties of NSDAs were affected by both storage temperature and packaging conditions during storage.

3.5 Changes in microbial counts

After fumigation of NSDAs, no live insects were observed in the samples during 12-months storage. The effect of fumigation on the microbial flora of NSDAs was also determined. Before fumigation, TMAB, TPAB, YM, XYM, OSY, LAB, ENT, and SM counts were 3.93 (±0.07) and 2.75 (±0.20), 2.09 (±0.03), 2.65 (±0.11), <0.60, <0.60, <0.60 and <1.60 log cfu/g, respectively. The microbial counts after fumigation are presented at "month 0" column in Table 4. Results of counts for OSY, LAB, ENT, and SM were not given in Table 4 because they were generally below detection limit. Compared to the microbial of loads of the samples before (given above) and after fumigation (month 0 in Table 4) the number TAMB and TAPB decreased slightly by 0.4 and 0.3 log cfu/g (P>0.05), respectively, whereas LAB count significantly increased from <0.60 log cfu/g to <1.30 log cfu/g (P<0.05). However, no significant changes in YM, XYM, OSY, ENT, and SM were found (P>0.05). Similarly, Alagöz et al. (2015) reported that counts for TMAB and TPAB were unchanged, and the YM counts declined by 0.6 log cfu/g after fumigation. Overall, the fumigation process had no effect on the number of YM, XYM, but increased the number of LAB and slightly decreased that of TMAB and TPAB.

Following fumigation, a part of the samples was rehydrated from the NSDAs (13.7% MC) to 23.5 and 27.0% MC. After rehydration, initial numbers of each microbial groups for the samples at 23.5 and 27% MCs are presented at "month 0" columns in Table 4. When compared to the initial counts of the sample at 13.7% MC, TAMB counts increased by 0.84 and 1.01 log cfu/g in the samples at 23.5 and 27.0% MCs (Table 4), respectively (P<0.05). While the number of OSY was below the detection limit

before rehydration, the number increased by 1.99 and 1.91 log cfu/g for the samples at 23.5 and 27.0% MCs, respectively ($P<0.05$). However, rehydration did not increase the numbers of TAPB, YM, OSY, XYM, LAB, SM and ENT ($P>0.05$). Alagöz et al. (2015) reported that the number of YM decreased by 0.7 log cfu/g after rehydration of the samples to 27.2% MC while YM increased by 0.7 log cfu/g for the samples with 34.1% MC. Results of the present study indicated that a_w values (0.67–0.69) for the rehydrated NSDAs at 23.5 and 27.0% MCs were appropriate for the growth of TAMB and OSY.

Generally, counts of the sample at 13.7% MC for TAMB and TAPB, YM, XM, and OSY were not affected by the storage temperatures. During 12-months storage, almost no changes in microbial counts were observed for all storage temperatures and the microbial groups. Although very slight increases and decreases ($P>0.05$) in the counts were observed, they are negligible in practice. However, the number of TAMB and TAPB in the sample at 23.5% MC decreased during storage at all temperatures ($P<0.05$). Despite the fluctuation of the bacterial counts between month 4–9, the decrease in the number of TAMB and TAPB were in the range of 1.20–0.75 and 0.91–1.01 log cfu/g for the storage temperatures between 4–30 °C and 4–10 °C during 12-months storage period (Table 4). Similarly, the number of TAMB in the sample at 27.0% MC decreased ($P<0.05$) after 12 months for all the storage temperatures (Table 4). Also, the number of TAPB decreased by 0.96 and 1.02 log cfu/g after 12 months at 4 and 10 °C, respectively ($P<0.05$). The decrease in bacterial counts for the samples at 23.5 and 27.0% MCs could be explained by the greater decrease in MC and a_w with increasing storage temperature. Significant reduction in TAMB counts with decreasing a_w during 12 months in bulk stored sulfitted dried apricots (SDA) at 5–30 °C was reported by Türkyılmaz et al. (2013). However, Alagöz et al. (2015) reported that TAMB decreased slightly, while a_w values did not change during the 10-months storage of packaged SDAs at 4–30 °C.

While YM and XYM counts did not change in the sample at 13.7% MC during the storage at all temperatures, they decreased in the sample at 23.5 and 27.0% MCs in the same storage conditions ($P<0.05$). The YM and XYM counts for the sample at 23.5% MC were similar to the initial values and decreased after 3-months storage at 4 °C, ($P<0.05$). The YM and XYM counts of the stored samples at 10, 20 and 30 °C significantly decreased ($P<0.05$) after the 3rd, 2nd and 1st month, respectively while they remained constant for the samples stored at 4 °C.

As seen in Table 4, YM count for the sample at 27.0% MC increased to 6 and 4 log cfu/g level after the 2nd and 1st month of storage at 20 and 30 °C, respectively, exceeding the legal limit. YM counts for the sample at 27.0% MC did not change significantly until the 6th month at 4 and 10 °C ($P>0.05$), and then they decreased (1.2 log cfu/g) after 12-months storage ($P<0.05$). Reduction in YM counts with increasing storage temperatures (20 and 30 °C) increased as seen in Table 4. Interestingly, YM and XYM followed a similar pattern in that they increased rapidly in numbers during the first 2-months storage at 20 and 30 °C. After 2 months, sharp decreases of the counts for the samples at 27.0% MC were observed. Counts for YM and XYM were in the range between 3.85–2.53 and 3.77–2.5 log cfu/g, compared to that in the following 3rd month, at 20 and 30 ºC, respectively. Microbial growth is expected to increase with increasing storage temperature. However, in our study, microbial growth was low or limited as the moisture loss increased with increasing temperature and storage time.

OSY-counts in the 13.7% MC stored at all temperatures remained below the detection level (<0.60 log cfu/g) throughout 12-months storage. On the contrary, OSY-counts remained almost constant (except month 4) and remained below the detection level after 9th month at 4 and 10 °C, and after 6 and 2 months at 20 and 30 °C, respectively. In these samples, increase in microbial reduction due to increase in storage temperature was observed. A similar trend in OSY-counts for the sample at 27.0% MC to that of the sample at 23.5% MC was also observed. Counts for OSY followed similar pattern to that of YM and XYM increased to 6.49 log cfu/g level after 2-months storage at 20 °C for the sample at 27.0% MC. After 2 months, a sharp reduction was observed for the counts of YM, XYM, and OSY. This trend might be related to the sharp decrease of the MC from 27% (a_w= 0.69) to 9.1% (a_w= 0.55) in the first 2-months storage. In the following months, MC remained at between 8.0–6.3% until the end of the storage.

Table 4- Microbial counts* of the NSDAs at various MCs during storage

Microbial count (log cfu/g)*	*MC** (%)*	*Storage temp. (ºC)*	*Storage period (months)*							
			0	*1*	*2*	*3*	*4*	*6*	*9*	*12*
TMAB		4°C	3.56 ^{bc}	3.88 ^{a}	3.63 ^{ab}	3.41 ^{bc}	3.45 ^{bc}	3.32 ^{cd}	2.86 ^{e}	3.40 ^{d}
		10°C	3.56 ^{b}	4.70 ^{a}	3.28 ^{b}	3.43 ^{b}	3.56 ^{b}	3.53 ^{b}	3.42 ^{b}	3.45 ^{b}
		20°C	3.56 ^{cd}	4.04 ^{ab}	3.20 ^{a}	3.51 ^{cd}	3.60 ^{bc}	3.50 ^{cd}	3.21 ^{d}	3.28 ^{cd}
		30°C	3.56 ^{a}	3.63 ^{a}	3.56 ^{a}	3.88 ^{a}	3.70 ^{a}	3.83 ^{a}	4.32 ^{a}	3.71 ^{a}
TPAB		4°C	2.48 ^{cd}	1.79 ^{e}	1.51 ^{f}	2.59 ^{c}	2.35 ^{d}	3.34 ^{b}	4.00 ^{a}	2.34 ^{d}
		10°C	2.48 ^{b}	2.08 ^{cd}	2.41 ^{bc}	2.32 ^{b}	2.53 ^{b}	3.50 ^{a}	3.56 ^{a}	2.08 ^{d}
YM	13.7	4°C	2.23 ^{cd}	2.90 ^{b}	4.93 ^{a}	2.88 ^{b}	2.25 ^{c}	2.33 ^{c}	1.99 ^{d}	2.10 ^{cd}
	(NP)	10°C	2.23 ^{cd}	3.21 ^{a}	2.78 ^{b}	2.27 ^{cd}	2.01 ^{de}	2.35 ^{c}	2.00 ^{e}	2.14 ^{de}
		20°C	2.23 ^{ab}	2.04 ^{b}	2.40 ^{a}	2.00 ^{cd}	2.33 ^{a}	2.33 ^{a}	1.63 ^{c}	1.46 ^{c}
		30°C	2.23 ^{b}	2.31 ^{b}	2.28 ^{a}	2.28 ^{b}	2.10 ^{b}	2.10 ^{bc}	1.99 ^{c}	1.98 ^{c}
XYM		4°C	2.65 ^{b}	2.60 ^{b}	4.94 ^{a}	2.78 ^{b}	2.31 ^{c}	2.22 ^{c}	1.01 ^{d}	2.20 ^{c}
		10°C	2.26 ^{c}	3.17 ^{a}	2.81 ^{b}	2.14 ^{cd}	1.99 ^{d}	2.30 ^{c}	2.01 ^{cd}	2.11 ^{cd}
		20°C	2.26 ^{b}	2.04 ^{bc}	2.64 ^{b}	2.02 ^{bc}	2.21 ^{bc}	3.68 ^{a}	1.74 ^{c}	1.40 ^{c}
		30°C	2.26 ^{a}	2.34 ^{a}	2.34 ^{a}	2.34 ^{a}	2.09 ^{ab}	2.23 ^{a}	1.91 ^{b}	2.08 ^{ab}
TMAB		4°C	4.40 ^{bc}	4.31 ^{bc}	3.54 ^{d}	4.02 ^{c}	4.82 ^{a}	4.59 ^{ab}	3.43 ^{e}	3.20 ^{d}
		10°C	4.40 ^{a}	3.55 ^{bc}	3.81 ^{b}	3.55 ^{bc}	3.45 ^{bc}	3.52 ^{bc}	4.03 ^{bc}	3.25 ^{c}
		20°C	4.40 ^{b}	3.58 ^{bc}	3.59 ^{b}	3.36 ^{bc}	3.28 ^{bc}	5.13 ^{a}	2.50 ^{bc}	3.49 ^{bc}
		30°C	4.40 ^{a}	3.88 ^{b}	3.33 ^{c}	4.41 ^{a}	3.32 ^{c}	3.37 ^{c}	3.43 ^{c}	3.65 ^{bc}
TPAB		4°C	2.67 ^{cd}	2.52 ^{cd}	2.21 ^{de}	2.06 ^{ef}	3.06 ^{c}	4.41 ^{a}	3.55 ^{b}	1.76 ^{f}
		10°C	2.67 ^{c}	2.25 ^{de}	1.91 ^{e}	1.91 ^{de}	2.52 ^{cd}	3.63 ^{b}	4.12 ^{a}	1.66 ^{e}
YM	23.5	4°C	2.29 ^{b}	2.81 ^{a}	2.49 ^{ab}	1.95 ^{c}	1.79 ^{cd}	1.45 ^{e}	1.92 ^{c}	1.61 ^{de}
	(P)	10°C	2.29 ^{a}	2.31 ^{a}	4.64 ^{a}	1.40 ^{c}	1.90 ^{b}	1.08 ^{d}	0.60 ^{c}	1.00 ^{d}
		20°C	2.29 ^{ab}	2.64 ^{a}	1.92 ^{bc}	1.48 ^{c}	2.53 ^{ab}	1.41 ^{c}	1.04 ^{cd}	0.60 ^{d}
		30°C	2.29 ^{a}	1.04 ^{b}	1.11 ^{b}	1.08 ^{b}	1.00 ^{bc}	0.95 ^{bcd}	0.70 ^{d}	0.70 ^{cd}
XYM		4°C	2.39 ^{b}	2.89 ^{a}	2.58 ^{ab}	2.08 ^{c}	1.81 ^{cd}	1.52 ^{cd}	2.00 ^{c}	1.41 ^{d}
		10°C	2.39 ^{a}	2.36 ^{a}	4.65 ^{a}	1.38 ^{c}	1.71 ^{b}	1.18 ^{cd}	0.60 ^{d}	0.95 ^{d}
		20°C	2.39 ^{ab}	2.52 ^{a}	2.04 ^{b}	1.53 ^{b}	2.38 ^{ab}	1.30 ^{c}	1.15 ^{c}	0.78 ^{d}
		30°C	2.39 ^{a}	0.90 ^{b}	1.04 ^{b}	1.11 ^{b}	0.95 ^{bc}	0.78 ^{c}	0.85 ^{bc}	1.11 ^{b}
TMAB		4°C	4.57 ^{a}	3.84 ^{b}	3.52 ^{c}	3.48 ^{c}	3.92 ^{b}	3.34 ^{c}	3.47 ^{c}	3.43 ^{c}
		10°C	4.57 ^{a}	4.10 ^{b}	3.58 ^{cd}	3.79 ^{c}	3.21 ^{d}	3.79 ^{d}	2.59 ^{e}	3.47 ^{cd}
		20°C	4.57 ^{b}	4.04 ^{b}	4.07 ^{b}	3.74 ^{b}	3.78 ^{b}	5.13 ^{c}	3.38 ^{c}	4.10 ^{b}
		30°C	4.57 ^{a}	4.08 ^{b}	3.33 ^{cd}	3.25 ^{d}	3.49 ^{cd}	3.37 ^{cd}	3.85 ^{bc}	3.44 ^{cd}
TPAB		4°C	2.66 ^{b}	2.53 ^{b}	1.95 ^{d}	2.14 ^{c}	2.86 ^{b}	2.14 ^{a}	3.55 ^{a}	1.70 ^{d}
		10°C	2.66 ^{bc}	2.05 ^{de}	1.93 ^{ef}	3.19 ^{cd}	2.42 ^{cd}	3.19 ^{ab}	2.77 ^{a}	1.64 ^{f}
YM	27.0	4°C	2.42 ^{a}	2.56 ^{a}	2.14 ^{bc}	2.59 ^{a}	2.21 ^{b}	2.59 ^{a}	2.00 ^{c}	1.20 ^{e}
	(P)	10°C	2.42 ^{a}	2.18 ^{a}	2.13 ^{a}	2.12 ^{a}	1.82 ^{b}	2.12 ^{a}	0.78 ^{c}	1.23 ^{c}
		20°C	2.42 ^{bc}	5.25 ^{a}	6.23 ^{a}	2.38 ^{bc}	3.43 ^{b}	2.38 ^{bc}	1.20 ^{c}	0.95 ^{c}
		30°C	2.42 ^{b}	4.05 ^{a}	3.37 ^{a}	0.84 ^{c}	0.78 ^{c}	0.85 ^{c}	0.70 ^{c}	<0.60 ^{c}
XYM		4°C	2.44 ^{a}	2.63 ^{a}	2.07 ^{bc}	2.53 ^{a}	2.14 ^{bc}	1.57 ^{c}	2.00 ^{bc}	1.18 ^{c}
		10°C	2.44 ^{a}	2.21 ^{a}	2.16 ^{a}	2.23 ^{a}	1.79 ^{b}	1.84 ^{b}	0.95 ^{c}	1.18 ^{bc}
		20°C	2.44 ^{bc}	5.19 ^{a}	5.96 ^{a}	2.19 ^{bc}	3.35 ^{b}	1.36 ^{c}	1.23 ^{c}	1.11 ^{c}
		30°C	2.44 ^{c}	4.01 ^{a}	3.10 ^{b}	<0.60 ^{d}	0.78 ^{d}	0.70 ^{d}	0.60 ^{d}	0.84 ^{d}

*: Counts are expressed as mean (n=3), log cfu/g: colony forming unit, **: Moisture content, NP: bulk storage (non-packaged), P: packaged. TMAB: Total Mesophilic Aerobic Bacteria, TPAB: Total Psychrophilic Aerobic Bacteria, YM: Yeast-Mould, XYM: Xerophilic Yeast-Mould. $^{a-f}$ Values with different letters within same row indicate significant difference (Duncan test, $P< 0.05$). <0.60 log cfu/g values indicate that microbial count are below detection limits.

The initial number (1.30 log cfu/g) of LAB in the sample 13.7% MC decreased at the beginning (10 and 20 ºC) and after 1-month storage (4 and 30 ºC) and remained below the detection limit throughout the storage, which might be due to the low MC of the sample. However, the LAB-counts for the samples at 23.5 and 27.0% MCs decreased gradually and faster due to higher moisture loss of NSDAs with the increasing storage temperatures, and later remained below the detection level for the rest of the storage period. On the contrary to the LAB counts of the present study, Alagöz et al. (2015) reported no detectable LAB (<0.6 log cfu/g) in both sorbic acid treated and untreated dried apricots before and after storage. This difference may be due to higher initial LAB loads of the NSDAs, which were 1.3 log cfu/g for the sample at 13.7% MC, and 2.5–2.7 for the samples at 23.5 and 27.0% MCs, respectively, in the present study.

ENT and SM counts, which can be considered as the index for the level of hygiene during production and microbial quality of the product (Jay 2000), were below the detection limit (<0.60 log cfu/g) and no growth was detected prior to and during 12-months storage in all samples and storage temperatures. Similar findings were reported by Sağırlı et al. (2008) and Alagöz et al. (2015) in high moisture dried apricots containing SO_2 and sorbic acid, respectively. In general, as the a_w values of the NSDA

samples decreased with storage time, significant reductions ($P<0.05$) were observed in microbial loads of the samples at 23.5 and 27.0% MCs for all the storage temperatures studied.

According to FDA (2013), the maximum limit of YM counts for dried fruits should not exceed 3 log cfu/g (1 and 2 log cfu/g for yeasts and molds, respectively). In the present study, the YM count for the samples at 13.7 and 23.5% MCs were below the legal limits; therefore, rehydrated NSDAs containing up to 23.5% moisture were acceptable for consumption during storage at 4–30 °C. However, after 1-month storage at 20 and 30 °C, the YM counts of NSDAs containing 27.0% moisture exceeded the permitted legal limits (3 log cfu/g). Alagöz et al. (2015) observed visible yeast and mold colonies on the surface of NSDAs containing no preservative (34.3% MC) after 1-month storage at 20 and 30 °C. In the present study, none of the samples yielded visible colonies. In addition to YM count, OSY count in rehydrated sample (27.0% MC) exceeded to the legal limit of 4 log cfu/g after 1-month storage at 20 and 30 °C. Similarly, XYM counts of the same sample group were above 5 and 4 log cfu/g after 1-month storage at 20 and 30 °C, respectively. These findings suggested that rehydrated NSDAs above 23.0% MC has the potential to be spoiled mostly by osmophilic yeast and xerophilic yeast-molds during the storage at 20 and 30 °C.

3.6 pH and titratable acidity

The initial pH values of NSDAs were found between 5.12 and 5.21, while the initial titratable acidity values ranged between 1.12–1.53 g/100 g DW. Almost no changes in the pH and titratable acidity values were observed in NSDA samples stored at 4 and 10 °C. On the other hand, significant increases were observed in the titratable acidity of the NSDAs stored at 20 and 30 °C ($P<0.05$). The decreases in pH and increases in titratable acidity values of the samples stored at 20 and 30 °C were fitted to zero-order kinetic model. Increasing storage temperature from 20 to 30 °C caused 1.3–1.5 times faster decrease in the pH values, while titratable acidity of values increased 1.2–2 times. The increase in titratable acidity of the samples during higher MC and at 30 °C would be attributable to the increase in microbial counts. This could be easily understood from the very high correlations ($r=0.960–0.991$) between titratable acidity and microbial counts. Similar results were reported by Türkyılmaz et al. (2012) who found that titratable acidity values of sulfitted dried apricots (2899 mg SO_2/kg) showed an increase with the increase of the storage temperature from 10 to 30 °C.

4. Conclusions

Physicochemical and microbial qualities of NSDAs were affected by both MC and storage temperature. Colour deterioration in NSDAs occurred because of browning reactions during the storage. The browning of NSDAs occurred at a much faster rate above 20 °C. Below 10 °C, brown colour formation was limited. The increase in MC from 13.7 to 23.5% substantially accelerated brown colour formation. On the contrary, increasing MC from 23.5 to 27.0% decreased brown colour formation. These findings clearly showed that the fastest brown colour formation occurred at the intermediate MC (23.5%) samples, indicating the non-enzymatic Maillard browning. The content of β-carotene did not change during storage at all temperature. In general, significant decreases were found in microbial loads and a_w values of NSDA samples during storage with the increasing storage temperature. However, among the microorganisms tested, only the number of yeast and mould exceeded permitted limits in the samples at 27.0% MC after 2-months storage at 20 °C. The results suggest that the NSDAs over 23.5% MC need to be stored below 20 °C to preserve their microbial and physicochemical quality.

Acknowledgement

This study is in part of Dr. Güzel's Ph. D. thesis. The authors thank the Apricot Research, Development and Publicity Foundation, Malatya, Turkey for providing dried apricots. We also extent our thanks and appreciations to Mr. Rahmi Semiz from Kayseri for helping us to carry out the rehydration of dried apricots in his company.

References

Aguilera J M, Oppermann K & Sanchez F (1987). Kinetics of browning of sultana grapes. *Journal of Food Science* 52: 990-993

Alagöz S, Türkyılmaz M, Tağı Ş & Özkan M (2015). Effects of different sorbic acid and moisture levels on chemical and microbial qualities of sun-dried apricots during storage. Food Chemistry 174: 356-364

AOAC (2000). Association Official analytical Chemists. In W. Hortwitz (Ed.), AOAC Official analytical methods (17th ed.). Gaithersburg: AOAC International

APHA (American Public Health Association) (2002). In F. P. Downes & K. Ito (Eds.), Compendium of methods for the microbiological examination of foods (4th ed.). Washington: APHA

Asma B M (2007). Malatya: World's capital of apricot culture. Chronica horticulturae 47: 20-24

Baloch A K, Buckle K A & Edwards R A (1973). Measurement of non-enzymic browning of dehydrated carrot. *Journal of the Science of Food and Agriculture* 24: 389-398

Barreiro J A, Milano M & Sandoval A J (1997). Kinetics of colour change of double concentrated tomato paste during thermal treatment. *Journal of Food Engineering* 33: 359-373

Berry H, Lambert C & Larreta-Garde V (1996). Influence of environment modifications on enzyme catalysis: Comparison of macromolecular and molecular effects of cosolvents on lipoxygenase reactions. Annals of the New York Academy of Sciences 799: 290-296

Beveridge T & Harrison J E (1984). Nonenzymatic browning in pear juice concentrate at elevated temperatures. *Journal of Food Science* 49: 1335-1336

Castañón X, Argaiz A & López-Malo A (1999). Effect of storage temperature on the microbial and colour stability of banana purée with addition of vanillin or potassium sorbate. Food Science and Technology Internationa 5: 51-58

Chutintrasri B & Noomhorm A (2007). Colour degradation kinetics of pineapple puree during thermal processing. LWT-Food Science and Technology 40(2): 300-306

Coşkun A L, Türkyılmaz M, Turfan-Aksu O, Erkan-Koç B, Yemiş O & Özkan M (2013). Effects of various sulphuring methods and storage temperatures of the physical and chemical quality of dried apricots. Food Chemistry 141: 3670-3680

Codex Alimentarius Commission. Codex standard for dried apricots (Amended 2019). CodexStan 130–1981, 5 p. <www.fao.org>. Accessed 15.09.2021

Davis E G, McBean D Mc G, Rooney M L & Gipps P G 1973. Mechanisms of sulphur dioxide loss from dried fruits in flexible films. *Journal of Food Technology* 8: 391-405

Elmacı Y, Altuğ T & Pazır F (2008). Quality changes in unsulfured sun dried apricots during storage. *International Journal of Food Properties* 11: 146-157

FDA (Food and Drug Administration) (2013). Microbiological standard for processed foods.http://www.fda.gov.ph/attachments/article/71149/Annex%20I%20%20FC%202013010%20Microbiological%20Standard%20for%20Processed%20Food.pdf. Accessed 20 July 2016

Ihns R, Diamante L M, Savage G P & Vanhanen L (2011). Effect of temperature on the drying characteristics, colour, antioxidant and beta-carotene contents of two apricot varieties. *International Journal of Food Science and Technology* 46: 275-283

Jay J M (2000). Modern Food Microbiology. (2nd ed.), Aspen Publishers, Gaithersburg.

Joubert E, Wium G L & Sadie A (2001). Effect of temperature and fruit-moisture content on discolouration of dried, sulphured Bon Chretien pears during storage. *International Journal of Food Science and Technology* 36: 99-105

Nury F S, Taylor D H & Brekke J E (1960). Research for better quality in dried fruits. Apricots. United States Department of Agriculture, Agricultural Research Service 74-19. Albany, CA, U.S.A

Özkan M & Cemeroğlu B (2002). Desulfiting dried apricots by hydrogen peroxide. Food Chemistry and Toxicology 67: 1651-1635

Özkan M, Kırca A & Cemeroğlu B (2003). Effect of moisture content on CIE colour values in dried apricots. *European Food Research and Technology* 216: 217-219

Özkan M, Türkyılmaz M & Hamzaoğlu F (2016). The effects of SO_2 concentration, polyphenol oxidase activity and apricot contents on the browning of apricots during sun-drying and storage at various temperatures. The Scientific and Technological Research Council of Turkey (TUBİTAK), Project Number: 113O456, pp. 141, Ankara, Turkey

Piga A, Pinna I, Özer K B, Agabbio M & Aksoy U (2004). Hot air dehydration of figs (*Ficus carica* L.): drying kinetics and quality loss. *International Journal of Food Science and Technology* 39: 793-799

Rose A H (2007). Sulphur dioxide and other preservatives. *Journal of Wine Research* 4(1): 43–47

Sadler G, Davis J & Dezman D (1990). Rapid extraction of lycopene and β-carotene from reconstituted tomato paste and pink grapefruit homogenates. *Journal of Food Science* 55: 1460-1461

Sağırlı F, Tağı Ş, Özkan M & Yemiş O (2008). Chemical and microbial stability of high moisture dried apricots during storage. *Journal of the Science of Food and Agriculture* 88: 858-869

Türkyılmaz M, Tağı Ş & Özkan M (2013). Changes in chemical and microbial qualities of dried apricots containing sulphur dioxide at different levels during storage. Food and Bioprocess Technology 6: 1526-1538

TS 485 (2019). Turkish dried apricots standard. Turkish Standards Institution, 13, Ankara

Journal of Agricultural Sciences (Tarim Bilimleri Dergisi) 2022, 28 (4) : 704 - 710 DOI: 10.15832/ankutbd.795425

Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

Investigation of Flight Activity and Damage Status of European Grapevine Moth *Lobesia botrana* (Denis & Schiffermuller) (Lepidoptera: Tortricidae)

Ayşe ÖZDEM[a]*, Arzu AYDAR[a], Yasemin SABAHOĞLU[a]

[a]*Ministry of Agriculture and Forestry Plant Protection Central Research Institute, Ankara, TÜRKİYE*

ARTICLE INFO
Research Article
Corresponding Author: Ayşe ÖZDEM, E-mail: aozdem1@hotmail.com
Received: 15 September 2020 / Revised: 01 December 2021 / Accepted: 05 December 2021 / Online: 15 October 2022

Cite this article
ÖZDEM A, AYDAR A, SABAHOĞLU Y (2022). Investigation of flight activity and damage status of European grapevine moth *Lobesia botrana* (Denis & Schiffermuller) (Lepidoptera: Tortricidae). *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 28(4):704-710. DOI: 10.15832/ankutbd.795425

ABSTRACT
The present study was carried out between 2013 and 2014 in vineyards in Ankara province on the Kalecik karası grape variety. The aim was to determine the biological characteristics of *Lobesia botrana* (Denis & Schiffermuller, 1775) (Lepidoptera: Tortricidae) such as adult flight activity, the duration of its presence in nature and the number of generations per year. These characteristics are essential for the control, as well as for the evaluation of the damage status. During this study, the first adults of *L. botrana* were caught in April and the first peak of the pest was seen in the last week of April in both years. The second peak of the pest occurred in the second-third week of June and it produced two generations per year. It was also determined that the flight of adults ended in September. European grapevine moth adults were found to be active for about six months. Although there were European grapevine moths in the vineyard during the control and count at vegetation and harvest period, there were no eggs and larvae or damage on grapes during harvest of both years. The results of this study clearly demonstrate that it is not necessary to use chemicals to control of *L. botrana* in the vineyards of Kalecik karası.

Keywords: *Lobesia botrana*, population, Integrated pest management, Damage, Kalecik karası

1. Introduction

Turkey ranks among the top countries in terms of vineyards and grape production. The grape production area comprises of 4 054 307 decares, and 2 050 000 tons are produced for table grapes, 1 599 000 tons for dried grapes and 451 000 tons for wine production, making up the total production of 3 600 000 tons (TUIK 2019). Ankara province provides 1.2% of Turkey's wine production. Despite the decline in vineyard areas across Turkey, viticulture in Kalecik district of Ankara province has shown a rapid development with public and private sector investments in the field of viticulture. Kalecik karası is a standard quality grape variety, suitable for red wine, produced in the Central Anatolia Region (Karataş et al. 2010).

While many diseases and pests require chemical control in vineyards, *Lobesia botrana* is the main pest in vineyards (Davydov 1976; Altındişli & Kısmalı 1996; Anonymous 2008). The larva of the European grapevine moth causes damage in the vineyard by eating buds, flowers, unripe grape and ripe ones. It causes abscission during budding and flowering period as well as rotting during the unripe and ripe grape harvest (Anonymous 2008). It affects the product in terms of both quality and quantity. Wine produced from grapes damaged by *L. botrana* has low quality. *L. botrana* infestation negatively affects taste, smell and alcohol content of wine (Altındişli 2014). According to studies done in different regions, it has been observed that the damage of vineyards due to *L. botrana* range between 45-92% in the Aegean region (Önçağ 1975), and between 34-52% in Şanlıurfa in the Southeastern Anatolia region (Mamay & Çakır 2014). According to the studies performed in different countries, the European grapevine moth, which is a polyvoltine species, generates 2 to 4 generations annually depending on geographical areas and climatic conditions (Martin-Vertedor et al. 2010; Caffarra et al. 2012; Pavan et al. 2013; Gilioli et al. 2016). *L. botrana* generally has three generations in our country; however, the species produces two generations in some vineyards in the Central Anatolia Region where our study has been conducted (Anonymous 2008). Since *L. botrana* is the main pest in vineyards, many studies have been carried out from past to present on population change, biology and control of the pest in different regions of our country.

The present study was conducted in vineyards in Kalecik district of Ankara province between 2013 and 2014 and the adult flight activity, the main control against European grapevine moth and important criteria such as climate data and the phenology of vineyard were evaluated together and the damage status was investigated. This study is the first in-depth study on the European grapevine moth in the Central Anatolian Region and aims to obtain basic data on the management strategy of pest control.

2. Material and Methods

Trials were carried out in a vineyard of 15 da, in which Kalecik karası grape variety was cultivated, in the district of Kalecik of Ankara province. This area was infected with the European grapevine moth *Lobesia botrana* Den. & Schiff. (Lepidoptera: Tortricidae). The trial vineyard was established according to the two-tier cordon training system with a trunk of 70 cm and an offshoot height of 110-120 cm whereas the distance between rows was 3 m and the distance above rows was 1.5 m. Pherocon type (Trece® incorporated Pherocon® CAP) sex pheromone traps (E-7, Z-9-dodecadienyl acetate) used to monitor the flight activity of European grapevine moth and a METOS computerized climate device used to obtain climate.

Starting from January 1, when the sum of the maximum temperatures reached 1000 °C, 2 traps/da of sex pheromone traps were hung in the trial vineyard to identify the adult flight activity of *L. botrana* (Anonymous 2011). These traps were hung to the south of vine stocks, at the level of clusters and a height of approximately 50 cm from the ground in the prevailing wind direction. The pheromone traps were checked twice a week until the first adult was obtained and once a week after the first adult was obtained, and the number of adults was counted and recorded. The pheromone capsules were changed every 4-5 weeks or when the sticky tray of these traps got dirty.

After the first adults were caught, the presence of first generation eggs in the flower buds were checked if the temperature was above 15 °C for at least two consecutive nights and continued in the following days. Control of the first generation larvae was carried out when the total effective temperature reached 120 days degrees, starting from January 1. A loupe was used (40X) for the control of larvae. When it reached 520 degrees/days (d/d), a control was performed whether larvae of European grapevine moth were produced in the second generation. At least 150 clusters were checked at each count. During the harvest at least 150 clusters randomly selected from the inner and outer part of grapevines at four directions were checked for any larvae damage. Phenological periods of the grapevine were recorded in both years (Anonymous 2011). Climate data in the two years of the study was obtained using a METOS computerized climate device, 50 m away from the vineyard area.

3. Results and Discussion

Starting from January 1, when the maximum total of temperatures reached 1000 °C, 2 traps/ha of sex pheromone traps were hung to identify the adult flight activity of *L. botrana* on April 4, 2013, and on April 2, 2014. The first adults in pheromone traps were caught on April 22, 2013, and on April 17, 2014 The population trend is given in Figures 1 and 2.

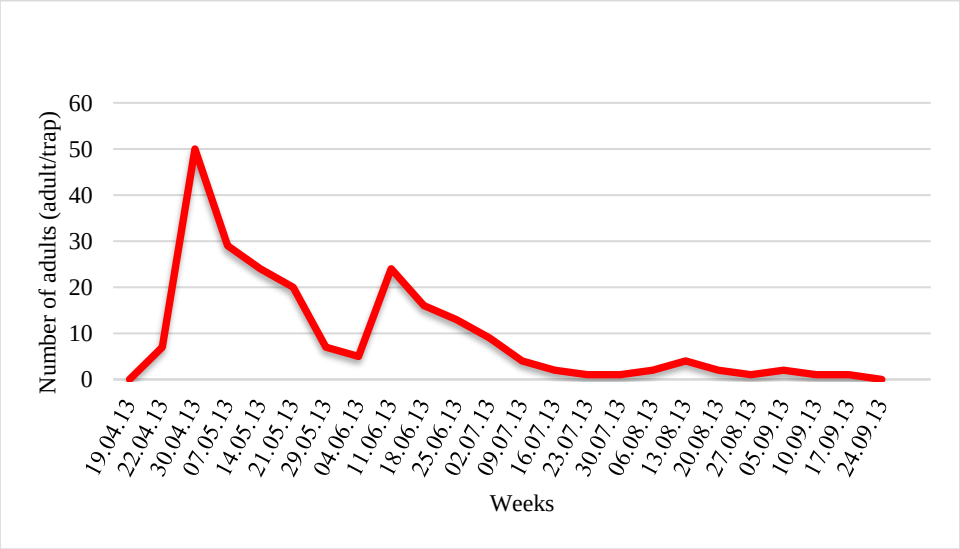


Figure 1- Flight activity of European grapevine moth in Ankara, Kalecik district in 2013

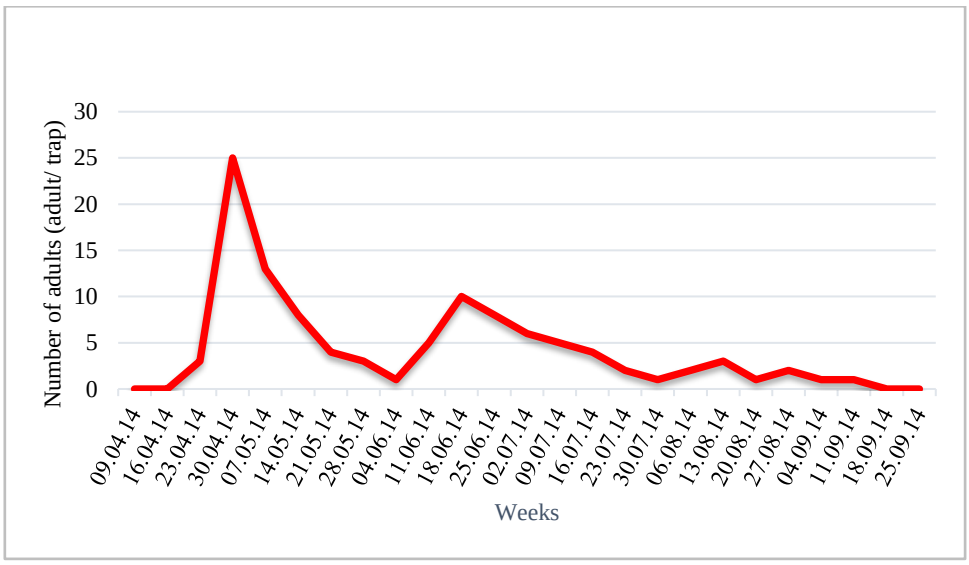


Figure 2- Flight activity of European grapevine moth in Ankara, Kalecik district in 2014

The first adult was obtained in the third week of April in both years. The date when the first adults of European grapevine moth were seen in vineyards was different in studies conducted in different provinces and regions.

In this context, Güçlü and Ünlü (2018) obtained the first adults in different districts of Manisa in late March, Mamay & Çakır (2014) in Şanlıurfa on May 19, Öztürk & Acıöz (2010) in Mersin, Tarsus district in late February-early March. Regional climate, ecological differences and plant phenology are considered to have affected the time when the first adult was seen.

Pentad temperature and relative humidity values were 13.8 °C and 48% (Figure 3) when the first adult was seen in 2013, while it was 17.1 °C and 47% in 2014 (Figure 4). It was recorded that pentad temperature values were 13.8-17.1 °C and relative humidity values were 47-48% when *L. botrana* adults were seen in these years. In a study performed in Tarsus district, Öztürk & Acıöz (2010) stated that the pentad temperature and relative humidity values were 14.1-13.5 °C and 66.5-70.3% respectively when *L. botrana* adults were first caught. Kovancı et al. (2005) stated that the pentad temperature values were 13.3-15.3 °C and relative humidity values were 67.0-71.0 when *L. botrana* adults were first caught in İznik vineyards. Figure 1 shows that the highest number of adults caught in traps in 2013 was on April 30, with 50 adults/trap/week and on June 11, with 24 adults/trap/week whereas the highest number of adults caught in traps in 2014 was on April 30, with 25 adults /trap/week and on June 18, with 10 adults /trap/week (Figure 2). At the first peak of the population, pentad air temperature and relative humidity were 19.5 °C and 44% in 2013, and 16.4 °C and 61% in 2014. At the second peak point of the population, pentad air temperature and relative humidity were 19.1 °C and 52% in 2013, and 21.4 °C and 58% in 2014. It was concluded that European grapevine adults had two peaks during the year, April 30 and June 11 in 2013 and April 30 and June 18in 2014. However, in both years, during the near-harvest period, there was an increase in the number of adults with 4 adults/traps/week in 2013 and 3 adults /trap/week in 2014 on August 13; which pointed out that there may be a third generation. In their study conducted in Ankara, Şereflikoçhisar district, Ataç et al. (1987) stated that the third generation of *L. botrana* had two small flights towards the end of August; however, there were not any eggs and larvae in cluster controls. It was observed that *L. botrana* generally produced 3-4 generations in Çanakkale and Şanlıurfa (Özpınar et al. 2004; Mamay & Çakır 2014), and 4 generations in Bursa, Tarsus, and Manisa (Kovancı et al. 2005; Öztürk & Acıöz 2010; Güleç & Ünlü 2018). In European vineyards, *L. botrana* is active from early spring to late summer (Noma et al. 2010). Abiotic factors may have a major effect on the population dynamics of *L. botrana* at all insect stages. In particular, temperature acting on adult and larval stages regulates female fecundity (Torres-Vila 1996). Adult activity, i.e. flight, feeding, calling, mating and egg-laying, is principally displayed at twilight, although some activity can also occur at daybreak or at any time on cloudy days. Water availability is necessary for adults to reach their potential reproductive capacity (Torres-Vila et al. 1996). The twilight temperature must be above 15-16 °C for two consecutive days and this condition must continue in the next days for the first generation adults of *L. botrana* to mate and lay eggs (Anonymous 2011). While it could be understood that the duration of European grapevine moths in nature varies according to the different ecology, it is thought that this may be due to climate conditions and ecological differences.

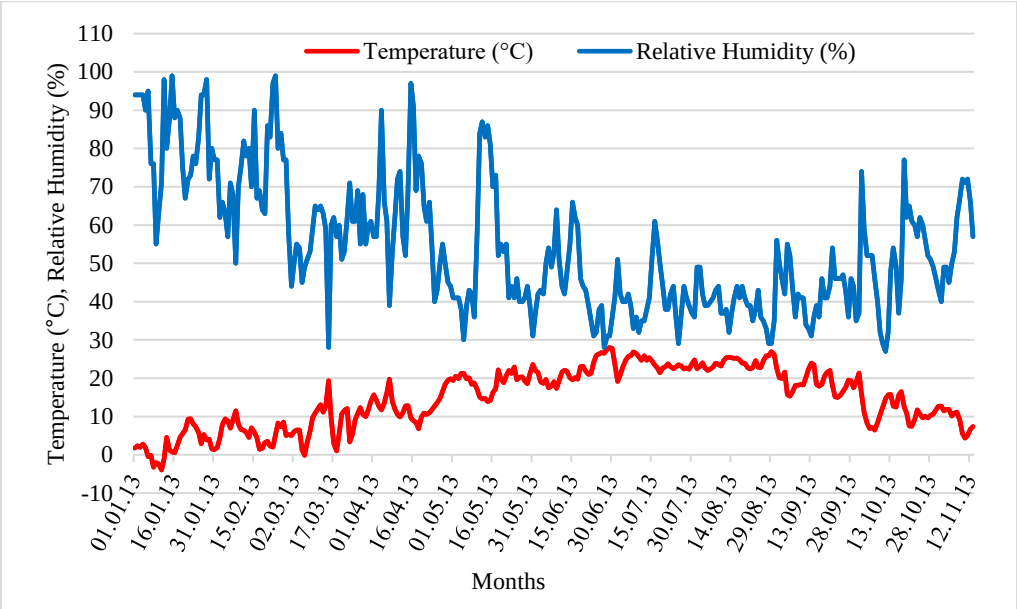


Figure 3- Temperature and relative humidity values in Ankara, Kalecik District in 2013 (January-November)

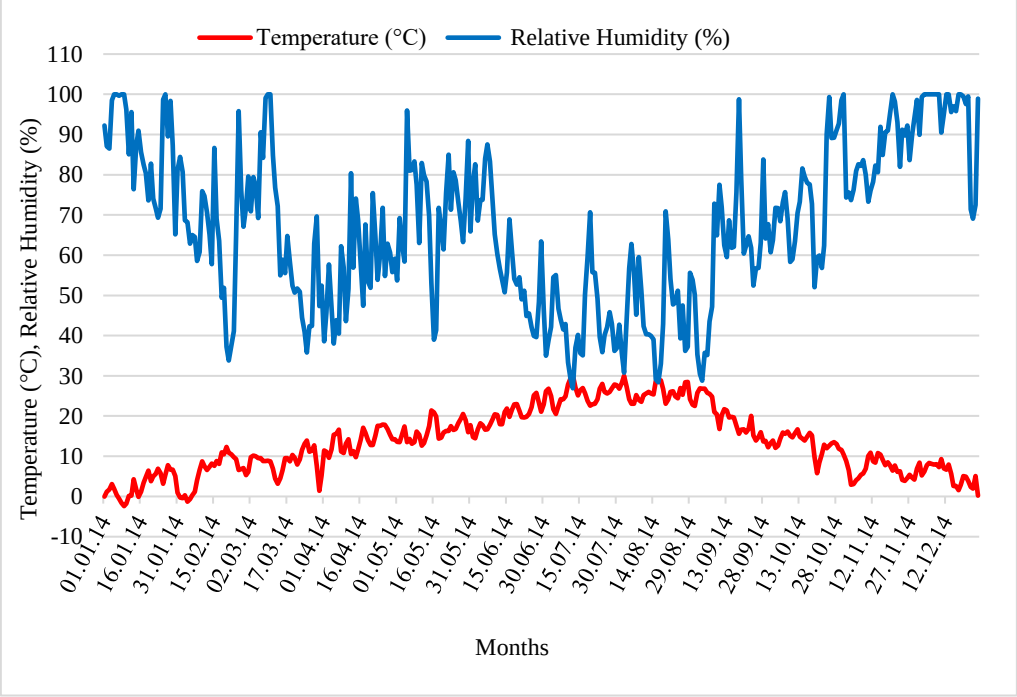


Figure 4- Temperature and relative humidity values in Ankara, Kalecik District in 2014 (January-December)

It was concluded that the dates when the first adult was caught in sex pheromone traps in both years coincided with the dates when leaves were observed phenologically in grapevines. It was also deduced that the adult population phenologically had its first peak during the budding period of clusters in grapevines and its second peak during the unripe grape period. In studies carried out in different regions, the adult population of the European grapevine moth was reported to have its first peak during the flowering period its second peak during unripe grape or the beginning of the ripe period (Mamay & Çakır 2014; Öztürk & Acıöz 2010).

The optimum laying temperature of *L. botrana* is 20-25 ºC, and the twilight temperature must be above and 15-16 ºC for two consecutive days and continue in this manner in the following days for adults in the first generation to mate and lay eggs (Anonymous 2008). The twilight temperature was over 15-16 ºC in 2013, on April 26 and 27. It was reported that during the dates when European grapevine moth adults were obtained and suitable twilight temperatures were observed, temperatures were usually below 20 ºC and this continued until the third week of May and went above 20 ºC for the next few days; however, temperatures were once again below 20 ºC in the first week of June (Figure 3). In 2014, it was observed that the average temperatures were generally below 20 ºC since the date when European grapevine moth adults were seen and that temperatures

were around 20 ºC only for a few days in the second week of May. Low temperatures continued until the second week of June, then started to rise above 20 ºC, and it was observed that the twilight temperature was above 15-16 ºC on May 15-16; however, this did not continue in the following days (Figure 4). In the study performed on the population dynamics of *L. botrana*, Briere & Pracros (1997) reported that the temperature must be between 8-32 ºC to provide the egg and larvae development of the pest. Starting from the days when the twilight temperatures were suitable for spawning of European grapevine moth in both years, pest eggs were searched in each lot, 150 clusters apiece; however, no eggs were found. Generally, it is thought that changes in temperature below 20 °C and unstable temperature changes affect the laying of *L. botrana*. The total of effective temperatures in Kalecik, starting from January 1, 2013, reached 118.67 d/d on May 6, 126.74 d/d on May 7, whereas the total of effective temperatures was close to 120 d/d on May 14 (119.2 d/d) and was 128.6 d/d on May 15. If the total of effective temperatures approach or reach 120 (d/d) as of January 1, it means that the first larvae have emerged and the cluster phenology was undergoing the budding period at that time. In addition to catching adults in traps on these dates, due to the suitability of a total of effective temperatures and cluster in phenological manner, European grapevine moth larvae were searched in each lot, 150 clusters apiece. However, larvae and larvae damage was not found in these clusters. Adults in the second generation of *L. botrana* reached the peak on June 11, 2013. The total of effective temperatures was found to be 520.86 d/d on June 11. It is reported that the most suitable flight temperature for adults is 20-27 °C with relative humidity of 40-70% (Anonymous 2011). Although the average temperature in June in Kalecik district was 21.2 °C and it was rainy between June 12-18, the average relative humidity was 43%. Adults in the second generation of *L. botrana* reached the peak on June 18, 2014. Starting from January 1, the total effective temperatures reached 517.5 d/d and the number of adults caught in traps rapidly decreased while the presence of eggs, larvae, and damage caused by larvae in the second generation could not be found as of July 5. Therefore, there was no spraying against the second generation. During this period, *L. botrana* eggs and larvae were searched in each lot, 150 clusters apiece; however, no eggs or larvae could be found. When this is evaluated together with climate data, temperature and relative humidity, values seemed close to the minimum value for adult flight, and this may affect adult flight, therefore, spawning. Harvesting in the trial vineyard in Kalecik was performed in the last week of August in both years. Cluster samples were collected to identify damaged ones during harvest, and no European grapevine moth egg or larva was found. Even though there were *L. botrana* adults in Kalecik both in 2013 and 2014, the reason why no egg or larvae or related damage were observed may be that unstable climate conditions can be effective as it is stated above. Moreover, it was concluded that these values were close to minimum value for adult flight; therefore, it may affect female butterflies to lay eggs. Coscollá & Dávila-Zurita (1983) concluded that there was a clear correlation between climate and the *L. botrana*'s biology and that the main determinant of the pest's population fluctuations was climatic factors, by using ecoclimatograms.

It was reported that abiotic factors could have a major effect on population dynamics of *L. botrana*'s at all biological stages, and in particular, that the effective temperature regulated fertility during the adult and larva stage (Torres-Vila 1996), adult activity and longevity (Bovey 1966), and egg deaths (Coscollá & Dávila-Zurita 1983). Although in literature it has been discussed that the ecological conditions in the study area may affect the *L. botrana*'s egg laying and damage status, ecological conditions alone are not considered to have an effect on its egg laying and damage status. It has been determined that the damage status of *L. botrana* is different from Kalecik karası in some grape varieties grown under the same ecological conditions.

Around the vineyard, where the trial was conducted, there were 2-3 decares of grape varieties such as Yalova pearl and Black muscat (Muscat Hamburg). During the years when the study was conducted, it was observed that these grape varieties were infested with European grapevine moths, though at a low density. Falling of buds were seen in clusters due to the damage caused by the first generation larva. Also, damage in grapes was seen by the second generation. In addition to adult flight, considering the overall temperatures and phenological features of the grapevine, it was observed that there were egg and larvae phases of European grapevine moth in these grape varieties and that there was damage during the budding and unripe grape period. It could be deduced that adults caught in traps established in vineyards of Kalecik karası variety flew to vineyard areas of Yalova pearl and Black muscat varieties and they formed populations there.

Even though adult flight activity of European grapevine moth was detected in vineyard areas of Kalecik karası grape variety, as it was mentioned before about this variety, there were no eggs or larvae damage. In a study performed on the preference of grape varieties of *L. botrana*, it was reported that the females had several effects on the host selection in four grape varieties (Sharon et al. 2009). Similarly, Birgücü et al. (2015), demonstrated that *L. botrana* was observed the most in Yalova pearl and the least in Alevsiz variety, among grape varieties of Yalova Pearl, Alevsiz Sultana, and Red Seedless. In another study, researchers found that *L. botrana*'s 1st generation caused the highest damage to Barış grapes, 2nd generation to the Yalova İncisi-Trakya İlkeren, and 3rd generation to the Hönüsü-Italia grape varieties (Aslan & Candan 2018). Although the preference was not investigated in our study, there is are indications that there might be a variety of preference. As it is supported by findings in Yalova pearl and Black muscat harvested around vineyard areas close to Kalecik karası grape variety were damaged by *L. botrana* and that Kalecik karası was not. Studies were done in different oviposition stimulants or deterrents of *L. botrana* on different grape varieties. The mated females of *L. botrana* typically oviposit at twilight and lay a series of single eggs, responding to olfactory cues (Gabel &Thiéry 1994; Hurtrel & Thiéry 1999; Masante-Roca et al. 2002) and taste stimuli (Maher & Thiéry 2004; Maher et al. 2006). The contact-chemoreceptors from both the tarsae and ovipositor were determined to respond to different oviposition stimulants or deterrents (Maher & Thiéry 2004; Maher et al. 2006). In oviposition choice assays, non-volatile polar compounds extracted from ripe grape berries of different grapevine varieties stimulate the oviposition of the *L. botrana* female (Maher & Thiéry 2004), whereas fatty acids and their derivatives were shown to be deterrent (Gabel & Thiéry

1996). In a study carried out in our country, it was identified that there is a total of 5 organic acids (citric, tartaric, malic, succinic and fumaric acids) and 14 anthocyanins (5 monoglycosides, 5 acetyls and 4 coumaryls) in Kalecik karası. In addition, a total of 8 phenol compounds (gallic acid, protocathic acid, catechin, epicatechin, B1, B2, B3 and B4 dimers) were detected in the seeds of Kalecik karası berries (Kelebek 2009). Kalecik karası grape berries have a deep purple, almost blue color, thick skins, medium size and frequent cluster. Snjezana (2004) stated that damage of *L. botrana* to cultivars can be said to be attributable to cluster compactness, epidermis thickness and sugar content of grapes.

So far, no study has been conducted on the relationship between the morphological and histochemical features of this variety with *L. botrana*. The results of the present study showed that *L. botrana* does not prefer the Kalecik karası grape variety, but detailed studies suggested that this variety is resistant to *L. botrana*. In conclusion, when both years are evaluated the study conducted on the damage status of European grapevine moth on Kalecik karası grape variety harvested in vineyard areas in Kalecik district for two consecutive years, revealed that even though there were European grapevine moth adults, no eggs, larvae, and therefore, no damage was present. When all the data of the study are evaluated together, it has been concluded that there is no need for a chemical control against European grapevine moth in vineyard areas where Kalecik karası grape variety is produced.

4. Conclusions

The results of this study clearly demonstrate that it is not necessary to use of chemicals to control *L. botrana* in the vineyards of Kalecik karası. Generally, when adults are caught using traps for European grapevine moth with a suitable total of effective temperatures and in phenological features, Provincial Directorate issues a warning on the matter and growers make the disinfestation. In vineyards of Kalecik karası, it can be advised to farmers not to apply chemical control against *L. botrana* through the provincial directorate's technical staff. Since Kalecik karası grape variety not only occupies an important place in wine-making in our country but also is consumed as a table grape variety, demonstrating that there is no need for spraying application under integrated pest management against *L. botrana* will contribute to risk-free agriculture for human health and environment wıthout any insecticide residue. In future studies, conduction of experiments to reveal the resistance of Kalecik karası against *L. botrana* under controlled and semi-field conditions is recommended. In addition, considering the changing of climatic conditions and the presence of different grape varieties grown in our country, the investigation of the damage status of *L. botrana* on different grape varieties is suggested.

Acknowledgements

This study was supported by the Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policy, Project No: TAGEM-BS-11/04-01/03-01.

References

Altındişli F Ö (2014). European grapevine moth and its control in vineyards. Journal of Turkey Seed Growers Association 3(11): 42-44

Altındişli F Ö & Kısmalı Ş (1996). Researches on the using possibilities of mass - trapping technique against Lobesia botrana Den. Schiff (Lepidoptera: Tortricidae) in the Agean Region. 3rd Turkish National Congress of Entomology, September 24-28 1996, Ankara, 91 p.

Anonymous (2008). European grapevine moth, Lobesia botrana Den.et Schiff. (Lepidoptera: Tortricidae) Plant Protection Technical Instruction, GDAR, Volume 4, pp: 334-343 (In Turkish)

Anonymous (2011). European grapevine moth, Lobesia botrana Den.et Schiff. (Lepidoptera: Tortricidae) Vineyard IPM Technical Instruction, GDAR pp: 15-27 (In Turkish)

Ataç Ö, Çevik T & Zeki C (1987). Investigations on the determination of chemical application time by using sex-pheremon traps against European Grape berrymoth Lobesia botrana Den. et Schiff. (Lep., Tortricidae) and the possibilities of the use of these traps in Agricultural warning and forecasting program. Proceedings 1st Turkish National Congress of Entomology, October 13-16 1987. No: 3 pp: 119-128

Aslan M M, & Candan G (2018). Determining the damage level of the European grapevine moth Lobesia botrana (Denis et Schiffermüller) in different grape varieties. KSU Journal of Agriculture and Nature 21(4): 482-488. DOI:10.18016/ksudobil.349828

Birgücü AK, Turanlı F, Gümüş E, Güzel B & Karsavuran Y (2015). The effect of grape cultivars on oviposition preference and larval survival of Lobesia botrana Den. & Schiff. (Lepidoptera: Tortricidae). Fresenius Environmental Bulletin 24 (1): 33-38

Bovey P (1966). Superfamille des Tortricoidea. In: Balachowsky AS, ed. Entomologie Appliquée à l'Agriculture Vol. 2 (1). Paris, France: Masson et Cie. pp: 859-887

Briere J F & Pracros P (1997). Modelisation de la dynamique des populations de i'eudemis de la vigne (Lobesia botrana Den.&Schiff.). Le Corum, Montpellier, France.Tome 2. Paris, France; ANPP pp: 373-380 (https://hal.inrae.fr/hal-02771667)

Caffarra A, Rinaldi M, Eccel E, Rossi V & Pertot I (2012). Modelling the impact of climate change on the interaction between grapevine and its pests and pathogens: European grapevine moth and powdery mildew. Agriculture, Ecosystems & Environment 148: 89-101. DOI: 10.1016/j.agee.2011.11.017

Coscollá R & Dávila-Zurita M (1983). Influence of climatic factors on the severity of attack by Lobesia botrana in vineyards in the Valencia region. OEPP/EPPO Bulletin 13(2): 97-102

Davydov G N (1976). The phenology of the grape moth. Zashchita Rastenii 2: 52

Gabel B & Thiéry D (1994). Non-host plant odour (Tanacetum vulgare; Asteraceae) affects the reproductive behaviour of Lobesia botrana (Lepidoptera: Tortricidae). Journal of Insect Behaviour 7: 149-157. DOI: 10.1007/BF01990077

Gabel B & Thiéry D (1996). Oviposition response of Lobesia botrana females to long-chain free fatty acids and esters from its eggs. Journal of Chemical Ecology 22: 161-171. DOI: 10.1007/BF02040207

Gilioli G, Pasquali S & Marchesini E (2016). A modelling framework for pest population dynamics and management: An application to the grape berry moth. Ecological modelling 320: 348-357. DOI: 10.1016/j.ecolmodel.2015.10.018

Güleç F & Ünlü L (2018). Determination of population development and infestation ratio of Lobesia botrana Den. & Schiff. (Lep.: Tortricidae) in Ahmetli and Turgutlu (Manisa) districts. Anadolu Journal of Agricultural Sciences 33: 191-201. DOI: 10.7161/omuanajas.391372

Hurtrel B & Thiéry D (1999). Modulation of flight activity in Lobesia botrana Den & Schiff (Lepidoptera: Tortricidae) females studied in wind tunnel. Journal of Insect Behaviour 12: 199-211. DOI: 10.1023/A:1020914800170

Karataş H, Değirmenci D & Ağaoğlu Y S (2010). The effects of fruiting cane direction on grape yield and quality of Kalecik Karası grape cultivar (Vitis vinifera L.). Journal of Agricultural Faculty of Uludag University 24 (1):37-46. (https://dergipark.org.tr/tr/download/article-file/154102)

Kelebek H (2009). Researches on the phenolic compounds profile of öküzgözü, bogazkere and kalecik karasi cultivars grown in different regions and their wines. Çukurova University, Institute of Science, PhD Thesis (unpublished), Adana-Turkey 255 pp.

Kovancı B, Türkmen C & Kumral N A (2005). Investigations on the adult population fluctuation of the harmful European grapevine moth [Lobesia botrana Den. & Schiff. (Lep.: Tortricidae)] in the vineyards in Iznik (Bursa) district. 6th Turkey Viticulture Symposium, September 19-23 2005, Tekirdağ-Turkey 1: 289-296

Maher N & Thiéry D (2004). A bioassay to evaluate the activity of chemical stimuli from grape berries on the oviposition of Lobesia botrana (Lepidoptera: Tortricidae). Bulletin of Entomological Research 94(1): 27-33. DOI: 10.1079/BER2003276

Maher N, Thiéry D & Stadler E (2006). Oviposition by Lobesia botrana is stimulated by sugars detected by contact chemoreceptors. Physiological Entomology 31: 14-22. DOI: 10.1111/j.1365-3032.2005. 00476.x

Mamay M & Çakır A (2014). Determination of adult population fluctuation and infestation rate of European grapevine moth [Lobesia botrana Denis & Schiffermüller (Lepidoptera: Tortricidae)] in the vineyards in Şanlıurfa Central county. Plant Protection Bulletin (Bitki Koruma Bülteni) 54(2): 103-114 (https://dergipark.org.tr/tr/download/article-file/428373)

Martin-Vertedor D, Ferrero-Garcıa J J & Torres-Vıla L M (2010). Global warming affects phenology and voltinism of Lobesia botrana in Spain. Agricultural and Forest Entomology 12: 169-176. DOI: 10.1111/j.1461-9563.2009. 00465.x

Masante-Roca I, Gadenne C & Anton S (2002). Plant odour processing in the antennal lobe of male and female grapevine moths, Lobesia botrana (Lepidoptera: Tortricidae). Journal of Insect Physiology 48: 1111-1121. DOI: 10.1016/s0022-1910(02)00204-4

Noma M, Colunga-Garcia M, Brewer J, Landis A & Gooch P (2010). European grapevine moth Lobesia botrana. Michigan State University's invasive M. species factsheets pp.1-2. (https://www.canr.msu.edu/ipm/uploads/files/Forecasting_invasion_risks/ european GrapevineMoth.pdf)

Önçağ G (1975). Studies on identification, distribution, biology, damage, natural enemies and chemical control possibilities of the European grapevine moth in the Aegean Region. İzmir Directorate of Agricultural Quarantine. Technical Bulletin No: 26, 68 p.

Özpınar A, Albayrak A & Görür S E (2004). Determination of population development of European grapevine moth [Lobesia botrana Den. & Schiff. (Lepidoptera: Tortricidae)] and the number of generation in the vineyard areas of Çanakkale province. Proceedings 1st Turkish National Congress of Plant Protection, September 08-10 2004, Samsun-Turkey pp.101

Öztürk N & Acıöz S 2010. The adult population dynamics of the European grapevine moth [Lobesia botrana Den.&Schiff. (Lepidoptera: Tortricidae)] in the vineyards in Tarsus (Mersin-Turkey). Plant Protection Bulletin (Bitki Koruma Bülteni) 50(3): 111-120 (https://dergipark.org.tr/tr/download/article-file/41596)

Pavan F, Floreani C, Barro P, Zandigiacomo P & Dalla Montà L (2013). Occurrence of two different development patterns in Lobesia botrana (Lepidoptera: Tortricidae) larvae during the second generation. Agricultural and Forest Entomology 14: 398-406. DOI:10.1111/afe.12027

Sharon R, Zahavi T Soroker V & Harari A (2009). The effect of grape vine cultivars on Lobesia botrana (Lepidoptera: Tortricidae) population levels. Journal of Pest Science 82(2): 187-193. DOI: 10.1007/s10340-008-0238-5

Snjezana H (2004). Susceptibility of Some Grapevine Cultivars in Area of Vineyards of Podgorica on the Attack of European Grape Berry Moth- Lobesia botrana Den. & Schiff. (Lepidoptera: Tortricidae). Acta Horticulture 652: 355-358 DOI: 10.17660/ActaHortic.2004.652.46

Torres-Vila L M (1996). Efecto de la temperatura de desarrollo preimaginal sobre el potencial biótico de la polilla del racimo de la vid, Lobesia botrana (Denis y Schiffermuller, (1775)) (Lepidoptera: Torticidae). SHILAP Revista de Lepidopterologia 24:197-206

Torres-Vila L M, Stockel J & Rodriguez-Molina M C (1996). Efecto de la indisponibilidad de agua sobre el potencial biótico de la polilla del racimo Lobesia botrana Den. y. Schiff. (Lepidoptera: Tortricidae). Boletn de Sanidad Vegetal, Plagas 22:443-449 (https://www.mapa.gob.es/ministerio/pags/biblioteca/revistas/pdf_plagas/BSVP-22-02-443-449.pdf)

TUIK (2019). Crop Production Statistics, Grape, Retrieved in June 8 2020 from (http://www.tuik.gov.tr/PreTablo.do?alt_id=1001)

Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

Determination of Germination Threshold Value of Chickpea Varieties with GGE Biplot Method Under Different Irrigation Water Salinity Conditions

Ismail TAS[a]*, Tulay TUTENOCAKLI[b], Yalcin COSKUN[b], Mevlut AKCURA[c]

[a]*Department of Agricultural Structures and Irrigation, Agriculture Faculty, Canakkale Onsekiz Mart University, Canakkale, TÜRKİYE*

[b]*Lapseki Vocational College, Canakkale Onsekiz Mart University, Canakkale, TÜRKİYE*

[c]*Department of Field Crops, Agri. Faculty, Canakkale Onsekiz Mart University, Canakkale, TÜRKİYE*

ARTICLE INFO

Research Article
Corresponding Author: Ismail TAS, E-mail: tas_ismail@yahoo.com
Received: 8 February 2021; Revised: 3 December 2021, Accepted: 5 December 2021 / Online: 15 October 2022

Cite this article

TAS I, TUTENOCAKLI T, COSKUN Y, AKCURA M (2022). Determination of Germination Threshold Value of Chickpea Varieties with GGE Biplot Method Under Different Irrigation Water Salinity Conditions. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 28(4):711-722. DOI: 10.15832/ankutbd.876362

ABSTRACT

For sustainable agricultural production, besides drought, plant resistance to irrigation water and soil salinity should be investigated. Researchers mostly focused on salinity and drought resistance of common species such as wheat, barley, maize, etc. However, the number of studies on chickpea with various uses is quite limited. In the present study, 11 chickpea varieties (Aksu, Arda, Hasanbey, Azkan, Cagatay, Aslanbey, Inci, Seckin, 21C, 42C and EN1867) were subjected to germination tests under different levels of irrigation water salinity (ECi) conditions to identify irrigation water salinity resistant and sensitive varieties. In germination tests, besides control treatment (0 dS/m), five different EC levels (6, 8 10, 12 and 16 dS/m) were used in germination solution. The sodium absorption ratio of saline waters was adjusted to be below 3. GGE biplot method was used for visual assessment of genotype response to saline irrigation waters. The threshold salinity value for germination was identified as 12 dS/m based on germination ratios and seedling dry weights, 8 dS/m based on seedling stem lengths and 10 dS/m based on seedling root lengths. Based on germination ratios and seedling dry weights, Azkan cultivar was identified as the most resistant and Cagatay cultivar was identified as the most sensitive cultivar to irrigation water salinity.

Keywords: Irrigation water quality, Germination ratios, Total salinity

1. Introduction

Chickpea is used in human nutrition and animal feeding in various parts of the world. Chickpea kernels are quite rich in protein, so constitute a good source of nutrient. Chickpea straw is a valuable forage source. Chickpea does not require too much water during the growing period and therefore it is considered as a drought-resistant plant. The drought level increases with the effect of the increasing global warming from year to year, and chickpea becomes advantageous compared to many other plant species. Together with increasing populations and rapid economic growth, water shortage has become a fundamental and chronic problem for sustainable agriculture especially in arid and semi-arid regions. Meanwhile, irrigation water quality is continuously deteriorated (Jiang et al. 2012). Water deficits in arid and semi-arid regions have made the use of saline water an inevitable component of irrigations (Assouline et al. 2006; Letey et al. 2007). However, the use of saline or brackish water increases the risk of soil salinization due to salt accumulation within the root zone (Pereira et al. 2002; Min et al. 2014).

Because saline water is among the most limiting factors in agriculture, it is important to study the potential effects of salt stress on plant growth and yield (Sozen & Karadavut 2018; Yurtseven et al. 2018). Soil salinity induces osmotic stress by increasing both the accumulation of toxic concentration of Na and Cl ions and the prevention of uptake of essential nutrients such as K, Ca and NO_3 (Munns 2002; Okhovatian-Ardakani et al. 2010; Peykanpour et al. 2016). Such negative impacts of salinity then alter soil flora and fauna, impairs germination and mergence and ultimately reduce yield and quality. In sustainable agriculture, the primary target is to supply optimum conditions to plants from seeding to harvest without generating any environmental problems. Natural supply of sufficient quantity and quality irrigation water is getting more difficult every day and water quality has become more important problem than water supply. Increasing pollution factors reduce water quality and use of low-quality waters has become an essential issue in irrigations. Such low-quality waters then result in soil salinity and reduce available land resources.

Irrigation water is the primary source of salts accumulated in soils. Inappropriate irrigation water quality directly influences fertilizer uptake from the soil. Plant nutrient uptake through root system depends on nutrient concentrations in soil. In case of

use of low-quality irrigation waters, plant characteristics should be taken into consideration. Both irrigation water pollution and increasing soil salinity in agricultural lands are the most important limiting factors for cultivation of culture crops. To meet increasing needs, marginal waters should be used and agricultural lands should be used for production. Saline lands could also be used in production through the use of salt-resistant plants.

Salinity studies generally are not conducted under field conditions since i) precipitation and groundwater levels are not able to be controlled, ii) soil salinity vary vertically and horizontally in short distances, salt concentrations and soil characteristics may also varies, iii) plant salt uptake and sensitivity may vary with the species and the environmental conditions (light intensity, temperature, relative humidity). Such studies should be conducted in multi replicates, thus require quite large areas and discordant results are obtained in most cases. Just because of these difficulties, generally more practical and reliable greenhouse and laboratory experiments are conducted. However, outcomes of such studies under controlled conditions should be proved under field conditions, then appropriate selection methods should be offered to breeders (Yeo et al. 1990; Ekiz et al. 2000; Koyuncu 2008).

Biplot was developed for the first time by Gabriel (1971) to present significant traits of a data matrix. Then, biplot started to be applied in various disciplines and proved to be highly practical method in visual presentation of experimental data. Biplot method was used in biomedical researches (Gabriel & Odoroff 1990), in multivariate process data (Sparks et al. 1997), two-way cross tables (Bradu & Gabriel 1978, Gabriel 1995), robust methods (Daigle & Rivest 1992), growth curve analysis (Ojeda & Juarez-Cerrillo 1996) and suitability analysis (Greenacre 1984; 1993).

Yan (2014) indicated that GGE Biplot analysis method could be used in analysis of all two-way data, in identification of which genotype is well adapted to which environment and which genotypes yielded better outcomes in all environments. Different genetic variation sources should be investigated with the use of developed methods and selection criteria should be developed in breeding programs. Genotypic/cultivar differences in germination play an important role in identification of salt resistance (Saxena et al. 1994). In this sense, seed germination in saline media in petri dishes is largely used to determine salt resistance rapidly (Jana & Slinkard 1976). Salt tolerance of beans (Goertz & Coons, 1989; Guvenc & Kantar 1996; Elkoca & Kantar 2003), sorghum (Esechie 1994), bread wheat (Kirtok et al. 1994; Coskun & Tas 2017) and different vegetables (Cucci et al. 1994) were tested in petri dishes with different salt solutions. Germination test results should be proven with small plot and field experiments.

In present study, different chickpea varieties were subjected to germination tests under saline irrigation water conditions to identify irrigation water salinity resistant and sensitive varieties. It is expected that the present results will provide important contributions for the breeding of chickpea varieties resistant to irrigation water salinity.

2. Material and Methods

Eleven chickpea cultivars commonly cultivated in Turkey were selected as the plant material of the study (Table 1). Experiments were conducted in randomized blocks - split plots experimental design with 3 replicates in laboratories of Canakkale Onsekiz Mart University Faculty of Agriculture. Pre-tests were conducted with randomly selected cultivars and germination threshold salinity level was determined as 10 dS/m. Present treatments were selected as two levels below and two levels above this threshold.

Table 1- Chickpea genotypes used in this study

Genotype No	*Cultivar name*
G1	Aksu
G2	Arda
G3	Hasanbey
G4	Azkan
G5	Cagatay
G6	Aslanbey
G7	Inci
G8	Seckin
G9	21C
G10	42C
G11	EN1867

Besides the control treatment, irrigation waters were prepared at 6, 8, 10, 12 and 16 dS/m electrical conductivity (ECi), SAR was adjusted to be below 3. Different salt sources (Na, Ca and Mg) were used to prepare saline irrigation waters. Ca/Mg ratio was adjusted to be greater than 2. Twenty seeds of each cultivar were placed into 15 cm diameter petri dishes. Petri dishes were supplemented with 45 mL of saline germination water. Seeded Petri dishes were monitored for 7 days. Along with the recommendations of Wang et al. (2009) and Kusvuran (2015), seeds were considered as germinated when the rootlets were

emerged. Germinated seeds were dried in an oven at 60 °C for 48 hours and weighed to get dry weights. Germinated seeds of each petri dish were converted into percentage to get germination ratios as recommended by Atak et al. (2006).

Descriptive statistics was used to determine germination ratio, root length, stem length and seedling dry weights. Regression and GGE biplot graphs were used for visual presentation of the experimental data (Alkan 2011). GGE biplot method was used to identify the best genotypes at each salinity level, the genotypes tolerant to entire salinity levels and germination threshold salinity level of the genotypes. In GGE biplot analysis of genotype x environment interactions, irrigation water salinity levels were considered as environments. GGE biplot graphs were generated with different perspectives (Yan 2014).

3. Results and Discussion

3.1. Germination ratio

Decreasing germination ratios were observed with increasing irrigation water salinity levels (Table 2). Except for one genotype (G8), germination was observed in all genotypes at 16 dS/m ECi level. Regression graphs for changes in germination ratios of the genotypes based on ECi levels are presented in Figure 1a and Genotype - ECi Biplot graph indicating which genotype had the greatest germination ratio at which ECi level and which genotype had the greatest germination ratio at all ECi levels is presented in Figure 1b. As can be seen in Figure1a, germination ratios linearly decreased with increasing ECi levels. However, genotypes G5 and G8 had greater decreases as compared to the other genotypes.

Table 2- Change in germination ratios of chickpea geneotype with ECi levels

Genotype	*0 dS/m*	*6 dS/m*	*8 dS/m*	*10 dS/m*	*12 dS/m*	*16 dS/m*
G1	73.01 ± 15.06	62.23 ± 7.15	58.83 ± 7.09	52.70 ± 6.45	37.85 ± 14.27	25.58 ± 4.57
G2	75.30 ± 12.51	71.79 ± 12.31	58.00 ± 12.59	51.47 ± 8.47	44.45 ± 7.14	12.58 ± 5.84
G3	71.72 ± 12.63	58.62 ± 4.74	52.45 ± 2.40	47.69 ± 2.24	44.85 ± 5.71	28.49 ± 7.93
G4	76.21 ± 5.59	66.87 ± 0.18	64.31 ± 3.73	62.32 ± 2.16	52.91 ± 6.63	39.58 ± 5.79
G5	39.03 ± 8.39	29.93 ± 4.32	17.50 ± 3.81	16.20 ± 3.58	12.68 ± 2.92	8.86 ± 4.47
G6	54.85 ± 1.97	48.62 ± 6.21	38.32 ± 4.32	35.03 ± 7.27	24.10 ± 13.37	14.23 ± 3.69
G7	67.73 ± 3.10	53.57 ± 15.08	47.17 ± 10.54	37.87 ± 4.41	31.03 ± 7.08	20.40 ± 0.87
G8	21.99 ± 4.42	18.44 ± 5.89	17.72 ± 5.17	13.21 ± 4.67	12.55 ± 5.09	0.00 ± 0.00
G9	47.53 ± 6.19	42.56 ± 4.46	39.03 ± 2.64	31.87 ± 5.62	28.96 ± 5.54	5.64 ± 1.37
G10	76.19 ± 6.68	69.81 ± 12.73	67.18 ± 13.05	61.08 ± 10.24	43.78 ± 5.32	19.32 ± 21.39
G11	41.05 ± 1.78	40.83 ± 1.87	33.33 ± 8.94	31.08 ± 10.56	22.86 ± 11.67	6.70 ± 0.75
Genotype	**	**	**	**	**	**
Average	58.60	51.21	44.89	40.05	32.37	16.49
Minimum	21.99	18.44	17.50	13.21	12.55	0.00
Maximum	76.21	71.79	67.18	62.32	52.91	39.58
LSD 0.01	19.18	18.75	17.78	15.17	19.50	17.64

**: Genotypes are statistically different (P<0.01)

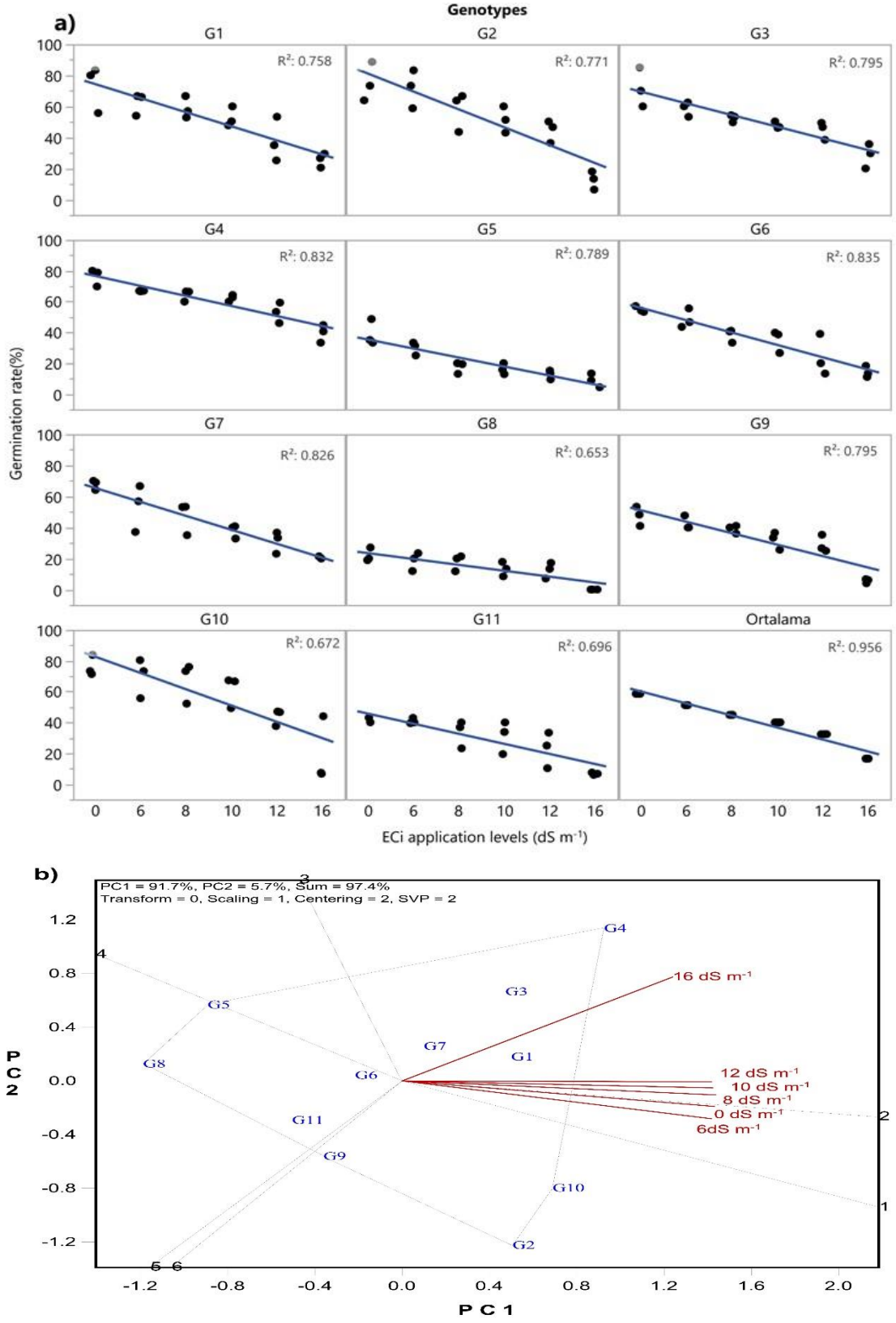


Figure 1- Germination ratios at different ECi levels a) regression b) GGE biplot graph

These genotypes also had quite lower germination ratios than the others in control treatments. Germination ratio of G5 and G8 genotypes in control treatments varied between 39.03 - 21.99%. These values were lower than the germination ratios of the other genotypes at different ECi levels.

A biplot graph was generated for visual presentation of change in germination ratios of the genotypes with ECi levels. In this graph (Figure 1b), salt treatments were gathered under two groups. The first group included 0 and 6 dS/m ECi levels and the second group included 8, 10, 12 and 16 dS/m ECi levels. However, 16 dS/m ECi level had weak correlations with the other

levels. In other words, in terms of germination ratio, genotype response was quite distinctive in 16 dS/m treatments. In terms of germination ratio, the diagonal genotype G4 of the second group (8, 10, 12 and 16 dS/m ECi), G1, G7, G3 genotypes of this group and G10 genotype with the closest position to the first group (6, 0 dS/m) were found to be superior over the others. In terms of germination ratio, the genotype G5, G8 and G9 had the most negative response to salinity (Figure 1b).

3.2. Seedling root length

Decreasing seedling root lengths were observed with increasing irrigation water salinity levels (Table 3). Regression graphs for changes in seedling root lengths of the genotypes with ECi levels are presented in Figure 2a. GGE biplot graph indicating the genotypes with the greatest seedling root length at each salinity level and the genotype with the greatest seedling root length at all ECi levels is presented in Figure 2b. A biplot graph was generated for visual presentation of change in seedling root lengths of the genotypes with ECi levels. In this graph, salt treatments were gathered under two groups. The first group included 0, 6 and 8 dS/m ECi levels and the second group included 10,12 and 16 dS/m ECi levels.

However, 12 and 16 dS/m treatments of the second group were placed at further position to 10 dS/m treatment. In these treatments, the greatest seedling root length was observed in G9 genotype. In the first group (0, 6, 8 dS/m), the greatest root length was observed in G2 and G4 genotypes. In terms of root lengths, the genotype G5 and G1 had the most negative response to salinity (Figure 2b).

Table 3- Change in seedling root lengths of chickpea genotype with ECi levels

Genotype	*0 dS/m*	*6 dS/m*	*8 dS/m*	*10 dS/m*	*12 dS/m*	*16 dS/m*
G1	36.58 ± 7.88	20.81 ± 5.33	17.87 ± 7.08	13.30 ± 5.86	1.63 ± 0.56	1.86 ± 0.81
G2	65.99 ± 10.80	49.67 ± 23.79	33.77 ± 3.39	22.51 ± 13.32	2.22 ± 0.29	1.63 ± 0.56
G3	54.65 ± 4.52	46.24 ± 6.68	32.48 ± 3.60	8.32 ± 1.09	0.00 ± 0.00	0.00 ± 0.00
G4	59.35 ± 6.25	47.61 ± 8.66	23.72 ± 16.16	21.46 ± 1.24	0.97 ± 0.04	0.00 ± 0.00
G5	28.10 ± 5.74	19.52 ± 3.77	12.89 ± 3.69	11.55 ± 4.28	3.93 ± 2.05	0.00 ± 0.00
G6	53.50 ± 8.92	44.11 ± 9.74	30.84 ± 15.37	6.46 ± 1.00	0.00 ± 0.00	0.00 ± 0.00
G7	50.23 ± 0.55	43.83 ± 1.62	28.10 ± 2.97	11.73 ± 6.17	0.00 ± 0.00	0.00 ± 0.00
G8	53.95 ± 3.11	35.81 ± 4.33	21.37 ± 7.16	15.36 ± 7.32	0.00 ± 0.00	0.00 ± 0.00
G9	59.31 ± 5.89	47.28 ± 11.78	32.30 ± 12.69	18.87 ± 14.81	11.43 ± 14.28	2.55 ± 3.47
G10	43.60 ± 3.40	25.61 ± 1.61	16.90 ± 2.64	14.10 ± 0.60	2.38 ± 0.54	0.00 ± 0.00
G11	44.06 ± 6.95	26.14 ± 8.15	15.39 ± 9.99	8.83 ± 4.90	5.54 ± 6.55	0.00 ± 0.00
Genotype	**	**	**	**	**	**
Average	49.94	36.97	24.15	13.86	2.55	0.40
Minimum	28.10	19.52	12.89	6.46	0.00	0.00
Maximum	65.99	49.67	33.77	22.51	11.40	2.55
LSD 0.01	14.81	22.51	20.97	16.56	11.01	2.47

**: Genotypes are statistically different (P<0.01)

3.3. Seedling stem length

Decreasing seedling stem lengths were observed with increasing irrigation water salinity levels (Table 4). Regression graphs for changes in seedling stem lengths of the genotypes based on ECi levels are presented in Figure 3a and Genotype - ECi Biplot graph indicating which genotype had the greatest seedling stem length at which ECi level and which genotype had the greatest seedling stem length at all ECi levels is presented in Figure 3b. In this graph, salt treatments were gathered under three groups.

The first group included 0, 6 and 8 dS/m ECi levels, the second group included 10 and 12 dS/m ECi levels and the third group included 16 dS/m ECi level. In the first group, the greatest stem length was observed in G2 and G6 genotypes. In the second group, G9 and G4 genotypes were prominent for stem lengths. These genotypes were placed at diagonals. The 16 dS/m ECi treatment constituting the third group alone had the lowest vector length. In this treatment, stem lengths of the genotypes were quite low and the greatest values were observed in G10 and G5 genotypes (Figure 3b).

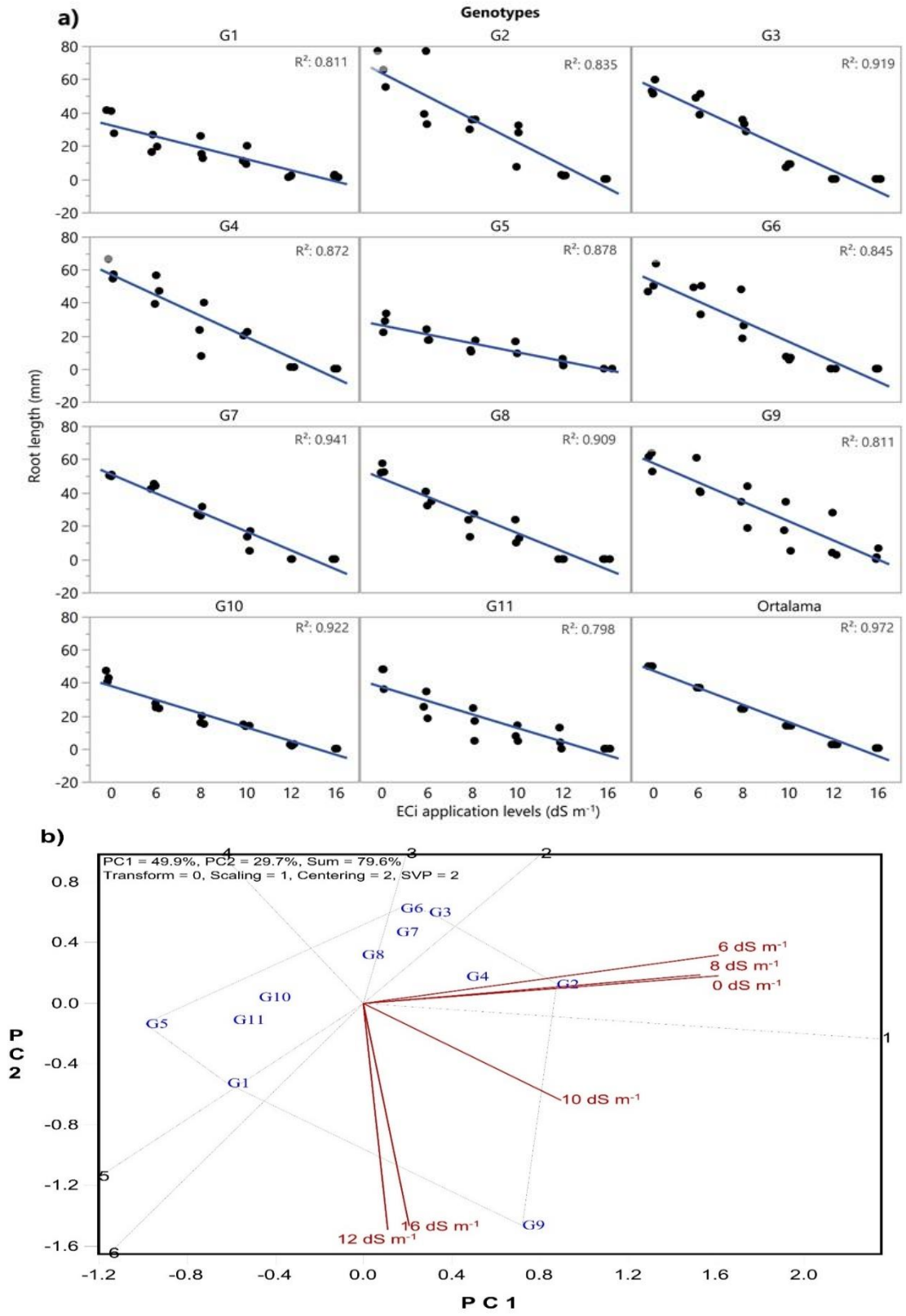


Figure 2- Seedling root lengths at different ECi levels a) regression b) GGE biplot graph

However, in the second group, diagonal ECi levels of 12 and 16 dS/m were placed at further position to 10 dS/m treatment. The greatest stem length in these treatments was observed in G9 genotype. In the first group (0, 6, 8 dS/m), the greatest seedling stem length was observed in G2 and G4 genotypes. In terms of stem lengths, the genotype G5 and G1 had the most negative response to salinity (Figure 3b).

Table 4- Change in seedling stem lengths of chickpea genotype with ECi levels

Genotype	*0 dS/m*	*6 dS/m*	*8 dS/m*	*10 dS/m*	*12 dS/m*	*16 dS/m*
G1	74.99 ± 5.84	56.06 ± 4.04	52.85 ± 7.61	45.58 ± 11.18	25.15 ± 3.38	4.65 ± 1.51
G2	102.31 ± 3.76	85.45 ± 6.87	79.91 ± 10.62	36.57 ± 22.53	32.62 ± 20.91	0.64 ± 1.12
G3	114.39 ± 4.15	84.23 ± 5.77	56.68 ± 23.36	32.69 ± 22.63	7.72 ± 2.86	2.58 ± 4.46
G4	75.19 ± 5.54	57.51 ± 14.14	48.93 ± 7.58	44.92 ± 3.75	29.94 ± 4.90	1.48 ± 2.06
G5	56.82 ± 11.02	44.71 ± 6.10	38.75 ± 11.28	34.97 ± 11.03	17.06 ± 2.34	1.24 ± 2.16
G6	104.51 ± 7.65	96.29 ± 14.72	73.91 ± 13.82	53.37 ± 24.60	23.58 ± 1.03	2.56 ± 0.34
G7	94.33 ± 15.23	87.27 ± 17.26	58.17 ± 25.66	30.10 ± 9.81	17.13 ± 3.22	5.33 ± 1.53
G8	68.29 ± 2.13	51.36 ± 5.21	42.52 ± 7.81	21.86 ± 0.98	20.56 ± 1.71	0.00 ± 0.00
G9	95.64 ± 5.73	78.10 ± 12.53	65.32 ± 10.98	54.02 ± 11.44	31.40 ± 7.35	4.34 ± 1.33
G10	52.68 ± 2.34	47.54 ± 5.82	41.82 ± 5.16	25.95 ± 2.63	23.34 ± 4.59	6.68 ± 1.29
G11	80.71 ± 4.49	59.64 ± 15.25	49.89 ± 7.98	42.63 ± 11.62	19.89 ± 2.35	5.40 ± 0.36
Genotype	**	**	NS	NS	**	**
Average	83.62	68	55.3	38.4	22.6	3.2
Minimum	52.68	44.7	38.8	21.9	7.72	0
Maximum	114.4	96.3	79.9	54	32.6	6.7
LSD 0.01	16.58	25.01	NS	NS	16.73	4.29

**: Genotypes are statistically different (P<0.01)

3.4. Seedling dry weight

Decreasing seedling dry weights were observed with increasing irrigation water salinity levels (Table 5). In biplot graph (Figure 4b) generated for visual presentation of change in seedling dry weights of the genotypes with ECi levels, salinity treatments were gathered under two groups. The first group included 0, 6, 8 and 10 dS/m ECi levels and the second group included 12 and 16 dS/m ECi levels.

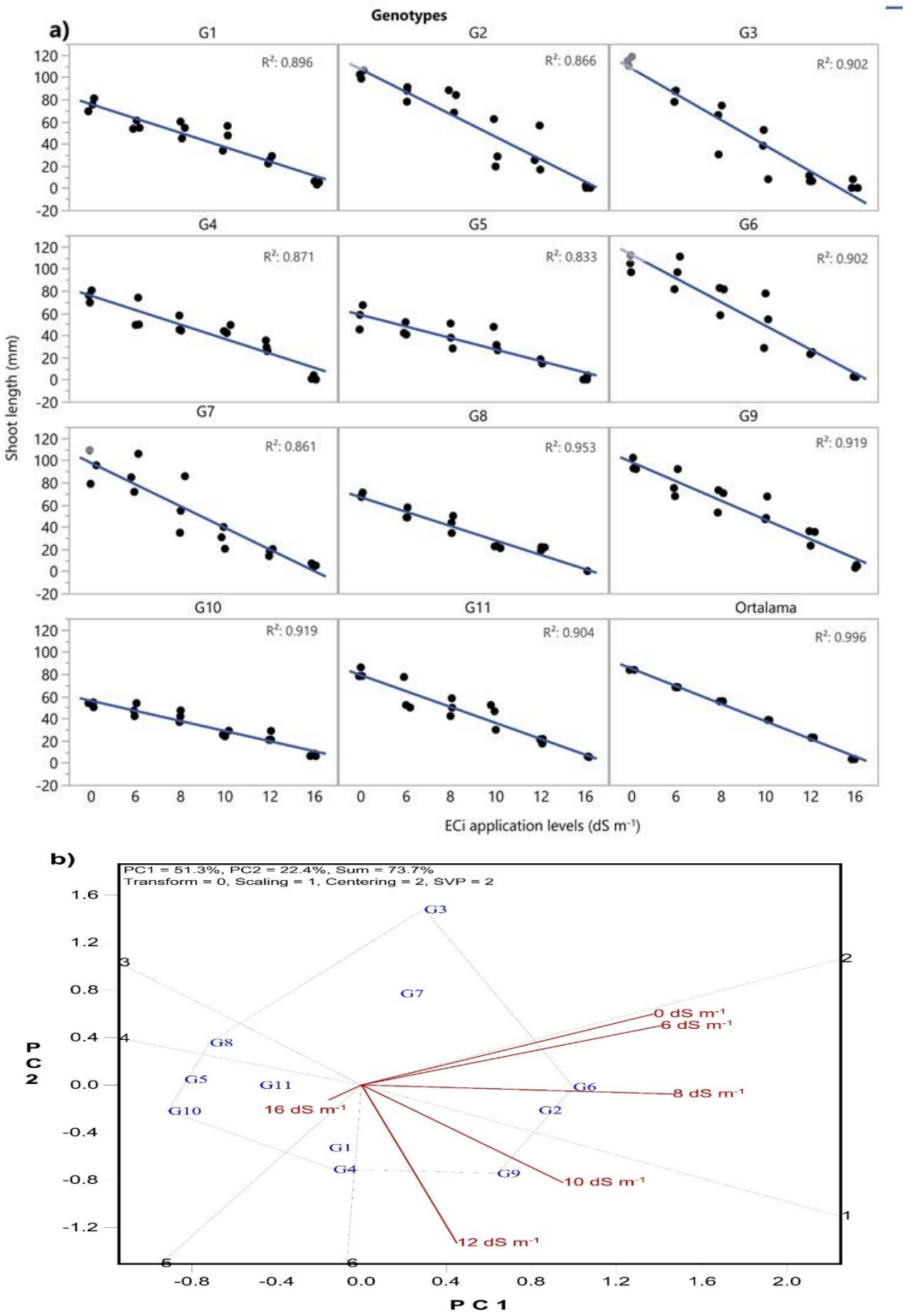


Figure 3- Seedling stem lengths at different ECi levels a) regression b) GGE biplot graph

The greatest seedling dry weight was observed in G4 of the first group and G10 of the second group. These genotypes were placed at diagonals. The greatest decreases in seedling dry weights with ECi levels were observed in G8, G5 and G9 genotypes (Figure 4b).

Table 5- Change in seedling dry weights of chickpea genotype with ECi levels

Genotype	*0 dS/m*	*6 dS/m*	*8 dS/m*	*10 dS/m*	*12 dS/m*	*16 dS/m*
G1	1.13 ± 0.28	1.09 ± 0.29	1.04 ± 0.31	0.87 ± 0.25	0.70 ± 0.22	0.27 ± 0.10
G2	1.57 ± 0.04	1.34 ± 0.20	1.05 ± 0.03	0.83 ± 0.27	0.61 ± 0.17	0.22 ± 0.15
G3	1.06 ± 0.14	0.84 ± 0.09	0.76 ± 0.04	0.71 ± 0.05	0.63 ± 0.12	0.36 ± 0.02
G4	2.41 ± 0.10	1.59 ± 0.24	1.40 ± 0.27	1.27 ± 0.31	0.97 ± 0.45	0.40 ± 0.41
G5	0.74 ± 0.05	0.71 ± 0.04	0.49 ± 0.06	0.44 ± 0.08	0.38 ± 0.08	0.33 ± 0.05
G6	1.12 ± 0.11	1.00 ± 0.06	0.93 ± 0.03	0.78 ± 0.14	0.59 ± 0.21	0.47 ± 0.24
G7	1.23 ± 0.21	0.97 ± 0.23	0.87 ± 0.15	0.67 ± 0.06	0.50 ± 0.00	0.30 ± 0.10
G8	0.41 ± 0.04	0.32 ± 0.04	0.30 ± 0.05	0.25 ± 0.01	0.18 ± 0.06	0.00 ± 0.00
G9	1.57 ± 0.10	1.03 ± 0.35	0.84 ± 0.27	0.73 ± 0.23	0.55 ± 0.29	0.13 ± 0.02
G10	1.72 ± 0.01	1.59 ± 0.13	1.29 ± 0.09	1.16 ± 0.12	1.00 ± 0.12	0.71 ± 0.50
11	1.12 ± 0.27	0.91 ± 0.09	0.83 ± 0.07	0.73 ± 0.04	0.64 ± 0.03	0.39 ± 0.23
Genotype	**	**	**	**	**	**
Average	1.28	1.04	0.89	0.77	0.61	0.33
Minimum	0.41	0.32	0.30	0.25	0.18	0.06
Maximum	2.41	1.59	1.40	1.27	1.00	0.71
LSD 0.01	0.35	0.43	0.37	0.40	0.47	0.53

**: Genotypes are statistically different (P<0.01)

In cultivar development studies with breeding methods, different genetic variation sources should be used through modern methods, significant traits should be investigated with the use of proper methods, selection criteria should be developed/specified and characteristics of developed cultivars should be well-defined.

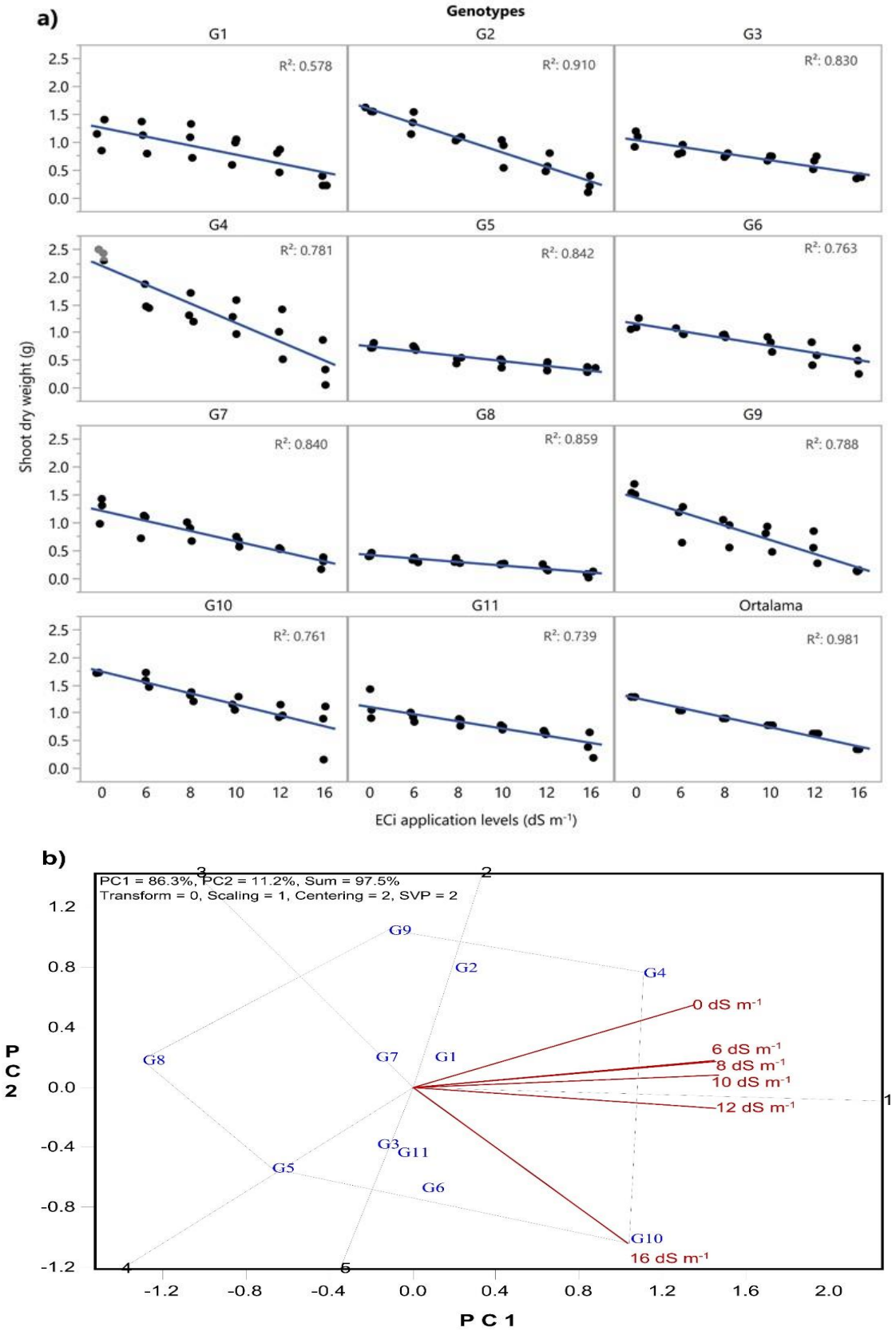


Figure 4- Seedling dry weights at different ECi levels a) regression b) GGE biplot graph

Already limited water resources will get even more deficit in the future. Such deficit nature of water resources will necessitate the use of poor or low-quality waters in irrigations. A sustainable production model should be generated while using these poor-quality waters. For sustainable plant production, resistance to stress factors, especially sensitivity/resistance of the plants to water and soil salinity should be determined and threshold salinity values should be identified. Present findings may offer significant information to further breeding studies about salt tolerance of the plants.

Genotypic differences in germination play an important role in identification of salt tolerance (Saxena et al. 1994). Therefore, seed germination in Petri dishes with saline irrigation water solutions are generally used for rapid identification of plant salt tolerance (Jana & Slinkard 1976). Previous researchers used petri dishes with different NaCl salt solutions in beans (Goertz & Coons 1989; Guvenc & Kantar 1996; Elkoca & Kantar 2003), sorghum (Esechie 1994), bread wheat (Kirtok et al. 1994; Coskun & Tas 2017) and different vegetables (Cucci et al. 1994) and identified salt tolerant/resistant cultivars in a short time. Citak & Toprak (2016) reported chickpea kernel yield was 351 kg/da at full irrigation and 281 kg/da at deficit irrigation (50%) treatment. Ozcan et al. (2000) supplemented 500 g pot soils with 68 mmol/kg NaCl to generate salt stress on three different chickpea cultivars under greenhouse conditions and reported that Damla chickpea cultivar had lower dry weight, Na and Cl content than the other cultivars under NaCl salt stress.

Present study revealed that, in the germination tests, besides control treatment (0 dS/m), five different ECi levels (6, 8 10, 12 and 16 dS/m) were applied. Threshold salinity value for germination was identified as 12 dS/m based on germination ratios and seedling dry weights, 8 dS/m based on seedling stem lengths and 10 dS/m based on seedling root lengths. According to these results, it is understood that chickpeas are quite resistant to salinity.

In terms of germination ratios and seedling dry weights, G4 genotype was identified as the most resistant and G5 was identified as the most sensitive cultivar to irrigation water salinity. The greatest seedling root and stem lengths were obtained from G2 and G4 genotypes. The present findings revealed that G5, G8 and G9 genotypes for germination rate and seedling dry weight, and G5 and G1 genotypes for seedling root length and seedling stem length were more sensitive to salinity.

4. Conclusions

The GGB biplot method can be used safely in determining the threshold value of irrigation water salinity in germination tests. Threshold salinity value for germination was identified as 12 dS/m based on germination ratios and seedling dry weights, 8 dS/m based on seedling stem lengths and 10 dS/m based on seedling root lengths. Based on germination ratios and seedling dry weights, Azkan cultivar was identified as the most resistant and Cagatay cultivar was identified as the most sensitive cultivar to irrigation water salinity.

Acknowledgments

We thank to Canakkale Onsekiz Mart University Scientific Research Coordination Unit. Project number: FBA-2019-2805.

References

Alkan B B (2011). Biplot Technique in Multivariate Statistical Methods. Ankara University Graduate School of Natural and Applied Sciences Department of Statistics. Ph.D. Thesis. Ankara (Unpublished).

Assouline S, Moller M, Cohen S, Ben-Hur M, Grava A, Narkis K & Silber A (2006). Soil-plant system response to pulsed drip irrigation and salinity bell pepper case study. *Soil Science Society of America Journal* 70: 1556-1568. https://doi.org/10.2136/sssaj2005.0365

Atak M, Kaya M , Kaya G, Cikili Y & Ciftci C Y (2006). Effects of NaCl on Germination, Seedling Growth and Water Uptake of Triticale. *Turk. J. Agric. For* 30: 39-47

Bradu D & Gabriel K R (1978). The Biplot as a Diagnostic Tool for Models of Twoway Tables. Technometrics Vol. 20. 47-68. https://doi.org/10.1080/00401706.1978.10489617

Citak G & Topak R (2016). The Effect of Different Irrigation Programs on the Yield and Quality of the Chickpea. *Selcuk Journal of Agriculture and Food Sciences* 3(2): 298-303

Coskun Y & Tas I (2017). Respons of wheat species to irrigation water salinity. Genetika-Belgrade Vol. 49(2). 435-444. https://doi.org/10.2298/GENSR1702435C

Cucci G, De Caro A, Ciciretti L & Leoni B (1994). Salinity and seed germination of some vegetable crops. Acta Hortic 362: 305-308. https://doi.org/10.17660/actahortic.1994.362.37

Daigle G & Rivest L P (1992). A robust biplot. *Canadian Journal of Statistics* 20(3): 241-255. https://doi.org/10.2307/3315312

Ekiz H, Bagci S A, Yilmaz A, Caglayan N & Bozoglu S (2000). Determination of salt tolerance degrees of different cereal varieties and lines. Ministry of Agriculture and Rural Affairs Agricultural Research Gen. Director, Bahri Dağdaş International Winter Wheat Res. Central Manager Publications, no: SR2000–6, 1-59 pp. Konya. (In Turkish)

Elkoca E & Kantar F (2003). The Effects of Different NaCl Concentrations on Germination and Seedling Growth of Dry Bean (Phaseolus vulgaris L.) Genotypes. *Atatürk University Journal of Agricultural Faculty* 34(1): 1-8

Esechie H A (1994). Interaction of salinity and temperature on the germination of sorghum. *J.Agron. and Crop Sci* 172: 194-199. https://doi.org/10.1111/j.1439-037x.1994.tb00166.x

Gabriel K R (1971). The Biplot Graphic Display of Matrices with Application to Principal Component Analysis. Biometrika 58(3): 453-467. https://doi.org/10.1093/biomet/58.3.453

Gabriel K R & Odoroff C L (1990). Biplots in Biomedical Research. Statistic in Medicine 9: 469 - 485. https://doi.org/10.1002/sim.4780090502

Gabriel K R (1995). MANOVA Biplots for Two-way Contingency Tables, Recent Advances in Descriptive Multivariate Analysis, Oxford. Clarendon Pres.

Goertz S H & Coons J M (1989). Germination response of tepary and navy beans to sodium chloride and temperature. *Hortsci* 24: 923-925

Greenacre M J (1984). Theory and applications of correspondence analysis. Academic Press, London.

Greenacre M J (1993). Biplots in correspondence analysis. *Journal of Applied Statistics* 20: 251-269

Guvenc I & Kantar F (1996). Determination of salt tolerant bean genotypes (Phaseolus vulgaris L.). *SDU Journal of Agriculture Faculty* 9(11): 144-153

Jana M K & Slinkard A E (1976). Screening for salt tolerance in lentil. Lens Newsletter 6: 5-27

Jiang J, Huo Z, Feng S & Zhanga C (2012). Effect of irrigation amount and water salinity on water consumption and water productivity of spring wheat in Northwest China. Field Crops Research 137: 78-88. https://doi.org/10.1016/j.fcr.2012.08.019

Kirtok Y, Veli S, Tukel S, Duzenli S & Kilinc M (1994). Evaluation of salinity stress on germination characteristics and seedling growth of 3 bread wheats (Triticum aestivum L.). Field Crops Congress 25-29 April 1994 Izmir. (In Turkish)

Koyuncu N (2008). Determination of In Vitro Salt Tolerance in Turkish Common and Durum Wheat (Triticum spp.) cultivars. Ankara University Graduate School of Natural and Applied Sciences Department of Field Crops. Ph.D. Thesis. Ankara. (Unpublished)

Kusvuran A (2015). The effects of salt stress on the germination and antioxidative enzyme activity of Hungarian vetch (Vicia pannonica Crantz.) varieties. Agricultural Research Communication Centre 38(1): 51-59. https://doi.org/10.5958/0976-0571.2015.00009.0

Letey J & Feng G L (2007). Dynamic versus steady-state approaches to evaluate irrigation management of saline waters. Agricultural Water Management 91: 1-10. https://doi.org/10.1016/j.agwat.2007.02.014

Min W, Hou Z, Ma L, Zhang W, Ru S & Ye J (2014). Effects of water salinity and N application rate on water- and N-use efficiency of cotton under drip irrigation. *J Arid Land* (2014) 6(4): 454-467. https://doi.org/10.1007/s40333-013-0250-3

Munns R (2002). Comparative physiology of salt and water stress. Plant, Cell and Environment 25: 239-250. https://doi.org/10.1046/j.0016-8025.2001.00808.x

Ojeda M M & Juarez-Cerrillo S F (1996). Biplot display for diagnostic in a two-level regression model for growth curve analysis. *Computational Statistics and Data Analysis*, Vol. 22, pp. 583-597. https://doi.org/10.1016/0167-9473(96)00013-8

Okhovatian-Ardakani A R, Mehrabanian M, Dehghani F & Akbarzadeh A (2010). Salt tolerance evaluation and relative comparison in cuttings of different pomegranate cultivars. Plant Soil Environ 56(4): 176-185. https://doi.org/10.17221/158/2009-pse

Ozcan H, Turan M A, Koc O, Cikili Y & Taban S (2000). Growth and Variations in Proline, Sodium, Chloride, Phosphorus and Potassium Concentrations of Chickpea (Cicer arietinum L. cvs.) Varieties Under Salinity Stress. *Turk J Agric For* 24: 649-654

Pereira L S, Oweis T & Zairi A (2002). Irrigation management under water scarcity. Agricultural Water Management 57: 175-206. https://doi.org/10.1016/s0378-3774(02)00075-6

Peykanpour E, Ghehsareh A M, Fallahzade J & Najarian M (2016). Interactive effects of salinity and ozonated water on yield components of cucumber. Plant Soil Environ 62(8): 361-366

Saxena N P, Saxena M C, Ruckenbauer P, Rana RS, El-Fouly M M & Shabana R (1994). Screening techniques and sources of tolerance to salinity and mineral nutrient imbalances in cool season food legumes. Euphytica 73: 85-93. https://doi.org/10.1007/978-94-011-0798-3_27

Sparks R, Adolphson A & Phatak A (1997). Multivariate Process Monitoring Using the Dynamic Biplot. International Statistical Review 65(3): 325-349. https://doi.org/10.2307/1403375

Sozen O & Karadavut U 2018. Determination of Genotype x Environment Interactions of Some Chickpea (Cicer arietinum L.) Genotypes by Using Different Stability Methods. *Journal of Agricultural Sciences* 24(2018) 431-438. https://doi.org/10.15832/ankutbd.490930

Wang W B, Kim Y H, Lee H S, Kim K Y, Deng X P & Kwak S S (2009). Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. Plant Physiology and Bioch 47(7): 570-577. https://doi.org/10.1016/j.plaphy.2009.02.009

Yan W (2014). Crop variety trials. Data management and analysis. Wiley-Blackwell, Hoboken, New Jersey, 349 p.

Yeo A R, Yeo M E, Flowers S A & Flowers T J (1990). Screening of rice (Oryza sativa L.) genotypes for physiological characters contributing to salinity resistance and their relationship to overall performance. Theoretical and Applied Genetics 79: 377-384. https://doi.org/10.1007/bf01186082

Yurtseven E, Colak M S, Ozturk A & Ozturk H S 2018. Drainage water salt load variations related to the salinity and leaching ratios of irrigation water. *Journal of Agricultural Sciences* 24(2018): 394-402. https://doi.org/10.15832/ankutbd.456667

Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

Secondary Metabolite Changes in Tedara (*Bituminaria bituminosa* L.) Genotypes in Different Growing Period

Zeki ACAR[a], Erdem GÜLÜMSER[b*], Sema LEBLEBİCİ[c], İlknur AYAN[a], Cihan DARCAN[c]

[a]*Department of Field Crop, Faculty of Agriculture, University of Ondokuz Mayıs, Samsun, TURKEY*

[b]*Department of Field Crops, Faculty of Agriculture and Natural Sciences, University of Bilecik Seyh Edebali, Bilecik, TURKEY*

[c]*Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Bilecik Şeyh Edebali University, Bilecik, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Erdem GÜLÜMSER, E-mail: erdem.gulumser@bilecik.edu.tr
Received: 19 August 2021 / Revised: 07 December 2021 / Accepted: 11 December 2021 / Online: 15 October 2022

Cite this article
ACAR Z, GÜLÜMSER E, LEBLEBİCİ S, AYAN İLKNUR, DARCAN C (2022). Secondary Metabolite Changes in Tedara (*Bituminaria bituminosa* L.) Genotypes in Different Growing Period. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 28(4):723-731. DOI: 10.15832/ankutbd.984689

ABSTRACT
In this study, secondary metabolite amounts of leaf samples belonging to 12 different *Bituminaria bituminosa* (L.) C.H. Stirtion genotypes were determined at the beginning of growth, budding and at the beginning of flowering periods. Leaf samples belonging to *B. bituminosa* genotypes were dried under natural conditions and extracted using a microwave system (NEOS). Furanocumarins (psoralen and angelicin), isoflavonoids (daidzein, genistein) and isoflovan glycosides (daidzin and genistin) obtained by LC-MS / MS. While the secondary metabolite contents determined in *B. bituminosa* differed among growth periods, genotype difference was found to be more effective on these metabolite contents. As a result, all genotypes performed remarkably in terms of secondary metabolites. Besides, the number 12 genotype (Samsun-Kavak) was prominent in terms of angelicin, daidzein, genistein, daidzin, and genistin compared to the other genotypes.

Keywords: Animal health, Furanocoumarin, Isoflavonoid, Tedera

1. Introduction

Bituminaria bituminosa (L.) C.H. Stirtion (*B. bituminosa)* is a perennial species and is a member of the *Leguminosea* family is originated in Mediterranean. However, it shows a wide spreading in the natural flora of Turkey, South Europe, Crimea, Western Syria, Cyprus, the Caucasus, Israel, the North Africa, Portugal and Spain (Davis 1965).

B. bituminosa is a plant species which has rich content in terms of secondary metabolites. The amount of secondary metabolites that give the plant a distinctive odour varies significantly according to genotype and ecological conditions (Pecetti et al. 2007; Correal 2012; Real 2012). While these metabolites play a role in the defence mechanism of the plant, they also have beneficial effects on human physiology and diseases due to their bioactive molecules. Secondary metabolite content of *B. bituminosa* such as furanocoumarins, isoflavonoids and pterocarbans have pharmacological effects (Martínez et al. 2010). These substances are used in the cosmetic industry and in the treatment of vitiligo, psoriasis, fungal, eczema, sunburn, skin cancer, colon cancer, Mediterranean anemia, allergen diseases and relieving inflammation (Pistelli et al. 2003; Pazos -Navarro et al. 2013). While secondary metabolites and antioxidant substances in the plant positively affect the health of animals, they also improve the quality of animal products (Pecetti et al. 2007).

The literature shows that secondary metabolites of *B. bituminosa* may change depending on the growth periods. Study of Tava et al. (2007) determined in the leaves and stems of *B. bituminosa* plant psoralen and angelicin content. Walker et al. (2006) analysed these metabolites in April, August and November and the highest values were found to be in the samples collected in August. This is related to temperature and increases the furanocoumarin content of the plant with increasing UV light intensity (Bourgaud et al. 1995). In previous studies, the presence of pterocarpan and daidzein were determined in the *Psoralea* species (Boardley et al. 1986; Bouque et al. 1998; Pistelli et al. 2003).

In the present study, secondary metabolites (psoralen and angelicin, daidzein, genistein, daidzin and genistin) were determined in beginning of growth, budding, and in the beginning of flowering periods of 12 different *B. bituminosa* genotypes and changes in the amounts of these secondary metabolites were determined in three different physiological periods.

2. Material and Methods

Seeds of *B. bituminosa* collected in 2012 from Samsun (8), Sinop (2) and Kastamonu (1) were used as plant material in the study. In addition, one genotype of Spain origin was also used in the study (Table 1).

Table 1- Localities of *B. bituminosa* genotypes

No	*Collection Location*	*Locations*	
		North	*East*
G1	Spain	-	-
G2	Kastamonu İnebolu	41° 58' 32.8"	33° 46' 10.4'
G3	Samsun-Çatalzeytin	41° 57' 48.4"	34° 09' 07.8'
G4	Sinop Kanlıçay	41° 40' 40.3"	35° 22' 22.8'
G5	Samsun-Kozağzı	41° 28' 05.1"	35° 49' 56.8'
G6	Samsun-Çarşamba	41° 04' 35.1"	36° 40' 09.0'
G7	Samsun-Bağkur	41° 18' 39.0"	36° 20' 02.5'
G8	Samsun- Baruthane	41° 19' 08.5"	36° 19' 13.6'
G9	Samsun-Nebyan	41° 23' 35.9"	35° 59' 06.2'
G10	Samsun-Kurupelit	41° 22' 16.0"	36° 11' 46.7'
G11	Sinop-Tıngıroğlu	41° 47' 41.0"	35° 00' 23.0"
G12	Samsun-Kavak	41° 03' 14.4"	35° 56' 59.8"

The collected seeds were firstly sown into seed trays. Then, they were transplanted to Experimental Field of Agriculture Faculty of Samsun Ondokuz Mayıs University with 70x70 cm spaces in autumn during 2016. The properties of experimental area soil which were gathered from 0-15 cm deep were determined as a pH of 6.45, 7.90% $CaCO_3$ and saltless (0.052 mmhos/cm). Plants were not irrigated and fertilized during the study. In the 2018 vegetation period; at the beginning of growth, budding and at the beginning flowering, the plants were harvested and the leaves were separated (Table 2). Besides, while sampling the leaves, genotypes with the same habitus were taken into account.

Table 2- Harvest dates of *B. bituminosa* genotypes

Genotypes	*Beginning of growth*	*Budding*	*Beginning of flowering*
Spain	10.05.2018	31.05.2018	07.06.2018
Kastamonu İnebolu	10.05.2018	24.05.2018	31.05.2018
Samsun-Çatalzeytin	10.05.2018	24.05.2018	31.05.2018
Sinop Kanlıçay	10.05.2018	24.05.2018	31.05.2018
Samsun-Kozağzı	10.05.2018	24.05.2018	31.05.2018
Samsun-Çarşamba	10.05.2018	24.05.2018	31.05.2018
Samsun-Bağkur	10.05.2018	24.05.2018	31.05.2018
Samsun- Baruthane	10.05.2018	24.05.2018	31.05.2018
Samsun-Nebyan	10.05.2018	24.05.2018	31.05.2018
Samsun-Kurupelit	10.05.2018	24.05.2018	31.05.2018
Sinop-Tıngıroğlu	10.05.2018	24.05.2018	31.05.2018
Samsun-Kavak	10.05.2018	24.05.2018	31.05.2018

B. bituminosa leaf samples taken from the genotypes were dried under natural conditions and then extracted with NEOS device (microwave system) in the ECO-Biotechnology Laboratory of Bilecik Şeyh Edebali University, Biotechnology Application and Research Centre. Secondary metabolite contents of the extracts were determined by LC-MS / MS. Secondary metabolites determined in leaf samples of *B. bituminosa* were psoralen, angelicin, daidzein, genistein, daidzin and genistin. Prior to the statistical analysis of the data obtained, transformation was applied and its suitability for normal distribution was examined. The results of the study were analysed by using the MSTAT-C statistical package program with regards to the split pilot design. Differences between genotype and physiological periods were determined by Duncan multiple comparison test. Besides, the correlation coefficients among all observed components were calculated by the in SPSS version 18.0, while the Principal component analysis (PCA) was carried out using the statistical software package PASW (18) Statistics Data Editor Program.

3. Results and Discussion

Mean squares and coefficient of variance for secondary metabolites and *B. bituminosa* genotypes are given Table 3. Accordingly, the difference between the growth periods, genotypes and the interaction between genotype x growth period were found to be significant at the 1% probability level for all secondary metabolites.

Table 3- Mean squares and coefficient of variance for secondary metabolites and *B. bituminosa* genotypes

Source of variation	*df*	*Psoralen*	*Angelicin*	*Daidzein*	*Genistein*	*Daidzin*	*Genistin*
Growth period (GP)	2	42.09**	7.17**	138.33**	9.81**	278.07**	172.38**
Genotype (G)	11	15.94**	2.08**	327.98**	7.16**	100.34**	98.56**
GPXG	22	9.32**	2.59**	115.86**	2.08**	73.78**	68.69**
CV%		0.46	7.52	3.73	3.37	0.50	1.05

**: P≤0.01

The highest psoralen content was obtained at the beginning of flowering and genotype number 11 with 118.47 ppm; the lowest psoralen content was obtained in budding and genotype number 12 with 3.88 ppm. Psoralen content in growth periods was determined as the highest in the beginning of flowering, lower in budding and the lowest in the beginning of growth. When the genotypes were compared, the highest psoralen content was determined in genotype number 8 (89.87 ppm) (Table 4).

Table 4- Psoralen contents of *B. bituminosa* (ppm)

Genotypes	*Beginning of growth*	*Budding*	*Beginning of flowering*	*Average***
G1	53.89 q	85.19 h	64.49 mn	67.85 f
G2	34.51 x	24.14 B	83.56 j	47.40 h
G3	12.36 D	25.03 A	43.29 v	26.89 k
G4	26.19 z	49.78 s	43.31 v	39.76 i
G5	47.11 t	107.16 c	91.48 f	81.91 b
G6	41.36 w	25.93 z	45.55 u	37.61 j
G7	23.15 C	104.66 d	84.09 ij	70.63 d
G8	72.44 l	87.40 g	109.77 b	89.87 a
G9	50.52 r	84.63 hi	79.34 k	71.49 c
G10	79.57 k	62.86 o	63.76 n	68.73 e
G11	31.13 y	57.06p	118.47 a	68.89 e
G12	65.28 m	3.88 E	95.52 e	54.89 g
Average**	**44.79 c**	**59.81 b**	**76.89 a**	

**: P≤0.01 (While the interactions were lettered, capital letters were continued after the lowercase letters were finished). G1: Spain; G2: Kastamonu İnebolu; G3: Samsun-Çatalzeytin; G4: Sinop Kanlıçay; G5: Samsun-Kozağzı; G6: Samsun-Çarşamba; G7: Samsun-Bağkur; G8: Samsun- Baruthane; G9: Samsun-Nebyan; G10: Samsun-Kurupelit; G11: Sinop-Tıngıroğlu; G12: Samsun-Kavak.

The angelicin contents of the genotypes ranged from 0.11 ppm to 19.61 ppm. The highest angelicin content was determined in genotype number 6 in the budding and the lowest angelicin content was determined in genotype number 5 in the beginning of flowering. When the growth periods were compared, the highest angelicin content was found in the budding (4.29 ppm) and the lowest angelicin content was found in the beginning of flowering (0.60 ppm) (Table 5).

Table 5- Angelicin content of *B. bituminosa* (ppm)

Genotypes	*Beginning of growth*	*Budding*	*Beginning of flowering*	*Average***
G1	0.16 tu	0.34 q	0.22 stu	0.24 i
G2	0.97 i-l	5.18 e	0.37 p-s	2.17 e
G3	1.85 g	13.57 b	0.71 k-n	5.38 b
G4	1.82 g	0.81 j-m	0.52 n-q	1.05 f
G5	0.68 l-o	1.02 ijk	0.11 u	0.60 gh
G6	0.32 qrs	19.61 a	0.47 o-r	6.80 a
G7	9.57 c	0.58 m-p	0.86 j-m	3.67 c
G8	1.07 hij	1.02 ijk	0.76 j-n	0.95 f
G9	0.63 mno	0.16 tu	1.41 h	0.73 g
G10	0.78 j-n	0.34 qrs	0.29 rst	0.47 h
G11	3.70 f	1.20 hi	1.23 hi	2.04 e
G12	0.37 p-s	7.67 d	0.26 rst	2.77 d
Average**	**1.83 b**	**4.29 a**	**0.60 c**	

**: P≤0.01. G1: Spain; G2: Kastamonu İnebolu; G3: Samsun-Çatalzeytin; G4: Sinop Kanlıçay; G5: Samsun-Kozağzı; G6: Samsun-Çarşamba; G7: Samsun-Bağkur; G8: Samsun- Baruthane; G9: Samsun-Nebyan; G10: Samsun-Kurupelit; G11: Sinop-Tıngıroğlu; G12: Samsun-Kavak.

Different researchers reported that *Psoraleae* varieties have a rich content of psoralen and angelicin, and they are used traditional medicine (Rakhmankulov & Korotkova 1975; Cappelletti et al. 1984). Baskaran et al. (2001) and Milesi et al. (2001) indicated that the psoralen is used for the photochemotherapy of vitiligo and skin diseases such as psoriasis, mycosis fungoidies, and eczema. Besides, psoralen inhibits cancer (Oliveira et al. 2006). Angelicin is used for determining DNA/RNA structures in cells and microorganisms, and the treatment of psoriasis (Kittler et al. 1980). Previous researches showed greater variability in psoralen and angelicin contents between *Psoralea* varieties. This may be due to genetic differences and environmental factors. Li et al. (2018) found that the psoralen and angelicin contents of *Psoralea gingivalis* were 6250 and 3125 ppm, respectively, while Ahandani et al. (2013) determined that psoralen and angelicin contents of *Psoraleae plumose* were at 2744 and 3022 ppm, respectively. Psoralen and angelicin contents may vary differences in the organs of plants. For example, Del Río et al. (2010) determined that the psoralen and angelicin content of *Psoralea bituminosa* leaves ranged between 2204-3416 and 2530-3918 ppm, respectively. On the other hand, Ahandani et al. (2013) indicated that the seeds of *Psoralea corylifolia* content of psoralen and angelicin were at 7800 and 2300 ppm, respectively. The angelicin and psoralen contents in this study are different from previous studies. This may be due to the varieties and ecological conditions.

The contents of daidzein and genistein determined in *B. bituminosa* leaf extracts are given in Tables 6 and Table 7. The daidzein content of genotypes was ranged from 3.66 ppm (genotype number 1 at the beginning of flowering) to 37.57 ppm (genotype number 5 at the budding period). When the growth periods were compared, the highest daidzein content was found at the beginning of growth (18.27 ppm) and the lowest daidzein content was found in the budding period (14.78 ppm) (Table 6). The highest genistein content was determined in genotype number 9 at the beginning of growth (6.34 ppm), while the lowest genistein content was determined in genotype number 8 in budding period (1.27 ppm). The genistein content in the growth periods from highest to lowest was at the beginning of growth, beginning of flowering and in the budding, respectively. When the genotypes were compared, the highest genistein content was determined in genotype number 9 (4.99 ppm) (Table 7).

Table 6- Daidzein contents of *B. bituminosa* (ppm)

Genotypes	*Beginning of growth*	*Budding*	*Beginning of flowering*	*Average***
G1	9.74 op	6.76 r	3.66 t	6.72 j
G2	10.63 o	5.42 s	13.24 n	9.76 i
G3	20.22 h	12.99 j	27.81 c	20.34 c
G4	9.30 pq	18.05 n	15.30 m	14.21 g
G5	19.44 hi	37.57 a	17.56 jk	24.86 b
G6	17.59 jk	9.53 pq	26.84 cd	17.99 e
G7	21.30 g	16.06 lm	15.39 lm	17.58 ef
G8	26.26 d	7.21 r	18.62 ij	17.36 f
G9	30.92 b	24.27 e	30.90 b	28.70 a
G10	18.38 ij	8.50 q	16.57 kl	14.48 g
G11	16.10 lm	8.60 q	15.47 lm	13.39 h
G12	19.36 hi	22.43 f	15.56 lm	19.12 d
Average**	**18.27 a**	**14.78 c**	**18.08 b**	

**: P≤0.01. G1: Spain; G2: Kastamonu İnebolu; G3: Samsun-Çatalzeytin; G4: Sinop Kanlıçay; G5: Samsun-Kozağzı; G6: Samsun-Çarşamba; G7: Samsun-Bağkur; G8: Samsun- Baruthane; G9: Samsun-Nebyan; G10: Samsun-Kurupelit; G11: Sinop-Tıngıroğlu; G12: Samsun-Kavak.

Table 7- Genistein contents of *B. bituminosa* (ppm)

Genotypes	*Beginning of growth*	*Budding*	*Beginning of flowering*	*Average***
G1	1.92 qr	1.60 st	1.71 st	1.74 h
G2	2.29 op	3.12 jk	3.49 gh	2.96 de
G3	5.65 b	3.32 hi	4.69 d	4.55 b
G4	1.76 rs	2.65 n	3.60 g	2.67 f
G5	3.92 f	2.44 o	2.77 mn	3.04 d
G6	3.83 f	2.20 p	3.02 kl	3.01 d
G7	2.22 p	2.29 op	2.27 op	2.26 g
G8	3.80 f	1.27 u	3.01 kl	2.69 f
G9	6.34 a	5.26 c	3.38 hi	4.99 a
G10	3.29 ij	1.57 t	4.13 e	2.99 de
G11	3.84 f	1.96 q	2.87 lm	2.89 e
G12	4.18 e	2.90 lm	3.00 kl	3.36 c
Average**	**3.59 a**	**2.55 c**	**3.16 b**	

**: P≤0.01. G1: Spain; G2: Kastamonu İnebolu; G3: Samsun-Çatalzeytin; G4: Sinop Kanlıçay; G5: Samsun-Kozağzı; G6: Samsun-Çarşamba; G7: Samsun-Bağkur; G8: Samsun- Baruthane; G9: Samsun-Nebyan; G10: Samsun-Kurupelit; G11: Sinop-Tıngıroğlu; G12: Samsun-Kavak.

Adlercreutz et al. (1991) and Setchell et al. (1981) explained that the daidzein has reduced breast cancer occurrence, and play a key role in the protection against colon cancer. Bouquet et al. (1998) reported that the daidzein concentration of Psoraleae varieties is greater in the stems than in the leaves, and it was ranged between 174-6072 ppm. Genistein is a secondary matter an estrogenic preventing bone resorption and promotes increased bone density (Anderson et al. 1995; Arjmandi et al. 1996; Alekel et al. 2000). Hsu et al. (2001) reported that the genistein content of *Psoralea corylifolia* was at 7800 ppm. Mustonen et al. (2018) reported daidzein and genistein content of red clover were ranged between 250-310 and 490-550 ppm, respectively. Our results showing a great difference between the previous studies. Sivesind & Seguin (2005) indicated that if there are environmental effects (soil) and plant phenological traits (plant age and growth period), the variety choice will influence most on isoflavonoid amounts. Besides, Kallela et al. (1988) and Saloniemi et al. (1995) reported that the isoflavone content of the plants was higher in the early growth periods than in the late periods. Similarly, in this study, daidzein and genistein content of the beginning of growth was higher than other growing periods.

The contents of daidzin and genistin determined in *B. bituminosa* leaf extracts were given in Tables 8 and Table 9. When Table 8 is examined, the daidzin content of *B.bituminosa* genotypes showed change among growth periods. The daidzin content of the plant between 0 ppm and 381.66 ppm. Accordingly, the highest daidzin content was determined in genotype number 6 at the beginning of flowering. When the growth periods were compared, the highest daidzin content was found at the beginning of flowering (113.05 ppm) and the lowest daidzin content was found at the beginning of growth (25.46 ppm) (Table 8). Genistin contents of genotypes ranged from 0 ppm to 330.14 ppm, while the highest genistin was obtained from genotype number 6 and in the beginning of growth (Table 9). Genistin content was not found in many genotypes especially in the budding period. Besides, when the genotypes were compared, the highest genistin content was determined in genotype number 3 (137.82 ppm) (Table 9).

Table 8- Daidzin contents of *B. bituminosa* (ppm)

Genotypes	*Beginning of growth*	*Budding*	*Beginning of flowering*	*Average***
G1	000 F	55.52 q	1.92 A	19.14 j
G2	3.55 y	000 F	8.93 u	4.16 l
G3	102.78 m	0.83 C	18.65 s	40.75 h
G4	1.18 B	231.53 c	222.29 e	151.66 b
G5	3.33 y	275.09 b	224.47 d	167.63 a
G6	0.31 E	000 F	381.66 a	127.32 d
G7	79.76 o	112.98 l	146.07 h	112.94 e
G8	0.96 C	43.89 r	2.15 z	15.66 k
G9	8.33 v	182.78 g	4.31 x	65.14 g
G10	7.53 w	75.70 p	15.86 t	33.03 i
G11	89.71 n	115.10 k	190.99 f	131.93 c
G12	8.06 v	126.85 j	139.34 I	91.41 f
Average**	**25.46 c**	**101.69 b**	**113.05 a**	

**: P≤0.01 (While the interactions were lettered, capital letters were continued after the lowercase letters were finished). G1: Spain; G2: Kastamonu İnebolu; G3: Samsun-Çatalzeytin; G4: Sinop Kanlıçay; G5: Samsun-Kozağzı; G6: Samsun-Çarşamba; G7: Samsun-Bağkur; G8: Samsun- Baruthane; G9: Samsun-Nebyan; G10: Samsun-Kurupelit; G11: Sinop-Tıngıroğlu; G12: Samsun-Kavak.

Table 9- Genistin contents of *B. bituminosa* (ppm)

Genotypes	*Beginning of growth*	*Budding*	*Beginning of flowering*	*Average***
G1	000 r	000 r	30.96 n	10.32 j
G2	130.31 f	38.11 l	130.30 f	99.57 e
G3	40.89 k	241.67 b	130.89 ef	137.82 a
G4	167.31 c	163.98 d	15.85 o	115.71 c
G5	244.51 b	000 r	85.57 h	110.02 d
G6	330.14 a	000 r	34.95 m	121.69 b
G7	6.86 p	000 r	000 r	2.28 k
G8	000 r	000 r	52.48 I	17.49 h
G9	000 r	000 r	131.09 ef	43.69 g
G10	000 r	000 r	44.97 j	14.99 I
G11	133.29 e	000 r	15.19 o	49.49 f
G12	38.02 l	0.23 q	109.35 g	49.20 f
Average*	**90.94 a**	**37.00 c**	**65.13 b**	

**: P≤0.01. G1: Spain; G2: Kastamonu İnebolu; G3: Samsun-Çatalzeytin; G4: Sinop Kanlıçay; G5: Samsun-Kozağzı; G6: Samsun-Çarşamba; G7: Samsun-Bağkur; G8: Samsun- Baruthane; G9: Samsun-Nebyan; G10: Samsun-Kurupelit; G11: Sinop-Tıngıroğlu; G12: Samsun-Kavak.

Daidzin and genistin is a natural organic compound in the class of phytochemicals known as isoflavones. *Bituminaria bituminosa* was less than daidzein and genistein content compared to the previous studies. Lojza et al. (2004) reported that daidzein and genistein content of soybean ranged between 249-468 and 280-618 ppm, respectively. On the other hand, the harvest time has influenced daidzin and genistin. This indicates that the plant secretes metabolites at different times.

Table 10 shows binary relationships among secondary metabolites in terms of the mean values of *B. bituminosa* genotypes. Accordingly, a negative and very important relationship was found between psoralen with angelicin (r = - 0.690) and genistin (r = 0.755). In other words, increased psoralen in genotypes lead to a decrease in angelicin and genistin and vice versa. Besides, while there was a negative and significant relationship between furanocoumarins (psoralen and angelicin), a significant and positive relationship was found between isoflavonoids (daidzein, genistein) and isoflovan glycosides (daidzin and genistin).

Table 10- Bilateral relationships between secondary metabolites of *B. bituminosa* genotypes

	Angelicin	***Daidzein***	***Genistein***	***Daidzin***	***Genistin***
Psoralen	- 0.690**	0.230	- 0.343	- 0.121	- 0.755**
Angelicin		0.032	0.232	0.157	0.439
Daidzein			0.078	- 0.208	- 0.018
Genistein				0.460	0.415
Daidzin					0.216

**: P≤0.01.

To determine the multivariate relationships between the secondary metabolite values of *B. bituminosa* genotypes, biplot analysis, which is the principal component analysis (PCA) of both genotypes and secondary metabolites, was carried out. Biplot analysis was performed by comparing PC1 and PC2 values. When the biplot graph is examined, PC1 (Main component 1) is 42.0% and PC2 (Main component 2) is 26.6% and the total variation is 68.6%. In the first group, since the angle value between the vectors belonging to the properties of angelicin and genistin is 90º lower, these two properties are in the same group. In the second group, the angle between the vectors belonging to genistein, daidzein and daidzin content is found to be less than 90º and these properties are in the same group. The third group is the psoralen vector. In the biplot graph, genotype number 2, 3, 4 and 6 are prominent in terms of content of angelicin and genistin. In the second group, genotype number 9 and 12 are prominent in terms of content of genistein, daidzein and daidzin. Genotypes number 5, 7, 8 and 10 fall into the same group in terms of psoralen (Figure 1).

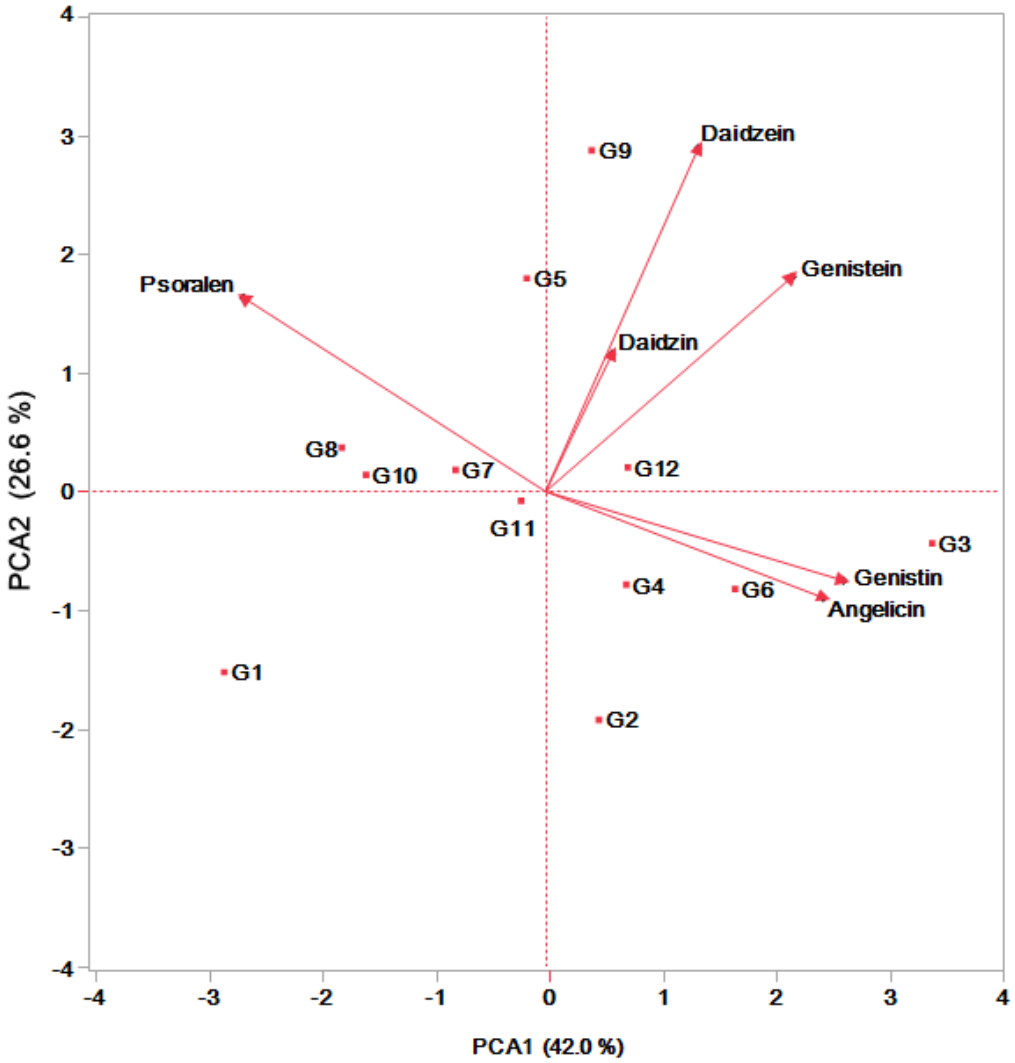


Figure 1- Principal Component Analysis of secondary metabolites of *B. bituminosa*
G1: Spain; G2: Kastamonu İnebolu; G3: Samsun-Çatalzeytin; G4: Sinop Kanlıçay; G5: Samsun-Kozağzı; G6: Samsun-Çarşamba; G7: Samsun-Bağkur; G8: Samsun- Baruthane; G9: Samsun-Nebyan; G10: Samsun-Kurupelit; G11: Sinop-Tıngıroğlu; G12: Samsun-Kavak

4. Conclusions

Secondary metabolites determined in leaf samples of *B. bituminosa* were found to be different both in genotypes and growth periods. When the growth periods were compared, it was found that furanocoumarins (psoralen and angelicin) and isoflovan glycosides (daidzin and genistin) showed a change in *B. bituminosa* leaves and these varied in three different growth periods. However, isoflavonoids (daidzein, genistein) were found to be higher in the beginning of growth than the other two periods.

On the other hand, as a result, all genotypes performed remarkably in terms of secondary metabolites. Besides, the number 12 genotype (Samsun-Kavak) was prominent in terms of angelicin, daidzein, genistein, daidzin, and genistin compared to the other genotypes.

In the literature, there is limited study on the changes in the amount of secondary metabolites both in different genotypes and in different growth periods of *B. bituminosa*. In this respect, the present study fills this gap. At the same time, the study will shed light on future studies about *B. bituminosa*.

Acknowledgements

The authors wish to thank the Scientific and Technological Research Council of Turkey (TUBİTAK) for financial support of this project under Grant No. TOVAG 118O047. This text has been proofread and edited by the School of Foreign Languages of staff, Bilecik Şeyh Edebali University.

References

Adlercreutz H, Mousavi Y, Loukovaara M & Hämäläinen E (1991). Lignans, isoflavones, sex hormone metabolism and breast cancer. In: R B Hochberg & F Naftolin (Eds.) *The New Biology of Steroid Hormones*, New York, Raven Press, pp.145–154

Ahandani E A, Abdel Gawwad M R & Yavari A (2013). Extraction and preparation of Psoralen from different plant part of Psoralea corylifolia and Psoralen increasing with some elicitors. *Journal of Plant Biology Research* 2(1): 25-37

Arjmandi B H, Alekel L, Hollis B W, Amin D, Stacewicz-Sapuntzakis M, Guo P & Kukreja S C (1996). Dietary soybean protein prevents bone loss in an ovariectomized rat model of osteoporosis. *The Journal of Nutrition* 126(1): 161–67. https://doi.org/ 10.1093/jn/126.1.161.

Alekel D L, Germain A S, Peterson C T, Hanson K B, Stewart J W, Toda T (2000). Isoflavone- rich soy protein isolate attenuates bone loss in the lumbar spine of perimenopausal perimenopausal women. *The American Journal of Clinical Nutrition* 72(3): 679–80. https://doi.org/10.1093/ajcn/72.3.844.

Anderson J J, Ambrose W W & Garner S C (1995). Orally dosed genistein from soy and prevention of cancellous bone loss in two ovariectomized rat models. *The Journal of Nutrition* 125: 799 S

Baskaran P I & Jayabalan N (2001). Effect of growth regulators on rapid micropropagation and psoralen production in Psoralea corylifolia L. Acta Physiologiae Plantarum 30:345–351. https://doi.org/10.1007/s11738-007-0129-z.

Boardley M, Stirton C H & Harborne J B (1986). A chemotaxonomic survey of the tribe Psoralea in Africa. *Biochemical Systematics and Ecology* 14(6): 603–613

Bouque V, Bourgaud F, Nguyen C & Guckert A (1998). Production of daidzein by callus cultures of Psoralea species and comparison with plants. Plant Cell, Tissue and Organ Culture 53: 35–40. https://doi.org/10.1023/A:1006057211490

Bourgaud F, Nguyen C & Guckert A (1995) XXII. Psoralea species: in vitro culture and production of furanocoumarins and other secondary metabolites. In Y P S: Bajaj (Eds.) *Biotechnology in Agriculture and Forestry*, Berlin, Springer-Verlag pp. 388–411

Cappelletti E M, Innocenti G & Caporale G (1984). Furocoumarins localization in the fruit and seed of Psoralea corylifolia L. Plantes Medicinales-etPhytotherapie 18: 181-1189

Correal E (2012). Bituminaria bituminosa Workshop, 28-30 November, Sassari/Italy

Davis P H (1965). Flora of Turkey and The East Aegean Islands. Edinburgh University Press, Edinburgh.

Del Río J A, Ortuño A, Pérez I, Bennett R G, Real D (2010). Furanocoumarin content in *Bituminaria bituminos* a varieties and Cullen species. In: C Porqu eddu & S Ríos (Eds.). *The contributions of grasslands to the conservation of Mediterranean biodiversity.* Option s Méditerranéennes: Série A. Sémin aires Méditerranéen, pp. 69-70

Hsu Y T, Wu C J, Chen J M, Yang Y C & Wang S Y (2001). The presence of three isoflavonoid compounds in *Psoralea corylifolia*. *Journal of Chromatographic Science* 39: 441-444. https://doi.org/10.1093/chromsci/39.10.441

Kallela K, Saastamoinen I, Huokuna E & Hakkola H (1988). Variation in plant oestrogen content between certain red clover cultivars in northern and southern Finland. Suomen Eläinlääkärilehti 94: 287−291

Kittler L, Hradecna Z & Suhnel J (1980). Cross-link formation of phage lambda DNA in situ photochemically induced by the furocoumarin derivative angelicin. Biochim Biophys Acta 607: 215-220. https://doi.org/10.1016/0005-2787(80)90074-x

Li X, Yu C, Hu Y, Xia X, Liao Y, Zhang J, Chen H, Lu W, Zhou W & song Z (2018). New application of psoralen and angelicin on periodontitis with anti-bacterial, anti-inflammatory, and osteogenesis effects. Front Cell Infect Microbiol 5: 8:178. https://doi.org/10.3389/fcimb.2018.00178

Lojza J, Schulzová V & Hajšlová J (2004). Changes of phytoestrogens daidzein, genistein and their glycosides daidzin and genistin and coumestrol during processing of soyabeans. *Czech Journal of Food Sciences* 22 (Special Issue): 223-226. https://doi.org/10.17221/10666-CJFS

Martínez S, Correal E, Real D, Ortuño A Río JA (2010). *Bituminaria bituminosa*: a source of furanocoumarins of pharmaceutical interest. Drug plants I: 307-322

Milesi S, Massot B, Gontier E, Bourgaud F & Guckert A (2001). Ruta graveolens L.: A promising species for the production of furanocoumarins. Plant Science 161:189-199. https://doi.org/10.1016/S0168-9452(01)00413-7

Mustonen E, Tuori M, Kurki P, Isolahti M, Taponen J & Vanhatalo A (2018). Variety, time of harvest and conditions during growing season have impact on red clover isoflavone content. *Agricultural and Food science* 27: 102–109. https://doi.org/10.23986/afsci.69781

Oliveira A M A G, Manuela M, Raposo M, Oliveira-Campos A M F, Machado A E H, Puapairoj P, Pedro M, Nascimento M S C, Portela C, Afonso C & Madalena P (2006). Psoralen analogues: synthesis, inhibitory activity of growth of human tumor cell lines and computational studies. *European Journal of Medicinal Chemistry* 41: 367–372. https://doi.org/10.1016/j.ejmech.2005.10.016

Pazos-Navarro M, Del Rı´o J A, Ortun˜o A, Romero-Espinar P, Correal E & Dabauza M (2013). Plant regeneration from different explant types of *Bituminaria bituminosa* and furanocoumarin content along plant regeneration stages. Plant Growth Regulation 70: 123–129. https://doi.org/10.1007/s10725-013-9784-9

Pecetti L, Tava A, Pagnotta M A & Russi L (2007). Variation in forage quality and chemical composition among Italian accessions of *Bituminaria bituminosa* (L.) Stirt. *Journal of the Science of Food and Agriculture* 87: 985–991. https://doi.org/10.1002/jsfa.2792

Pistelli L, Noccioli C, Appendino G, Bianchi F, Sterner O & Ballero M (2003). Pterocarpans from *Bituminaria morisiana* and *Bituminaria bituminosa*. *Phytochemistry* 64: 595–598. https://doi.org/10.1016/s0031-9422(03)00190-0

Rakhmankulov U & Korotkova E E (1975). Dynamics of the content of furocoumarins in various organs of *Psoralea drupacea*. *Rastitel, nye-Resursy* 11: 98-104

Real D (2012). Tedera a modern model of species domestication and breeding. Booklet. Department of Agriculture and Food Government of Western Australia.

Saloniemi H, Wähälä K, Nykänen-Kurki P, Kallela K & Saastamoinen I (1995). Phytoestrogen content and estrogenic effect of legume fodder. Proceedings of the Society for Experimental Biology and Medicine 208: 13−17. https://doi.org/10.3181/00379727-208-43825

Setchell K D R, Lawson A M, Borriello S P, Harkness R, Gordon H, Morgan D M, Kirk D N, Adlercreatz H, Anderson L C & Axelson M (1981). Lignan formation in man-microbial involvement and possible roles in relation to cancer. The Lancet 2: 4–7. https://doi.org/10.1016/S0140-6736(81)90250-6

Sivesind E & Seguin P (2005). Effects of the environment, cultivar, maturity, and preservation method on red clover isoflavone concentration. *Journal of Agricultural and Food Chemistry* 53: 6397-6402. https://doi.org/10.1021/jf0507487

Tava A, Pecetti L, Ricci M, Pagnotta M A & Russi L (2007). Volatile compounds from leaves and flowers of *Bituminaria bituminosa* (L.) Stirt. (Fabaceae) from Italy. *Flavour Flavour and Fragrance* Journal 22: 363–370. https://doi.org/10.1002/ffj.1806

Walker D J, Moñino I & Correal E (2006). Genome size in *Bituminaria bituminosa* (L.) C.H. Stirton (Fabaceae) populations: Separation of "true" differences from environmental effects on DNA determination. Environmental and Experimental Botany 55: 258-265. https://doi.org/10.1016/j.envexpbot.2004.11.005

Journal of Agricultural Sciences (Tarim Bilimleri Dergisi) 2022, 28 (4) : 732 - 739 DOI: 10.15832/ankutbd.937717

Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

Effects of Row Spacing and Sowing Rate on Quality Performance of Alfalfa (*Medicago sativa* L.) Under Tokat Ecological Conditions

Mahir OZKURT[a]*, Yaşar KARADAG[a]

[a]*Department of Plant Production and Technologies, Faculty of Applied Sciences, Mus Alparslan University, Muş, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Mahir OZKURT, E-mail: mahirozkurt@gmail.com
Received: 15 May 2021 / Revised: 16 December 2021 / Accepted: 18 December 2021 / Online: 15 October 2022

Cite this article
OZKURT M, KARADAG Y (2022). Effects of Row Spacing and Sowing Rate on Quality Performance of Alfalfa (Medicago sativa L.) Under Tokat Ecological Conditions. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 28(4):732-739. DOI: 10.15832/ankutbd.937717

ABSTRACT
This research was conducted to determine the effects of row spacing and sowing rate on the hay quality characteristics of alfalfa under Tokat-Kazova ecological conditions during the years of 2014-2016. Alfalfa cultivar Bilensoy-80 was sown at four different row spacings (15, 30, 45 and 60 cm) and six different sowing rates (5, 10, 15, 20, 25 and 30 kg ha^{-1}). The experimental design was randomized complete block design in split plots with four replications. Row spacings were main plots and sowing rates were sub-plots. The highest crude protein content (% 20.8) and relative feed value (147.8) were obtained from 5 kg ha^{-1} sowing rate and 15 cm row spacing. It was concluded based on present findings that Belinsoy-80 alfalfa cultivar should be sown at 15 cm row spacing and 25 kg ha^{-1}sowing rate for high hay quality under Tokat-Kazova ecological conditions.

Keywords: *Lucerne, Sowing rate, Crude protein rate, ADF, RFV, Anatolia*

1. Introduction

Alfalfa is the most common forage plant worldwide. So, called as the queen of forage crops, alfalfa has a higher forage yield than almost all the other forage crops. Hay and green herbage of alfalfa with quite a high protein yield per unit area are delicious and nutritious for all kinds of animals. Alfalfa herbage is rich in vitamins. Due to its deep roots, it can easily benefit from soil water and nutrients. Since alfalfa is a leguminous plant, it is able to fixate free atmospheric nitrogen into the soils through root nodules (Acıkgöz 2001). Relative feed value is generally used to assess quality of alfalfa hay and herbage (Yavuz 2005). The relative feed value is calculated with the use of acid detergent fiber (ADF) and neutral detergent fiber (NDF) values. In animal feeding, ADF has been used as an energy indicator especially in ruminant rations (Tekce & Gül 2014) and NDF has been used as an indicator of how much feed an animal will consume within 24 hours (Budak & Budak 2014). In other words, while ADF value gives an idea about the quality of the feed, NDF value gives an idea about the size-thickness of the feed (Kutlu 2008). In calculating the relative feed value, digestible dry matter and dry matter intake are determined and protein content is not included in the calculation (Güney et al. 2016). Protein ratio itself is a quality criterion.

Inter-row and intra-row spacing designate solar radiation and consequent biomass production of alfalfa plants. Row spacing alone influences the yield and quality of the plants and it is an easy-to-apply agronomic practice (Mattera et al. 2013). Plant losses in the year of establishment are generally higher in high sowing rate than in low sowing rate (Volenec et al. 1987).

Seed cost and seed supply constitute the most important problems in cultivation of forage crops. Alfalfa cultivated lands are renewed generally in every four years. In 2017, alfalfa seed need of Turkey was 3297.0 tons, but certificated alfalfa seed production was 887.4 tons. That means only 26.9% of alfalfa seed needs were met (Anonymous 2018). The remaining 75% was supplied from the seeds produced in alfalfa cultivated lands of farmers and growers. In Turkey, although varied with the regions, alfalfa sowing rate vary between 40 - 180 kg ha^{-1}. Following the first winter after sowing, it is desired to have a plant density of 130 plants/m^2 (Rashidi et al. 2009). With a simple calculation, approximately 9 kg ha^{-1} seed is sufficient to attain this plant density after the first winter. Thousand grain weight of alfalfa is approximately 2.4 g. It is known that approximately 50-60% of the seeds sown form the seedlings and 60-80% of these seedlings die after the first winter (Rashidi et al. 2009). In this case, the sowing rate per hectare is 9 kg. When it considered that half of the seeds planted will not emerge since the field conditions were not optimal, soil was not well prepared and problems were encountered in germination of the seeds, it will be sufficient to use 18 kg of seed per hectare to achieve optimum plant density. In this sense, sowing rate (amount of seed to be sown per unit area) has become an important issue in alfalfa cultivation.

Numerous researches have been completed on sowing rate and plant density in many parts of the world. Chocarro & Lloveras (2015) reported in their research that narrower row spacings provided higher dry matter yields. Yilmaz et al. (2015) conducted a study under ecological conditions of Kahramanmaras province and reported that narrow row spacing was more advantageous in terms of herbage and seed yields. Caddel et al. (2017) indicated "variety selection", "seed quality" and "sowing rate" as the cost items in alfalfa cultivation and pointed out that these issues should not be considered separately.

Although the number of seeds is low in different regions in Turkey and the distance between rows has been studied, no research has been found on the seed amount and row spacing of Bilensoy 80 alfalfa cultivar in Tokat Kazova, which is located in the transition zone climate zone. This study was conducted to determine the effects of different row spacings and sowing rates on the hay quality characteristics of Bilensoy-80 alfalfa cultivar under Tokat-Kazova ecological conditions.

2. Material and Methods

The research was conducted under ecological conditions of Tokat-Kazova located at between 40° 19` North latitude and 40° 19` East longitudes and with an altitude of 595 m between the years 2014-2016. Experimental soils were rich in lime and potassium, clay-loam in texture, alkaline, but poor in organic matter (Anonymous 2014). Bilensoy-80 alfalfa cultivar was used as the plant material. This widely-grown and well-adapted cultivar was developed by Ankara Field Crops Central Research Institute and registered in 1984. It is listed in 4-5 dormancy group. Experiments were conducted in randomized complete block design in split plots with 4 replications. Experimental treatments are composed of 6 different sowing rates (5, 10, 15, 20, 25 and 30 kg ha^{-1}) and 4 different row spacings (15, 30, 45 and 60 cm). Row spacings were placed in main plots and sowing rates were placed in sub-plots. Experimental plots were 5 m long and each plot had 6 rows. Based on row spacings, plot sizes were arranged as 0.15 x 6 x 5 = 4.5 m^2, 0.30 x 6 x 5 = 9 m^2, 0.45 x 6 x 5 = 13.50 m^2, 0.6 x 6 x 5 = 18 m^2. When the plants reached 10% flowering, two side rows and 50 cm sections from the top and bottom of each plot were omitted as to consider side effects and harvest was performed from the remaining plot area (Avcıoglu et al. 2009).

Since alfalfa is a perennial crop and the first year is its establishment year (2014), observation and measurements were not taken in the first year. Observations and measurements were made in the 2nd (2015) and 3rd (2016) years (Anonymous 2001). Quality analyses were conducted in accordance with the studies of Sleugh et al. (2000); Bulgurlu & Ergül (1978); Van Soest et al. (1991) and Sheaffer et al. (1995). Variance analysis was applied to the experimental data by using the MSTAT-C statistical software in accordance with the randomized complete block design in split plots. Significant means were compared with the use of Duncan's multiple comparison test (Yurtsever 2011).

3. Research Findings and Discussion

3.1. Crude protein ratio (%)

According to variance analysis of separate years data, row spacing, sowing rate and row spacing x sowing rate interactions had significant effects on crude protein ratio in the first year of the study; sowing rate and row spacing x sowing rate interactions had significant effects on crude protein ratio in the second year of the study. According to variance analysis of combined years data, year, year x row spacing, row spacing x sowing rate, year x sowing rate and row spacing x year x sowing rate interactions had significant effects on crude protein ratio. Average crude protein ratios for different row spacing-sowing rate combinations in the experimental years and as averaged values of two years are provided in Table 1.

Table 1- Averaged crude protein ratios (%) for different row spacings and sowing rates in the experimental years and in the combination of two years

Years	*Row Spacing*	*Sowing Rate*						
		5 kg ha^{-1}	*10 kg ha^{-1}*	*15 kg ha^{-1}*	*20 kg ha^{-1}*	*25 kg ha^{-1}*	*30 kg ha^{-1}*	*Average*
2015	15 cm	20.4 f-n[2]	20.3 g-p	20.5 d-m	19.5 o-t	20.2 h-p	19.2 q-t	20.0 D[*]
	30 cm	20.1 l-q	19.2 rst	19.7 m-t	19.4 p-t	19.7 m-t	19.0 t	19.5 E
	45 cm	19.9 k-s	20.8 b-k	19.6 n-t	20.4 f-n	19.9 l-s	19.5 o-t	20.0 D
	60 cm	20.0 j-r	19.1 st	20.1 ı-q	20.6 c-l	20.2 g-o	20.1 ı-p	20.0 D
	Avg.	20.1 C[+]	19.8 CD	20.0 C	20.0 C	20.0 C	19.5 D	19.9 B[1]
2016	15 cm	21.3 b-e	21.4 bc	21.3 bcd	21.1 b-g	20.6 c-l	21.5 ab	21.2 A
	30 cm	20.7 c-l	20.2 h-p	21.3 bcd	20.4 e-m	21.6 ab	22.2 a	21.1 AB
	45 cm	19.7 m-t	20.6 c-l	21.3 bcd	21.0 b-h	20.8 b-k	20.4 e-m	20.6 C
	60 cm	21.2 b-f	20.8 b-k	20.3 g-o	20.9 b-ı	20.8 b-j	20.9 b-ı	20.8 BC
	Avg.	20.7 B	20.7 B	21.1 AB	20.9 B	20.9 AB	21.3 A	20.9 A
Combined Years	15 cm	20.8 ab[3]	20.8 ab	20.9 a	20.3 b-f	20.4 a-f	20.4 a-f	20.6
	30 cm	20.4 a-f	19.7 g	20.5 a-e	19.9 efg	20.6 abc	20.6 abc	20.3
	45 cm	19.8 fg	20.7 abc	20.5 a-e	20.7 abc	20.3 a-f	20.0 d-g	20.3
	60 cm	20.6 abc	19.9 efg	20.2 c-g	20.8 abc	20.5 a-d	20.6 a-d	20.4
	Avg.	20.4	20.3	20.5	20.4	20.5	20.4	20.4

*: Means indicated with the similar capital letters in the same column are not significantly different at P≤0.05 according to Duncan's test; [+]: Averaged values of year x sowing rate combinations indicated with similar capital letters are not significantly different at P≤0.01 according to Duncan's test; [1]: The averages of the years indicated with different letters are not significantly different; [2]: Row spacing x year x sowing rate interactions indicated with similar lower case letters are not significantly different at P≤0.01 according to Duncan's test; [3]: Averaged values of row spacing x sowing rate combinations indicated with similar lower-case letters are not significantly different at P≤0.01 according to Duncan's test.

The average crude protein ratio was determined as 19.9% in the first year and 20.9% in the second year of the study. There were significant differences in average crude protein ratios of the years (Table 1).

In the first year of the study, row spacings significantly affected crude protein ratio. On the other hand, significant row spacing x year interactions revealed that effects of row spacings on crude protein ratio varied with the years. In terms of year x row spacing interactions, the lowest value (19.5%) was obtained from 30 cm row spacing of 2015 and the greatest values (21.2 and 21.1%) were respectively obtained from 15 and 30 cm row spacings of 2016.

Separate analyses of the years revealed that sowing rates significantly affected crude protein ratios. On the other hand, significant year x sowing rate interactions revealed that effects of sowing rates on crude protein ratios varied with the years. In the first year, row spacing of 30 cm resulted in significantly lower crude protein ratio than the other row spacings tested while row spacing of 15 cm gave significantly higher crude protein ratio than the other row spacings with the exception of 30 cm in the second year. In terms of crude protein ratio, while the sowing rate of 5 kg ha^{-1} was found to be the optimum sowing rate in the first year of the study, 30 kg ha^{-1} sowing rate was identified as the optimum sowing rate in the second year of the study. At sowing rate of 5 kg ha^{-1}, population density was lower, thus greater number of branches and consequently greater crude protein ratios were achieved. On the other hand, in the second year of the study, change in population resulted in greater crude protein ratio at 30 kg ha^{-1} sowing rate. Similarly, Hansen & Krueger (1973) obtained the highest crude protein ratio from the low sowing rate (45 kg ha^{-1}) in the first year and from the higher sowing rate (135 kg ha^{-1}) in the second year.

Karadag et al. (2011) conducted a study with different alfalfa cultivars under the same ecological conditions as in this study and indicated Bilensoy-80 cultivar as prominent with crude protein ratio. Stout (1998) reported insignificant differences in crude protein ratios of alfalfa under different row spacings.

Present findings on crude protein ratios are in agreement with the findings of earlier studies (Hansen & Kreuger 1973; Stout 1998; Avci et al. 2009; Scholtz 2009; Saruhan & Kusvuran 2011; Yavuz 2011; Cinar 2012; İnal 2015; Acikbas et al. 2017; Erdel 2017).

3.2. Acid detergent fiber content (%)

Variance analysis revealed that sowing rates had significant effect on ADF ratio in the first year of the study. Additionally, significant differences were observed in ADF ratio depending on the year. Combined analysis of the years revealed that year x sowing rate and row spacing x year x sowing rate interactions were statistically significant for this character.

Acid detergent fiber ratios of the years and two-year averages under different row spacings and sowing rates are provided in Table 2.

The average ADF ratio was determined as 35.5% in the first year and 30.9% in the second year of the study. ADF ratio significantly varied with the years (Table 2).

In the first year of the study, sowing rates significantly affected ADF ratios. On the other hand, significant year x sowing rate interactions revealed that effects of sowing rates on ADF ratios varied with the years.

Table 2- ADF Ratios (%) for different row spacings and sowing rates in the experimental years and in the combination of two years

Years	*Row Spacing*	*Sowing Rates*						
		5 kg ha^{-1}	*10 kg ha^{-1}*	*15 kg ha^{-1}*	*20 kg ha^{-1}*	*25 kg ha^{-1}*	*30 kg ha^{-1}*	*Average*
2015	15 cm	34.5 cd[1]	34.6 cd	35.3 a-d	35.7 a-d	35.6 a-d	36.1 a-c	35.3
	30 cm	36.1 abc	35.0 bcd	35.6 a-d	35.2 a-d	35.9 a-d	36.3 ab	35.7
	45 cm	36.0 abc	35.4 a-d	36.7 a	35.9 a-d	35.2 a-d	35.5 a-d	35.8
	60 cm	34.6 cd	35.2 a-d	36.0 abc	35.2 a-d	34.3 d	35.5 a-d	35.1
	Avg.	35.3 AB[+]	35.1 B	35.9 A	35.5 AB	35.3 AB	35.9 A	35.5 A[*]
2016	15 cm	30.3 fg	30.8 ef	31.1 ef	30.9 ef	30.7 ef	30.7 ef	30.8
	30 cm	31.7 ef	31.0 ef	30.6 ef	31.1 ef	30.6 ef	29.0 g	30.7
	45 cm	30.5 efg	31.5 ef	32.0 e	31.5 ef	31.9 ef	31.8 ef	31.5
	60 cm	30.8 ef	30.4 efg	30.4 efg	31.3 ef	31.5 ef	30.3 fg	30.8
	Avg.	30.8 CD	30.9 CD	31.0 CD	31.2 C	31.1 CD	30.5 D	30.9 B
Combined Years	15 cm	32.4	32.7	33.2	33.3	33.2	33.4	33.0
	30 cm	33.9	33.0	33.1	33.2	33.3	32.7	33.2
	45 cm	33.3	33.5	34.4	33.7	33.6	33.7	33.7
	60 cm	32.7	32.8	33.2	33.3	32.9	32.9	33.0
	Avg.	33.1	33.0	33.5	33.4	33.2	33.2	33.2

[*]: Means indicated with different capital letters are significantly different from each other; [+]: Year x sowing rate combinations indicated with similar capital letters are not significantly different at P≤0.05 according to Duncan's test; [1]: Row spacing x year x sowing rate combinations indicated with similar lower case letters are not significantly different at P≤0.05 according to Duncan's test.

Thus, optimum sowing rate for a low ADF content of the forage was 10 kg ha^{-1} in the first year while it was 30 kg ha^{-1} in the second year (Table 2). Combined analysis of the years revealed that row spacing x year x sowing rate interactions had significant effects on ADF ratios. Such a case indicated that effects of row spacing x sowing rate interactions varied with the years. Thus, optimum row spacing x sowing rate combination for a low ADF content in the first year was 60 cm x 25 kg ha^{-1} while 30 cm x 30 kg ha^{-1} in the second year.

Stout (1998) indicated insignificant differences between of 15 cm row spacing and 30 cm row spacing in ADF ratio and reported that the lowest ADF ratio was obtained at sowing rate of 22.4 kg ha^{-1}. Besides, Min et al. (2000) indicated that high stand densities (278 plants/m^2 or more) did not increase herbage yield and forage quality of alfalfa compared with low plant population densities (100 plants/m^2 or less). Thus, using lower seeding rates may enable alfalfa producers to reduce the establishment costs and increase their marginal profit.

Present findings on ADF ratios of alfalfa plants under different sowing rates and row spacings are in agreement with the results of earlier studies (Scholtz 2009; Avci et al. 2011; Yavuz 2011; Yücel et al. 2011; Yüksel 2012; Gündel et al. 2014; İnal 2015; Engin 2016; Yilmaz & Albayrak 2016; Acıkbas et al. 2017; Erdel 2017).

3.3. Neutral detergent fiber content (%)

Sowing rates and sowing rate x row spacing interactions had significant effects on NDF ratio in the first year and row spacings and row spacing x sowing rate interactions had significant effects on NDF ratios in the second year of the study. Combined analysis of the years revealed that row spacings, years, row spacing x sowing rate, year x sowing rate and row spacing x year x sowing rate interactions had significant effects on NDF ratio.

The NDF ratios in the experimental years and average of two years under different row spacings and sowing rates are provided in Table 3.

The averaged NDF ratio was determined as 42.3% in the first year and 41.5% in the second year, and there were significant differences between the averaged NDF values of the years (Table 3).

Table 3- NDF Ratios (%) for different row spacings and sowing rates in the experimental years and in the combination of two years

Years	*Row Spacing*	*Sowing Rate*						
		5 kg ha^{-1}	*10 kg ha^{-1}*	*15 kg ha^{-1}*	*20 kg ha^{-1}*	*25 kg ha^{-1}*	*30 kg ha^{-1}*	*Average*
2015	15 cm	41.8 b-ı[2]	40.7 f-j	42.1 b-h	42.5 a-g	42.4 b-g	42.8 a-d	42.1
	30 cm	42.6 a-f	42.1 b-h	42.0 b-h	40.7 f-j	43.2 abc	43.4 ab	42.3
	45 cm	42.2 b-h	42.7 a-f	44.3 a	42.2 b-h	42.2 b-h	43.4 ab	42.8
	60 cm	42.4 b-g	42.3 b-g	42.7 a-e	42.5 a-g	40.6 g-j	41.5 b-j	42.0
	Avg.	42.3 AB[+]	42.0 AB	42.8 A	42.0 B	42.1 AB	42.8 A	42.3 A[1]
2016	15 cm	40.0 ıj	39.7 ı	40.8 e-j	40.3 hıj	40.7 f-j	40.5 g-j	40.3 C[*]
	30 cm	42.1 b-h	42.0 b-h	41.0 d-j	43.4 ab	42.0 b-h	41.0 d-j	41.9 AB
	45 cm	42.3 b-g	43.4 ab	42.3 b-h	42.2 b-h	41.6 b-j	41.1 d-j	42.2 A
	60 cm	41.1 d-j	41.4 c-j	41.9 b-h	41.6 b-j	41.6 b-ı	41.1 d-j	41.5 B
	Avg.	41.4 BC	41.6 BC	41.5 BC	41.9 B	41.5 B	40.9 C	41.5 B
Combined Years	15 cm	40.9 fg[3]	40.2 g	41.5 def	41.4 def	41.6 def	41.7 c-f	41.2 B[4]
	30 cm	42.4 bcd	42.1 b-e	41.5 def	42.1 b-e	42.6 abc	42.2 bcd	42.1 A
	45 cm	42.3 bcd	43.1 ab	43.3 a	42.2 bcd	41.9 c-f	42.3 bcd	42.5 A
	60 cm	41.8 c-f	41.9 c-f	42.3 bcd	42.1 cde	41.1 efg	41.3 def	41.7 AB
	Avg.	41.8	41.8	42.1	41.9	41.8	41.9	41.9

[*]: Means indicated with the similar capital letters in the same column are not significantly different at P≤0.01 according to Duncan's test; [1]: Averaged values of the Years indicated with different capital letters are significantly different; [+]: Year x sowing rate combinations indicated with the similar capital letters are not significantly different at P≤0.05 according to Duncan's test; [2]: Row spacing x year x sowing rate combinations indicated with the similar lowercase letters are not significantly different at P≤0.05 according to Duncan's test; [3]: Row spacing x sowing rate combinations indicated with the similar lowercase letters are not significantly different at P≤0.01 according to Duncan's test; [4]: Means indicated with the similar capital letters in the same column are not significantly different at P≤0.05 according to Duncan's test.

Although there were no significant differences among the mean NDF values of row spacings in the first year, row spacings significantly affected NDF ratio in the second year of the study (Table 3). According to two-year averages, the lowest NDF ratio (41.2%) was obtained from 15 cm row spacing and the highest (42.5%) from 45 cm row spacing. Present findings revealed that 15 cm row spacing could be optimal row spacing for a low NDF content of forage in alfalfa.

In the first year of the study, sowing rates had significant effects on NDF ratios. On the other hand, significant year x sowing rate interactions (Table 2) revealed that effects of sowing rates on NDF ratios varied with the years. Thus, optimal sowing rate for a low NDF content was 20 kg kg ha^{-1} in the first year while the optimal sowing rate was 30 kg ha^{-1} in the second year.

Combined analysis of the years revealed that row spacing x sowing rate interactions had significant effects on NDF ratios. Such a case indicated that effects of sowing rates on NDF ratios varied with the row spacings. Significant row spacing x year x sowing rate interactions revealed that effects of row spacing x sowing rate interactions on NDF ratios varied with the years. Accordingly, optimal combination of row spacing and sowing rate was 60 cm row spacing with a sowing rate of 25 kg ha^{-1} in the first year while it was 15 cm row spacing with a sowing rate of 10 kg ha^{-1} in the second year of the study (Table 3).

Low plant population in narrow row spacing resulted in taller plants leading to an increase in leaf / stem ratio and lower NDF ratios. Iwaasa et al. (1996) also indicated that sowing rates did not have significant effects on NDF ratio.

Present findings on NDF ratios under different row spacings and sowing rates are in agreement with the results of previous studies (Min et al. 2000; Scholtz 2009; Avci et al. 2011; Yücel et al. 2011; Albayrak & Türk 2013; Engin 2016; Yilmaz & Albayrak 2016; Acikbaş et al. 2017; Erdel 2017).

3.4. Relative feed value

Sowing rates had significant effects on relative feed values in the first year while row spacings had significant effects on relative feed values in the second year. Row spacing x sowing rate and year x sowing rate interactions had significant effects on relative feed values in both years.

Averaged relative feed values in the experimental years and averages of two years under different row spacings and sowing rates are provided in Table 4. The average relative feed value was determined as 137.8 in the first year and 148.7 in the second year of the study. There were significant differences between the averaged relative feed values of the years (Table 4).

In the second year of the study, row spacings greater than 15 cm caused significant decreases in relative feed values. As the average of two years, the highest relative feed value (145.8) was obtained from 15 cm row spacing and the lowest one (141.0) from 45 cm row spacing.

Table 4- Relative feed values for different row spacings and sowing rates in the experimental years and in the combination of two years

Years	*Row Spacing*	*Sowing Rate*						
		5 kg ha^{-1}	*10 kg ha^{-1}*	*15 kg ha^{-1}*	*20 kg ha^{-1}*	*25 kg ha^{-1}*	*30 kg ha^{-1}*	*Average*
2015	15 cm	141.6	144.2	138.7	137.4	137.5	136.0	139.2
	30 cm	135.0	141.7	138.8	143.1	134.8	131.3	137.5
	45 cm	136.9	136.3	128.9	138.7	139.8	135.1	136.0
	60 cm	137.4	139.7	135.0	136.6	145.2	138.1	138.7
	Avg.	137.7 BCD^{+}	140.5 B	135.4 CD	139.0 BC	139.3 B	135.1 D	137.8 B*
2016	15 cm	154.0	154.2	150.6	152.0	151.5	151.5	152.3 A
	30 cm	145.4	148.4	152.3	142.7	147.9	152.8	148.3 B
	45 cm	147.3	141.5	144.9	147.1	147.3	148.4	146.1 B
	60 cm	150.0	148.2	147.1	146.9	146.4	149.8	148.1 B
	Avg.	149.2 A	148.1 A	148.7 A	147.2 A	148.3 A	150.6 A	148.7 A
Combined Years	15 cm	147.8 ab^{1}	149.2 a	144.7 b-e	144.7 b-e	144.5 b-f	143.8 b-f	145.8 A^{2}
	30 cm	140.2 fgh	145.1 b-e	145.6 a-d	142.9 c-g	141.4 d-g	142.1 c-g	142.9 AB
	45 cm	142.1 c-g	138.9 gh	136.9 h	142.9 c-g	143.6 b-f	141.8 c-g	141.0 B
	60 cm	143.7 b-f	144.0 b-f	141.1 efg	141.8 c-g	145.8 abc	144.0 b-f	143.4 AB
	Avg.	143.5	144.3	142.0	143.1	143.8	142.9	143.3

*: Averaged values of the years indicated with different letters are significantly different; $^{+}$: Year x sowing rate combinations indicated with the similar capital letters are not significantly different at P≤0.05 according to Duncan's test; ^{1}: Row spacing x sowing rate combinations indicated with the similar lowercase letters are not significant different at P≤0.01 according to Duncan's test; ^{2}: Means indicated with the similar capital letters in the same column are not significantly different at P≤0.01 according to Duncan's test.

Sowing rate had significant effect on relative feed value while relative feed value was not significantly influenced by sowing rate in the second year (Table 4). Therefore, year x sowing rate interaction was statistically significant. In the first year of the study, the optimal sowing rate for a high relative feed value was 10 kg ha^{-1}.

According to combined analysis of the years, row spacing x sowing rate interaction was statistically significant. Thus, the optimal combination of row spacing and sowing rate for a high relative feed value was 15 cm row spacing with a 10 kg ha^{-1} sowing rate. Since relative feed value is calculated with the use of ADF and NDF values, as it was expected, the treatments with low ADF and NDF values yielded high relative feed values since there is a negative relationship between ADF - NDF ratios and relative feed value.

Present findings revealed that under present ecological conditions, alfalfa plants should be sown at 10 kg ha^{-1} sowing rate and 15 cm row spacing for high relative feed values. Present are in agreement with the results of Albayrak & Türk (2013), Engin (2016) and Acikbas et al. (2017).

4. Conclusions

From the results of the study conducted two years under Tokat-Kozova conditions, it was concluded that 15 cm row spacing and 10 kg ha^{-1} sowing rate should be applied in order to obtain satisfactory results in terms of hay quality of alfalfa plants under ecological conditions of Tokat-Kazova and in similar ecologies. Present findings revealed that agronomic practices played a great role in alfalfa cultivation and such practices varied considerably with the regions and ecological conditions. Further research is recommended to be conducted on different alfalfa cultivars with different dormancy groups.

Acknowledgments

This study was supported by the Scientific Research Projects Department of Tokat Gaziosmanpaşa University, Turkey (Project No: BAP 2014/101) and produced from the Ph.D. thesis of the first author.

References

Acikbas S, Albayrak S & Türk M (2017). Determination of forage yield and quality of some alfalfa (*Medicago sativa* L.) genotypes collected from natural vegetation. *Turk Journal of Agricultural Research* 4(2): 155-162. doi.org/10.19159/tutad.293446

Acikgöz E (2001). Forage Crops. VIPAS Edit No: 58, 584 pp. Bursa

Albayrak S & Türk M (2013). Changes in the forage yield and quality of legume-grass mixtures throughout a vegetation period. *Turkish Journal of Agriculture and Forestry* 37(2): 139-147 DOI: 10.3906/tar-1202-73

Anonymous (2001). Ministry of Agriculture and Forestry. Seed Registration and Certification Center Directorate. Technical Instruction for Trials to Measure Agricultural Values of Alfalfa Species (Medicago L. Species) Ankara.

Anonymous (2014). Tokat Gaziosmanpasa University Agriculture Faculty. Soil Science and Plant Nutrition Department. Soil Analysis Laboratory. Tokat.

Anonymous 2018. Turkish Statistical Institute.

Avci M, Cinar S, Kizil S, Aktas A, Yücel C, Hatipoglu R, Yücel H, Kilicalp N, İnal İ & Gültekin R (2009). Research on the hay yields and hay qualities of some alfalfa cultivars under Cukurova conditions. Turkey VIII. Field Crop Congress, Oct 19-22, Volume I. Hatay, pp. 666-670

Avcioglu R, Geren H, Tamkoc A & Karadag Y (2009). Alfalfa (*Medicago sativa* L.). In: Forage Crops, Legume Forage Crops, Volume II, Ed. Avcioglu R., Hatipoglu R. & Karadag, Y, Ministry of Agriculture and Rural Affairs Publication (in Turkish), pp. 290-336

Budak F & Budak F (2014). Quality on forage plants and factors effecting forage quality. Turkish Journal of Scientific Reviews (7)1:1-6

Bulgurlu S & Ergül M (1978). Physical, Chemical and Biological Analysis Methods of Feeds. Ege University Faculty of Agriculture Publication. No: 127. Ege University Press. Bornova-İzmir, Turkey pp. 58-76

Caddel J, Huhnke R, Stritzke J & Johnson G (2017). Alfalfa stand establishment. http://pods.dasnr.okstate.edu/docushare/dsweb/Get/Rendition-3517/unknown.

Chocarro C & Lloveras J (2015). The effect of row spacing on alfalfa seed and forage production under irrigated Mediterranean agricultural conditions. Grass Forage Science 70(4): 651-660. DOI: 10.1111/gfs.12146

Cinar S (2012). Performances of Pure Growings and Mixture of Some Warm Season Perennial Grasses and Alfalfa (*Medicago sativa* L.) Under Lowland Conditions of Cukurova. PhD Thesis, (Published). Cukurova University, Institute of Natural and Applied Sciences Department of Field Crops, Adana Turkey.

Engin B (2016). Determination of hay yield and some quality traits of alfalfa (*Medicago sativa* L.) cultivars in Yozgat Ecologycal conditions. Master of Science Thesis, Yozgat Bozok University. Institute of Natural and Applied Sciences Department of Field Crops, Yozgat, Turkey.

Erdel B 2017. Determination of Forage Yield and Quality Characteristics of Some Alfalfa (*Medicago sativa* L.) Cultivars Under Bursa Ecological Conditions. Master of Science Thesis, Uludag University. Institute of Natural and Applied Sciences Department of Field Crops, Bursa, Turkey.

Gündel F D, Karadag Y & Cınar S (2014). A research on yield, quality and adaptation of some warm season perennial legumes under Çukurova ecological conditions. Tokat Gaziosmanpasa University, *Journal of Agricultural Faculty of Gaziosmanpasa University* 31(3): 10-19

Güney M, Bingöl N & Aksu T (2016). Relative Feed Value (RFV) and Relative Forage Quality (RFQ) used in the classification of forage quality. Atatürk University Journal of Veterinary Sciences 11(2): 254-258. https://doi.org/10.17094/avbd.50526

Hansen L H & Krueger C R (1973). Effect of establishment method, variety, and seeding rate on the production and quality of alfalfa under dryland and irrigation. *Agron. J* 65(5): 755-759. doi: 10.2134/agronj1973.00021962006500050024x

Iwaasa A D, Beauchemin K A, Acharya S N, Bowley S R & Buchanan-Smith J G (1996). Shearing Force Of Alfalfa Stems as Affected by Seeding Rate. *Can. J. plant Sci* 76: 321-328

İnal N (2015). Determination of yield and quality characteristics of some alfalfa varieties under Kirsehir conditions. Master of Science Thesis, Kırsehir Ahi Evran University. Institute of Natural and Applied Sciences Department of Field Crops, Kırşehir, Turkey.

Karadag Y, İptaş S, Kır H & Akbay, S (2011). The Determination of the Yield and Quality Characteristics of Some Alfalfa Cultivars Under Tokat-Kazova Conditions. IX. Turkey Field Crops Congress, 12-15 September 2011, Bursa.

Kutlu H R (2008). Feed Evaluation and Analysis Methods. Cukurova University Department of Animal Husbandery, Course Note, Adana.

Mattera J, Romero L A, Cuatrín A L, Cornaglia P S & Grimoldi A A (2013). Yield components, light interception and radiation use efficiency of lucerne (*Medicago sativa* L.) in response to row spacing. *Eur. J. Agron* 45(1): 87-95. DOI: 10.1016/j.eja.2012.10.008

Min D H, King J R, Kim D A & Lee H W (2000). Stand density effects on herbage yield and forage quality of alfalfa. *Asian-Aus. J. Anim. Sci* 13(7): 929-934. https://doi.org/10.5713/ajas.2000.929

Rashidi M, Zand B & Gholami M (2009). Effect of different seeding rates on seed yield and some seed yield components of alfalfa (*Medicago sativa* L.). *International Journal of Agriculture and Biology*. 11(6): 779-78

Saruhan V & Kusvuran A (2011). Determination of yield performances of some lucerne cultivars and genotypes under the Güneydogu Anadolu Region conditions. *Journal of Agriculture Faculty of Ege University* 48(2): 131-138

Scholtz G D J, Merwe H J V D & Tylutki T P (2009). The nutritive value of South African Medicago sativa L. hay. *South African Journal Animal Science* 39(1): 179-182. DOI: 10.4314/sajas.v39i1.61269

Sheaffer C C, Peterson M A, Mccalin M, Volene J J, Cherney J H, Johnson K D, Woodward W T & Viands D R (1995). Acid Detergent Fiber, Neutral Detergent Fiber Concentration and Relative Feed Value, North American Alfalfa Improvement Conference, Minneapolis.

Sleugh B, Moore K J, George J R & Brummer E C (2000). Binary Legume-Grass Mixtures Improve Forage Yield, Quality, and Seasonal Distrubition. *Agronomy Journal* 92: 24-29. https://doi.org/10.2134/agronj2000.92124x

Stout D G (1998). Effect of high lucerne (*Medicago sativa* L.) sowing rates on establishment year yield, stand persistence and forage quality. *J. Agron. Crop Sci* 180(1): 39-43. DOI: 10.1111/j.1439-037X.1998.tb00367.x

Tekce E & Gül M (2014). The importance of NDF and ADF in ruminant nutrition. Atatürk University Journal of Veterinary Sciences. 9(1): 63-73

Van Soest P J, Robertson J B & Lewis B A (1991). Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *J. Dairy Sci* 74 pp. 3583-3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Volenec J J, Cherney J H & Johnson K D (1987). Yield components, plant morphology, and forage quality of alfalfa as influenced by plant population. Crop Sci 27(2): 321-326. https://doi.org/10.2135/cropsci1987.0011183X002700020040x

Yavuz M (2005). Detergent Fiber System. *Journal of Agricultural Faculty of Gaziosmanpasa University* 22(1): 93-96

Yavuz T (2011). Karadeniz determination of artificial pasture mixtures for dryland conditions of transitional climate zone of black sea region. PhD Thesis (Published). Tokat Gaziosmanpasa University, Institute of Natural and Applied Sciences Department of Field Crops, Tokat, Turkey

Yilmaz M F, İnal İ, Kara R, Dalkılıc A Y & Avci M (2015). Different sowing frequencies on alfalfa (*Medicago sativa* L.) of effects on herb and seed yield in Kahramanmaras conditions. XI. Turkey Field Crops Congress, 07-10. Sept, Çanakkale, pp.391-392

Yilmaz M & Albayrak S (2016). Determination of forage yield and quality of some alfalfa (*Medicago sativa* L.) cultivars under Isparta Ecological conditions. Journal of Central Research Institute for Field Crops 25(1): 42-47. https://doi.org/10.21566/tbmaed.91487

Yurtsever N (2011). Experimental statistical methods ministry of agriculture and forestry. Soil Fertilizer and Water Resources Central Research Institute Publications, No.121/56. Volume 2, Ankara.264-271 pp.

Yücel H, Avci M, Cinar S, Aktaş & Kökasık F D (2011). Determining hay yield and hay quality characteristics of different alfalfa cultivars. IX. Turkey Field Crops Congress, 12-15 Sept, Bursa, pp.1883-1886.

Yüksel O (2012). The determination of proper forage grasses and their most suitable mixture rates for artificial pasture established with alfalfa (Medicago sativa L.). PhD Thesis, Süleyman Demirel University, Institute of Natural and Applied Sciences Department of Field Crops, Isparta, Turkey.

Journal of Agricultural Sciences (Tarim Bilimleri Dergisi) 2022, 28 (4) : 740 - 750 DOI: 10.15832/ankutbd.995878

Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

Transplanting and Seed Hydro-priming Affects Yield, Water Use Efficiency, and Grain Quality of Maize Cultivars under Delayed Planting

Amir GHALKHANI[a], Farzad PAKNEJAD[a]*, Ali MAHROKH[b], Mohammad Reza ARDAKANI[a], Farid GOLZARDI[b]

[a]*Department of Agronomy, Karaj Branch, Islamic Azad University, Karaj, IRAN*

[b]*Seed and Plant Improvement Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, IRAN*

ARTICLE INFO
Research Article
Corresponding Author: Farzad Paknejad, E-mail: farzadpaknejad@yahoo.com
Received: 18 September 2021 / Revised: 11 December 2021 / Accepted: 11 December 2021 / Online: 15 October 2022

Cite this article
GHALKHANI A, PAKNEJAD F, MAHROKH, ARKADANI R Z, GOLZARDI F (2022). Transplanting and Seed Hydro-priming Affects Yield, Water Use Efficiency, and Grain Quality of Maize Cultivars under Delayed Planting. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 28(4):740-750. DOI: 10.15832/ankutbd.995878

ABSTRACT
To evaluate the effect of planting method (PM), planting date (PD), and cultivar (CV) on the grain and herbage yield, irrigation water use efficiency for herbage (IWUEH) and grain (IWUEG), and grain quality of maize, a two-year field study was conducted at the Research Farm of Seed and Plant Improvement Institute, Karaj, Iran, during the 2017 and 2018 growing seasons. The experiment was arranged as split-plot factorial with three planting dates, including PD1 (July 1st), PD2 (July 11th), and PD3 (July 23rd) as main plots. Three planting methods, including direct seeding (DS), seed hydro-priming (HP), and transplanting (TP), were factorially combined with two maize cultivars (S.C.704 and S.C.260) as sub-plots. The highest herbage and grain yields (76685 and 7369 kg ha^{-1}, respectively) and the maximum IWUE for herbage and grain production (13.1 and 1.21 kg m^{-3}, respectively) were found in the TP. The CV S.C.704 obtained a higher grain yield (GY) on PD1 than that of S.C.260, but as planting was delayed, the GY of S.C.704 was affected more negatively in both years. Delay planting enhanced IWUEH, but not IWUEG. The highest IWUEG was obtained from TP on each PD. The CV S.C.260 in delay planting resulted in better IWUEG in both years. The highest and lowest contents of crude protein, starch, ash, crude fiber, and oil were obtained from hydro-primed and transplanted maize, respectively. In conclusion, the yield in the transplanting method had superiority over DS and HP. Also, HP was found to be the optimal PM to enhance the grain quality of maize in delayed planting. Furthermore, selecting S.C.260 as a high-yielding hybrid on delayed planting is suggested.

Keywords: Planting method, Planting date, *Zea mays* L, Crude protein, Semi-arid region

1. Introduction

Maize (*Zea mays* L.) is one of the main cultivated cereals in the world (FAO 2019). As the consumption of maize increases due to its vital role in human and animal diet, the countries' demand for grain maize has grown fast (Loy & Lundy 2019; Ünay et al. 2021). On the other hand, the production of crops such as maize declined remarkably in arid and semi-arid regions worldwide (Golzardi et al. 2017). The high percent of grain maize cultivated in the second cropping; nevertheless, water shortage and short growing season in the semi-arid regions are the main problems of farmers to produce high-yield maize (Khalily et al. 2010). The shortage of growing season in the second cropping causes the maize to be harvested too early with high moisture content, which reduces the maize grain yield (GY) and consequently causes economic losses for the farmers in storage conditions (Moradi et al. 2013). Indeed, delayed planting will expose the maize to different conditions of temperature, water availability, photoperiod, and solar radiation. Several studies reported that delayed planting of maize significantly reduced the GY and herbage yield (HY) as well as irrigation water use efficiency (IWUE) (Srivastava et al. 2018; Cao et al. 2019).

Germination is particularly important for determining the final plant density. Delayed cultivation of maize reduces its GY by postponing the germination and growth stages (Long et al. 2017). Choosing a suitable planting method (PM) that could lead to the early maturity of maize in delayed planting might result in lower maize yield losses. Grain maize producers require more information on how PM and planting date (PD) affect the yield of grain maize in the delayed sowing.

Transplanting (TP) is a planting strategy used for higher crop yield, especially when the condition is not suitable for direct seeding (DS). Fanadzo et al. (2009) reported that transplanting can be used as a planting method to compensate for yield loss in delayed planting of maize by improving its establishment and germination and reducing the growth period and flowering time. Furthermore, another planting method that can improve the yield of maize is seed hydro-priming (HP). This method is based on

controlled irrigation technology, and hydro-primed seeds go through the first (physical absorption of water) and second (initiation of biological processes and carbohydrate hydrolysis) stages of germination, though failing to pass the third stage (carbohydrate intake by the embryo and rootlet growth) (Nazari et al. 2017). Rehman et al. (2015) reported that hydro-priming as an important physiological method could accelerate and boost the strength of germination processes. Furthermore, previous studies revealed that transplanted and hydro-primed maize could use available water more efficiently (San Miguel-Chávez & Larqué Saavedra 1996; Rockström et al. 2007).

It was hypothesized that transplanting and hydro-priming increased maize grain yield and quality and IWUE compared to direct seeding in delayed planting. Recently, due to the increasing need for more grain maize production in arid and semi-arid regions, determining the optimum planting method and planting date to improve the IWUE, quantity, and quality of grain maize in delayed planting has received considerable critical attention. Although many studies have investigated the effect of transplanting and hydro-priming on grain and herbage yield, the impacts of these planting methods on IWUE and grain quality under delayed planting are not well documented. Furthermore, previous reports did not compare the transplanting and hydro-priming methods regarding their effects on grain maize yield, quality, and IWUE. Therefore, the present study was carried out to evaluate the impact of transplanting and hydro-priming on the IWUE and grain quality besides grain yield of early- and late-maturing cultivars of maize to determine the most suitable planting method in delayed planting for a semi-arid environment.

2. Material and Methods

This study was carried out at the Research Farm of Seed and Plant Improvement Institute, Karaj, Iran (35° 47' N, 50º 54' E, 1250 m. a.s.l.), during the 2017 and 2018 cropping seasons. The climate of this region is characterized as semi-arid, with a long-term average annual rainfall of 251 mm and annual evaporation of 2184 mm. The long-term average air temperature was 13.5 °C, and the average soil temperature was 14.5 °C in Karaj. The meteorological characteristics of the two years of the study sites are presented in Table 1. The physical and chemical characteristics of the soil of the study location are shown in Table 2.

Table 1- The monthly meteorological data of experimental sites during 2017 and 2018 growing seasons

Year	*Month*	*Mean soil temp (°C)*	*Max air temp (°C)*	*Min air temp (°C)*	*Mean air temp (°C)*	*Evaporation (mm)*	*Precipitation (mm)*	*Relative Humidity (%)*
2017	June	13.41	34.16	17.6	26.08	338.6	0.00	32.30
	July	16.65	36.41	20.29	28.88	367.8	0.41	29.54
	August	16.26	34.88	19.35	27.44	355.5	0.00	32.57
	September	12.53	31.59	15.92	23.72	267.6	0.00	34.37
	October	7.00	23.87	10.59	17.02	175.5	4.80	38.55
	November	2.10	17.26	6.148	11.53	89.3	0.64	43.48
2018	June	15.53	33.81	17.35	25.58	334.3	7.23	37.27
	July	21.16	38.87	23.31	31.91	471.8	0.00	22.06
	August	17.87	36.18	20.08	28.41	425.6	0.00	33.73
	September	13.70	31.56	16.79	23.99	266.1	0.81	34.9
	October	9.03	22.62	11.07	16.62	137.6	29.12	52.71
	November	3.80	13.24	5.45	9.06	70.3	65.91	73.17

Temp: temperature; Max: maximum; Min: minimum.

Table 2- Physicochemical properties of the soil (0–30 cm) at the experimental site during 2017 and 2018 growing seasons

Year	*Soil texture*	*Clay (%)*	*Silt (%)*	*Sand (%)*	*N (%)*	*P (mg kg^{-1})*	*K (mg kg^{-1})*	*OM (%)*	*EC (ds/m)*	*pH*
2017	Clay loam	28	49	23	0.06	12.0	256	0.55	2.21	7.22
2018	Clay loam	29	48	23	0.07	12.2	254	0.57	2.20	7.21

OM: organic matter; EC: electrical conductivity

A split-plot factorial design was set up based on a randomized complete block design with three replications. The planting date at three levels (July 1, 11, 23) was allocated to the main plots, and factorial of planting methods at three levels (HP, TP and DS) and cultivars (S.C.704, S.C.260) were allocated in the subplots. Each subplot consists of 3-rows spaced 75 cm apart and measuring 6 m long. Spaces between plants in the rows were 18 cm for S.C.704 (planting density of 7.5 plants m^{-2}) and 16 cm for S.C.260 (planting density of 8.3 plants m^{2}).

Seedbed prepared by plowing, disc, and leveling. Chemical fertilizers were applied based on soil analysis (Table 2) and the nutritional needs of maize. A total of 250 kg ha^{-1} di-ammonium phosphate (DAP) and 200 kg ha^{-1} urea were added to the soil before cultivation. In addition, when the plants reached the stage of 6-8 leaves, urea was applied as topdressing with a rate of 200 kg ha^{-1}.

Seeds for TP were sown in a tray with holes full of soil (60% soil, 20% animal manure, 20% sand). Crops were fertilized with a dose of fertilizer (20:20:20; N: P_2O_5: K_2O kg ha^{-1}) + humic acid, two times, first after the emergence of primary leaves (BBCH=12), then one week later. After attaining the age of three weeks (2-3 leaves stage), seedlings were uprooted manually and transplanted to the main field on the same day. The hydro-primed seeds were laid out in distilled water, and the surfaces of seeds were dried at optimum temperature and, finally, were planted in the PD according to experimental treatments (Rashid et al. 2006).

The irrigation system was drip irrigation. For determining irrigation water volume, sampled from the plot soil up to deep root development, and determined soil moisture percentage. Irrigation water volume was calculated by Penman-Monteith (Allen et al. 1998). The volume of water consumed in each PD is shown separately in Table 3. It should be noted that the amount of water used to produce seedlings has also been added to the amount of water used in the field. Also, for each treatment, the time interval between planting and harvesting date is reported in Table 4. The growing degree days (GDD) was calculated using the equation 1, where T_{max}, T_{min}, and T_{base} are the maximum temperature, minimum temperature, and 10 °C base temperature, respectively (McMaster & Wilhelm 1997):

$$GDD = \sum_{0}^{n} \left[\frac{T_{max} + T_{min}}{2} \right] - \boldsymbol{T_{base}} \quad (1)$$

Table 3- Total volume of irrigation water used (m^3 ha^{-1}) in each treatment during2017 and 2018 growing seasons

Planting method	*Cultivar*	*2017*			*2018*		
		July 1	*July 11*	*July 23*	*July 1*	*July 11*	*July 23*
Direct seeding	260	6459	5672	4826	6928	6012	5261
	704	6459	5672	4826	6928	6012	5261
Hydro-priming	260	6459.05	5672.05	4826.05	6928.05	6012.05	5261.05
	704	6459.05	5672.05	4826.05	6928.05	6012.05	5261.05
Transplanting	260	6480	5694	4850	6950	6035	5285
	704	6480	5694	4850	6950	6035	5285

Table 4- Growing degree days and days from planting to harvest in different treatments during 2017 and 2018 growing seasons

Year	*Planting method*	*Cultivar*	*July 1*		*July 11*		*July 23*	
			GDD	*DAP*	*GDD*	*DAP*	*GDD*	*DAP*
2017	Direct seeding	260	1407.8	106	1333.3	108	1234	104
		704	1534.5	122	1423.6	119	1246.9	106
	Hydro-priming	260	1448.6	113	1306.9	105	1234	104
		704	1511.6	120	1423.6	119	1246.9	106
	Transplanting	260	1245.3	86	1241	94	1249.3	91
		704	1448.6	113	1380.3	112	1246.9	106
2018	Direct seeding	260	1543.9	112	1457.8	118	1252.1	106
		704	1583.4	117	1457.8	118	1252.1	106
	Hydro-priming	260	1530.6	110	1457.8	118	1252.1	106
		704	1615.1	125	1457.8	118	1252.1	106
	Transplanting	260	1452	98	1396.2	104	1252.1	106
		704	1599.7	119	1457.8	118	1252.1	106

GDD: growing degree days; DAP: days after planting.

For yield determination, plants were harvested from an area of 3.75 m^2 in each plot. The herbage yield was measured at the dough stage, and grain yield was measured at the physiological maturity stage. The irrigation water use efficiency was calculated as yield divided by irrigation water (Golzardi et al. 2017). One kilogram of grain was randomly selected from every treatment to determine grain quality, then samples were milled, and crude fiber (CF), ash, water-soluble carbohydrates (WSC), crude protein (CP), starch, oil, and digestible dry matter (DDM) were measured by NIR (near infra-red) device (DICKEY-gun model) (Siesler et al. 2002; Baghdadi et al. 2017).

Homogeneity of experimental errors in two years was tested following Bartlett's test (Bartlett 1937), and data were imposed to combine analysis of variance. The statistical model was $Y_{ijkl}= \mu + R_l + A_i + E_{il} + B_j + (AB)_{ij} + C_k + (AC)_{ik} + (BC)_{jk} + (ABC)_{ijk} + E_{ijkl}$, where μ, R_l, A_i, E_{il}, B_j, C_k, and E_{ijkl} were the total mean, the effects of the block, planting date, the error of main factor (E_a),

planting methods, cultivar, and the error of sub-factors (E_b), respectively. Year and block were considered as random effects, whereas the planting date, planting methods, and cultivar were deemed to be fixed effects. Statistical analysis was performed using SAS software version 9.4, and the means were compared by Duncan's multiple range test ($P<0.05$).

3. Results and Discussion

3.1. Herbage yield

The results indicated that HY was influenced by PD, and shortening the growing season could significantly reduce it (Table 5). The highest HY was recorded on PD1 (74407 kg ha^{-1}), and the lowest HY was recorded on PD3 (67261 kg ha^{-1}) (Table 6). Delayed planting from PD1 to PD2 and PD3 decreased the available GDD by about 174.5 and 364.1, respectively (Table 4), and consequently reduced the HY by 5% and 10% (Table 6).

Table 5- *P*-Values obtained from ANOVA for the effect of planting date (July 1, July 11, and July 23), planting method (hydro-priming, transplanting, and direct seeding) and cultivar (S.C. 704 and S.C.260) on yield and irrigation water use efficiency of maize

Source of variation	*df*	*Herbage yield*	*IWUE$_H$*	*Grain yield*	*IWUE$_G$*
Year (Y)	1	0.1643	0.3335	0.6986	0.9097
Rep (Year)	4	0.4096	0.4307	0.7571	0.7968
Planting Date (PD)	2	0.0277	0.027	0.0077	0.0084
Y×PD	2	0.7243	0.9944	0.4503	0.5692
Rep (Y×PD)	8	0.7252	0.6085	0.1532	0.2320
Planting Method (PM)	2	0.0278	0.0427	0.0378	0.0420
PD×PM	4	0.1195	0.0628	0.0366	0.0466
Y×PM	2	0.2603	0.2451	0.5472	0.5226
Y×PD×PM	4	0.2678	0.2915	0.6745	0.6283
Cultivar (CV)	1	0.0534	0.0767	0.4900	0.3335
Y×CV	1	0.4004	0.2525	0.5246	0.5772
PD×CV	2	0.2297	0.8420	0.1302	0.1298
Y×PD×CV	2	0.2610	0.1976	0.0441	0.0348
PM×CV	2	0.0015	0.0023	0.2503	0.2086
Y×PM×CV	2	0.9603	0.9338	0.2295	0.2434
PD×PM×CV	4	0.1538	0.0862	0.1911	0.1584
Y×PD×PM×CV	4	0.9923	0.992	0.4438	0.483

df: degree of freedom; IWUE$_H$: irrigation water use efficiency for herbage production; IWUE$_G$: irrigation water use efficiency for grain production

Table 6- The effect of year, planting date, planting method and cultivar on yield, irrigation water use efficiency

Experimental Factors		*Herbage yield*	*Grain yield*	*IWUE$_H$*	*IWUE$_G$*
		(kg ha^{-1})		*(kg m^{-3})*	
Year	**2017**	69505a	6775a	12.4a	1.16a
	2018	72318a	6565a	12.0a	1.04a
Planting Date	**July 01**	74407a	8890a	11.1b	1.32a
	July 11	71067a	8004a	12.2b	1.37a
	July 23	67261b	3115b	13.3a	0.62b
Planting Method	**DS**	66741b	6189b	11.4b	1.02b
	HP	69308b	6451b	11.9b	1.07b
	TP	76685a	7369a	13.1a	1.21a
Cultivar	**S.C.260**	65481a	6786a	11.2a	1.13a
	S.C.704	76342a	6553a	13.1a	1.07a

DS: direct seeding; HP: hydro-priming; TP: transplanting; IWUE$_H$: irrigation water use efficiency for herbage production; IWUE$_G$: irrigation water use efficiency for grain production. Means within each column followed by the same lowercase letter(s) are not significantly different ($P\leq0.05$) according to Duncan's multiple range test

Plant growth rate depends on light absorption and the efficiency of converting this light into dry matter. The increase in the HY on PD1 can be explained by plants' more efficient use of sunlight at the beginning of the planting season (Aziz et al. 2007).

On the other hand, the delayed cultivation of maize reduces its photosynthetic activities due to its sensitivity to lower temperatures. These results are consistent with those of other studies and suggest that HY was affected by photoperiod, and delayed cultivation resulted in shorter plants with fewer and smaller leaves through shortening of photoperiod (Srivastava et al. 2018; Cao et al. 2019).

The herbage yield was significantly affected by the planting method and cultivar (Table 5). The Highest and lowest herbage yield was produced from TP of S.C.704 (80490 kg ha^{-1}) and DS of S.C.260 (60582 kg ha^{-1}), respectively (Figure 1a). In line with our findings, Andonova et al. (2014) observed that transplanted plants increased maize biomass accumulation compared to the direct-seeded ones. The mechanisms associated with increased yield in transplanted plants are improving light interception, leaf area development, and changes in anatomical features such as the phloem to xylem ratio and the vascular bundle to mesophyll ratio related to TP (Aziz et al. 2007).

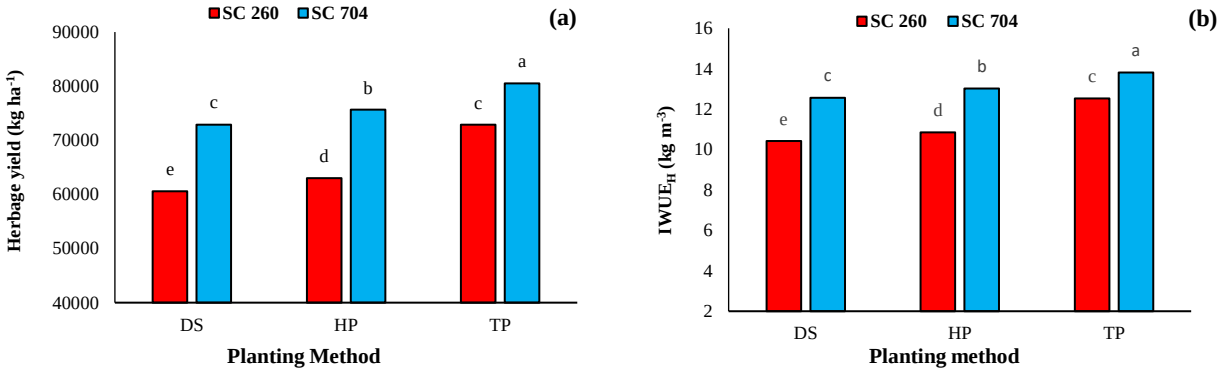


Figure 1- The effect of planting method × cultivar on the herbage yield (a), and irrigation water use efficiency for herbage production (b) DS, direct seeding; HP, seed hydro-priming; TP, transplanting; $IWUE_H$, irrigation water use efficiency for herbage production.

On the other hand, based on our results, the S.C.704 as a late-maturing cultivar showed greater herbage yield than the S.C.260 as an early-maturing cultivar in all planting methods (Figure 1a). These results corroborate with other authors, who mention that the herbage yield is influenced by genotype. In comparing cultivars, the herbage yield of late-maturing significantly was higher than early-maturing ones. Late maturing cultivars took more days to maturity and hence had a better chance to utilize more nutrients and more photosynthetic activity, resulting in higher herbage yield (Aziz et al. 2007; Hassan et al. 2020a).

3.2. Irrigation water use efficiency for herbage production

Water deficit is one of the main limiting factors in maize production in different regions of the world. Due to declining irrigation sources, improvement of IWUE of crops becomes more essential nowadays. IWUE depends on several factors, including genotype, planting time, and method of planting.

The $IWUE_H$ was significantly affected by planting date, as shown in Table 5. Based on the result of this study, the highest and lowest $IWUE_H$ (13.3 and 11.1 kg m^{-3}) were recorded on PD3 and PD1, respectively (Table 6). Delayed planting significantly improved $IWUE_H$ by 16.5% on PD3 compared to PD1 (Table 6). Increased $IWUE_H$ on PD3 can be explained by temperature decreases, therefore, reduces evaporation in delayed cultivation. The present findings seem consistent with other research, which found a significant increase in $IWUE_H$ in the delayed planting of maize (Feyzbakhsh et al. 2015).

$IWUE_H$ was also remarkably influenced by the planting method and cultivar interaction, and the highest and lowest $IWUE_H$ (13.806 and 10.415 kg m^{-3}) was obtained from transplanting S.C.704 and direct seeding of S.C.260 (Figure 1b). Compared with S.C.260, the late-maturing variety (S.C.704) significantly extended the maize growing cycle, increasing the HY and the $IWUE_H$ in all three PMs (Bu et al. 2015). Since various cultivars of crops have different IWUE, recognizing and cultivation cultivars with higher IWUE could improve the water productivity per unit area. In line with our finding, Rafiee & Kalhor (2016) reported that the late-maturing hybrids such as S.C.704 had a higher IWUE than mid-maturing hybrids. On the other hand, the transplanted maize used available water most efficiently. In previous research, TP of maize increased the IWUE by 66% or more over the DS (San Miguel-Chávez & Larqué Saavedra 1996). Overall, choosing appropriate maize hybrids and a suitable planting method will contribute to the effective use of agricultural water resources.

3.3. Grain yield

The GY was significantly affected by the interaction of planting date and planting method (Table 5). Results revealed that the maximum GY (9655 kg ha^{-1}) was obtained from TP on PD1 and the minimum GY (2646 kg ha^{-1}) obtained from DS on PD3 (Figure 2a). According to the findings, delayed planting using any of the three PMs (DS, HP, and TP) significantly reduced the

GY (Figure 2a). The current experiment results also presented the superiority of TP over HP and DS to achieve higher GY. The TP obtained more GY than HP by 11.3%, 12.5% and 16.5% on PD1, PD2 and PD3, respectively (Figure 2a). Comparison of HP and DS on PD3 indicated that hydro-priming with 18.8% more grain yield was a better planting method than direct seeding.

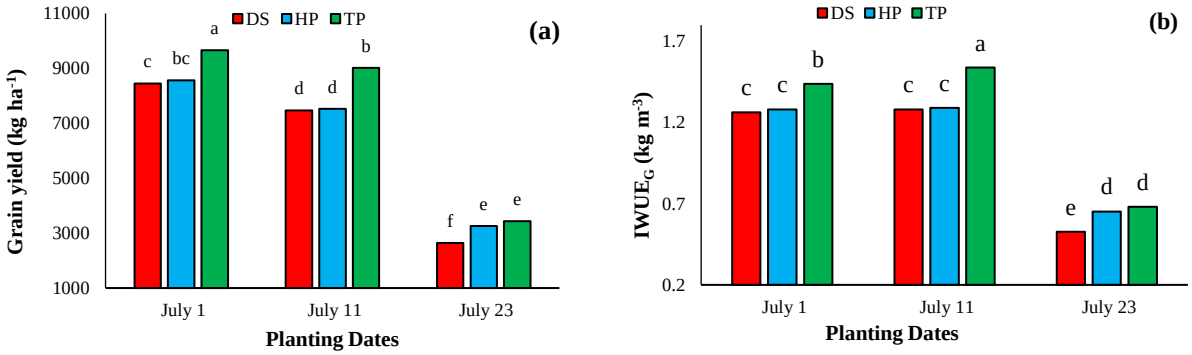


Figure 2- Effect of planting date × planting method on the grain yield (a), and IWUEG (b) DS, direct seedling; HP, seed hydro-priming; TP, transplanting; $IWUE_G$, irrigation water use efficiency for grain production.

Our results align with previous studies, which also reported that delayed cultivation had reduced the grain yield (Feyzbakhsh et al. 2015; Cao et al. 2019). The decrease in grain yield of delayed planting crops could be attributed to lower nutrient uptake and reduced photosynthetic translocation in the developing seeds. Srivastava et al. (2018) concluded that delayed planting reduced overall growth stage duration from 143 days in regular cultivation to 127 days in delayed cultivation, resulting in lower grain yield.

The data revealed that transplanting maize as a strategy for crop management increased grain yield by shortening the growth period. These results match those observed in the earlier study, in which the growth period of maize had cultivated using TP was shorter than DS in a way that it reached flowering 11-15 days earlier (Fanadzo et al. 2009). Despite the higher GY of TP than HP on PD3, this difference was not significant. During manual uprooting of seedlings to the main field on PD3, breaking some of the seminal roots had coincided with high temperature, and transplanting shock got worse. This might result in the inability of maize roots to regenerate after TP and GY reduction (Andonova et al. 2014).

Moreover, GY is highly affected by the interaction of Year×PD×CV (Table 5). The highest GY in 2017 and 2018 (9193 kg ha^{-1} and 9609 kg ha^{-1}, respectively) was obtained from S.C.704 on PD1. Further, the lowest GY in 2017 (2604 kg ha^{-1}) and 2018 (2104 kg ha^{-1}) were achieved from S.C.704 on PD3 (Figure 3a). As planting was delayed from 1 to 11 July, the GDD decreased by 169.4 and 180.8 in 2017 and 2018, respectively. This GDD reduction decreased the grain yield of S.C.704 by about 8% and 24% in two consecutive years; however, the grain yield of S.C.260 wasn't affected (Figure 3a, Table 4). In addition, we found a 70% (2017) and 71% (2018) reduction in GY of S.C.704 when planting was delayed from 1 to 23 July. However, the GY of S.C.260 was reduced by 48% (2017) and 60% (2018) when planting was delayed 3 weeks in two consecutive years. It means that two hybrids responded differently to delayed planting and available GDD since the GDD on PD3 was about 345.2 and 384.7 fewer than 1 July in 2017 and 2018, respectively (Figure 3a, Table 4).

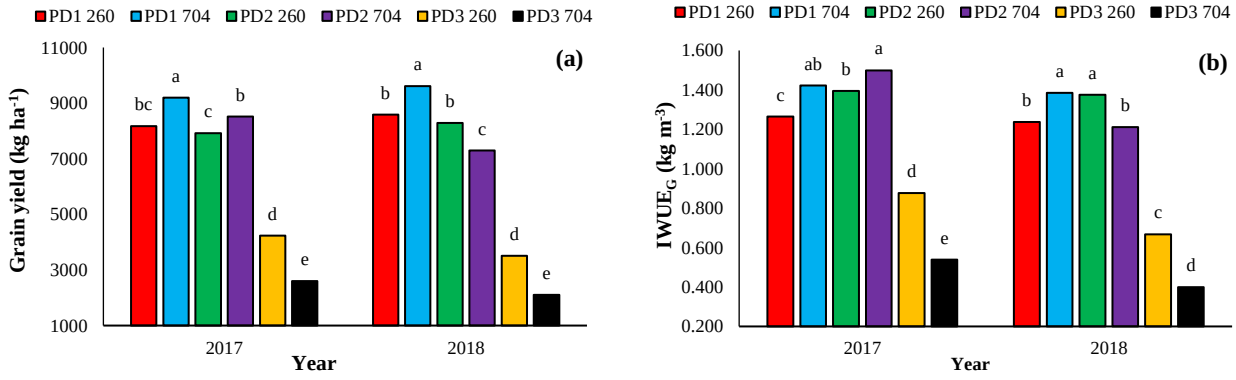


Figure 3- Effect of year × planting date × cultivar on the grain yield (a), and $IWUE_G$ (b) sliced on year
PD1, July 1; PD2, July 11; PD3, July 23; 260, cultivar S.C.260; 704, cultivar S.C.704; $IWUE_G$, irrigation water use efficiency for grain production.

Each maize cultivar has its optimal planting date; according to the results, S.C.704 obtained significantly higher GY on PD1 than S.C.260 in both years. The late-maturing varieties of maize resulted in higher GY if cultivated on optimized PD since these

varieties genetically require more time for the grain-filling period and could obtain more water resources for plant growth and grain production (Rafiee & Kalhor 2016). However, as planting was delayed, the GY of S.C.704 affected more negatively than S.C.260 in both years since S.C.260 (early-maturing) obtained required GDD in a shorter duration compared to S.C.704 (late-maturing). Moreover, despite late-maturing cultivars' potential to produce high yields when planted on a suitable date, delayed planting causes them to enter into the flowering phase earlier due to their low growth rate (Hassan et al. 2020b). We found that S.C.260 is superior in GY to S.C.704 in delayed cultivation. In line with our findings, Biswas (2015) and Koca and Canavar (2014) reported that various cultivars of maize in different PD had different amounts of GY.

Furthermore, there was a significant difference in GY of S.C.704 on PD2 (11 July) between two years, which was likely related to the 10% drop in temperature at the end of the growing season in 2018 compared to 2017 (Table 1), which coincided with the grain maturity and grain-filling period of S.C.704 and subsequently reduced GY on PD2 in 2018 (Figure 3a).

3.4. Irrigation water use efficiency for grain production

The effects of PD×PM on $IWUE_G$ were significant (Table 5). The highest $IWUE_G$ (1.53 kg m^{-3}) was recorded from TP on PD2, and the lowest $IWUE_G$ (0.52 kg m^{-3}) was obtained from DS on PD3 (Figure 2b). These results also revealed that delayed cultivation from PD2 to PD3 caused reductions of $IWUE_G$ 60%, 50%, and 56%, respectively, in DS, HP, and TP. Since there is a direct association between GY and $IWUE_G$, decreasing the GY in delayed planting led to a remarkable reduction of $IWUE_G$ on PD3 in all three planting methods.

On the other hand, TP in all three PD could obtain the highest $IWUE_G$. The superiority of TP to achieve higher WUE in this study was mainly due to the shorter growth stage period, about 10-12 days, compared to HP and DS, which decreased the requirement for irrigation at least 700 m^3 in this study. In line with the present study, investigating the effects of TP on cotton and maize showed that TP could lead to an increase in IWUE (Biswas 2015).

Examining the effects of Year×PD×CV interactions on $IWUE_G$ showed that the highest $IWUE_G$ (1.49 kg m^{-3}) was obtained from cultivating S.C.704 on PD2 in 2017, and the lowest $IWUE_G$ (0.39 kg m^{-3}) was obtained from S.C.704 on PD3 in 2018 (Figure 3b). $IWUE_G$ of cultivars in all planting dates decreased in 2018 compared to 2017 since the higher temperature in 2018 increased evapotranspiration (Figure 3b). Delay in planting to 23 July in both years significantly decreased $IWUE_G$ of S.C.704 and S.C.260. The reduction of $IWUE_G$ under delayed planting was due to a higher reduction in maize GY than the consumptive water use (Bu et al. 2015).

On the other hand, $IWUE_G$ of S.C.260 significantly increased about 10% as cultivation was delayed from 1 to 11 July in both years (Figure 3b). A possible explanation for these results may be the insignificant differences in GY of S.C.260 when planting was delayed for 10 days (Figure 3a), besides less evapotranspiration occurred on PD2 rather than PD1, which led to enhancement of $IWUE_G$. In comparing hybrids, S.C.260 in delayed planting dates resulted in better $IWUE_G$ in both years. This result agrees with the findings of other studies, which reported that the WUE of maize depends on multiple factors, such as genotype. The shorter growing period in early-maturing cultivars like S.C.260 than late-maturing ones may lead to lower evapotranspiration, which increased $IWUE_G$ (Rockström et al. 2007). Overall, the appropriate CV selection is essential to improve $IWUE_G$ in delayed planting.

3.5. Grain quality

Maize is an important crop, and concerns about grain quality are increasingly important. According to the findings of the present study, grain quality parameters were significantly affected by sowing date (Table 7). As shown in Table 8, delayed planting negatively affects qualitative traits of grain maize such as CP, DDM, starch, and WSC. The highest CP, DDM, starch, WSC were obtained on PD1, and the lowest contents were obtained on PD3. Delaying cultivation from PD1 to PD3 decreased CP, DDM, starch and WSC by 11.5%, 11%, 8% and 13%, respectively (Table 8).

Table 7- *P*-Values obtained from ANOVA for the effect of planting date (July 1, July 11, and July 23), planting method (hydro-priming, transplanting, and direct seeding) and cultivar (late-maturing S.C. 704 and early-maturing S.C.260) on grain quality of maize

Source of variation	*df*	*CP*	*DDM*	*Starch*	*Ash*	*CF*	*WSC*	*Oil*
Year (Y)	1	0.8953	0.5056	0.324	0.6855	0.6412	0.7718	0.8994
Rep(Year)	4	0.0220	0.0960	0.2172	0.3475	0.7471	0.8101	0.1849
Planting Date (PD)	2	0.0500	0.0311	0.0153	0.0006	0.0290	0.0444	0.1114
Y×PD	2	0.8999	0.5870	0.4223	0.9155	0.1683	0.7395	0.2345
Rep(Y×PD)	8	0.6368	0.8559	0.6711	0.1098	0.2984	0.2452	0.2597
Planting Method (PM)	2	0.0405	0.8900	0.0227	0.0281	0.0046	0.8813	0.0500
PD×PM	4	0.0879	0.2762	0.7511	0.0243	0.0184	0.6477	0.2102
Y×PM	2	0.7287	0.8196	0.7708	0.5495	0.9783	0.1379	0.646
Y×PD×PM	4	0.5261	0.5358	0.1769	0.0618	0.9140	0.1066	0.5342
Cultivar (CV)	1	0.1411	0.1958	0.9028	0.0277	0.0891	0.7247	0.2966
Y×CV	1	0.2324	0.9485	0.1961	0.7083	0.7744	0.5981	0.5766
PD×CV	2	0.5780	0.3245	0.4409	0.0013	0.0068	0.1678	0.1894
Y×PD×CV	2	0.2323	0.3706	0.1434	0.6866	0.9136	0.2575	0.5014
PM×CV	2	0.4090	0.2284	0.9036	0.0883	0.7547	0.1628	0.3779
Y×PM×CV	2	0.1270	0.7774	0.5438	0.1963	0.2572	0.9536	0.5627
PD×PM×CV	4	0.4921	0.3587	0.5389	0.0134	0.7898	0.218	0.6734
Y×PD×PM×CV	4	0.9647	0.2119	0.4745	0.9980	0.7710	0.8939	0.6040

df: degree of freedom; CP: crude protein; DDM: digestible dry matter; CF: crude fiber; WSC: water-soluble carbohydrate

Table 8- The effect of year, planting date, planting method and cultivar on grain quality of maize

Experimental Factors		*CP*	*DDM*	*Starch*	*Ash*	*CF*	*WSC*	*Oil*
		%						
Year	**2017**	8.87a	82.08a	68.5a	1.40a	2.92a	3.59a	3.71a
	2018	10.51a	80.81a	69.7a	1.38a	2.90a	3.65a	3.70a
Planting Date	**July01**	9.86a	86.5a	71.5a	1.63a	3.23a	3.90a	3.89a
	July11	9.26ab	80.6b	70.0a	1.60b	3.11a	3.58ab	3.69a
	July23	8.72b	77.3b	65.9b	1.34c	2.77b	3.40b	3.53a
Planting Method	**DS**	9.30ab	81.6a	69.5a	1.51b	3.04b	3.57a	3.74a
	HP	9.48a	81.7a	69.6a	1.56a	3.09a	3.68a	3.75a
	TP	9.06b	80.9a	68.1b	1.51b	2.99c	3.62a	3.61b
Cultivar	**S.C.260**	8.95a	81.3a	69.2a	1.50b	3.00a	3.61a	3.74a
	S.C.704	9.61a	81.6a	69.1a	1.55a	3.08a	3.63a	3.67a

DS, direct seeding; HP, hydro-priming; TP, transplanting; CP, crude protein; DDM, digestible dry matter; CF, crude fiber; WSC, water-soluble carbohydrate. Means within each column followed by the same lowercase letter(s) are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Maize grains mainly consist of endosperm and embryo, containing protein, starch, oil, and other grain quality parameters (Motto et al. 2012). As we explained later, delayed planting could disrupt maize grain growth and development, reducing grain quality. In accordance with the present results, previous studies have demonstrated that delayed sowing of maize negatively affects the grain quality since there is a high probability of experiencing lower temperature during the grain-filling period and reduction of rate and duration of grain filling in delayed cultivation (Koca and Canavar. 2014; Cao et al. 2019). Grain nutritional contents reduction in delayed sowing supports the idea of down-regulation of enzymatic activities in delayed planting dates, which results in lower assimilate convention and grain starch, oil, and protein reduction.

Based on our findings, the effect of the planting method on some qualitative traits such as CP, starch, and oil was significant. The hydro-priming method had the highest CP, starch, and oil (9.48, 69.6, and 3.75%, respectively). The lowest contents of CP, starch, and oil were obtained from transplanted maize (Table 8). According to the results, HP was found to be the optimal PM to enhance grain quality. Furthermore, assessing the interactions of PD×PM on the CF (Table 7) indicated that the highest CF (3.27%) was obtained from HP on 1 July and the lowest CF (2.65%) obtained from TP on 23 July (Figure 4a). Delayed planting

from PD1 to PD2 decreased CF by 2.8%, 3.4%, and 4.9% in DS, HP, TP, respectively. Likewise, CF content was reduced when planting delayed from PD1 to PD3 in DS (11.6%), HP (13.1%), and TP (18.2%). As shown in Figure 4a, CF obtained from TP is more negatively affected by delayed sowing than the other two planting methods. As mentioned in the previous literature review, the maize grain quality could be remarkably affected by planting method and date (Mason & D'croz-Mason 2002). Seed priming of maize could affect grain quality through various physiological strategies and improve grain quality under different PD. Indeed, seed priming triggers a range of biochemical reactions and enhances photoassimilates translocation (Bakhtavar et al. 2015).

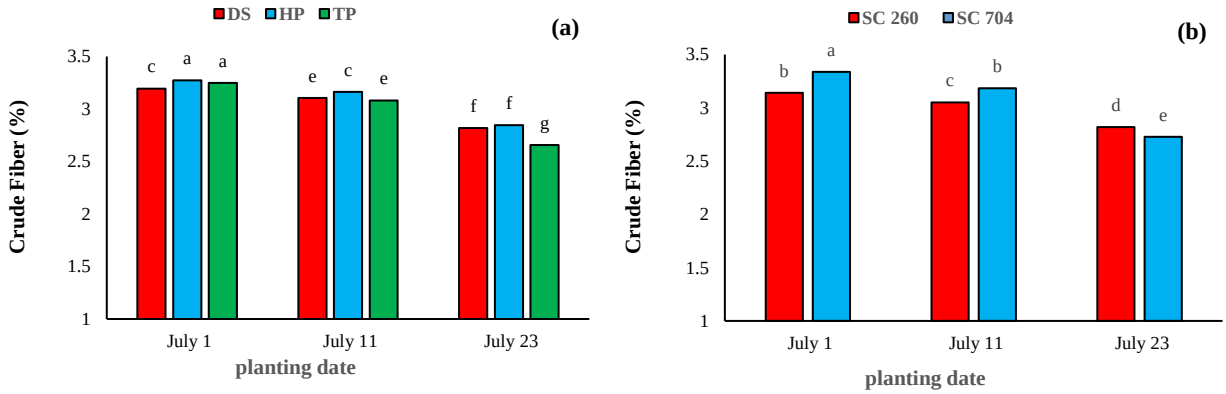


Figure 4- Effect of planting date × planting method (a), and planting date × cultivar (b) on crude fiber content
DS, direct seedling; HP, seed hydro-priming; TP, transplanting; 260, S.C.260; 704, S.C.704.

The CF is one of the main parameters influencing the quality of maize. Based on the findings, the interactions of PD×CV on CF were significant (Table 7). The highest and lowest CF were obtained from S.C.704 on PD1 and PD3, respectively. Our findings revealed that CF of S.C.704 and S.C.260 decreased by 18.3% and 10.2% in delayed sowing from 1 to 23 July, respectively. Variation in grain quality parameters among different hybrids in delayed planting is in line with Buriro et al. (2015), who reported that grain quality parameters such as protein, starch, and oil content were significantly affected by genetic differences among maize cultivars and different sowing dates.

In addition, the interaction of PD×PM×CV on ash was significant (Table 7). In this study, transplanted S.C.704 on PD1 had maximum ash content (1.71%) whereas, from transplanting variety S.C.704 on PD3 minimum ash (1.26%) was obtained (Figure 5). Comparing three PD revealed that the lowest ash content of all CV and PM was achieved on PD3. The highest ash content on PD3 (1.42%), obtained from hydro-priming S.C.260.

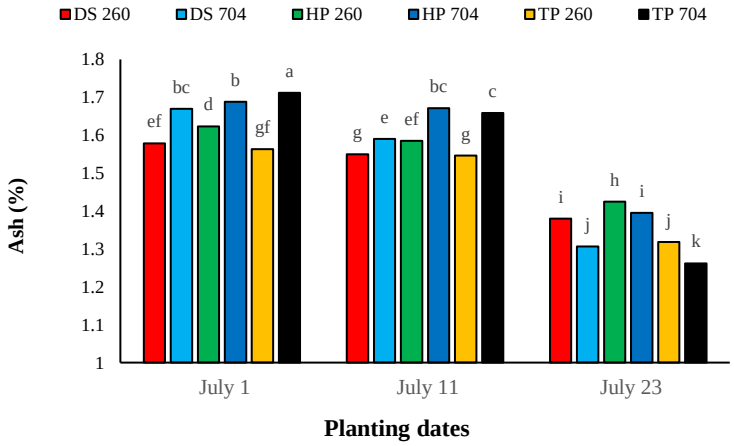


Figure 5- Effect of planting date × planting method × cultivar on ash content DS, direct seedling; HP, seed hydro-priming; TP, transplanting; 260, S.C..260; 704, S.C.704

4. Conclusions

To our knowledge, this is the first report of the effect of transplanting and hydro-priming on grain yield, herbage yield, irrigation water use efficiency, and grain quality of late- and early-maturing maize cultivars under delayed planting. It was concluded from the findings of the present research that all quantity and quality traits of grain maize except $IWHE_H$ remarkably decreased in delayed planting. Most agronomic and qualitative characteristics were significantly affected by the planting date, planting method, and cultivar interactions. Our data revealed the superiority of transplanting to achieve high grain and herbage yield among other planting methods. On the other hand, hydro-priming was the optimal planting method to enhance grain quality in delayed planting. Furthermore, selecting S.C.260 as a suitable hybrid on delayed planting is suggested. Choosing an appropriate planting method and cultivar could help farmers achieve a higher maize GY, HY, IWUE in delayed planting. Further investigations are necessary to validate the kinds of conclusions that can be drawn from this study.

Acknowledgments

This research was a part of PhD thesis of the first author and funded and supported by Seed and Plant Improvement Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran. (Project No: 0-03-03-012- 960188).

Abbreviations

PD, planting date; PD1: July 1st; PD2: July 11th; PD3: July 23rd; PM, planting method; TP, transplanting; HP, hydro-priming; DS, direct seeding; CV, cultivar; GY, grain yield; HY, herbage yield, IWUE, irrigation water use efficiency; $IWUE_G$, IWUE for grain production; $IWUE_H$, IWUE for herbage production; CP, crude protein; CF, crude fiber; DDM, digestible dry matter; WSC: water-soluble carbohydrate.

References

Allen R G, Pereira L P, Raes D & Smith M (1998). Crop evapotranspiration-Guidelines for computing crop water requirements- FAO Irrigation and drainage paper 56. FAO, Rome 300(9): D05109

Andonova P S, Rattin J & Di Benedetto A (2014). Yield increase as influenced by transplanting of sweet maize (*Zea mays* L. saccharata). *Journal of Experimental Agriculture International* 4(11): 1314-1329 DOI: 10.9734/AJEA/2014/11077

Aziz A, Rehman H U & Khan N (2007). Maize cultivar response to population density and planting date for grain and biomass yield. *Sarhad Journal of Agriculture* 23(1): 25-30

Baghdadi A, Balazadeh M, Kashani A, Golzardi F, Gholamhoseini M & Mehrnia M (2017). Effect of pre-sowing and nitrogen application on forage quality of silage corn. *Agronomy Research* 15(1): 11-23

Bakhtavar M A, Afzal I, Basra S M, Ahmad A U & Noor M A (2015). Physiological strategies to improve the performance of spring maize (*Zea mays* L.) planted under early and optimum sowing conditions. *PloS one* 10(4): e0124441 DOI: 10.1371/journal.pone.0124441

Bartlett M S (1937). Properties of sufficiency and statistical tests. *Proceedings of the Royal Society of London. Series A-Mathematical and Physical Sciences* 160(901): 268-282 DOI: 10.1098/rspa.1937.0109

Biswas M (2015). Direct seeded and transplanted maize: Effects of planting date and age of seedling on the yield and yield attributes. *Journal of Experimental Agriculture International* 5(5): 489-497 DOI: 10.9734/AJEA/2015/13594

Bu L, Chen X, Li S, Liu J, Zhu L, Luo S, Hill RL & Zhao Y (2015). The effect of adapting cultivars on the water use efficiency of dryland maize (*Zea mays* L.) in northwestern China. *Agricultural Water Management* 148: 1-9 DOI: 10.1016/j.agwat.2014.09.010

Buriro M, Bhutto T, Gandahi A, Kumbhar I & Shar M (2015). Effect of sowing dates on growth, yield and grain quality of hybrid maize. *Journal of Basic and Applied Sciences* 11: 553-558 DOI: 10.6000/1927-5129.2015.11.73

Cao Q, Li G, Yang F, Jiang X, Diallo L, Zhang E & Kong F (2019). Maize yield, biomass and grain quality traits responses to delayed sowing date and genotypes in rain-fed condition. *Emirates Journal of Food and Agriculture* 27: 415-425 DOI: 10.9755/ejfa.2019.v31.i6.1969

Fanadzo M, Chiduza C & Mnkeni P N S (2009). Comparative response of direct seeded and transplanted maize (*Zea mays* L.) to nitrogen fertilization at Zanyokwe irrigation scheme, Eastern Cape, South Africa. *African Journal of Agricultural Research* 4(8): 689-694 DOI: 10.5897/AJAR.9000116

FAO (2019). Crops and livestock products. Retrived in Junuary, 1, 2021 from http://www.fao.org/faostat/en/#data/TP

Feyzbakhsh M T, Kamkar B, Mokhtarpour H & Asadi M E (2015). Effect of soil water management and different sowing dates on maize yield and water use efficiency under drip irrigation system. *Archives of Agronomy and Soil Science* 61(11): 1581-1592 DOI: 10.1080/03650340.2015.1019345

Golzardi F, Baghdadi A & Afshar R K (2017). Alternate furrow irrigation affects yield and water-use efficiency of maize under deficit irrigation. Crop and Pasture Science 68(8): 726-734

Hassan M U, Chattha M U, Barbanti L, Mahmood A, Chattha M B, Khan I, Mirza S, Aziz S A, Nawaz M & Aamer M (2020a). Cultivar and seeding time role in sorghum to optimize biomass and methane yield under warm dry climate. *Industrial Crops and Products* 145: 111983 DOI: 10.1016/j.indcrop.2019.111983

Hassan M U, Chattha M U, Khan I, Chattha M B, Barbanti L, Aamer M, Iqbal M M, Nawaz M, Mahmood A, Ali A & Aslam M T (2020b). Heat stress in cultivated plants: nature, impact, mechanisms, and mitigation strategies-a review. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology* 155(2): 211-234 DOI: 10.1080/11263504.2020.1727987

Khalily M, Moghaddam M, Kanouni H & Asheri E (2010). Dissection of drought stress as a grain production constraint of maize in Iran. Asian Journal of Crop Science 2(2): 60-69 DOI: 10.3923/ajcs.2010.60.69

Koca Y & Canavar O (2014). The effect of sowing date on yield and yield components and seed quality of corn (*Zea mays* L.). *Sci Papers Series A Agron* 57: 227-223

Long N V, Assefa Y, Schwalbert R, Ciampitti I A (2017). Maize yield and planting date relationship: A synthesis-analysis for US high-yielding contest-winner and field research data. Frontiers in Plant Science 8: 2106 DOI: 10.3389/fpls.2017.02106

Loy D D & Lundy E L (2019). Nutritional properties and feeding value of corn and its coproducts. In: *Corn (Elsevier)* AACC International Press, pp. 633-659 DOI: 10.1016/B978-0-12-811971-6.00023-1.

Mason S C & D'croz-Mason N E (2002). Agronomic practices influence maize grain quality. *Journal of Crop Production* 5(1-2): 75-91 DOI: 10.1300/J144v05n01_04

McMaster G S & Wilhelm W W (1997). Growing degree-days: one equation, two interpretations. *Agric. Forest Meteorol* 87: 291–300 DOI: 10.1016/ s0168-1923(97)00027-0

Moradi R, Koocheki A, Mahallati M N & Mansoori H (2013). Adaptation strategies for maize cultivation under climate change in Iran: irrigation and planting date management. *Mitigation and Adaptation Strategies for Global Change* 18(2): 265 284 DOI: 10.1007/s11027-012-9410-6.

Motto M, Hartings H, Fracassetti M & Consonni G (2012). Grain quality-related traits in maize: gene identification and exploitation, *Maydica* 56(3): 291-314.

Nazari S, Aboutalebian M A & Golzardi F (2017). Seed priming improves seedling emergence time, root characteristics and yield of canola in the conditions of late sowing. *Agronomy Research* 15(2): 501-514

Rafiee M & Kalhor M (2016). Economic water use efficiency of corn (*Zea mays* L.) hybrids as affected by irrigation regimes: a case study in West Iran. *Archives of Agronomy and Soil Science* 62(6), 781-789 DOI: 10.1080/03650340.2015.1105360

Rashid A, Hollington P A, Harris D & Khan P (2006). On-farm seed priming for barley on normal, saline and saline–sodic soils in North West Frontier Province, Pakistan. *European journal of agronomy* 24(3): 276-281 DOI: 10.1016/j.eja.2005.10.006

Rockström J, Lannerstad M & Falkenmark M (2007). Assessing the water challenge of a new green revolution in developing countries. Proceedings of the National Academy of Sciences 104(15): 6253-6260 DOI: 10.1073/pnas.0605739104

San Miguel-Chávez R & Larqué Saavedra A L (1996). Increased water use efficiency and grain yield by transplanting maize, *Drought-and Low N-Tolerant Maize* 25: 179

Siesler H W, Ozaki Y, Kawata S & Heise H M (Eds.) (2002). Near-infrared spectroscopy: principles, instruments, applications. *John Wiley & Sons*, Germany

Srivastava R K, Panda R K, Chakraborty A & Halder D (2018). Enhancing grain yield, biomass and nitrogen use efficiency of maize by varying sowing dates and nitrogen rate under rainfed and irrigated conditions. *Field Crops Research* 221: 339-49 DOI: 10.1016/j.fcr.2017.06.019

Ünay A, Sabanci I, Cinar V M (2021). The Effect of Maize (Zea mays L.)/Soybean (Glycine max (L.) Merr.) Intercropping and Biofertilizer (Azotobacter) on Yield, Leaf Area Index and Land Equivalent Ratio. *Journal of Agricultural Sciences* 27(1): 76-82 DOI: 10.15832/ankutbd.572495

Rehman H, Iqbal H, Basra S M, Afzal I, Farooq M, Wakeel A & Ning W A (2015). Seed priming improves early seedling vigor, growth and productivity of spring maize. *Journal of Integrative Agriculture* 14(9): 1745-1754 DOI: 10.1016/S2095-3119(14)61000-5

Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

Association of Satellite RNA with *Grapevine fanleaf virus* in its Geographical Origin and Sequence Characteristics

Afsaneh DELPASAND KHABBAZI[a*], Nemat SOKHANDAN BASHIR[a], Shaheen NOURINEJHAD ZARGHANI[b]

[a]*Department of Plant Protection, Faculty of Agriculture, The University of Tabriz, Tabriz 51666, IRAN*
[b]*Department of Entomology and Plant Pathology, College of Abouraihan, The University of Tehran, Tehran 14174, IRAN*

ARTICLE INFO
Research Article
Corresponding Author: Afsaneh DELPASAND KHABBAZI, E-mail: afsaneh_delpasand@yahoo.com
Received: 04 April 2018 / Revised: 06 January 2020 / Accepted: 10 January 2020 / Online: 15 October 2022

Cite this article
DELPASAND KHABBAZI A, SOKHANDAN BASHIR N, NOURINEJHAD ZARGHANI S (2022). Association of Satellite RNA with *Grapevine fanleaf virus* in its Geographical Origin and Sequence Characteristics. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 28(4):751-758. DOI: 10.15832/ankutbd.549511

ABSTRACT
The region between The Caspian Sea and Black Sea has been hypothesized as the origin of grapevine. Likewise, an extensive study on *Grapevine fanleaf virus* (GFLV) from this region suggests this region as a possible origin of the virus. However, as yet there is no information as to whether or not the virus variants from this region accompanied with the virus satellite RNA (satRNA) and if so, how diverse theses satRNAs can be. To answer these questions, *Grapevine fanleaf virus* isolates were collected from different vineyards in the northwest region of Iran for the occurrence of the satellite RNA. A total of 421 samples including *Vitis vinifera*, *Chenopodium quinoa*, *C. amaranticolor*, *Cynodon dactylis*, *Medicago sativa* were initially screened against GFLV by RT-PCR with the coat protein primers (Cp433, Cp912). When the GFLV-infected plants (36 samples) were screened by RT-PCR with the newly designed satRNA primers Gf750 and Gr750, three samples appeared to possess the satRNA. The satellite cDNA fragments were cloned and sequenced and when the resulting data were compared with sequences of previously-reported satRNA of GFLV and *Arabis mosaic virus* (ArMV), 70-98% and 69-71% similarities were found, respectively. Phylogenetic studies revealed distinctness of the satRNAs from Iran. This may suggest coevolution of the satRNA with the helper virus because GFLV isolates from this region also form a distinct branch. Among previously reported corresponding satRNA sequences to those from Slovenia and South Central Europe were the closest. This study also reports *C. amaranticolor* as the natural host for GFLV, which was previously reported only as an experimental host.

Keywords: *Arabis mosaic virus*, Double-stranded RNA, *Grapevine fanleaf virus*, Satellite RNA

1. Introduction

Grapevine fanleaf virus (GFLV) is responsible for one of the important diseases of grapevine (*Vitis vinifera* L.), fan leaf, worldwide and causes economic losses (Martelli & Boudon-Padieu 2006). This virus has two genomic RNA molecules each coding for a viral polyprotein (Andret-Link et al. 2004; Sokhandan Bashir et al. 2009). Some GFLV isolates have an extra RNA molecule known as satellite RNA (satRNA). SatRNAs are thought to be originated from similar helper viruses through mutation events such as deletion, insertion, recombination or reassortment (Cepin et al. 2016). GFLV satRNA was initially reported in GFLV isolate F13 which caused severe symptoms on *Chenopodium quinoa* (Pinck et al. 1988). Later on, satRNA was also detected in other nepoviruses such as *Arabis mosaic virus* and *Tomato black ring virus* (Cepin et al. 2016).

SatRNAs associated with nepoviruses are divided into three groups: large linear, small linear and circular satRNAs (Roossinck et al. 1992; Palukaitis 2016). The large satRNAs usually encode nonstructural proteins. They all encode a protein which is necessary for the replication of satRNA associated with nepoviruses (Hu et al. 2009; Cepin et al. 2016). In contrast, the small satRNAs are highly structural and have internal base pairing. Small satRNAs of nepoviruses are packaged as linear RNAs; however, during replication in plants they form a circular RNA (Roossinck et al. 1992; Palukaitis 2016).

SatRNAs of nepoviruses vary in size from 1.1 to 1.5 kb and have end structures (5′-Vpg and 3′-poly A) like that of the helper virus genome. These satRNAs encode a 37-48 kDa non-structural protein which is necessary, but not sufficient, for the satRNA replication (Roossinck et al. 1992). SatRNAs might affect the symptoms incurred by the helper virus (Simon et al. 2004); which could be due to satRNA's potential in multiplication and accumulation level in host plant (Betancourt et al. 2011). This role of satRNAs involves some aspects of RNA silencing and its suppression as described by Palukaitis (2016). A comparative analysis

of GFLV isolates containing satRNA or not, showed no obvious effect on virus accumulation and symptom expression in *C. quinoa* (Gottula et al. 2013).

This study was carried out for the first time to determine presence of satRNA in the GFLV isolates from vineyards in the northwest region of Iran. Vineyards in this region have previously been shown to be widely infected with GFLV. Also, phylogenetic analyses have shown that GFLV variants from this region form a distinct clade. This region is located between The Caspian Sea and Black Sea and hypothesized to be the origin of the grapevine and GFLV (Raski et al. 1983). Therefore, we were interested to find out if the GFLV isolates from this region are accompanied with the satellite and, if so, what would be the differences between satRNA of theses isolates compared to that of GFLVs reported from elsewhere. Association of satRNA with viruses may have an important implication as to the biology of helper virus and its potential use in the virus control.

2. Material and Methods

2. 1. Primer design

Initially, previously-published (Fuchs et al. 1989; Cepin et al. 2016) primer pairs, Gsat191f/Gsat1005r and Fp3/Rp, were used for detection of GFLV satRNA in RT-PCR before designing new primers. Previously reported GFLV satRNA sequences were retrieved from GenBank (Sequences which reported by Fuchs et al. 1989; Liu et al. 1990; Gottula et al. 2013; Cepin et al. 2016; Chiumenti et al. 2016) and incorporated in the design of new primers (Table 1) by the use of Primer 3 software (Primer3web version 4.1.0, http://primer3.ut.ee/).

Table 1- Features of the GFLV coat protein and satellite RNA specific primers tried in this study

Primer name	*Primer sequence*	*Expected product size (bp)*	*The amplified fragment*	*Reference*
Cp433 Cp912	F: 5 ´-GAACTGGCAAGCTGTCGTAGAA-3´ R: 5 ´- GCTCATGTCTCTCTGACTTTGACC-3´	350	GFLV coat protein	Izadpanah et al. 2003
Gf750 Gr750	F: 5 ´-ACACAAACAGCAGTCTGATGGA-3´ R: 5 ´-GCTGAGGAAAAACTGTTCGGA-3´	700-750	GFLV satellite	Newly designed Primers
Rp Fp3	R: 5 ´-TAAWGAGCAACCAAAATCCCA-3´ F: 5 ´-GTGGSCCCGCRAGTGT-3´	870-900	GFLV satellite	Cepin et al. 2016
Gf1000 Gr1000	F: 5 ´-GCTTGTATTGTCGTGTAAGCAC-3´ R: 5 ´-AGTTGGCTAATGAGCAACC-3´	1000	GFLV satellite	Newly designed Primers
Gsat191f Gsat1005r	F: 5 ´-CCGAGACCGAAATGGGAGTAAAACA-3´ R: 5 ´-ACAGAAGCAACCGTGGGGATACAC-3´	850-900	GFLV satellite	Fuchs et al. 1989

2. 2. Virus sources, extraction of total RNA and RT-PCR

First, 421 samples including grapevine and several weeds were subjected to total RNA extraction according to Rowhani et al. (1993) with modifications as described elsewhere (Sokhandan et al. 2007; Khabbazi et al. 2017) and then screened by RT-PCR with GFLV coat protein primers to determine the virus infection. Then, 36 GFLV- infected plants (Table 2) including grapevine (30 samples), *Chenopodium quinoa* (4 samples), *C. amaranticolor* (1 sample) and *C. dactylis* (1 sample) were subjected to RT-PCR with the satellite primers.

RT-PCR was initially conducted to confirm the GFLV entity of the extracted total RNA by the use of Cp433/Cp912 primers (Table 1). Accordingly, cDNA was synthesized with the reverse primer, Cp912 following the RevertAid First Strand cDNA Synthesis Kit instructions (Thermo Fisher Scientific Inc., Waltham, USA). Subsequently, PCR was carried out using the Cp433 and Cp912 primer pairs under a thermal profile of an initial denaturation at 94 °C for 1 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 30 s, and a final polymerization step at 72 °C for 7 min.

cDNA synthesis was also performed with Gsat1005r, GR1000, Rp or Gr750 primer to detect the virus satRNA, respectively. Then, the generated cDNA was used as the template to amplify the expected fragment by PCR using Gsat191f/Gsat1005r, Gf1000/Gr1000 or Fp3/Rp primer pairs, respectively. PCR amplification was performed by using one Unit of *Taq* DNA Polymerase (SinaClon Co., Tehran, Iran) in a total reaction mixture of 20 µl containing 1× reaction buffer, 1.5 mM $MgCl_2$, 1 mM dNTPs and 0.25 pmol of each primer. A gradient PCR (for all the satRNA primer pairs) was initiated with an initial denaturation at 94 ˚C for 1 min and the reaction was progressed in 30 cycles of denaturation at 94 ˚C for 30 secs, annealing at 52, 54, 56, 58 and 60 ˚C for 30 sec and extension at 72 ˚C for 1 min. The amplification was ended with the elongation step of 72 ˚C for 7 min. In PCR with the new primer set Gf750/Gr750, an annealing temperature at 60 °C for 30 secs was applied. A 5 µl-aliquot of the products were electrophoresed on 1% agarose.

Table 2- List of samples tested for GFLV and its satRNA

Sample code	*Host*	*Symptoms*	*Origin*	*GFLV*	*satRNA*
M3	C. quinoa	Local lesions	Khelejan, E. Azar.1	+	+
A2	V. vinifera cv. Keshmeshi	Mosaic	Urmia, W. Azar.2	+	+
G3,G4,G1,Kh2	V. vinifera cv. Keshmeshi	Vein banding	Khalatpoushan, E. Azar.	+	-
G2	V. vinifera cv. Keshmeshi	Vein banding and leaf deformation	Khalatpoushan, E. Azar.	+	-
Kh3, Kh6	V. vinifera cv. Shahani	Vein banding	Khalatpoushan, E. Azar.	+	-
Kh4	C. quinoa	Local lesions	Khalatpoushan, E. Azar.	+	-
Kh7, Kh8	V. vinifera cv. Shahani	Mosaic	Khalatpoushan, E. Azar.	+	-
S2	V. vinifera cv. Asgari	Mosaic	Sardrud, E. Azar.	+	-
S3	V. vinifera cv. Asgari	Vein banding	Sardrud, E. Azar.	+	-
S4, S6, S10	V. vinifera cv. Keshmeshi	Vein banding	Sardrud, E. Azar.	+	-
A6	V. vinifera cv. Hoseini	Mosaic and yellowing	Ajabshir, E. Azar.	+	-
A9	V. vinifera cv. Hoseini	No symptoms	Ajabshir, E. Azar.	+	-
A2, A11	V. vinifera cv. Asgari	No symptoms	Ajabshir, E. Azar.	+	-
A14	C. quinoa	Local lesions and yellowing	Ajabshir, E. Azar.	+	-
T.H	V. vinifera cv. Keshmeshi	Mosaic	Tabriz, E. Azar.	+	-
L2, L3	V. vinifera cv. Keshmeshi	Vein banding	Lahroud, Ardebil	+	-
U3, U4	V. vinifera cv. Sahebi	No symptoms	Urmia, W. Azar.	+	-
U6, U7	V. vinifera cv. Keshmeshi	No symptoms	Urmia, W. Azar.	+	-
M.ch	C. quinoa	Local lesions	Mayan, E. Azar.	+	-
M.cy	C. dactylis	Mosaic	Mayan, E. Azar.	+	-
B2, B10	V. vinifera cv. Qizil uzum	Vein banding	Bonab, E. Azar.	+	-
N1, N5	V. vinifera cv. Keshmeshi	No symptoms	Khelejan, E. Azar.	+	-
T1	C.amaranticolor	Local lesions	Khelejan, E. Azar.	+	+

1: East Azarbaijan, 2: West Azarbaijan

2. 3. Cloning and sequence analysis

DNA fragment amplified by the use of the satellite primers (Gf750, Gr750) were excised from agarose gel and purified using Thermo Fisher DNA Gel Extraction kit (Waltham, USA). The purified fragment (150 ng) from each sample was ligated into pTG19-T cloning vector (50 ng) according to the manufacturer's protocol (SinaClon Corp., Tehran, Iran) and transformed into 100 µl home-prepared competent *Escherichia coli* DH5α cells through heat-shock method (Chung et al. 1989). The transformed bacteria were grown on LB medium containing ampicillin (100 mg/L), X-GaL (20 mg/ml) and IPTG (0.1 M). Three desired colonies each from an isolate (RT-PCR product species) were selected by colony- PCR screening (Sokhandan et al. 1997) and the plasmids were extracted following the alkaline lysis method (Green & Sambrook 2012). To confirm that the satellite fragment is cloned into the vector (pTG19-satRNA), *Bam*HI restriction analysis was conducted. Subsequently, recombinant plasmids from the desired colonies were sequenced (Refgen Corp., Ltd., Ankara, Turkey) by the use of satRNA specific primers, Gf 750 and Gr 750. Analysis of the sequences was performed to assess variation between the satRNA sequences. Nucleotide (nt) alignment and phylogenetic trees were constructed using GeneDoc (Nicholas & Nicholas, 1997) and CLC sequence viewer 7.6.1 program (CLC Bio-Qiagen, Aarhus, Denmark), respectively. The SIAS online tool (Immunomedicine Group, UCM, Spain) was used for calculation of pairwise identity percentages. A 700 nt data from each of the three satRNA isolates were aligned with satellite sequences of GFLV and ArMV which were retrieved from GenBank. The alignment numbering was relative to the satRNA strain R2 (accession number: KC162000.1) to find out the position of highly conserved regions.

2. 4. Glasshouse studies

Glasshouse studies were performed to gather further evidence for association of the satellite with GFLV. It was hypothesized that if the GFLV possesses the satRNA, it may still be accompanied with the virus after seed transmission. Accordingly, *C. quinoa* (50 pots) and *V. vinifera* (20 pots) were used in this study. One healthy control was included for every five inoculated samples. Three-week old *C. quinoa* and newly germinated leaves (2 cm long) of 4-month old *V. vinifera* were subjected to mechanical inoculation. The leaves were cleaned with a piece of wet cloth before being dusted with carborundum and rubbed with sap of GFLV-infected grapevine leaves ground in 0.1 M phosphate buffer (pH 7.2) containing 2.5% (v/v) nicotine. The inoculated leaves were rinsed with water after 10 minutes of the inoculation and the plants were kept at 24 ± 2 ̊C for 10 to 14 days in glasshouse until appearance of the symptoms. The infected plants were tested by total RNA extraction and RT-PCR for infection with GFLV and presence of the satRNA.

The infected *C. quinoa* plants were grown to flowering and allowed to produce seeds. The resultant seeds were germinated and maintained under glasshouse conditions for the appearance of any viral symptom before being checked by total RNA extraction and RT-PCR for the presence of the virus and satRNA.

3. Results and Discussion

3. 1. Detection of GFLV and satRNA

When total RNA samples were tested by RT-PCR, only 36 samples (8.6%) appeared to have an infection with GFLV. The expected 350 bp and 700 bp fragments were amplified with the coat protein (Cp912, 433) and satRNA (gf 750, gr 750) primers, respectively (Figure 1). Among the GFLV-infected plants, satRNA was detected only in three plant samples (Table 2) including *V. vinifera*, *C. quinoa,* and *C. amaranticolor*. However, no satisfying result was achieved using the primers already reported in the literature.

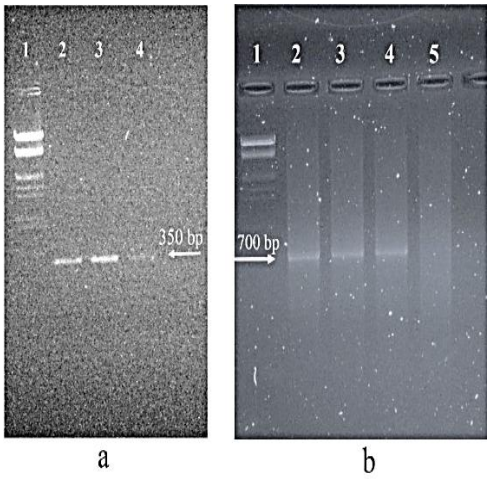


Figure 1- Electrophoresis of products from RT-PCR with GFLV primers (Cp912, Cp433) [a] and GFLV satellite (gf750 and gr750) primers [b] on 1% agarose gel. Lane 1(a, b): Lambda DNA *EcoR*I + *Hind*III; (a) Lanes 2, 3 and 4: infected *C.quinoa*, *V. vinifera,* and *C. amaranticolor*, respectively. (b)Lanes 2, 3 and 4: infected *C.quinoa*, *V. vinifera,* and *C. amaranticolor* containing satRNA, respectively; Lane 5: GFLV-infected sample lacking satRNA

3. 2. Sequencing and phylogenetic analysis

When RT-PCR-amplified satRNA fragment (700 bp) was ligated into pTG19-T and the resulting recombinant plasmid was used for transformation of *E. coli*, the desired colonies were found among which three colonies (A2, M3, and T1) were randomly selected for further analyses. A subsequent *Bam*HI restriction digestion also confirmed cloning of the 700 bp satRNA cDNA into the pTG19-T vector. When the recombinant plasmids were subjected to sequencing and the resultant sequence data were aligned, homologies of 91-97% were observed between the three satRNA isolates. Maximum (97%) homology was between the isolates A2 and M3 and the least homology (91%) between T1 and M3. By Comparison of these sequences to previously recorded GFLV satRNA sequences, homologies of 70-98% were revealed between them. In addition, comparison of the newly-generated GFLV satRNA isolates with ArMV satRNA sequences (accession number D00664.1 and NC_003523.1) revealed homologies of 59-61% (Table 3). Further, there were 80-85% homologies between the new isolates and that reported from Slovenia and South Central Europe (Cepin et al. 2016). This considerably big difference (15-20%) between these satRNAs could be due to the differences in lineages of the helper virus or independent events in evolutionary history such as deletion or insertion of nucleotides.

Table 3- Similarity analysis of the isolated satRNA sequences performed by SIAS program; A2, M3 and T1 are satRNAs isolated in this study, L1, L2 and C1-C22 are codes for the sequences reported earlier. Numbers represent the similarity percentage between the isolates

Code of isolates	*A2*	*M3*	*T1*
A2	-	97%	94%
M3	97%	-	91%
T1	94%	91%	-
L1, L2		59-61%	
C1-C22		80-85%	

In the cladogram constructed on the basis of the satRNA sequences (Table 4), there were two clades so that the newly-generated GFLV satRNA sequences (A2, T1, and M3) were clustered in a distinct position together with previously-reported GFLV and ArMV satRNAs (Figure 2). Thus, the isolates from Iran were clustered in the same clade together with the isolates

reported from Slovenia and South Central Europe. Sequences of GFLV satRNA isolates A2, T1, and M3 were placed in a common subclade although the latter (from *C. quinoa*) was on a different branch in the same subclade. The first two isolates shared 95% similarity. GFLV satRNA-isolate A2 from *V. vinifera* was from a different geographical region far away from that of GFLV satRNA-T1 from *C. amaranticolor*. GFLV satRNA-M3 and T1 were from the same region, but it (M3) shared 99% similarity with satGFLV-A2 and 96% with GFLV satRNA- T1. The accession numbers provided by GenBank for the sequences of GFLV satRNA (A2, M3 and T1) are MK248516, MK248517 and MK248518, respectively.

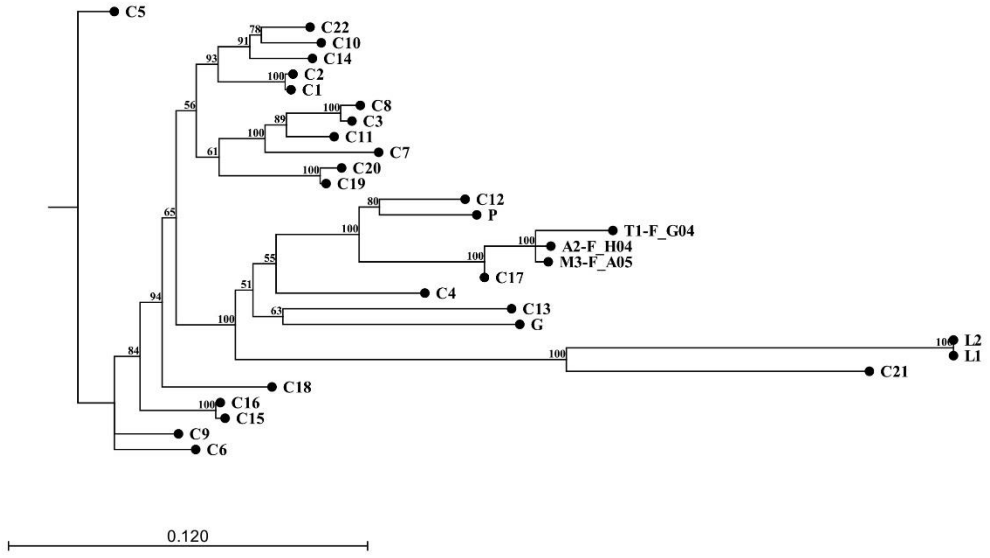


Figure 2- Phylogenetic relationship between sequences of satRNA obtained in this study and that of previously reported GFLV satellite RNA retrieved from GenBank. T1, A2, and M3 are the newly-generated sequences

Table4- List of the satRNA sequences retrieved from NCBI gene bank and incorporated in this study

Code	*Viral isolate*	*Accession number*	*Reference*
C1	Zup_2_1 clone c2 P3*	KR014664.1	Cepin et al. 2015
C2	Zup_2_1 clone c1 P3*	KR014663.1	
C3	Zelen_1_2_10 clone c1 P3*	KR014655.1	
C4	SORO1 clone c2 P3*	KR014653.1	
C5	Sla_1_3 clone c2 P3*	KR014651.1	
C6	SauDUK_2_27 clone c2 P3*	KR014647.1	
C7	Sau_h clone c3 P3*	KR014645.1	
C8	RefKE1_10_2 clone c3 P3*	KR014642.1	
C9	RefDUC_4_10 clone c8 P3*	KR014626.1	
C10	Rec_4_25 clone c2 P3*	KR014606.1	
C11	Racuk_B6_18 clone c1 P3*	KR014604.1	
C12	PP_21 clone c1 P3*	KR014602.1	
C13	P22 clone c1 P3*	KR014600.1	
C14	Mal_o_5 clone c4*	KR014599.1	
C15	LR_6_30 clone c3 P3*	KR014586.1	
C16	LR_6_30 clone c2 P3*	KR014585.1	
C17	ITA3 clone c1 P3*	KR014579.1	
C18	Cividin_5_51 clone c1 P3*	KR014571.1	
C19	B2_7 clone c1 P3*	KR014570.1	
C20	A2_25 clone c2 P3*	KR014569.1	
C21	GFLV103 clone c3 P3*	KR014565.1	
C22	Vol_2_55 clone c6 P3*	KR014557.1	
P	Panse Precoce, clone 7FL-19*	LN890580.1	Chiumenti et al. 2015
G	Strain R2*	KC162000.1	Gottula et al.2012
L1	ArMV large satRNA**	D00664.1	Liu et al. 2000
L2	ArMV large satRNA**	NC_003523.1	

*, Host: *Vitis vinifera;* **, Host: *Syringa vulgaris*

3. 3. Glasshouse studies

Among the 50 plants of *C. quinoa* 40 plants (80%) demonstrated evident symptoms including the local lesions and leaf deformation. Among the inoculated *V. vinifera* species, five of twenty plants (25%) showed reduction in growth and mottling of

the leaves whereas in the remaining plants the disease symptoms were not visible. However, total RNA from all the inoculated plants were subjected to RT-PCR to verify the results. 350 and 700 bp fragments were obtained by the use of GFLV coat protein (Cp 433/Cp 912) and satellite primers (Gf750/Gr750), respectively.

Based on the assessments of GFLV+satRNA transmission to *C. quinoa*, it could be concluded that the plants infected with satRNA-containing viruses indicate normal growth with local lesions only whereas the satRNA-free GFLV infection causes stunting, chlorosis, and local lesions as well (Figure 3).When the seeds of *C. quinoa* from the plants infected with satRNA-possessing GFLV were germinated and the progeny plants were compared to the healthy plants, obvious difference in plant height was evident, validating the virus transition to the next progeny. A subsequent RT-PCR assays confirmed the presence of GFLV and satRNA in the progeny (Figure 4).

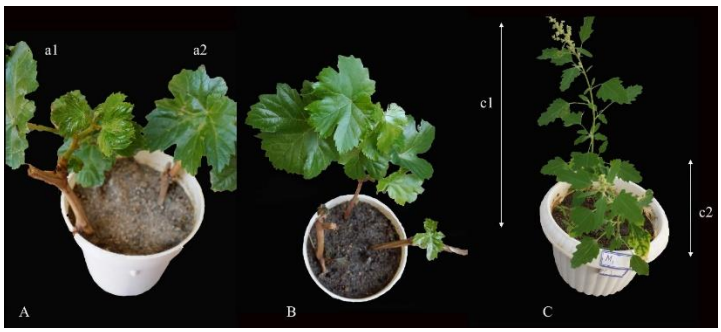


Figure 3- A (a1): Grapevine leaves, inoculated with satRNA-free GFLV, showing leaf deformation and growth reduction; A (a2): Grapevine leaves inoculated with GFLV isolate containing satRNA (T1) showing no obvious symptoms; B: Healthy plant; C (c1): *C. quinoa* inoculated with GFLV isolate containing satRNA (T1) shows local lesions on leaves with normal growth; C (c2): *C. quinoa* inoculated with GFLV isolate lacking satRNA shows stunting, chlorosis in lower leaves and local lesions in upper leaves

In this study, samples were collected in late spring, early summer and early autumn. This timing was because of the symptom disappearance in hot summer days (Francki et al. 1985). In addition to different samples with various GFLV symptoms, asymptomatic samples from *V. vinifera* and weeds were also collected, because it has been documented that some *V. vinifera* varieties are tolerant to GFLV (Martelli & Boudon-Padieu 2006). Thus, they could be infected with GFLV although asymptomatically, as 9 out of 71 asymptomatic samples appeared to be carrying GFLV although none of them had the satRNA.

The distribution pattern of the satRNA among the GFLV-infected vines suggests that the satRNA could be absent in a GFLV-infected plant adjacent to others which contain the satRNA (Gottula et al. 2013). Likewise, in the present study, satRNA-containing GFLV was detected in *C. amaranticolor* grown in vineyards, but not in the surrounding grapevines in the same vineyard.

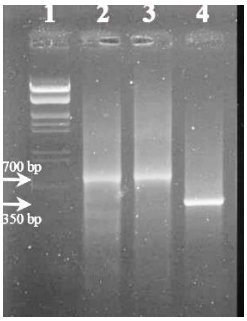


Figure 4- Surveying seed transmission of the satellite RNA in *C. quinoa*; electrophoresis of the RT-PCR products on 1.2% agarose gel. Lane 1: Lambda DNA *Eco*RI+*Hind*III; Lane 2: RT-PCR product from satRNA in the progenies; Lane 3: RT-PCR product from satRNA in the parental plant; Lane 4: RT-PCR product from the GFLV coat protein gene in the progenies as the control

To confirm the detection of GFLV and its satRNA, RT-PCR with GFLV coat protein and satRNA primers were applied in this study. SatRNA detection was carried out using different primer pairs, of which three pairs had earlier been utilized in similar researches elsewhere in the world; however, detection of satRNA was not successful with the previously published primers (Gsat191f/Gsat1005r, Fp3/Rp) which might be due to the satellite genetic variation. The genetic variation in the flanking sequences of satRNA and failure in the primer annealing process might be the reason for unsuccessful RT-PCR amplification once other primers were used. Also, previous studies on Iran isolates of GFLV have revealed that they possess distinct genotypes, consequently the primers which were designed based on exotic GFLV isolates could not be efficient in annealing the local

isolates (Sokhandan et al. 2012). Among the newly designed primers, Gf750/Gr750 had a better amplification thus were utilized in the study. The other newly designed primers (Gf1000/Gr1000) were not useful in the detection and the expected amplified fragment (1000 bp) was very faint and the PCR conditions could not be optimized further.

Comparison of results from the two different primer sets (Gf1000/Gr1000 and Gf750/Gr750) suggested that the satRNA may possess some conserved regions internally rather than at the ends because only Gf750/Gr750 satisfactionally gave the expected band.

The main objective of this study was to determine presence of the satRNA and investigate its diversity in virus isolates of GFLV from Iran. To confirm further that the GFLV is accompanied with the satRNA, seeds from GFLV+satRNA infected *C. quinoa* plants were germinated and a subsequent analysis of the progeny plants revealed that the transmitted GFLV contained the satellite (Figure 4). This gave a further evidence for association of the satRNA with the GFLV isolates (causing vein banding and mosaic). The results also indicated that the satRNA did not affect severity of symptoms which is in contrast to the previous report that satRNAs impact on their helper viruses and the induced symptoms (Roossinck et al. 1992). In general, the effect of satRNA on virus symptoms is influenced by helper virus, host plant and features of satRNA (Roossinck et al. 1992). As reported by Lamprecht et al. (2013), helper virus was the determinative factor in the infection of *C. quinoa* plants when they were mechanically inoculated with satRNA-containing GFLV isolates. These GFLV isolates were infectious only when co-inoculated with GFLV as the helper virus but not when co-inoculated with ArMV-NW.

C. quinoa seeds, infected with GFLV+satRNA, germinated one month later than the control and also showed reduction in plantlet growth in 50% of the replicates when compared to the control plants which were in agreement with the results reported by Saldarelli et al. (1993) and Gottula et al. (2013). However, with the *V. vinifera* plants no obvious symptom appeared when they were inoculated with the GFLV-infected samples containing satRNA. Accordingly, the effect of satRNA on infection and symptom appearance might be dependent on the host species as well.

Among 36 infected plant samples, GFLV isolates harboring the satRNA (8.33%) were detected in three cases which demonstrates the low frequency of the satRNA in the studied region. In a similar study conducted by Saldarelli et al. (1993) only five isolates from among 34 GFLV-infected samples were determined to contain the satRNA (14.70%). Likewise, in another study more than 100 plants were screened but the satRNA was found in two samples only. Therefore, it appears that the occurrence of satRNA is a rare event (Lamprecht et al. 2013) which is speculated that helper virus and host plant are the factors involved in satRNA origination (Roossinck et al. 1992; Saldarelli et al. 1993; Lamprecht et al. 2013).

This study also dealt with the variation of the satRNA compared to the previously reported GFLV satRNA. Accordingly, phylogenetic studies demonstrated that GFLV isolates M3 (*C. quinoa*) and T1 (*C. amaranticolor*) were placed on different branches in the same subclade although they were collected from the same region (Khelejan, East Azarbaijan, Iran). As such, there appears that geographical location is not the only determination factor for satRNA grouping in phylogenetic trees. As this is the case for the helper virus as well (Lamprecht et al. 2013; Cepin et al. 2016). Comparison of the satRNA sequences from Iran with the previously reported satRNA sequences suggested that the former were phylogenetically close to that reported from Slovenia and Central Europe, however, the reported satRNA sequences in this study are placed in a distinct sub clade on the phylogenetic tree. The previous studies on CP and MP genes of GFLV in Iran have shown the distinctness of Iran isolates among all the reported GFLV sequences from around the world (Sokhandan et al. 2012). So, the distinct positions of satRNAs from Iran in the phylogenetic tree could be because of the distinctness of the helper virus. This also could be strengthening the previous hypothesis that Iran is the origin of GFLV and from there has spread to all over the world (Vuittenez 1970). The present study is the first study of GFLV satRNA in the origin of grapevine, Iran, which provides new insights into the occurrence and genetic diversity of GFLV satRNA. Results achieved pave the way for future studies of satRNA and development of genetically modified GFLV-resistant varieties of grapevine.

4. Conclusions

In this study, GFLV satellite RNA was detected and characterized for the first time in the northwest region of Iran by RT-PCR. A heterogeneity of 2-30% was revealed between the three newly-reported satRNAs and the previously-reported isolates and it was also demonstrated that the GFLV satRNA sequences from Iran are phylogenetically close to that reported from Slovenia and Central Europe. Though they have close relationship phylogenetically but the Iran isolates formed distinct sub clade.

References

Andret-Link P, Laporte C & Valat L (2004). *Grapevine fanleaf virus*: still a major threat to the grapevine industry. *Journal of Plant Pathology* 86: 183-95 doi:10.4454/JPP.V86I3.987

Betancourt M, Fraile A & Garcia-Arenal F (2011). *Cucumber mosaic virus* satellite RNA that induce similar symptoms in melon plants show large differences in fitness. *Journal of General Virology* 92(8): 1930-1938 DOI: 10.1099/vir.0.032359-0

Cepin U, Guti_errez-Aguirre I, Ravnikar M & Pompe-Novak M (2016). Frequency of occurrence and genetic variability of *Grapevine fanleaf virus* satellite RNA. *Plant Pathology* 65 (3): 510-520 https://doi.org/10.1111/ppa.12428

Chiumenti M, Mohorianu I, Roseti V, Saldarelli P, Dalmay T & Minafra A (2016). High throughput sequencing based identification of a GFLV satellite RNA in *Vitis vinifera*. *Archieves of Virology* 161(5): 1401-1403

Chung C T, Niemela S L & Mille R H (1989). One step preparation of competent *Escherchia coli*: Transformation and storage of bacterial cells in the same solution. In: *Proceeding of National Academics Science*, April, USA pp. 2172-2175 doi: 10.1073/pnas.86.7.2172

Francki R I B, Randles J W, Chu P W G, Rohozinski J & Hatta T (1985). Viroid-like RNAs incorporated in conventional virus capsids. In: K Maramorosch & J McKelvey (Eds.), *Subviral Pathogens of Plants and Animals: Viroids and Prions*, Jr. London Academic Press pp. 265-297

Fuchs M, Pinck M, Serghini M A, Ravelonandro M, Walter B & Pinck L (1989). The nucleotide sequence of satRNA in Grapevine fanleaf virus, strain F13. *Journal of General Virology* 70:955-962

Gottula J, Lapato D, Cantilina K, Saito S, Bartlett B & Fuchs M (2013). Genetic variability, evolution, and biological effects of Grapevine fanleaf virus satellite RNAs. *Phytopathology* 103 (11): 1180-1187

Green R M & Sambrook J (2012). Molecular Cloning: a laboratory manual. 4th ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York 1885 pp.

Hu C C, Hsu Y H & Lin N S (2009). Satellite RNAs and satellite viruses of plants. *Viruses* 1: 1325-1350

Khabbazi A D, Bashir N S, Khabbazi S D & Ighani H (2017). Extraction and molecular detection of viral dsRNA from different infected plants. *Journal of Scientific Agriculture* pp. 197-203

Lamprecht R L, Spaltman M, Stephan D, Wetzel T & Burger J T (2013). Complete nucleotide sequence of a South African isolate of *Grapevine fanleaf virus* and its associated satellite RNA. *Viruses* 5: 1815-1823 doi: 10.3390/v5071815

Liu Y Y, Hellen C U, Cooper J I, Bertioli D J, Coates D & Bauer G (1990). The nucleotide sequence of a satellite RNA associated with Arabis mosaic nepovirus. *Journal of General Virology* 71: 1259-1263 doi: 10.1099/0022-1317-71-6-1259

Martelli G P & Boudon-Padieu E (2006). Directory of infectious diseases of grapevines. *Options Méditerranéennes* 55: 157-194

Nicholas K B & Nicholas H B Jr (1997). GeneDoc: a tool for editing and annotating multiple sequence alignments. Distributed by the authors. http://www.psc.edu/biomed/genedoc

Palukaitis P (2016). Satellite RNAs and satellite viruses. *Molecular Plant-Micobe interaction* 29(3): 181-186

Pinck L, Fuchs M, Pinck M, Ravelonandro M & Walter B (1988). A satellite RNA in *grapevine fanleaf virus* strain F13. *Journal of General Virology* 69: 233-239

Raski D J, Goheen A C, Lider L A & Meredith C P (1983). Strategies against grapevine fanleaf virus and its nematode vector. *Plant Disease* 67: 335-339

Roossinck M J, Sleat D & Palukaitis P (1992). Satellite RNAs of plant viruses: structures and biological effects. *Microbiology Review* 56: 265-279

Rowhani A, Chay C, Golino D A & Falk B W (1993). Development of a polymerase chain reaction technique for the detection of *grapevine fanleaf virus* in grapevine tissue. *Phytopathology* 83: 749-753

Saldarelli P, Minafra A & Walter B (1993). A survey of Grapevine fanleaf nepovirus isolates for the presence of satellite RNA. *Vitis* 32: 99-102

Simon A E, Roossinck M J & Havelda Z (2004). Plant virus satellite and defective interfering RNAs: new paradigms for a new century. *Annual Review of Phytopathology* 42: 415-437 https://doi.org/10.1146/annurev.phyto.42.040803.140402

Sokhandan N B, Gillings M R & Bowyer J W (1997). Polymerase chain reaction detection and assessment of genetic variation in New South Wales isolates of Passionfruit woodiness potyvirus. *Australasian plant pathology* 26: 155-164

Sokhandan N B, Nourinejhad Sh & Hejazi M S (2007). Diversity of *Grapevine fanleaf virus* isolates from Iran. *Virus Research* 28: 144-148

Sokhandan N B, Houshmand A & Khabbazi A D (2012). Molecular Characterization of Phylogenetically Distinct Isolates of Grapevine fanleaf virus from Iran Based on 2A(HP) Gene. *Indian Journal of Virology* 23(1): 50-56

Sokhandan Bashir N, Delpasand Khabbazi A & Torabi E (2009). Isolation of the gene coding for movement protein from Grapevine fanleaf virus. *Iranian Journal of Biotechnology* 7(4): 258-261

Vuittenez A (1970). Fanleaf of grapevine. In: N W Frazier (Ed.), *Virus diseases of small fruits and grapevine*, California: University of Berkeley pp. 217-228