ISSN 1300 - 0225

T.C.
GIDA TARIM VE HAYVANCILIK
BAKANLIĞI

AARI

ANADOLU

EGE TARIMSAL ARAŞTIRMA ENSTİTÜSÜ DERGİSİ

JOURNAL OF AEGEAN AGRICULTURAL RESEARCH INSTITUTE

İZMİR
TURKEY

CİLT
VOLUME 28

SAYI
NUMBER 1

2018

ANADOLU

ISSN 1300 - 0225

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Cumhuriyet Mah. Çanakkale Asfaltı No: 57
PO Box 9 Menemen
35661 IZMIR, TURKEY

ISSN 1300 - 0225

ANADOLU

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Banka Hesabı	:	Ziraat Bankası Menemen Şubesi Hesap No: 8445877-5001 IBAN No: TR75 0001 0001 4608 4458 7750 01			
Basım Yeri	:	Meta Basım 87 Sokak No: 4/B Bornova - İZMİR			
Basım Tarihi	:	15.05.2018			

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Dr. Ayfer TAN'a teşekkür ederiz.

Editorial Board of Anadolu would like to express its sincere thanks to
Dr. Ayfer TAN for her valuable review of manuscripts in this issue

ANADOLU (ISSN 1300-0225)

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MFAL

Wild Plants Used as Herbal Tea in Antakya and Defne Provinces of Hatay

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Received (Geliş tarihi): 06.06.2017 *Accepted (Kabul tarihi): 29.01.2018*

ABSTRACT: *In the present study, wild plants that are members of the local flora and used traditionally as herbal tea in 32 villages of Antakya and Defne provinces of Hatay were compiled. Although all of them have some medicinal properties and are also used for healing, the main difference of these plants from other medicinal herbal tea plants is that they are consumed as tea for pleasure mostly without any medical purpose in daily life. All information has been compiled by face-to-face interviews with 182 local people as a part of an ethnobotanical study in Antakya province. 33 species belong to 9 families were determined as herbal teas that consumed for pleasure in daily life. Reminding, cultivation and marketing of these herbal teas should be encouraged in terms of evaluating our natural resources efficiently.*

Keywords: *Ethnobotany, herbal tea, Antakya, Defne, Hatay.*

Hatay'ın Antakya ve Defne İlçelerinde Bitki Çayı Olarak Kullanılan Yabani Bitkiler

ÖZ: *Bu çalışmada, Hatay'ın Antakya ve Defne ilçelerinden 32 köyde, yerel floranın elemanları olup geleneksel bitki çayı olarak kullanılan bitkiler derlenmiştir. Tamamının bazı tıbbi özellikleri olmasına ve şifa için de kullanılmalarına rağmen, bu bitki çaylarının diğer şifalı bitki çaylarından başlıca farkı, tıbbi bir amaç olmaksızın, günlük yaşam rutininde keyif amaçlı olarak içiliyor olmalarıdır. Bütün bilgiler, Antakya ilçesinde yürütülen etnobotanik çalışması kapsamında yüzyüze görüşülen 182 yerli kişiden derlenmiştir. Günlük yaşamda, keyif amaçlı çay olarak içilen 9 familyaya mensup 33 tür tespit edilmiştir. Doğal kaynaklarımızı verimli bir şekilde değerlendirmek açısından bu bitki çaylarının hatırlanması, yetiştirilmesi ve pazarlanması teşvik edilmelidir.*

Anahtar Sözcükler: *Etnobotanik, bitki çayı, Antakya, Defne, Hatay.*

INTRODUCTION

Wild plants that are members of the Hatay flora and used traditionally as herbal tea in Defne and Antakya provinces were compiled as a part of an ethnobotanical study on this multicultural and historical city (Guzelsemme, 2014). Located in the most southern region of Turkey, Hatay is a province that is situated on the coast of the Eastern Mediterranean. Within the province of Hatay, where Antakya and Defne is located, there are approximately 1900 specific and sub specific taxa. The rate of endemism is 11.8% (Davis, 1965-1985; Davis *et al*., 1988; Guner *et al*., 2000; Kayıkcı *et al*., 2012). Due to its rich flora, multicultural structure and deep-rooted historical background, Hatay is an interesting city in terms of ethnobotany.

MATERIALS AND METHODS

All information has been compiled by face-to-face interviews with 182 local people from 32 villages of Antakya and Defne provinces of Hatay/Turkey

(Guzel *et al.,* 2015). Wild plants that are members of the local flora and used traditionally as herbal tea without any medicinal purpose were asked to the interviewees. Interviewees were carefully encouraged to show the plants for which they were providing information to eliminate confusion that may have stemmed from multiple common names. Voucher specimens of plant materials were deposited in the Herbarium of Faculty of Sciences, Mustafa Kemal University (MKUH) and identified by first author.

RESULTS AND DISCUSSION

33 species belong to 9 families were determined as herbal teas that consumed mainly for pleasure in daily life (Table 1). All of them have also various medicinal and edible properties (Guzel *et al.,* 2015). Such ethnobotanical uses of the each herbal tea are given in the Table 1 with local names, used parts and preparation of herbal teas.

The most widely used family is Lamiaceae, with 14 species and second family is Rosaceae with 6 species. *Sideritis* and *Helichrysum* are the most used genera with 4 and 3 species respectively. Two endemic taxa (one species and one subspecies) that belong to Sideritis are also used as herbal tea widely.

According to interviewed local people, modern day's popular tea, *Camellia sinensis*, was not very common in this region about fifty years ago. In those days, these plants were collected from the wild and consumed as herbal tea. Although it is not common as the old days, this routine is still continues today.

CONCLUSION

All of these herbal teas have medicinal benefits also and used widely for these beneficial purposes as well as for pleasure. Especially Asteraceae, Rosaceae and Malvaceae members of below mentioned plants have smooth floral aroma that will suit everyone's taste. Herbal teas of Lamiaceae, the most videly used family have pleasant, pungent aroma as well as significant medicinal values. All of these herbal teas are collected from the nature. Especially *Sideritis*, *Salvia*, *Origanum* and *Helichrysum* are collected intensively to sell at herbal markets. This is a very objectionable application especially for endemic taxa. Cultivation is the only way to ensure sustainable use of these natural resources. Some of the plants, such as *Thymbra spicata*, are widely cultivated today. Cultivation of the other plants also should be encouraged and collections from nature should be prevented. Reminding, cultivation and marketing of these herbal teas will provide an important source of revenue to the region where unemployment is a big problem and will also provide natural, healthy and alternative drink sources to the people.

Table 1. Wild plants used as herbal tea in Antakya and Defne provinces of Hatay with local names, used parts, preparations and other ethnobotanical uses.
Çizelge 1. Hatay'ın Antakya ve Defne ilçelerinde bitki çayı olarak kullanılan bitkiler, yerel adları, kullanılan kısımları, hazırlanma şekilleri ve diğer etnobotanik kullanımları.

Family Aile*	Local names Yerel adlar	Used parts Kullanılan kısımlar	Preparation Hazırlama	Other ethnobotanical uses Diğer etnobotanik kullanımlar
Apiaceae Maydanozgiller				
Foeniculum vulgare Mill. Rezene	Şımra Rezene Şimura	Fruits	Docoction (boiling)	Medicinal tea for digestive problems; As spice especially for local pastries such as 'katıklı ekmek' 'yağlı börek' etc.
Asteraceae Papatyagiller				
Cota palaestina Reut. Ex. Unger & Kotsch (Syn.:*Anthemis palestina* Reut. Ex. Boiss.) Kuru babuççа	Papatya Babaniç Beybuneç Kuhen	Flowers (capitulas)	Infusion (steeping in hot water)	Multipurpose medicinal tea
Helichrysum plicatum subsp. *plicatum* DC. Mantuvar	Gudame Ölmez çiçek	Flowers (capitulas)	Infusion	Multipurpose medicinal tea
Helichrysum stoechas (L.) Moench Kudama	Gudame Ölmez çiçek	Flowers (capitulas)	Infusion	Multipurpose medicinal tea
Helichrysum sanguineum (L.) Kostel Kırmızı guddeme	Kırmızı gudame Kırmızı ölmez çiçek	Flowers (capitulas)	Infusion	Multipurpose medicinal tea
Cistaceae Ladengiller				
Cistus creticus L Laden	Laden	Flovers Leaves	Infusion	Medicinal tea for urinary and gastrointestinal diseases also used externally for acne and oily skin treatments.
Eleagnaceae İğdegiller				
Elaeagnus angustifolia L. İğde	Barsin Zeyisfun İğde	Flowers	Infusion	Medicinal tea for cough and urinary diseases
Fabaceae Baklagiller				
Glycyrrhiza glabra L. var. *glandulifera* (Waldst. et Kit.) Boiss. Meyan	Meyan Peyam	Roots	Cold infusion Drunk cold as sherbet	Multipurpose medicinal tea
Ceratonia siliqua L. Keçiboynuzu	Harnup Keçi boynuzu	Fruits	Decoction of fruits Also by diluting its molasse	Medicinal tea for anemia and invigoration Its molasse, 'harnup pekmezi' eaten at breakfast

*Turkish plant names in the Table are based on Guner *et al.* (2012) and Anonim (2017).
*Türkçe bitki adları Güner ve ark. (2012) ve Anonim (2017) temel alınarak yazılmıştır.

Table 1. Continued.
Çizelge 1. Devam.

Family Aile*	Local names Yerel adlar	Used parts Kullanılan kısımlar	Preparation Hazırlama	Other ethnobotanical uses Diğer etnobotanik kullanımlar
Lamiaceae Ballıbabagiller				
Mentha pulegium L. Yarpuz	Yarpız Kırneyya	Aerial parts	Infusion	Medicinal tea for cystitis, colic spasms and muscle joint pains
Clinopodium serpyllifolium (M.Bieb.) Kuntze. Taş nanesi	Taş nanesi	Aerial parts	Infusion	Medicinal tea for colic spasms also used externally for inflamed or suppurating wounds
Micromeria graeca (L.) *subsp. graeca* (L.) Bentham ex Reichb. Boğumcuk	Zevfa Dağ çayı	Aerial parts	Infusion	Medicinal tea for skin disorders
Micromeria myrtifolia Boiss. et Hohen. Boğumlu çay	Zevfa Dağ çayı	Aerial parts	Infusion	Medicinal tea for skin disorders
Origanum syriacum subsp. bevanii (Holmes) Greuter & Burdet Hababa	Zahter Halil Halil İbrahim kekiği	Aerial parts	Infusion	Medicinal tea for colds-flu, cough, colic spasms and menstrual pains, and as spice
Lavandula stoechas subsp. stoechas L. Karabaş	Ebruh Eşek zahteri	Inflorescences	Infusion	Multipurpose medicinal tea
Salvia aramiensis Rech. Fil. Pohur	Adaçayı Buhur ağacı Yara otu	Leaves	Infusion	Medicinal tea for cough, cold and flu and diabetes
Salvia tomentosa Mill. Şalba	Adaçayı	Leaves	Infusion	Medicinal tea for cough, cold and flu and stomach pain
Sideritis libanotica subsp. *libanotica* Labill. Gevreğen	Ana baba bohuru	Aerial parts	Infusion	As appetizer, carminative and sedative medicinal tea
Sideritis syriaca subsp. *nusairiensis* (Post) Hub.-Mor. Endemic subspecies Amanos çayı	Amanos dağ çayı	Aerial parts	Infusion	Medicinal tea for cough, cold and flu and diabetes
Sideritis huber-morathii L. Endemic species Şenköy çayı	Şenköy çayı	Aerial parts	Infusion	Medicinal tea for cough, cold and flu and diabetes
Sideritis perfoliata L. Fincan çayı	Dağ çayı	Aerial parts	Infusion	Medicinal tea for digestive problems and colds-flu
Thymbra spicata var. *spicata* L. Zahter	Zahter	Aerial parts	Infusion	Multipurpose medicinal tea, One of the most important ingredients of local cuisine
Thymus cilicicus Boiss. & Balansa Kılçık kekiği	Dağ kekiği	Aerial parts	Infusion	Medicinal tea for digestive problems and colds-flu
Malvaceae Ebegümecigiller				
Alcea setosa (Boıss.) Alef. Hıtmiye çiçeği	Hıttayme Hatmi	Flowers	Decoction	Medicinal tea for cough
Lavatera punctata All. Saracak	İnce hatmi	Flowers	Decoction	Medicinal tea for cough
Tilia tomentosa Moench Gümüşi ıhlamur	Ihlamur	Flowers	Decoction	Medicinal tea for colds-flu

*Turkish plant names in the Table are based on Guner *et al.* (2012) and Anonim (2017).
*Türkçe bitki adları Güner ve ark. (2012) ve Anonim (2017) temel alınarak yazılmıştır.

Table 1. Continued.
Çizelge 1. Devam.

Family Aile*	Local names Yerel adlar	Used parts Kullanılan kısımlar	Preparation Hazırlama	Other ethnobotanical uses Diğer etnobotanik kullanımlar
Rosaceae Gülgiller				
Crataegus azarolus var. *aronia* (L.) Bosc. ex DC. Müzmüldek	Alıç Zaarur	Leaves and flowers	Decoction	Medicinal tea for jaundice and hypertension
Crataegus monogyna subsp. *monogyna* Jacq. Yemişen	Kırmızı alıç Masmus	Leaves and flowers	Decoction	Medicinal tea for palpitation and hypertension
Eriobotrya japonica (Thunb.) Lindl. Yenidünya	Yeni dünya Gidinya	Flowers	Decoction	Medicinal tea for cough
Rosa canina L. Kuşburnu	Kuşburnu	Flowers and fruits	Decoction	Medicinal tea for colds-flu, hemorrhoid and intestinal worms
Rosa x damascena Mill. Isparta gülü	Nisan gülü	Flowers	Infusion of petals or Rose water obtained by distillation of the petals	Rose water also used externally for skin diseases
Rubus sanctus Schreber Böğürtlen	Böğürtlen Dis	Fruit	Decoction or molasse	Besides fruits, flowers and leaves are used for preparing multipurpose medicinal teas
Violaceae Menekşegiller				
Viola odorata L. Kokulu menekşe	Menekşe Minefsec	Flowers	Infusion	Medicinal tea for cough

*Turkish plant names in the Table are based on Güner *et al.* (2012) and Anonim (2017).
*Türkçe bitki adları Güner ve ark. (2012) ve Anonim (2017) temel alınarak yazılmıştır.

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ANADOLU, J. of AARI
ISSN: 1300 - 0225
28 (1) 2018, 6 - 12
MFAL

Reassessment of Mentha Species from Kunhar River Catchment Using Morphological and Molecular Markers

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Received (Geliş tarihi): 08.06.2017 Accepted (Kabul tarihi): 02.02.2018

ABSTRACT: ***Mentha*** *specimens collected from Kunhar River catchment of Hazara region were analyzed through numerical and molecular markers. For numerical analysis twenty two traits were used. Dendrogram analysis of morphological traits assorted the 25* ***Mentha*** *collections into 4 groups viz, Group-A, B, C and D. Group-A showed 98% similarity (****M. longifolia****). Group-B showed 98% similarity (****M. spicata****). Group-C showed 92% similarity (****M. arvensis****) and Group-D showed 89% similarity (****M. royleana****). Molecular analyses were carried out through 11 RAPD primers. Primers amplification revealed high level of genetic diversity (0-100%) existed among the* ***Mentha*** *genotypes. Dendrogram analyses based upon the genetic distance estimates conferred the cluster analysis of the morphological traits. Furthermore, the variations among populations of* ***M. royleana*** *need further elaboration through additional marker assisted discrimination for establishing their taxonomic status.*

Key words: Mentha*, CTAB, DNA, PCR, RAPD, dendrogram, trait.*

INTRODUCTION

The genus *Mentha* of family Lamiaceae comprising 25-30 species is widely distributed in the temperate areas (Kokkini *et al*., 1995; Dorman *et al*., 2003; Celenk *et al.,* 2008). The herbaceous, perennial mint plants are cultivated for their essential oils (produced as secondary metabolites) characterized by strong odor and widely used for medicinal and aromatic purposes (Betts, 2001; Bowles, 2003; Broza *et al*., 2009; Pichersky *et al*., 2006). The quality and quantity of the oils may change according to climate, soil composition, plant organ, age and stage in the vegetative cycle (Masotti *et al*., 2003; Angioni *et al.,* 2006).

In Pakistan *Mentha* is represented by six species that is *Mentha pulegium*, *M. arvensis*, *M. longifolia*, *M. piperita*, *M. spicata* and *M. royleana* (Ali and Nasir, 1990; Hedge, 1994). Out of these six species, four species i.e. *M.longifolia*, *M. arvensis*, *M. royleana* and *M. spicata* are commonly found in Kunhar river catchment. The river Kunhar catchment is administratively placed in District Mansehra Khyber Pakhtunkhwa Province located on 34° 14' to 35° 11' N latitudes, and 72° 49' to 74° 08'E longitudes, spreading over an area of 4,579 km^2 (Figure 1). The catchment area is bound by Azad Jammu and Kashmir on the Eastern as well as on Southern side, Chilas and Gilgit on the North and Nandyar Khwarr-Siran River catchment in the West (Jan *et al*., 2008; Haq *et al*., 2010). Climatically the catchment lies in temperate region with distinct seasonal variation (Sultana and Qureshi, 2007).

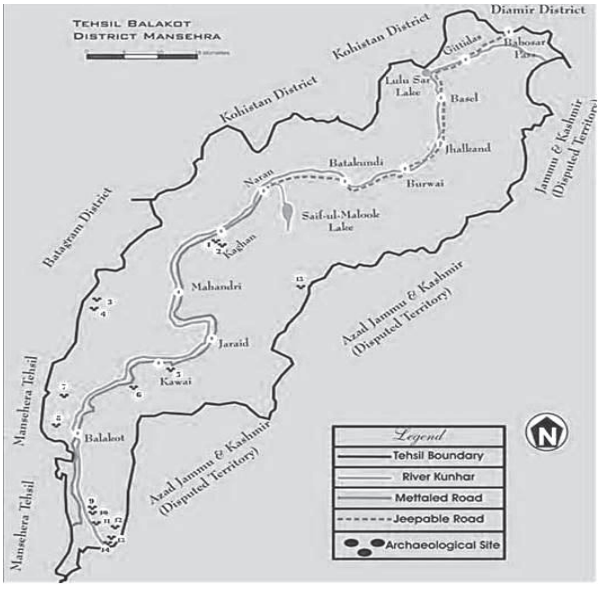

Figure 1. Map of Kunhar River catchment.

Classification of *Mentha* is very complex due to high polymorphism in morphology and great diversity in essential oil composition (Gobert *et al*., 2002). Different methods have been used in the past to examine the diversity of *Mentha* using morphological cytological, chromosomal and chemical markers (Malinvaud, 1880; Singh and Sharma, 1986; Harley and Brighton, 1977; Lawrence, 1978). The polymorphism of the genus needs careful taxonomic reassessment with modern technologies. The present research was conducted to optimize protocol for the DNA isolation from *Mentha* plants and to carry out DNA fingerprinting and phylogenetic elaboration of *Mentha* of Kunhar catchment through morphological and DNA markers.

MATERIALS AND METHODS

Plant specimens were collected from 25 different localities of river Kunhar catchment Mansehra Pakistan. The specimens were rightly pressed, dried, poisoned and mounted on the Herbarium sheets of size 28.75 cm x 32.50 cm. Field data including GPS coordinates, locality, and altitude were documented and submitted into Herbarium Women University of AJK, Bagh (WUAJKB) for future references. The specimens were provisionally identified with the help of Herbarium specimens and available literature (Stewart, 1972; Nasir and Ali, 1975; Xi- Ven and Hedge, 1994).

DNA isolation

The plant specimens were dried and then grinded with pestle and mortar. Genomic DNA was isolated with the help of modified protocols of Doyle and Doyle (1990) and Lodhi, *et al*. (1994). For DNA isolation 0.041 g of the powder material of each specimen were taken in 1.5 ml Eppendorf tube and added 500 µl of Cetyl tri methyl ammonium bromide (CTAB) buffer. The reagent was incubated for 35 minutes at 65°C, cooled at room temperature and added 500 µl of Chloroform: Isoamyl alcohol (24:1 v/v), mixed gently for 1-2 minutes and centrifuged at 6000 rpm for 15 min., supernatant was transferred to another eppendorf and was added to 250 µl of 5 M NaCl. The reagent was then added 500 µl of ice cold PCR grade ethanol and kept at 4-6 °C for 15-20 minutes. It was then centrifuged at 3000 rpm for 3 minutes and at 8000 rpm for 5 minutes. The supernatant was discarded and the pellet was washed with 70% ethanol. The DNA pellet then obtained was dried, dissolved in 20 µl TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0) and preserved at 4 ^{0}C. The DNA was treated with 1 µl RNaseA at 37 ^{0}C to remove the RNA. Quality and quantity of the DNA was checked on 1% agarose / TBE gel. For gel preparation 0.5 g agarose powder was dissolved in 50 ml TBE. The mixture was boiled on hotplate at 100 ^{0}C till the agarose was dissolved completely. The suspension was then cooled at room temperature. 5 µl Ethidium Bromide was added to it. The gel was casted in a Gel Tray with inserted comb. The suspension was allowed to solidify which was then placed in gel tank containing 1X TBE. For loading DNA, 3.00 µl DNA of each sample was mixed with 2 µl loading dye and was then loaded in the wells. Gel was then run at constant voltage of 80 volt and 120 mA for 30 minutes. The DNA was visualized under UV light using "Uvitech" Gel Documentation System.

RAPD analysis

The isolated DNA was checked with Gel electrophoresis and was subjected to dilution according to the quality and quantity of the DNA. The DNA which is visually concentrated for PCR reaction was diluted twice or thrice as needed. For each sample a pre-mix of 12.5 µl was prepared on ice for PCR. The pre-mix for PCR was prepared in 0.5 ml PCR tubes with 0.25 µl of each dNTP (10 mM), 0.5 µl of genomic DNA, 0.5 µl of primer, 1 µl of 10X buffer, 1 µl $MgCl_2$ and 0.5 µl of Taq Polymerase. The volume in the tube was made 12.5 µl by adding 8 µl of H_2O. The PCR reaction was carried out in Applied Biosystems 2720 thermal cycler. All the specimens were run independently with each primer for PCR amplification. Thermocycling condition of PCR was set as 94 ^{0}C for 4 minutes as denaturation temperature, 94 ^{0}C for 1 minute, 30 ^{0}C for 2 minutes, 72 ^{0}C for 1 minute. The cycling was continued for 35 cycles, which amplified a desired DNA sequence. The PCR product was mixed with 3 µl loading dye and electrophoresed in a 1.5 % Agarose gel mixed with Ethidium Bromide for 45 minutes to resolve the bands clearly. Eleven RAPD primers were used for DNA amplification. Details of the primers are given in Table 1.

Quantitative trait analysis

For numerical analysis hierarchical clustering was performed using the Euclidean distance index and Multivariate Cluster Analysis with the computer package MINITAB (Anonymous, 1996). Each taxon was treated as Operational Taxonomic Unit (OTU). Weightage was given to the key characters which are thought to be more informative than others (Abbott *et al*., 1985). For the analysis, total of twenty two characters were selected. These characters were assorted into two groups as quantitative characters and qualitative characters.

Quantitative characters

Seventeen quantitative characters including macromorphological and micromorphological characters were used for characterization. Macromorphological characters comprised of leaf length, leaf width, petiole length, spike length, pedicel length, bract length, peduncle length, leaf length of middle leaf, leaf width of middle leaf, Number of longitudinal veins per leaf and internode length, while micromorphological characterization comprised of length of sepal, length of petal, width of petal, length of style, length of anther and length of filament. For quantitative characters their average values were used (Abid and Qaiser, 2006).

Qualitative characters

Total of five qualitative characters were selected. Qualitative characters were included stem color, flower color, inflorescence, leaf dentation and leaf apex. Qualitative characters were recorded in binary state and in some cases in multiple states. The binary characters were recorded as 1 and 2. Multiple state characters were recorded as 1, 2, 3 and 4.

RESULTS AND DISCUSSION

The PCR amplification results produced from GLA- 11, GLA-15, Gt-2, Gt-4, Gt-5 and Gt-6 gave a total of 492 bands. Only 6 were monomorphic and the remaining 486 were polymorphic bands (98.7%). Gt-4 primer produced 102 (maximum) bands out of which 3 were monomorphic, Gt-5 produced 93 bands, Gt-6 produced 87 bands out of which 3 were monomorphic, Gt-2 gave 85 bands, GLA-11 gave 72 bands with 1 monomorphic while GLA-15 produced 51 (minimum) numbers of bands. The total accessions (1-25) of *Mentha* were clearly differentiated from one another. Further analysis of RAPD could clearly differentiate three varieties of *Mentha royleana* i.e *Mentha royleana var. afghanica, M. royleana var. royleana and M. royleana var. tugidus.*

Gel samples of the PCR amplification of 25 genotypes of *Mentha* collections (1-25) are provided in Figure 2, 3. The similarity coefficient matrix of 25 *Mentha* genotypes based on Multivariate Analysis is shown in Table 2. The

data of 6 RAPD primers using POPGENE 32 software to construct a dendrogram presented in Figure 4. On the base of dendrogram 25 genotypes were assorted into 4 groups (A, B, C, and D). Group A comprised of twelve genotypes; all of them belong to same species (*M. royleana*) but of different geographical regions. Group B comprised of nine genotypes of same species (*M. arvensis*). Group C comprised of two genotypes of same species *(M. longifolia*) and group D also comprised of two genotypes belong to same species (*M. spicata).*

Morphological similarity was estimated among 25 specimens of *Mentha* based on the numerical characterization using MINITAB software to construct dendrogram presented in Figure 5. 25 specimens of *Mentha* were assorted into 4 groups (A, B, C and D). Group A comprised of two specimens which show maximum similarity 98% belong to same species (*M. longifolia*). Group B also comprised of two specimens which also show maximum similarity 98% belong to same species (*M. spicata*). Group C comprised of nine specimens which show similarity up to 92% belong to same species (*M. arvensis*) and group D comprised of 12 specimens, which show similarity up to 89% belong to same species (*M. royleana*).

It is a common observation that isolation of high quality DNA from medicinal plants generally had troublesome due to secondary metabolites which directly or indirectly react with enzymatic reactions to reduce the yield and quality of extracted DNA (Weishing *et al*., 1995). Polysaccharides often react with DNA and thus reduce the action of restriction enzymes, DNA polymerase and ligase during DNA isolation (Sharma *et al*., 2002).

For over all genetic diversity studies, eleven Randomly Amplified Polymorphic DNA (RAPD) primers were used (GLA-11, GLA-15, GLA-18, GLB-12, GLB-14, GLC-20, Gt-2, Gt-4, Gt-5 Gt-6 and Gt-7). Among them, five primers (GLA-18, GLB-12, GLB-14, GLC-20 and Gt-7) did not optimize PCR. The RAPD primers produced different levels of genetic polymorphism. Over all genetic distances ranged from 0 to 66%. The findings strengthened earlier reports that RAPDs can be used for studying genetic polymorphism and tagging of useful genes. It is also evident from the PCR based assays, that RAPD-markers can be used effectively to estimate genetic variability in *Mentha* and could be considered as an easy diagnostic analysis for identifying the over lapping traits. It can also be noted that more molecular analysis is required to reach on a better conclusion regarding genetic variability and more detailed mapping of the *Mentha* genome.

Table 1. Basic information of RAPD primers used for DNA amplification.

S. No	Primer	Sequence (5'-3')	Polymorphic bands	Monomorphic bands	Polymorphism (%)
1	GLA-11	CAATCGCCGT	74	1	
2	GLA-15	TTCCGAACCC	51	-	
3	GLA-18	AGGTGACCGT	-	-	
4	GLB-12	CCTTGACGCA	-	-	
5	GLB-14	TCCGCTCTGG	-	-	
6	GLC-20	ACTTCGCCAC	-	-	
7	Gt- 2	TGCGCGATCG	85	-	
8	Gt- 4	GCGAATTCCG	102	3	
9	Gt- 5	GTGCAATGAG	93	-	
10	Gt- 6	GGATCTGAAC	87	2	
11	Gt- 7	GGACTCCACG	-	-	
Total			492	6	98.7

Source: Alpha DNA Company Canada.

Table 2. Means of Genetic Distances estimated in all the *Mentha* collections analyzed through DNA amplification.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	-																								
2	0.5	-																							
3	0.5	0.25	-																						
4	0.44	0.22	0.08	-																					
5	0.5	0.22	0.25	0.22	-																				
6	0.5	0.2	0.12	0.05	0.2	-																			
7	0.47	0.51	0.31	0.32	0.51	0.37	-																		
8	0.55	0.63	0.45	0.54	0.65	0.58	0.5	-																	
9	0.5	0.55	0.51	0.49	0.55	0.48	0.61	0.7	-																
10	0.5	0.53	0.45	0.38	0.53	0.33	0.42	0.25	0.6	-															
11	0.61	0.26	0.16	0.19	0.33	0.16	0.41	0.62	0.47	0.5	-														
12	0.55	0.16	0.16	0.19	0.26	0.16	0.41	0.62	0.55	0.5	0.13	-													
13	0.47	0.23	0.04	0.04	0.23	0.09	0.27	0.5	0.50	0.42	0.14	0.14	-												
14	0.44	0.44	0.30	0.22	0.44	0.27	0.15	0.54	0.54	0.38	0.41	0.41	0.26	-											
15	0.41	0.45	0.48	0.45	0.23	0.43	0.40	0.65	0.53	0.53	0.57	0.50	0.47	0.30	-										
16	0.44	0.42	0.28	0.2	0.42	0.25	0.26	0.54	0.52	0.38	0.39	0.39	0.24	0.11	0.38	-									
17	0.4	0.47	0.4	0.33	0.47	0.38	0.09	0.58	0.66	0.44	0.51	0.44	0.37	0.16	0.36	0.27	-								
18	0.47	0.40	0.20	0.21	0.40	0.26	0.16	0.5	0.56	0.42	0.30	0.30	0.16	0.24	0.45	0.31	0.26	-							
19	0.44	0.28	0.15	0.16	0.28	0.20	0.38	0.54	0.53	0.47	0.25	0.17	0.11	0.37	0.50	0.36	0.38	0.26	-						
20	0.55	0.5	0.5	0.52	0.6	0.5	0.47	0.29	0.66	0.16	0.46	0.33	0.47	0.52	0.6	0.52	0.5	0.47	0.44	-					
21	0.55	0.44	0.49	0.46	0.54	0.44	0.20	0.62	0.58	0.5	0.49	0.49	0.48	0.3	0.43	0.41	0.23	0.37	0.58	0.55	-				
22	0.41	0.23	0.48	0.45	0.45	0.43	0.40	0.65	0.61	0.53	0.50	0.40	0.47	0.42	0.22	0.38	0.36	0.45	0.50	0.5	0.33	-			
23	0.46	0.36	0.36	0.31	0.46	0.36	0.36	0.62	0.58	0.5	0.42	0.33	0.34	0.22	0.43	0.11	0.28	0.40	0.37	0.46	0.44	0.33	-		
24	0.5	0.2	0.45	0.42	0.42	0.4	0.23	0.65	0.66	0.53	0.46	0.36	0.43	0.33	0.38	0.44	0.2	0.40	0.55	0.5	0.16	0.16	0.38	-	
25	0.5	0.2	0.45	0.42	0.42	0.4	0.23	0.65	0.66	0.53	0.46	0.36	0.43	0.33	0.38	0.44	0.2	0.40	0.55	0.5	0.16	0.16	0.38	0	-

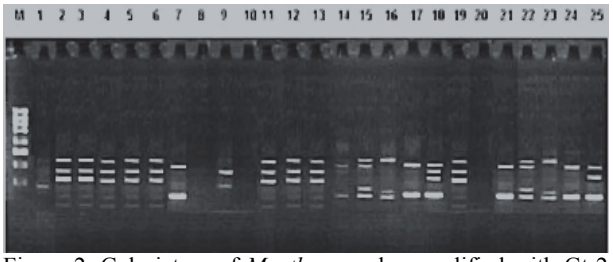

Figure 2. Gel picture of *Mentha* samples amplified with Gt-2 primer viewed under gel documentation system.

It is further evident from morphological study that numerical taxonomy can also be used as a best tool for identification in those species whose taxonomy is complicated by continuous polyploidy and stabilization of novel forms by ease of vegetative propagation. It is further noticed that there was no conclusion at varietal level in the present dendrogram. Therefore, it is strongly recommended that more morphological characters and molecular markers have to be used to refine the species up to varietal level.

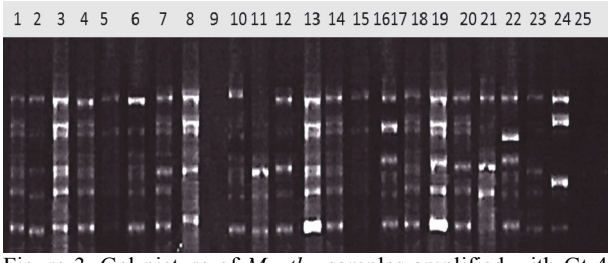

Figure 3. Gel picture of *Mentha* samples amplified with Gt-4 primer viewed under gel documentation system.

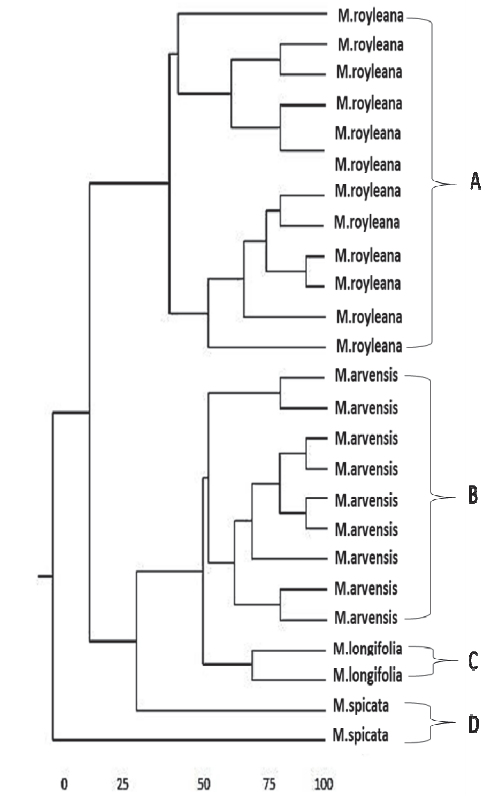

Figure 4. Dendrogram of *Mentha* collections obtained from RAPD-DNA bands analyzed by using Pop Gene 32.

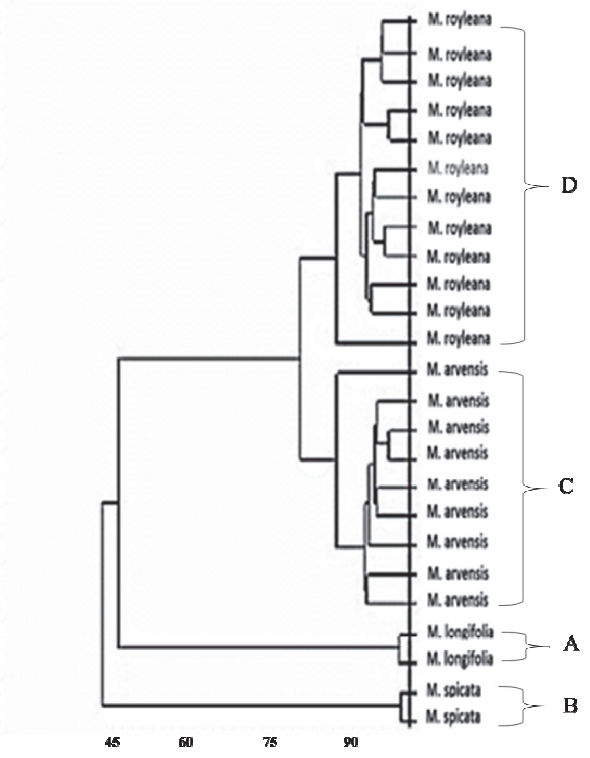

Figure 5. Dendrogram based on numerical data obtained *Mentha* collections computed by using MINITAB program.

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ANADOLU, J. of AARI
ISSN: 1300 - 0225
28 (1) 2018, 13 - 17
MFAL

Researches on Organic Black Myrtle Growing

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Received (Geliş tarihi): 20.04.2017 Accepted (Kabul tarihi): 30.01.2018

ABSTRACT: *Myrtle (**Myrtus communis** L.), is a characteristic plant of Mediterranean climate. The myrtle plant, known as 'Hambeles', which has a white and large fruit had been cultivated in the region for many years. The black myrtle plant on the other hand, grows wild in forest characterized by smaller fruit sizes. Up to now, there has not been any registered black myrtle variety in our country. The results of the observations made in Antalya province showed the presence of some black myrtle ecotypes with large fruits. The aim of this study was to examine the characteristics of black myrtle types; fruit yield and shoot growth performance of this plant grown in two different ecologies of Antalya province. The myrtle plant has been cultivated in two different ecological areas, such as high and low lands. The cultured types were budded on the wild plants under high land conditions by using the patch budding method, whereas under low land conditions, the plants were grown by using own-rooted cuttings. The number of shoots emerged from the patches ranged from 3.9 to 7.0. The shoot length increased up to 208 cm in second growing season, but decreased to 104 cm in the third year. Fruit yield has increased markedly from the third year and reached up to 10.6 kg / plant.*

Keywords: ***Myrtus communis*** *L., organic, black myrtle, yield, shoot growth.*

Organik Siyah Mersin Yetiştiriciliği Üzerine Araştırmalar

ÖZ: *Mersin bitkisi (**Myrtus communis** L.), Akdeniz ikliminin karakteristik bir meyvesidir. İri ve beyaz renkli meyveye sahip olan ve 'Hambeles' adıyla bilinen mersinlerin uzun yıllardır yetiştiriciliği bölgede yapılmaktadır. Siyah renkli mersinler ise doğada yabani olarak yetişmekte ve daha küçük meyvelidir. Siyah mersinde şimdiye kadar ülkemizde tescil edilmiş bir çeşit yoktur. Antalya yöresinde yapılan gözlemler sonucu nispeten iri meyveli tipler tespit edilmiştir. Bu çalışmanın amacı, farklı iki ekolojide yetiştirilen siyah mersin tiplerinin, meyve verimi ve sürgün gelişimiyle ilgili özellikleri incelemektir. Mersinler yayla ve sahil olmak üzere farklı iki ekolojide yetiştirilmiştir. Bitkiler yayla koşullarında yabani bitkiler üzerine kültür tiplerinin yama aşı metoduyla aşılanmasıyla tesis edilmiştir. Sahil koşullarında ise bitkiler çelikten yetiştirilmiştir. Yama aşılardan çıkan sürgün sayısı 3.9-7.0 arasında değişmiştir. Bu sürgünler ikinci yıl 208 cm kadar boy yapmışlardır. Üçüncü yılki sürgün uzunluğu ise en fazla 104 cm olmuştur. Meyve verimi üçüncü yıldan itibaren belirgin bir şekilde artmış ve 10.6 kg/bitki ye kadar yükselmiştir.*

Anahtar sözcükler*:* ***Myrtus communis*** *L., organik, siyah mersin, verim, sürgün gelişimi.*

INTRODUCTION

Myrtle (*Myrtus communis* L.) is an evergreen plant in the *Myrtaceae* family and contains about 100 genera and 3000 species. It grows in a wide geographical area from the Mediterranean basin to the northwest Himalayas (Surmaghi *et al*., 2014). In Turkey, the myrtle plant is cultivated in several parts of the coastal area, but the highest production areas are located in the Mediterranean region and are used for different purposes. The majority of the black and white colored fruits found in the nature belong to the wild myrtle plants. However, the

myrtle plant growing for fruit purposes named as 'Hambeles' type is mostly characterized by large and white colored fruit, this plant type is often obtained by means of grafting it on the wild myrtle plants growing at the edge of the fields. However, in this region, a few regular myrtle orchards could also be found. The black myrtle fruits are generally collected from the nature and consist of big fruits as well. Until now there has not been any work on the grafting of the big black-fruited myrtles on the wild myrtle type and shoot growth studies in Turkey. The myrtle plants have many benefits, in Turkey essential oils are often extracted from the leaves. There are very few works regarding the horticultural practices on myrtles plants in Turkey, but there are several works regarding the essential oil components research from fruits and leaves of myrtle plants. The vegetative propagation of myrtle plants are often carried out by methods such as grafting and cutting. There are a few literatures on the vegetative propagation of myrtles plants in Turkey (Uzun *et al*., 2014). Similarly, there are also very few studies on the establishment of black and white myrtle plant orchards as well as fruit yield relationship in Turkey. The majority of the myrtle plants selection researches are often obtained from the natural population. Under these natural conditions, the myrtle plants are exposed to harsh environmental conditions and for that reason, the plant growth and yield are often low as compared to selected domesticated types. A work carried out by Mulas and Cani (1999) regarding the myrtle shoot growth and development found the shoot length in the ranged of 2.8-19.2 cm. The myrtle plants often selected from the natural populations are usually used for the establishment of regular orchards. The planting distances of the black myrtle plants in the regular orchards were reported by Mulas *et al.* (2002b) to be 1x3.25 m. After three years of planting two varieties of myrtle plants in the regular orchards, they found that, the Barbara variety produced 0.8 kg fruit yield whereas, Daniela variety recorded 1.0 kg they also found the shoot length of the Barbara variety to be 11.4 cm whereas Daniela variety to be 11.6 cm. There are several criteria in the selection of the myrtle plants from the natural population, but in selection process, one has to take into consideration the fruit yield Mulas *et al.* (2002a). They did not provide any numerical value for fruit yield, but they categorized the fruit yield selection criteria of the myrtle plants into three groups as Low, Medium and High. As explained above, there are many gaps in the myrtle plant propagation methods and fruit yield researches. The purpose of this study was therefore, to examine the shoot growth and fruit yield performance after grafting of the black myrtle plant.

MATERIALS AND METHODS

The myrtle orchards were established in two different locations in the coastal and high hilly areas in Antalya province. The first location was the Yumaklar village under Aksu district in the hilly part of Antalya province. The graftings were carried out by using two genotypes namely, Yakup and Işlangıç black myrtles, which were grafted onto the wild types in nature by using patch grafting method in May 2011. The two orchards established in the Yumaklar village was carried out in two locations namely, Hayatözü with altitude of 354 m and Aktarla, 510 m high in Yumaklar village. The research was carried out by using three genotypes namely, Yakup and Işlangıç and Yumaklar black myrtles, which were propagated plant by using cuttings. The establishment of the orchard in the coastal area was carried out in the research plots in the Agricultural Faculty of Akdeniz University in the altitude of 50m in the year of 2010 by using planting distances of 3x3 m. Based on the results of the soil analysis, 3 ton/da fermented goat manure were applied to the soil. In all the three orchards established in both high hilly and the coastal part, no pest and disease problems were observed and for that matter, any pesticide and insecticide were not used. Additionally, in this study any chemical fertilizer didn't applied. The orchards established in the two different locations were organic in nature.

Grafting development: In the second year after grafting, the minimum and maximum shoot lengths were measured for monitoring shoot development performance. The shoot length measurements of the emerged grafted myrtle plants started in the third year after grafting and was measured from month to month (i.e. from May to December) until the experiment was terminated.

In the Yumaklar village myrtle plant orchards, which are wild in nature and established in nature, 10 plants were randomly chosen and grafted.

In the second location (coastal side), the orchard was established in a randomized block design with 3 replicates and every replicates has three tree.

Fruit yield: In the costal side, the orchard was established by using cuttings in 2010, Yumaklar village orchards on the other hand, were established on the hilly part of Antalya and by using patch grafting method. The fruits were harvested only from the coastal side because of the earlier plantation (in 2010) of the trees, but harvest was not carried out in the Yumaklar village because the trees were grafted in 2011.

It was determined that the data not normally distributed according to the results of the Kolmogorov Smirnov test. In order to make the parametric hypothesis tests the Rank transformation was performed for the data not Gaussian distributed. Then the analysis of variance was applied for comparing group averages and Duncan Test was performed for multiple comparisons (Narinc and Aygun, 2017).

RESULTS AND DISCUSSION

The results of the black myrtle types grafted on the wild myrtle shrubs in two locations of Yumaklar village, showing the numbers and shoot growth after two years of grafting were presented in Table 1 and Table 2. Additionally, because the myrtle types established in the plots (Akdeniz University research plots) were planted earlier on, the fruit yield could be monitored until three years. The yield values of the three years of myrtle types examined in the coastal area are provided in Table 3.

Table 1. The shoot growth of the grafted Yakup and Işlangıç black myrtle types in two locations in Yumaklar village in 2012.
Çizelge 1. Yumaklar köyünde iki lokasyonda aşılanmış Yakup ve Işlangıç siyah mersin tiplerindeki sürgün büyümesi (2012).

Locations Lokasyon	Yakup			Işlangıç		
	Shoot number (number / grafting) Sürgün sayısı (adet/aşılanan)	Maximum shoot length (cm) Mak. sürgün Uzunluğu (cm)	Minimum shoot length (cm) Min. sürgün Uzunluğu (cm)	Shoot number (number / grafting) Sürgün sayısı (adet/aşılanan)	Maximum shoot length (cm) Mak. sürgün Uzunluğu (cm)	Minimum shoot length (cm) Min. sürgün Uzunluğu (cm)
Aktarla	7.0	179.1	98.9	4.6	94.7	39.9
Hayatözü	5.3	208.0	78.4	3.9	153.9	90.7

Table 2. The total shoot length of the grafted Yakup and Işlangıç black myrtle types in Hayatöz location of Yumaklar village, third year after grafting in 2013, according to the months.
Çizelge 2. Yumaklar köyünde Hayatözü lokasyonunda aşılanmış Yakup ve Işlangıç siyah mersin tiplerinde aşılama sorası 3. yıldaki toplam sürgün uzunluğunun aylara göre gelişimi (2013).

Months Aylar	Shoot length / Sürgün uzunluğu (cm)			
	Yakup		Işlangıç	
	Minimum	Maximum	Minimum	Maximum
May / Mayıs	15.6	28.5	15.6	21.4
June / Haziran	25.2	36.1	21.0	35.4
July / Temmuz	31.3	48.8	30.7	77.0
August / Ağustos	33.5	57.2	33.5	92.6
September / Eylül	33.5	62.2	34.7	94.1
October / Ekim	33.6	66.9	34.9	102.2
November / Kasım	33.5	66.5	35.1	102.6
December / Aralık	33.5	66.9	35.1	104.6

Grafting Development

The values of the grafting development of the black myrtle orchard established in the high hilly area of Yumaklar village are provided below.

Generally, the most successfully grafting method on myrtle plant is patch grafting usually carry out in the month of May, because, during this month, the plant it is mostly active and it is easier to separate the bark from both the scion and the rootstock. The myrtle plant scions even with the same sizes of rectangular patches have a different number of dormant buds, and produce new shoots after grafting (Uzun *et al*., 2014). In this study, it was found that, the least number of shoots of the grafted Işlangıç genotype was 3.9 numbers per grafted plants in the Hayatözü location, whilest the highest shoot number of Yakup genotype was 7.0 numbers per grafted plants in Aktarla location (Table 1). There can be variations in the development in terms of plant length of the first shoots after grafting based on the plant vigor. Generally, in the first year of the shoot development, the plant usually develops single branch, sometimes there could also lateral shoots. For all the developed shoots, the average lengths of the shoots were not measured, instead, the minimum and maximum shoot lengths were measured, which can be useful for monitoring shoot development performance. For that reason, in this study, second year after grafting, the minimum shoot length of Işlangıç genotype was 39.9 cm in Aktarla location. The reason for these low shoot lengths recorded by the 'Işlangıç' genotype in Aktarla location, could be due to low soil fertility and steep slope nature of the land, whereas the maximum shoot length obtained by Yakup genotype was 208.0 cm in Hayatözü location. The differences between the maximum and the minimum shoot lengths of the myrtle plants in the same location of Hayatözü were about 3 times fold.

The results of the emerged shoots length from the grafted myrtle plants in the third year after grafting, was observed to increase progressively from month to month as shown in Table 2. As observed in this study, active shoot growth started in April, but the shoot length measurement was carried out in the month of May. The differences in the plant shoot length between the maximum and the minimum was low in the first months, but increased (2-3 times fold) progressively during the later months of shoot development. The least plant shoot lengths recorded by both genotypes in the May was 15.6 cm, whereas the highest shoot lengths were 21.4 and 28.5 cm in 'Işlangıç' and Yakup genotypes, respectively. The total shoot length in the December, which was the same as the harvest month recorded by the Yakup genotype was 33.5 and 66.9 cm for minimum and maximum, respectively. In the Işlangıç genotype it was 35.1 and 104.6 cm for minimum and maximum shoot length, respectively. The level of plant shoot growth in the third year after grafting decreased as compared to the growth during the second year. The reason for this decrease in the short length could be attributed to the rise or growth of the lateral shoot during the later period of plant development. There are very limited researches on the grafting and shoot development studies on the black myrtle plant in the World. In a related work carried out by Mulas *et al*. (2002b) in the spring season on the shoot length development of black myrtle plant, they found shoot length to between 11.4-11.6 cm depending on cultivars under consideration. The results of our study showed little high values with regards to the shoot development studies carried out by Mulas *et al*. (2002b). The reason could be due to ecological and genotypic differences carried in these two separate studies.

Fruit Yield

The fruit yield of all the three myrtle genotype plants established in the coastal part (Akdeniz University Agricultural Faculty Research plots), were obtained two years post-planting (Table 3). There was no statistical difference in fruit yield of all the three genotypes evaluated. The fruit yield in 2011 of all the two-year-old- genotypes were approximately 1 kg, the yield was found to increase sharply (7,670-10,600 g/plant) in the three-year-old plants in 2012, where as in 2013 it was found to be 7,666-9,226 g/plant (fourth year).

Table 3. Fruit yield of black myrtle plants obtained based on the years (coastal side) (g/plant).
Çizelge 3. Siyah Mersin bitkilerinin yıllara göre verimi (sahil kesimi) (g/bitki).

Year / Yıl		Mean±SE	P value
2011		940.56 ± 418.59 [b*]	
2012		9444.44 ± 418.59 [a]	0.000
2013		8242.22 ± 418.59 [a]	
Genotypes / Genotipler			
Işlangıç		5468.33 ± 418.59	
Yakup		6288.89 ± 418.59	0.084
Yumaklar		6870.00 ± 418.59	
Interaction / İnteraksiyon			
2011	Işlangıç	905.00 ± 725.02 [d]	0.000
	Yakup	1133.33 ± 725.02 [d]	
	Yumaklar	783.33 ± 725.02 [d]	
2012	Işlangıç	7666.67 ± 725.02 [c]	
	Yakup	10066.67 ± 725.02 [ab]	
	Yumaklar	10600.00 ± 725.02 [a]	
2013	Işlangıç	7833.33 ±725.02 [bc]	
	Yakup	7666.67 ±725.02 [c]	
	Yumaklar	9226.67 ±725.02 [abc]	

*Significant differences among the means were determined by Duncan's multiple range test and means with the same letter do not differ significantly at P≤0.05.
*Duncan testi ile tespit edilen ve aynı harfle gösterilen ortalamalar arasında önemli fark (P ≤ 0,05) yoktur.

The values obtained from closely-spaced black myrtle orchards in Italy by Mulas *et al*. (2002b) in the third year was 0.8 and 1.0 kg/plant, which was lower than the results obtained in this study. The differences observed in the fruit yields in the genotypes in these two separate studies could be due to differences in the genotypic structure, or the environmental conditions under which the genotypes were grown in Italy favors good plant growth and development as compared to the Antalya climatic condition Table 2.

CONCLUSION AND RECOMMENDATION

From the results of this study, we observed that the black myrtle plant grafted on to wild myrtle shrubs and the propagation by cutting method produced the successful results. The plant cuttings used to establish the orchard in this study, were obtained by using the cutting propagation method. The grafting method used in this study was successful, whereas the results of the shoot development were satisfactory. Furthermore, the vegetative propagated plants yielded early fruit set and at the same time provide large number of fruits per plant. We recommend that, further research on myrtle propagation should be carried out. In addition, in order to improve myrtle fruit cultivation, it is recommended that, the research on the regular myrtle orchards, should be established with the incorporation of practices such as irrigation, fertilization, and cultural practices in order close the research gabs.

ACKNOWLEDGEMENT

Part of this research was supported by Republic of Turkey Ministry of Food, Agriculture and Livestock with Project number TAGEM/10-ARGE-02.

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ANADOLU, J. of AARI
ISSN: 1300 - 0225
28 (1) 2018, 18 - 22
MFAL

The Density Status of Cyclotrichium niveum (Boiss.) Manden. & Scheng. Species Showing Natural Distribution in Adiyaman Province and the Comparison of Different Locality Populations in terms of Essential Oil Contents

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Received (Geliş tarihi): 17.04.2017 Accepted (Kabul tarihi): 23.01.2018

ABSTRACT: ***Cyclotrichium niveum*** *(Boiss.) Manden. & Scheng species which is a member of Lamiaceae family, being an endemic species to our country, is named as külotu (dag nanesi) by locals in its natural habitats and it is consumed as a herbal tea for various health problems. For this reason, a need has arisen to make such a study in order to determine the natural distribution and status of the plant within Adiyaman province borders. It has been determined that the species has a distribution near the west (860-963 m), and North-west 1040-1617 m of Nemrut Mountain and Ulubaba Mountain (1650-1750 m). Sociability and covering status in populations are averagely on 2 and 2 (22), respectively. In samples representing the population the plant height has varied between 27- 48 cm. Besides, it has been determined that essential oil contents of dry leaf are between 1.6- 3.6 % in the samples taken during the flowering period according to the distribution status and density of the plants. Taxon's IUCN danger category is "VU".*

Keywords: Cyclotrichium niveum*, covering, sociability, essential oil.*

Adıyaman İlinde Doğal Yayılış Gösteren Cyclotrichium niveum (Boiss.) Manden. & Scheng. Türünün Yoğunluk Durumu ve Farklı Lokalite Popülasyonlarının Uçucu Yağ Oranları Bakımından Karşılaştırılması

ÖZ: *Lamiaceae familyasının bir üyesi olan* ***Cyclotrichium niveum*** *(Boiss.) Manden.&Scheng türü ülkemiz için endemik bir tür olup, doğal yetiştiği alanlarda yöre halkı tarafından külotu (dağ nanesi) olarak isimlendirilmekte ve çeşitli rahatsızlıklar için bitkisel çay olarak tüketilmektedir. Bu nedenle, bitkinin Adıyaman ili sınırlarında doğal yayılış alanlarını ve bolluk- yoğunluk durumunu belirlemek ve farklı lokalitelerde bulunan popülasyonlarda uçucu yağ oranlarının karşılaştırılması amacıyla böyle bir çalışma yapma ihtiyacı doğmuştur. Türün Nemrut Dağı'nın Batısı (860-963 m), Kuzey Batısı 1040-1617 m) ve Ulubaba Dağı Batı yamaçlarında (1650-1750 m) yayılış gösterdiği belirlenmiştir. Lokalitelerdeki popülasyonlarda örtüş ve sosyabilite durumu ortalama sırasıyla 2 ve 2 (22) pozisyonundadır. Popülasyonu temsil eden örneklerde bitki boyu 27-48 cm arasında değişim göstermiştir. Bunun yanında bitkilerin yayılış durumları ve yoğunluğuna göre çiçeklenme döneminde alınan örneklerde kuru herbadaki uçucu yağ oranları % 1,6-3,6 arasında olduğu tespit edilmiştir. Taksonun IUCN tehlike sınıfı "VU" dur.*

Anahtar Kelimeler: Cyclotrichium niveum*, örtüş durumu, sosyabilite, uçucu yağ.*

INTRODUCTION

Turkey is a country with sources rich in terms of plant diversity and endemic species. Medicinal and aromatic plants among these sources have currently been used in several areas. Especially in recent years, the tendency towards these plants has increased due to various reasons, and has also continued to be increasing gradually. As in several countries in the world, unfortunately, in our country, most of the plants used as medicinal and

aromatic are picked from the nature. And this causes destruction of nature, and especially endemic plants face the danger of extinction.

Cyclotrichium genus is represented with 6 species in our country (*C. niveum, C. origanifolium, C. leucotrichum, C. stamineum, C. glabrescens,* and *C. longiflorum*). Two of them are endemic (*C. niveum and C. glabrescens*). One species is Mediterranean element (*C. origanifolium)*, and the other five species are Iranian-Turan phyto-geographical region elements (Davis, 1965-1988). Whereas *C. origanifolium* has a wide spread area in Mediterranean and Eastern Mediterranean regions rather than the other species, the other 5 species are known with a narrower spread in eastern and southeastern regions of Turkey. When the vertical distribution of this six species has been analyzed in relevant resources, the lowest level is noticed to be 1000 meter. Namely, *Cyclotrichium* species prefer high-altitude areas, and IUCN level of the taxa is "VU" (Vulnerable) (Ekim *et al*., 2000).

One of these species is *Cyclotrichium niveum* (Boiss.) Manden.&Scheng. *Cyclotrichium niveum* is a perennial, semi-brushy species endemic for Turkey as a member of Lamiaceae family growing up to 20- 50 cm on its natural area (Kaya and Baser, 1996). The plant is covered with dense white hair. Blooming period for the plant includes July and August. As the habitat, the plant prefers rocky and chalky slopes (step). It grows between 1200 and 1830 meter. The plant that is endemic for our country is Iranian-Turan phyto-geographical region element. Eastern Anatolia is the main spreading area for the plant, it does not grow out of our country, and the species' sample has been known from Turkey (B6 Malatya) (Davis, 1965-1988). The plant has a spread on C6 and C7 squares according to the Grid square system of Davis (1965-1988). It has an interrupted spread between approximately 1200 and 1830 meter within the provincial borders of Adıyaman, Malatya, Sivas and Erzincan.

The other localities the plant grows are 1400 m altitude on 40th km of Malatya-Gürün, 5 km southeastern of Darende at 1200 m, 5th km on Gürün-Sivas highway at 1700 m altitude, and at 1830 m altitude on Akdağ within the provincial borders of Malatya/Adıyaman and between Kemaliye and Kolsan. This species has been used as a herbal tea for flu, nausea and muscle pain complaints by the local people in the areas this plant grows (Gulcin *et al.,* 2008; Alim *et al.,* 2009; Göze *et al.,* 2010; İnan *et al.,* 2014). Because the smell of the plant resembles to the smell of mint, it is also called as "mountain mint, dog mint" by the local people (Baser *et al.,* 1996).

While investigating Ulubaba Mountain vegetation within the provincial borders of Adıyaman, Simsek (2015) reported that this species had a 10% and 40% covering status, and had 1 to 3 densities out of 5 in terms of its sociability. The presence of the same rates was also reported in the studies carried out in populations in Nemrut Mountain (Tel, 2009; Tel *et al.,* 2010). In the studies carried out upon *C. niveum*, 1.54-5.60% essential oil was determined in the plant (Baser et al., 1994; Cetinus et al., 2007; Gulcin *et al.,* 2008; Alim *et al.,* 2009; Goze *et al.,* 2010; Inan *et al.,* 2014). Inan and Tel (2014) determined in their study that essential oil rate in the plants they picked from different altitudes in western slopes of Nemrut Mountain (Adıyaman) varied between 4.1- 5.5%, and essential oil rate was affected from altitude. Furthermore, it was mentioned by some researchers that several positive or negative internal and external environmental factors were efficient upon the rate for essential oil (Telci *et al.,* 2009; Shahat *et al.,* 2012; Kirpik and Inan, 2016). In the studies carried out upon determining the effects of *C. niveum* essential oil, the essential oil was specified to have antioxidant (Baser *et al.,* 1994; Cetinus *et al.,* 2007), antispasmodic (Cetinus *et al.,* 2007), antimicrobial (Alim *et al.,* 2009; Gursoy *et al.,* 2009), and antiangiogenic (Goze *et al.*, 2010) effects.

In this study that was carried out within the provincial borders of Adıyaman located phyto-geographically on Iranian-Turan region, spreading

area, density, sociability and covering of *Cyclotrichium niveum* (Boiss.) Manden. & Scheng species; and essential oil rate in populations in the localities were determined and compared.

MATERIALS AND METHODS

In the study, the species of *Cyclotrichium niveum* (Boiss.) Manden.&Scheng spreading naturally within the provincial borders of Adıyaman was discussed as the material. The field survey was carried out in 2015, and the coordinates and the altitudes for the plant were determined.

The plant spreads on brown forest soils over the limestone bedrock. During the investigation of the plant ecology, the soil was determined to have nearly neutral basic pH value according to the physical and chemical analysis results of the soil sample taken from the sample parcel, and the value was measured to be 7.45. The texture class of the soil was determined to be "clayey-loamy." The data specified for the chemical structure of the soil were presented in Table 1 (Simsek, 2015).

Covering status

Braun-Blanquet (1932) measured the covering degree of each taxon in a parcel using a sample parcel. It started with "+," and each number between 1 and 5 in the scale had a % equivalent. Projection of the each canopy of each individual in the sample parcel was considered, and area covering rate was calculated. Presentation of the values related to each taxon in the sample parcel included 2 digits, and covering status was represented by the first of these.

Sociability

Braun-Blanquet (1932) measured the degree for the unity of the same taxon in the parcel with each other using a sample parcel. Each number in the scale between 1 and 5 had a density equivalent. Presentation of the each individual within the sample parcel included 2 digits, and sociability was represented by the second of these.

Essential oil rate

The plants of the species were observed to start blooming period in July. For that reason, the samples were started to be picked from the localities of blooming as of the beginning of July. After drying the picked samples under room conditions, leaf and scape were separated, and essential oil rates in the leaves were determined. For that purpose, the samples were boiled for 3 hours using Clevenger type tools according to the water vapor distillation method, and the essential oil measured volumetrically was transformed into percentage (Inan *et al.,* 2014).

RESULTS AND DISCUSSION

Spreading of the plants

The coordinates related to the spreading area of the species within the provincial borders of Adıyaman were presented in Table 1. The average length of the plants picked from the south of Nemrut Mountain (L_1) was 48 cm, the length of the plants picked from the southwestern (L_2) was 28 cm, and the length of the plants picked from the west of Ulubaba Mountain (L_3) was measured as 32 cm.

Table 1. Some results related to the chemical structure of the soil taken from the natural habitat of *Cyclotrichium niveum* plant.
Çizelge 1. *Cyclotrichium niveum* bitkisinin doğal yaşam alanlarından alınan toprağın kimyasal yapısına ilişkin bazı sonuçlar.

Name of the defined plant unity Tanımlanan bitki birliğinin adı	Fe (ppm)	Zn (ppm)	Mn (ppm)	Cu (ppm)	N (%)	pH	EC (µS/cm)	K_2O (kg/da)	Texture Tekstür
Cyclotrichido niveae / *Pennisetetum orientalis*	19.70	0.48	12.21	0.98	0.61	7.45	566	208.43	Clayey-Loamy

The coordinates, altitudes related to the plants, and some properties of the plants were presented in Table 2. L_1 location was 5 km away from Kahta Castle (Değirmenbaşı locality) on Malatya road on west of Nemrut Mountain (860-963m). Direction of the sample parcel was west. The rest of this road went up to Malatya-Nemrut Mountain highway reaching to Nemrut Mountain. L_2 location represented the location at 1040-1617 altitude that was 6-8 km away from the summit and that was on the Northwest of Nemrut Mountain. Directions of the sample parcels were west, southwest, and east. The species was noticed to be spreading on the right of the road from L_1 location to L_2 location (throughout nearly 10-20 km).

This unity was noticed away from 5 km from the west of Ulubaba Ziyaret Hill on L_3 location (Çelikhan). Slope of the area including the parcels where the samples were taken varied between 25% and 60%, and elevation from the sea level varied between 1650 and 1750 m. directions of the sample parcels were west, northwest, southeast and east.

Sociability and covering status

Covering status in the sample parcels and sociability grades varied between +1 and 33, and the sociability and covering in all localities we mentioned with their coordinates and we evaluated were the same and the value was 22 (20% and 40%). While calculating the density, population rate per square in the investigated localities was not calculated, and in subsequent studies, population rate in 1st, 3rd, 5th, 10th and further years would be followed.

Essential oil rate

There were differences between the essential oil rates of the samples taken from different localities. It was determined that there were decreases in essential oil rates as the altitude increased. The highest essential oil rate was determined as 3.6% in the plants in L_1 location, and the subsequent essential oil rates in the plants from the locations were 2.0% in L_2, and 1.6% in L_3 locations. Whereas the values determined in L_2 and L_3 localities were lower than the values of some researchers (Cetinus *et al,*. 2007; Alim *et al.,* 2009; Goze *et al.,* 2010; Inan and Tel, 2014), the essential oil rate we determined in L_1 location was between the values reported by the aforementioned researchers. It was reported by several researchers that various effects were efficient upon the essential oil rates in the plants (Telci *et al.,* 2009; Shahat *et al.,* 2012; İnan and Tel, 2014; Kirpik and İnan, 2016).

Table 2. Spreading and some properties of *Cyclotrichium niveum* (Boiss.) Manden. & Scheng. species within the provincial border of Adıyaman.
Çizelge 2. Adıyaman il sınırları içerisinde *Cyclotrichium niveum* (Boiss.) Manden. & Scheng. türünün yayılma durumu ve bazı özellikleri.

Localities Lokaliteler	Coordinate Koordinat	Altitude (m) Yükselti (m)	Plant Length (cm) Bitki Boyu (cm)	Essential Oil Rate (%) Uçucu Yağ Oranı (%)
West of Nemrut Mountain (L_1; Kahta) Nemrut Dağı Batısı (L_1; Kahta)	37°57'47" North 38°40'21" East	860 - 963	48	3.6
Northwest of Nemrut Mountain (L_2; Kahta) Nemrut Dağı Kuzeybatısı (L_2; Kahta)	38°00'16" North 38°45'22" East	1040 - 1617	28	2.0
West of Ulubaba Mountain (L_3; Çelikhan) Ulubaba Dağı Batısı (L_3; Çelikhan)	37°57'06" North 38°08'31" East	1650 - 1750	32	1.6

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ANADOLU, J. of AARI
ISSN: 1300 - 0225
28 (1) 2018, 23 - 29
MFAL

Estimation of Biodiversity of Eastern Black Sea Mixed Forests in Turkey

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Received (Geliş tarihi): 03.07.2017 Accepted (Kabul tarihi): 24.01.2018

ABSTRACT: *Biodiversity, the variety of life, has been recognized as one of the key components of environmental sustainability. Human actions, however, often lead to irreversible losses in terms of diversity of life on earth. So importance of measurement of biological diversity is increasing and need for biodiversity assessment methods to enable biodiversity to be measured is outlined. The biodiversity indices are statistical method which is planned to evaluate the variety of a data group consisting of different types of components. Eastern Black Sea Region of Turkey is rich in biodiversity compared to other regions and endemism is high. Eastern Black Sea forest is dominated by* ***Picea orientalis, Abies spp., Pinus sylvestris, Fagus orientalis, Quercus*** *ssp.****, Castanea satvia, Alnus glutinosa, Carpinus betulus*** *and* ***Fraxinus angustifolia****. Data were collected 40 temporary sample plots from the mixed stands of Karadeniz Technical University Research Forest in Eastern Black Sea Region. In this study, aim to compare biodiversity using biodiversity indices such as Shannon-Weaner Index, Simpson Index, MacArthur Index, Pielou Regularity Index. The calculations are based on species composition as well as basal area distribution.*

Keywords: *Biodiversity index, mixed forest, species composition, ratio of number of tree, ratio of basal area.*

Türkiye Doğu Karadeniz Karışık Ormanlarında Biyoçeşitliliğin Hesaplanması

ÖZ: *Yaşamın çeşitliliği olan biyoçeşitlilik, çevresel sürdürülebilirliğin ana bileşenlerinden biri olarak kabul edilmiştir. Bununla birlikte, insan eylemleri, çoğu zaman, yeryüzündeki canlı çeşitliliği açısından geri dönüşü olmayan kayıplara neden olmaktadır. Bu nedenle biyolojik çeşitliliğin ölçümünün önemi artmakta ve biyoçeşitliliğin ölçülebilmesi için özel yöntemlere ihtiyaç duyulmaktadır. Biyoçeşitlilik indeksleri, farklı bileşen türlerinden oluşan bir veri grubunun biyolojik çeşitliliğini değerlendirmek için geliştirilmiş istatistiksel bir yöntemdir. Doğu Karadeniz Bölgesi, biyoçeşitlilik bakımından diğer bölgelere göre zengin olup endemik tür sayısı fazladır. Doğu Karadeniz Bölgesi ormanları* ***Picea orientalis, Abies spp., Pinus sylvestris, Fagus orientalis, Quercus*** *ssp.****, Castanea satvia, Alnus glutinosa, Carpinus betulus*** *ve* ***Fraxinus angustifolia*** *türlerinden oluşmaktadır. Çalışmada kullanılan veriler, Doğu Karadeniz Bölgesi'ndeki Karadeniz Teknik Üniversitesi Araştırma Ormanı'ndaki karışık meşcerelerden alınan 40 adet geçici örnek alandan elde edilmiştir. Bu meşcerelerin Shannon-Weaner, Simpson, MacArthur ve Pielou Düzenlilik İndeksleri hesaplanarak biyoçeşitlilikleri karşılaştırılmıştır. Hesaplamalar hem sayı hem de göğüs yüzeyi bakımından türlerin karışım oranına göre yapılmıştır.*

Anahtar Kelimeler: *Biyoçeşitlilik indeksi, karışık meşcere, tür karışımı, ağaç sayısı oranı, göğüs yüzeyi oranı.*

INTRODUCTION

The earth is made up of ecosystems and ecological features which are supported by biodiversity; yet many people do not understand the meaning of biodiversity or what the impact of its loss would mean. The fact is that all the species of flora and fauna, including humans, are dependent on each other, and the extinction of any one of these species can trigger a domino effect on the other species, which are directly or indirectly depend on it.

Biodiversity has been shown to play a key role at all levels of the ecosystem service hierarchy (Mace *et al*., 2012; Gao *et al*., 2014). The diverse habitats

and microhabitats contained in forest ecosystems hold the majority of the world's terrestrial species (Ozanne *et al*., 2003; Gao *et al*., 2014). However, these biologically diverse systems are increasingly being threatened by deforestation and forest degradation via varied direct or indirect mechanisms (Singh *et al*., 2001; Dirzo and Raven, 2003; Gao *et al*., 2014). Therefore, conserving forest biodiversity has become a critical task at local, national and global level.

To discern the influence of forest management intervention on forest biodiversity conservation among management regimes, we need to explore the effects of environmental and human forest use variables on species richness, diversity and density (Hooper *et al*., 2005; Kalonga *et al*., 2016). Generally, easily accessible forests are more affected by human activities (Sassen and Sheil, 2013) depending on tree species (Ndangalasi *et al*., 2007); although effective forest management planning could reverse the situation (Ball, 2011; Kalonga *et al*., 2016).

Turkey is among few temperate countries with the highest diversity in its fauna and flora. Because of its special position between Asia and Europe, and Africa, fauna and flora comprises elements from these continents. Turkey is in the interface of three different biogeographically regions where consist from European-Siberian, Mediterranean and Irano-Turanian regions. Of the Euro-Siberian Biogeographical Zone contain Kolshic zone aka Eastern Black Sea. This is the climatic region with the highest rainfall and is largely covered with forests. Eastern Black Sea forest is dominated by *Picea orientalis, Abies spp., Pinus sylvestris, Fagus orientalis, Quercus ssp., Castanea satvia, Alnus glutinosa, Carpinus betulus* and *Fraxinus angustifolia*.

Various diversity indices are used to determine the biodiversity. Diversity index is a statistical method which is planned to evaluate the variety of a data group consisting of different types of components. Features of a population such as number of existing species (Richness), distribution of individuals equally (Evenness) and total number of existing individuals underlie the basis of diversity indices (Wilhm and Dorris 1968; Allan, 1975). Thus, any changes in any of these three features will affect the whole population, so that the diversity indices depending upon these features are used effectively to determine the changes in a population (Dügel 1995; Mandaville, 2002; Turkmen and Kazanci, 2010). In this study, aim to compare biodiversity using biodiversity indices such as Shannon-Weaner Index, Simpson Index, MacArthur Index, Pielou Regularity Index. The calculations are based on species composition and species distribution as well as basal area and number of tree.

MATERIALS AND METHODS

The data used in this study were collected from mixed forests in Karadeniz Technical University Research Forest in Eastern Black Sea Region of Turkey. Study area located 40° 48' 45" - 40° 43' 25" N and 39° 36' 41" - 39° 28' 39" E and average attitude 1420 m. In this area, from the various age and site classes 40 temporary sample plots which size range 400 m^2 to 800 m^2 were taken. Diameter at breast heights all trees was measured and identified to species in each sample plots. Ratios of basal area and number of tree to species in each sample plots were calculated. Shannon-Weaner Index, Simpson Index, MacArthur Index, Pielou Regularity Index were used for statistical analyses of biodiversity.

Shannon-Weaner Diversity Index

This is an index applied to biological systems by derived from a mathematical formula used in communication area by Shannon in 1948 (Mandaville, 2002). It's the most preferred index among the other diversity indices. The index values are between 0.0-5.0.

Results are generally between 1.5-3.5, and it exceeds 4.5 very rarely (Kocatas, 1992). The values above 3 indicate that the structure of habitat is stable and balanced but the values under

1indicate that there are pollution and degradation of habitat structure (Turkmen and Kazanci, 2010).

$$H = -\sum_{i=1}^{n} P_i \times \ln P_i \quad (1)$$

H: Shannon-Weaner Diversity Index

P_i: Ratios of each species according to basal area or number of tree

Simpson Diversity Index

It's a diversity indices derived by Simpson in 1949 (Mandaville, 2002). Simpson index values are between 0 - 1. But while calculating, final result is subtracted from 1 to correct the inverse proportion (Turkmen and Kazancı, 2010).

$$H = 1 - \sum_{i=1}^{n} P_i \quad (2)$$

H: Simpson Diversity Index

P_i: Ratios of each species according to basal area or number of tree

MacArthur Diversity Index

It was derived from Shannon-Weaner index by MacArthur in 1965.

$$H = \frac{1}{\sum_{i=1}^{n} P_i} \quad (3)$$

H: MacArthur Diversity Index

P_i: Ratios of each species according to basal area or number of tree

Pielou Regularity Index

It was derived from Shannon-Weaner index by Pielou in 1966. The ratio of the observed value of Shannon-Weaner index to the maximum value gives the Pielou Regularity Index result. The values are between 0- 1. When the value is getting closer to 1, it means that the individuals are distributed equally (Pielou, 1966; Turkmen and Kazanci, 2010).

$$R = \frac{H}{\ln S} \quad (4)$$

R: Pielou Regularity Index

H: Shannon-Weaner Diversity Index

S: Number of species

RESULTS

Biodiversity in 40 sample plots in mixed forest in Karadeniz Technical University Research Forest in Eastern Black Sea Region were calculated to use 1-4 formulas. In calculation, ratios of basal area and number of tree of species in sample plot were used. The calculation of one of the sample area has shown Table 1 and 2.

The values of Shannon-Weaner diversity index were between 0.38-1.35. The lowest value was for plot 495 and the highest value was for plot 467 (Table 3 and 4). All results were found under the expected ranges (1.5-3.5). Although plot 1007 has five species, it has lower value than plot 467. The reason of this is a behalf of one species has high ratio.

The values of Simpson diversity index were between 0.18-0.73. The lowest value was for plot 495 and the highest value was for plot 467 (Table 5 and 6). The result of this index and the result of Shannon-Weaner index were found highly resemble to each other. The values of the four plots (plot 467, 628, 339 and 745) which had the fourth highest values were the same in both indices.

Table 1. Biodiversity indices according to ratio of basal area in sample plot 214.
Çizelge 1. Göğüs yüzeyi oranına göre 214 nolu örnek alanın biyoçeşitlilik indeks değerleri.

Species Türler	Basal area Göğüs yüzeyi	Ratio of basal area Göğüs yüzeyi Oranı	Shannon-Weaner	Simpson	MacArthur	Pielou Regularity
Oriental spruce	1.61	0.72				
Oriental beech	0.62	0.28				
Oriental hornbeam	0.01	0.01				
Total	2.24		0.63	0.41	1.69	0.57

Table 2. Biodiversity indices according to using ratio of number of tree in sample plot 214.
Çizelge 2. Ağaç sayısı oranına göre 214 nolu örnek alanın biyoçeşitlilik indeks değerleri.

Species Türler	Number of species Türlerin Birey sayısı	Ratio of number of trees Türlerin birey sayısı oranı	Shannon-Weaner	Simpson	MacArthur	Pielou Regularity
Oriental spruce	23	0.58				
Oriental beech	15	0.38				
Oriental hornbeam	2	0.04				
Total	40		0.84	0.53	2.11	0.76

Table 3. Sample plots which have highest Shannon-Weaner index value.
Çizelge 3. En yüksek Shannon-Weaver indeks değerine sahip örnek alanlar.

Sample plot no Örnek alan no	Oriental spruce Ladin	Oriental beech Kayın	Oriental fir D.K.Göknarı	Alder Kızılağaç	Oriental hornbeam Gürgen	Hazelnut Fındık	Shannon-Weaner
Ratio of basal area (Göğüs yüzeyi oranı)							
467	0.24	0.30		0.32		0.14	1.35
1007	0.65	0.11	0.01	0.11	0.12		1.05
628		0.21		0.28	0.51		1.03
339	0.33	0.14		0.53			0.98
745	0.40	0.50			0.10		0.95

Table 4. Sample plots which have lowest Shannon-Weaner index value.
Çizelge 4. En düşük Shannon-Weaver indeks değerine sahip örnek alanlar.

Sample plot no Örnek alan no	Oriental spruce Ladin	Oriental beech Kayın	Oriental fir D.K.Göknarı	Oriental hornbeam Gürgen	Shannon-Weaner
Ratio of basal area (Göğüs yüzeyi oranı)					
463	0.19	0.81			0.49
246	0.86	0.12		0.02	0.48
718	0.05	0.87		0.08	0.45
1108	0.16		0.84		0.44
495	0.90	0.10			0.33

Table 5. Sample plots which have highest Simpson index values value.
Çizelge 5. En yüksek Simpson indeks değerine sahip örnek alanlar.

Sample plot no Örnek alan no	Oriental spruce Ladin	Oriental beech Kayın	Oriental fir D.K.Göknarı	Alder Kızılağaç	Oriental hornbeam Gürgen	Hazelnut Fındık	Simpson
Ratio of basal area (Göğüs yüzeyi oranı)							
67	0.24	0.30		0.31		0.15	0.73
628		0.21		0.28	0.51		0.62
339	0.33	0.14		0.53			0.59
745	0.40	0.50			0.10		0.58
971	0.33	0.11	0.56				0.57

Table 6. Sample plots which have to lowest Simpson index values.
Çizelge 6. En düşük Simpson indeks değerine sahip örnek alanlar.

Sample plot no Örnek alan no	Oriental spruce Ladin	Oriental beech Kayın	Oriental fir D.K.Göknarı	Alder Kızılağaç	Oriental hornbeam Gürgen	Simpson
Ratio of basal area (Göğüs yüzeyi oranı)						
272	0.82	0.08		0.10		0.31
1108	0.16		0.84			0.27
246	0.86	0.12	0.02			0.25
718	0.05	0.88			0.07	0.22
495	0.90	0.10				0.18

The values of MacArthur Diversity Index were between 1.22-3.73. The lowest value was for plot 495 and the highest value was for plot 467 (Table 7 and 8). Also result of this index and the result of Shannon-Weaner and Simpson index were found highly resemble to each other. The values of the four plots (no. of 467, 628, 339 and 745) which had the fourth highest values were the same in other (Shannon-Weaner and Simpson index) indices.

The values of Pielou Regularity Index were between 0.41-0.99. The lowest value was for plot 718 and the highest value was for plot 1050 (Table 9 and 10). Unlike other indices (Shannon-Weaner, Simpson and MacArthur indices) plot 467 doesn't have to highest value. The reason is that, Pielou is derived from Shannon-Weaner Diversity Index and even if the value of Shannon-Weaner index is high, due to the number of species is increased Pielou is decreased. In plot 465 and 1108, we found that the individuals evenly distributed into species.

Table 7. Sample plots which have highest MacArthur index values value.
Çizelge 7. En yüksek MacArthur indeks değerine sahip örnek alanlar.

Sample plot no Örnek alan no	Oriental spruce Ladin	Oriental beech Kayın	Oriental fir D.K.Göknarı	Alder Kızılağaç	Oriental hornbeam Gürgen	Hazelnut Fındık	MacArthur
Ratio of basal area (Göğüs yüzeyi oranı)							
467	0.25	0.30		0.32		0.13	3.73
628		0.21		0.28	0.51		2.63
339	0.33	0.14		0.53			2.46
745	0.40	0.50			0.10		2.40
971	0.32	0.12	0.56				2.30

Table 8. Sample plots which have to lowest MacArthur index values.
Çizelge 8. En düşük MacArthur indeks değerine sahip örnek alanlar.

Sample plot no Örnek alan no	Oriental spruce Ladin	Oriental beech Kayın	Oriental fir D.K.Göknarı	Alder Kızılağaç	Oriental hornbeam Gürgen	MacArthur
Ratio of basal area (Göğüs yüzeyi oranı)						
272	0.82	0.08		0.10		1.45
1108	0.16		0.84			1.34
246	0.86	0.12	0.02			1.34
718	0.05	0.86			0.09	1.29
495	0.90	0.10				1.22

Table 9. Sample plots which have highest Pielou Regularity index values value.
Çizelge 9. En yüksek Pielou Regularity indeks değerine sahip örnek alanlar.

Sample plot no Örnek alan no	Oriental spruce Ladin	Oriental beech Kayın	Oriental fir D.K.Göknarı	Oriental hornbeam Gürgen	Hazelnut Fındık	Pielou Regularity
Ratio of basal area (Göğüs yüzeyi oranı)						
1050	0.54		0.46			0.99
273	0.46	0.54				0.99
660	0.56	0.44				0.99
1029	0.60		0.40			0.97
467	0.24	0.30		0.32	0.14	0.97

Table 10. Sample plots which have to lowest Pielou Regularity index values.
Çizelge 10. En düşük Pielou Regularity indeks değerine sahip örnek alanlar.

Sample plot no Örnek alan no	Oriental spruce Ladin	Oriental beech Kayın	Oriental fir D.K.Göknarı	Alder Kızılağaç	Maple Akçaağaç	Ash Dişbudak	Oriental hornbeam Gürgen	Pielou Regularity
Ratio of basal area (Göğüs yüzeyi oranı)								
272	0.82	0.08		0.10				0.54
587	0.23	0.73			0.03	0.01		0.53
495	0.90	0.10						0.47
246	0.86	0.12	0.02					0.43
718	0.05	0.87					0.08	0.41

The increase in the distribution of species in the area in favor of a species has reduced the biodiversity indices (Table 11). While ratio of basal area of Spruce (L) %46 in sample plot 243, when this ratio increase to %90 in sample plot 495, biodiversity indices reduce.

Mean values of biodiversity indexes by species in sample plot shown Table 12. When number of species increased, biological diversity was also increased. But biological diversity decreased when number of species 4 and 5 because of lack of sample plots (Table 12).

CONCLUSIONS

Forest management include protect, develop and sustainability of biodiversity. So when forest managing, biodiversity is calculated using biodiversity indices. In this study, Shannon-Weaner Index, Simpson Index, MacArthur Index, Pielou Regularity Index were used for calculate to biodiversity.

As a result, as the number of species increase, as the distribution ratios of species approach each other, biodiversity indices have increased also. Biodiversity indices have increased in proportion to the number of species in regions where the ratio is behalf of a species. Biodiversity indices are higher in areas where the ratios are close to each other than areas have the ratios are distributed in behalf of one species. This result shows that, the distributions of ratios between species are important.

Table 11. Effect of species ratios on biodiversity indices.
Çizelge 11. Türlerin oransal dağılımının biyolojikçeşitlilik üzerindeki etkisi.

Sample plot no Örnek alan no	Oriental spruce Ladin	Oriental beech Kayın	Shannon-Weaner	Simpson	McArthur	Pielou Regularity
	Ratio of basal area (Göğüs yüzeyi oranı)					
243	0.46	0.54	0.69	0.50	1.99	0.99
495	0.90	0.10	0.33	0.18	1.22	0.47

Table 12. Average values of biodiversity indices regard to number of species.
Çizelge 12. Biyoçeşitlilik indekslerinin tür sayısına göre ortalama değerleri.

Number of Species Tür Sayısı	Shannon-Weaner	Simpson	MacArthur	Pielou Regularity	Number of sample plots Örnek alan sayısı
2	0.64	0.45	1.84	0.92	14
3	0.80	0.49	2.01	0.73	22
4	1.22	0.67	3.10	0.88	3
5	1.05	0.52	2.08	0.65	1

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ANADOLU, J. of AARI
ISSN: 1300 - 0225
28 (1) 2018, 30 - 36
MFAL

Intra-Genetic Variation within Olive Cultivar 'Nabali' in Palestine by Microsatellite and Random Amplified Polymorphic DNA

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Received (Geliş tarihi): 10.07.2017 Accepted (Kabul tarihi): 30.01.2018

ABSTRACT: *Over seventy olive trees (Nabali) from different regions in Palestine were used in this study. Intra-genetic variation within different olive Nabali variants were approved by SSR and RAPD markers. Four SSRs bands were monomorphic revealing a true-to-genotype of Nabali cultivar. Ten RAPD markers produced 60 reproducible bands with an average of 6 bands / marker. Only 24 were polymorphic. The percentage of polymorphic bands was 38% which is relatively high. Similarity matrix for studied populations ranged from moderate (0.610) for Jalkamous and Karawa Bani Zaid (2) to highly genetic similarity or even identity (1.000) in some cases as Aqraba and Aseerah (N). The interaction between different variants trends to be high. The effect of geographic location was absent in this study and has no significant contribution. Dendogram based on Jaccards coffiecnt revealed three main clusters, the biggest group consisted of the majority of variants including Bieta, Karawa Bani Zaid (1), Salfeet, Salfeet (h), Aqraba, Aseereh (N), Jalkamous, and Alaroub. Second group consisted of two variants Alaar and Nahaleen. The third group is containing only Karawa Bani Zaid (2). The relative high polymorphic bands of RAPD markers (40%) and moderate genetic similarity among different Nabali variants suggested attribution of genetic background. Selection for new traits within Nabali is suggested.*

Keywords*: Olive, Intra-Genetic Variation, SSR, RAPD.*

INTRODUCTION

Olive (*Olea europaea* L.) is as an important oil-producing crop in the Mediterranean region whose domestication occurred during the Chololithic period (5700–5500 years BC) in the Near-East (Zohary and Hopf, 1994). In Palestine, olive represents the most important fruit trees and growing for hundreds of years. Nabali is the most predominant cultivar and met around 75% of the total planted olive cultivars in Palestine. Nabali is adapted well with the environmental conditions with high productivity of olive oil (22-28%). The chemical parameters of its oil are met the International Olive Council (I.O.C.) standards for extra virgin olive oil except Δ-7-stigmastenolw which shows values higher than 0.5% (Qutub *et al*., 2010). Nabali is susceptible to olive leaf spot disease (*Spilocaea oleaginea*) and showed alternating bearing.

A high genetic diversity level and the presence of homonyms and synonyms cases observed in olive germplasm (Gomes *et al*., 2012), therefore, an efficient and rapid discriminatory methods are urgent. Molecular markers have been used successfully in olive characterization (Angiolillo *et al*., 1999; Khadari *et al*., 2003; Abdel hamid *et al*., 2012). Genetic variability among olive cultivars within a country was higher than olive cultivars from different countries (Belaj *et al*., 2003; Owen *et al*., 2005). Variability of 27 clones of the Portuguese olive cultivar was investigated by Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeat (SSR), and Inter Simple Sequence Repeat (ISSR) markers (Gomes *et al*., 2008), and the intra-variation within an olive cultivar has been approved (Muzzalupo *et al*., 2010; Ipek *et al*., 2012).

Though RAPD provides an inexpensive and reliable method for routine screening of large number of cultivars for olive germplasm collection (Belaj *et al*. 2004) and detecting genetic similarities in olive (Belaj *et al*. 2003). It's reproducibility still under question. Therefore, providing RAPD profile with other molecular technique could be a good option to support the obtained results. Recently, SSRs have become one of the most useful molecular markers in plant breeding, cultivar fingerprinting, and genome mapping genetic (Rallo *et al*., 2003).

In Palestine, genetic variation for Nabali has been studied and approved previously on eight olive trees from two sites only (Wiesman *et al*., 1998). No other studies were done to determine the intra-genetic variation within Nabali cultivar by using a large number of trees on large scale of area. Identification of genetic variation within Nabali could be a useful tool for breeding program in future in Palestine.

The objectives of this work are;

To identify by RAPD and SSR markers DNA fingerprints of Nabali cultivar, figure out the genetic relationship between them, and define the clones within the studied cultivar.

MATERIALS AND METHODS

Plant material consisted of fresh leaves of 72 trees tagged as Nabali cultivar. Trees were selected from thirty two villages located in eight governorates in Palestine (Table 1). The villages and trees were selected according to the extension agent's recommendations and knowledge. The samples were stored in paper bags in the field and at cold temperature in the lab until DNA extraction.

Genomic DNA was isolated from 100 mg leaf by ground into fine powder using pistil and mortar in the presence of liquid nitrogen. The leaf powder of each individual sample was then subjected to DNA extraction using DNeasy Plant Mini Kit (Qiagen). DNA quality was determined by visualization on 0.7% ethidium bromide agarose gel. Concentration of DNA for all samples was measured by S-30 spectrophotometer (BOECO, Germany) and uniformed to 20 ng/µl. Resulting DNA solutions were stored at -20 °C.

Amplification and genotyping four RAPD primers (opx-09, opj-05, opi-12, and opx-03) were tested (Table 2). All the 72 samples were amplified and analyzed with four RAPD primers. PCRs were performed in a total reaction mixture of 25 µL volumes containing 10 mM Tris-HCl, pH 8.2,50 mM KCl, 1.5 mM MgCl2, 200 mM primer, 0.2 unit of Taq DNA Polymerase (sigma) and 20 ng DNA. Amplifications were performed in PTC 100 thermocycler (MJ Research), initial denaturation at 94 °C for 1min, then 45 cycles at 92 °C for 1 min, 36 °C for 1 min, and 72 °C for 2 min and finally one cycle for 8 min at 72 C for elongation. Amplified products were fractionated by electrophoresis in 2% ethidium bromide Agarose gels (1× TAE buffer) and photographed.

Table 1. The Governorates and the villages where the leaves of Nabali olive trees were collected.

No	Governorate	Villages
1	Jenin	Biet Qad, dier Abu Daaf, Jalkamous, Maithloun, Sier, Alyamoun, Bourqeen, Yaabed, Arabeh, KuferRaa, and Markaa
2	Qalqelia	Jayous, BaqatAlhatab, and koufer Qadoum
3	Nablus	Qabalan, Aqraba, Biet Foureek, and Aseerah(N)
4	Tulkarem	Dier Ghosoun, Alaar, and Ras Rouman
5	Salfeet	Alwaraam, Salfeet[1], and Dierstya
6	Ramallah	Karawa Bani Zaid[2], Dier Gassaneh, and Biet Laqya
7	Bietlahem	Biet Jala, Nahaleen, and Tkouaa
8	Hebron	Alaroub and Dora,

[1]: In salfeet village two different olive variants were found. Thereafter in the text, salfeet and salfeet (h) used to distinguish between both.
[2]: In Karawa Bani Zaid village two different olive variants were found. Thereafter in the text, Karawa Bani Zaid (1), and Karawa Bani Zaid (2), used to distinguish between both.

Later on, in order to minimize the size of population (72 samples), only samples which showed at least a different RAPD profile bands or more were selected for regenotyping again with ten RAPD and six SSR primers in order to assess the genetic diversity (Table 2). Out of seventy two, eleven samples of Nabali were selected for second cycle of genotyping. SSR primers were described and used by Cipriani *et al*. (2002). SSR primers were amplified by PTC 100 thermocycler (MJ Research), initial denaturation at 94 °C for 2 min, then 35 cycles at 92 °C for 45s., 57 °C for 45s., and 72 °C for 45s., and finally one cycle for 8 min at 72 °C for elongation. Amplified SSR bands were fractionated by electrophoresis in 4% ethidium bromide garose gels (1×TAE buffer).

DATA analysis

Each SSR and RAPD fragments was treated as a unit character and was scored presence or absence of the band (1 or 0). The 1/0 matrix was prepared for all fragments scored and the data were used to generate Jaccard's similarity coefficients (1908) for RAPD bands depending on the following formula:

$$Sij = a / (a+b+c)$$

Where Sij: standard Jaccard between two individuals i and j;

a = bands shared by both individuals;

b = bands present in i but not in j; and

c = bands present in j but not in I

Jaccard's coefficients were subjected to unweighted pair-group method using arithmetical averages (UPGMA) to generate a dendrogram using linkage procedure. The RAPD data were analyzed using fingerprint analysis missing DATA (FAMD version1.25) (Schlüter and Harris, 2006).

RESULTS

The aim of this work is determining the genetic diversity within Nabali cultivar in Palestine. Seventy two variants of Nabali from different areas were scored with four RAPD markers. All produced RAPD profiles were selected and analysed in next step with SSR and RAPD markers. Eleven Nabali variants were selected as a result of first RAPD running.

Table 2. RAPD and Olive SSR primers characterized in the study.

Type of primers	primer	Primer sequences (5′ to 3′)	Size of sequenced alleles (bp)
RAPD	OPA-19	CAA ACG TCG G	
	OPK-16	GAG CGT CGS A	
	OPX 09	GGT CTG GTT G	
	OPJ-06	CTC AGT CGC A	
	OPZ-11	GGG AAT TCG G	
	OPF-06	CCA GGA GGA C	
	OPJ 05	CTC CAT GGG G	
	OPZ-07	TCG TTC CGC A	
	OPI 12	AGA GGG CAC A	
	OPX 03	TGG CGC AGT G	
SSR	UDO99-006	TCA GTT TGT TGC CTT TAG TGG A TTG TAA TAT GCC ATG TAA CTC GAT	172
	UDO99-008	AAA AAC ACA ACC CGT GCA AT AAA TTC CTC CAA GCC GAT CT	159
	UDO99-024	GGA TTT ATT AAA AGC AAA ACA TAC AAA CAA TAA CAA ATG AGC ATG ATA AGA CA	188
	UDO99-031	TAT CCT CTA TGT GGC GAT TTG GTT AAA AGC ATT GAT ACA	151
	UDO99-043	TCG GCT TTA CAA CCC ATT TC TGC CAA TTA TGG GGC TAA CT	174
	UDO99-039	AAT TAC CAT GGG CAG AGG AG CCC CAA AAG CTC CAT TAT TGT	170

Only four SSR markers produced readable bands (UDO99-006, UDO99-024, UDO99-039, and UDO99-043)**.** All SSR markers were monomorphic with the eleven Nabali variants. This outcome confirmed that all studied and selected olive trees were Nabali and any presented genetic variation due to intra-variation within Nabali rather than mis–selection with other cultivar. Ten RAPD primers produced 60 reproducible bands with an average of 6 bands / marker. Out 60 bands only 24 were polymorphic. The percentage of polymorphic bands was 40% which is relatively high.

Based on Jaccards coefficient, similarity matrix for studied populations was done. Similarity ranged from moderate (0.610) for Jalkamous and Karawa Bani Zaid (2) to highly genetic similarity or even identity in some cases such as Aqraba and Aseerah (N) (1.000) (Table 3). Interaction between different variants showed high trends. For example the interaction between Nabali variants from Jalkamous and Alaroub was high (0.925) the same trend was found between variants Salfeet and Salfeet (h) (0.953), Aqraba and Aseerah (N) (0.952). On other side some exceptions were found, the interaction for Nabali variants from Karawa Bani Zaid, Bieta and Alaroub was (0.675).

The effect of geographic location was absent in this study and has no significant contribution. For instance for two variants from Aqraba and Aseerah (N) the similarity was 1.000. As well variant from Nahaleen (in the south of Palestine) with variants from Akarab, Aseerah (N), and Alaar (North of Palestine) were 0.914, 0.914, and 0.912, respectively. On other side, Karawa Bani Zaid (1) and Karawa Bani Zaid (2) are two variants from the same village showed low genetic similarity (0.667). The high similarity in this study suggested a genetic similarity or even identity between different variants from different locations. This could be due to fact that these variants come from the same gene pool.

Dendogram based on Jaccards coffiecnt revealed three main groups (Figure 1), the biggest group consisted of the majority of variants including eight Nabali varints; Bieta, Karawa Bani Zaid (1), Salfeet, Salfeet (h), Aqraba, Aseerah (N), Jalkamous, and Alaroub. Second group consisted of two variants Alaar and Nahaleen. While the third group is contains only Karawa Bani Zaid (2).

DISCUSSION AND CONCLUSIONS

Two types of molecular markers were used in this study, SSR and RAPD markers. SSR markers were monomrphic and produced the same profile for all Nabali variants. In opposite, microsatellites were effective tool to discriminate on intra-varietal genotypes level (Muzzalupo *et al*., 2010; Ipek *et al*., 2012). The negative result of SSR may be due to the low number of used markers and the small size of the population. The same copies of profile of microsatellite for all samples confirmed that, all selected trees belong to the same olive cultivar Nabali. As well, any existed genetic variation within the olive population were due to the

Table3. Similarity matrix of Jaccard's coefficient of 11 olive trees (variants) in different sites in Palestine.

	1	2	3	4	5	6	7	8	9	10	11
1	1.000										
2	0.868	1.000									
3	0.610	0.667	1.000								
4	0.770	0.829	0.788	1.000							
5	0.925	0.892	0.675	0.842	1.000						
6	0.814	0.893	0.675	0.842	0.864	1.000					
7	0.878	0.917	0.692	0.865	0.864	0.826	1.000				
8	0.878	0.865	0.692	0.865	0.910	0.867	0.953	1.000			
9	0.829	0.914	0.730	0.914	0.860	0.864	0.952	0.952	1.000		
10	0.829	0.914	0.730	0.914	0.860	0.864	0.952	0.952	1.000	1.000	
11	0.780	0.757	0.722	0.912	0.773	0.778	0.860	0.860	0.857	0.857	1.000

Abberiviation: 1: Jalkamous, 2: Karawa bani zied (1), 3: Karaw abani zied (2), 4: Nahaleen, 5: Alaroub, 6: Bieta, 7:Salfeet, 8: Salfeet (h), 9: Aqraba, 10: Aseerah(N), and 11: Alaar.

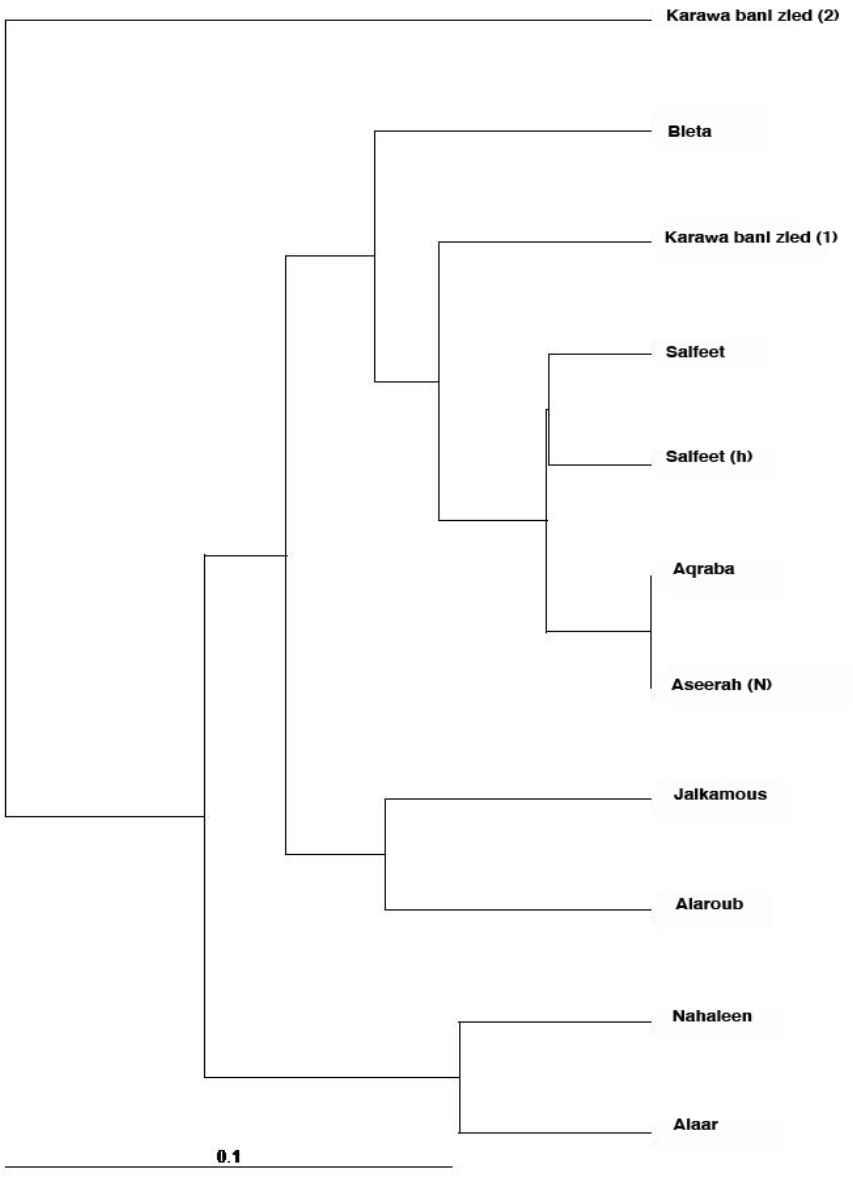

Figure 1. Dendogram based on Jaccards coefficient illustrating genetic similarity among eleven Nabali variants in Palestine based on 10 RAPD markers.

intra-genetic rather than inter-genetic variation level. In this study, RAPD were able to distinguish the genetic variation among different Nabali variants. Over seventy samples were screened with RAPD in first time. RAPD marker provides a cheap and fast tool for screening large population, which offers a good choice for researchers in developing countries (Belaj *et al*., 2003; Belaj *et al*., 2004). Combining two types of Markers SSR and RAPD in this study was good strategy to overcome the low reproducibility of RAPD.

Out of fifty eight, twenty two bands were polymorphic, the relative high polymorphic bands (40%) and moderate genetic similarity among different Nabali variants suggested attribution of genetic background. In Palestine, Nabali known as difficult rooting olive cultivar and grafting is the only and common vegetative propagation method. As well, sexual propagation is not used. Therefore, the variation due to somatic mutation within Nabli cultivar is possible since somatic mutations considered as an important source of intra-plant

genetic variation (Salomonson, 1996). Genetic contribution within Nabali variants or other olive cultivars was indicated before. (Wiesman *et al*., 1998; Muzzalupo *et al*., 2010; and Ipek *et al*., 2012). The low-moderate genetic variation obtained in this study could be due to two main reasons. First, the predominant propagation method for olive in Palestine is grafting. Second, Nabali is self-pollinated cultivar. Therefore, the maintenance of cultivar is expected and could lead to minimize the genetic diversity within Nabali olive trees in Palestine.

In this study, two Nabali olive trees from Akarab and Aseerah (N) were completely genetically identity (1.00). This may due to the fact that Aseerah is known as a high quality olive oil producer village in Palestine and containing very old olive orchards which back to the Roman period. Therefore, trees from Aseerah (N) are expected to be used as a good source for scions which are used in grafting in many olive orchards. Oppositely, the similarity for two Nabali trees from Karawa Bani Zaid was moderate (0.667). In other cases, the genetic similarity was high for two Nabali variants from too far distance areas, Nahaleen (south of Palestine) and Aqraba (North of Palestine) (0.914). Therefore, the geographic contribution in genetic variation within Nabali variants is absent and eliminated.

The intra- genetic variation within Nabali variants is approved in this study. Few groups or lines of Nabali were found. The main group contains the majority of studied lines (eight variants). These group representatives the predominant Nabali variant in Palestine. Other lines of Nabali were found in this study. The available of these lines or genotypes is less.

Because Nabali variant is adapted well to the local conditions and producing oil with high quality, Nabali still the preferred olive variant for farmers in Palestine. Nabali is known as susceptible to peacock disease and showed an alternative bearing phenomena. The fact that there intra-genetic variation is existed within Nabali variants, justifies the selection for new lines with promising traits. In this study, several new lines (variants) of Nabali were identified. These variants will be subject for more research in order to evaluate its performance against important agronomic traits such as, alternative bearing phenomena and resistant to peacock disease, consequently, could be integrated in future in national breeding program.

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ANADOLU, J. of AARI
ISSN: 1300 - 0225
28 (1) 2018, 37 - 44
MFAL

Identification and Evaluation of Propagation Techniques of Dianthus orientalis Adams.

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Received (Geliş tarihi): 29.05.2017 Accepted (Kabul tarihi): 15.02.2018

ABSTRACT: *The aim of the present study was identification and evaluation of propagation techniques of* ***Dianthus orientalis*** *Adams., a Turkish native plant which has good potentials as a landscaping plant with conspicuous pink flowers in autumn. Three different propagation methods were used in this study. These methods are the propagation of* ***D. orientalis*** *by seed, cuttings and* ***in vitro****. The seeds and cuttings of this species collected from wild were used as the experimental material. The germination rate of the seeds and the rooting rate of the cuttings were determined. An effective surface sterilization protocol for in vitro seed germination of* ***D. orientalis*** *was developed. The seeds were germinated on MS medium. Germination success of the seeds on MS medium was determined.*

Key words: Dianthus orientalis*, cutting, germination, rooting, seed,* ***in vitro.***

Dianthus orientalis Adams.'ın Üretim Tekniklerinin Belirlenmesi ve Değerlendirilmesi

ÖZ: *Bu çalışmanın amacı, Türkiye doğasında bulunan ve sonbaharda son derece dikkat çekici pembe çiçekleriyle peyzaj bitkisi olarak değerlendirilme potansiyeline sahip olan* ***Dianthus orientalis*** *Adams.(Yar karanfili)'ın üretim tekniklerinin belirlenmesi ve değerlendirilmesidir. Bu çalışmada üç farklı üretim yöntemi kullanılmıştır. Bu yöntemler* ***D. orientalis****'in tohumla, çelikle ve* ***in vitro*** *ortamda üretimidir. Deneme materyali olarak, bu türün doğadan toplanan tohumları ve çelikleri kullanılmıştır. Tohumların çimlenme oranı ve çeliklerin köklenme oranı belirlenmiştir****. D. orientalis****'in in vitro tohum çimlenmesi için etkili bir yüzey sterilizasyon protokolü geliştirilmiştir. Tohumlar MS ortamında çimlendirilmiştir. Tohumların MS ortamında çimlenme başarısı belirlenmiştir.*

Anahtar kelimeler: Dianthus orientalis*, yar karanfili, çelik, çimlenme, tohum,* ***in vitro.***

INTRODUCTION

The genus *Dianthus* L. belongs to Caryophyllaceae family and includes approximately 300 species distributed in Europe, Asia, Africa and North America (Reeve, 1967). The mainly center of genus diversity is the Mediterranean area (Bittrich, 1993). They are commonly known as pinks and carnations. The plants are annual, biennial or perennial. *Dianthus orientalis* Adams. is a perennial herbaceous species which has a wide distribution through South and East of Turkey. It is mostly seen in calcareous slopes, rocky areas, cliffs and lime-free rocks which have structure of the schist or gneiss and at altitudes ranging from 20 m to 200 m. It is a very attractive with pink flowers throughout autumn on 25-70 cm stems. Flowers are usually solitary, sometimes on the branches. It has a good potential of being landscape plant. This species is particularly blooming in the autumn when there are few flowers. It can also be a good erosion preventing plant on slopes, since it has extensive creeping woody base. Any scientific

research on propagation of *D. orientalis* was not found in literatures. Methods of propagation of this species should be known both in terms of conservation and utilization of its potential as an ornamental plant. Many *Dianthus* species can be propagated by seeds, cuttings and tissue culture.

Dianthus species such as *D. barbatus*, *D. chinensis*, *D. plumarius* and *D. gratinanopolitanus* were easily produced with both seed and cutting (Galbally and Galbally, 1997; Hartmann *et al.*, 2002).

Pre-treatments such as submersion in hot water, mechanical or chemical scarifications and hot air are used to break dormancy related to seed coat while the cold and warm stratifications are usually applied to break the dormancy caused by some restrictions at the embryo level (Landis *et al.*, 1996). Some enzymes and growth regulators, such as auxins, cytokinins and gibberellins have critical role on breaking dormancy and germination of seeds. Among these growth regulators, gibberellic acid has important influence on them (Riley, 1987). Exogenous GA stimulates amylase activity. Aleurone layer of endosperm is sensitive to GA (Fincher, 1989). GA also causes release of enzymes amylase and protease. These enzymes participate in the breakdown of stored starch to simple sugars (Gubler *et al.*, 1995). These sugars are then translocated to growing embryo where they provide energy for growth (Mayer and Poljakoff-Mayber, 1989).

Auxin-type growth regulators have been used to increase the percentage of cuttings that form roots, root initiation, increase the number and quality of roots per cutting and increase uniformity of rooting (Hartmann *et al*., 1990). Indole butyric acid (IBA) has been the most common rooting hormone for general use because it is generally not phytotoxic over a wide concentration range and is effective in promoting rooting a large number of plant species (Hartmann *et al.*, 1990). Exogenous auxin application improves rooting efficiency and quality of stem cuttings, while IBA and NAA stimulate adventitious rooting in cuttings (Copes and Mandel, 2000).

There is no previous report on propagation of *D. orientalis*.

The objective of this study was to determine rooting rate of their cuttings, germination success in greenhouse and *in vitro* propagation of *Dianthus orientalis* seeds, and to identify suitable propagation method for *D. orientalis.*

MATERIALS AND METHODS

Plant material: The cuttings and mature seeds of *D. orientalis* were collected from Kemer-Çamyuva-Turunçova locations (36^{o} 28’ 02’’E and 40^{o} 51’ 91’’N - 36^{o} 28’ 12’’E and 40^{o} 51’ 44’’N - 36^{o} 24’ 36’’E and 40^{o} 32’ 25’’N; 22-20-85 m, respectively) in Antalya Province. The seeds were removed from capsules and cleaned. Seeds with the same size, shape and color were selected for use in the experiments. In addition, their maturity was the same. In this study, the herbaceous cuttings of 7-10 cm length taken from the shoot tips of *D. orientalis* were used. The cuttings were transferred from the natural habitats in the ice box. Cuttings were taken on April.

Propagation techniques

Three propagation methods of *D. orientalis* were used in this study.

1. Rooting of cuttings: In this study, the herbaceous cuttings of 7-10 cm length taken from the shoot tips of *D. orientalis* were used. The cuttings were transferred from the natural habitats in the ice box. The basal ends of the cuttings were dipped into IBA solutions at 50, 100, 500, 1000, 2000 and 3000 mg/l for 10 seconds. Only the distilled water treatment was applied to the control group cuttings. After treatment, *D. orientalis* cuttings were planted into three different rooting media that contained peat, perlite and their mixture (1:1 / v:v). The study was carried out in a nursery belonging to a private company in Antalya. After 26 days from planting, the cuttings were removed from the rooting medium and rooting rates were determined. Each treatment had 3 replicates containing 20 cuttings.

2. Greenhouse seed germination: To determine the effect of pre-sowing treatment on seed germination, three different treatments were applied.

The treatments were;

i) Dipping in 10, 50, 100, 250, 500, 1000 and 2000 mg/l giberellic acid (GA_3) solutions for 24 hours,

ii) Soaking in hot water at 50, 60 and 70^oC for 2 minutes,

iii) Store at 5 and 10^oC for 20 and 40 days in the storage room. Control seeds were sown without any treatment.

The study was conducted in a commercial nursery in Antalya. The seeds were sown soon after fungicide (Thiram) treatment in styrofoam trays containing peat, perlite and their mixture (1:1 / v:v). A thin vermiculite layer was spread over cells of the trays to keep the moisture. The trays were irrigated and wrapped with plastic folia, before they were placed in a germination room which had 22 ^oC temperature, 60-70% relative humidity and darkness. In the third day, seeds started to germinate and then the trays were transferred to the nursery. Throughout the study, minimum temperatures were kept at 9 ^oC, maximum temperatures were at 33 ^oC and average relative humidity was 44 % in the greenhouse. Germination rates were recorded daily. Each treatment had 10 seeds in 3 replications.

3. *In vitro* seed germination: Pre-sowing treatments: In this study, three different pre-sowing treatments were applied in order to enhance *in vitro* seed germination:

i) Soaking in 0, 10, 50,100, 250 mg/l of gibberellic acid (GA_3) solutions for 24 hour,
ii) Soaking in hot water at 50, 60, 70^oC for 2 minutes and control,
iii) Storing at 5 and 10^oC for 30, 45, 60 days in a controlled storage room and control.

Sterilization: At first, pre-treated seeds were washed with tap water for 6 hours. Then, they were surface sterilized in 0.15% Benomyl (fungicide) including two drops of Tween 20 for 10 min and then rinsed three times in sterile distilled water. Finally, it was followed by surface sterilization with 10% sodium hypochlorite solution for 5 min. and again rinsed three times in sterile distilled water.

***In vitro* culture media and conditions:** The seeds were placed into glass jars, contained with 40 ml modified MS (Murashige and Skoog, 1962) basal nutrient medium. The cultures were maintained at 25^oC under a 16/8 h (light/dark) photoperiod with 3000 lux light irradiance provided by cool-white fluorescent tube. Germination rates were recorded daily. Each treatment had 3 replicates containing 10 seeds.

Statistical analysis: All the experiments were set up in a completely randomized design. Germination studies both in greenhouse and *in vitro* conditions had 3 replicates containing 10 seeds. Rooting study had 3 replicates containing 20 cuttings. Data were statistically analyzed using SAS (Anonymous, 1985). Means were compared by Duncan's multiple range tests at 5% probability (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Rooting of Cuttings

In this study, the effects of media ($P<0,001$), IBA concentrations ($P<0.001$) and media x IBA concentrations interactions ($P<0.01$) on rooting were found to be significant (Table 1).

The best result was obtained from cuttings which were treated with 2000 mg/l IBA and planted in peat medium (Figure 1). The rooting rate of these cuttings was 28%. Control cuttings gave the lowest rooting rate with 2%. The rooting of cuttings was found higher in peat medium than the other ones. In herbaceous cuttings, IBA treatments of 1000-2000 ppm usually yielded successful results (Johnson *et al.*, 2006). Pilon (2006) reported that *D. gratianopolitanus* 'Firewitch' cuttings were rooted in 3-4 weeks when they were immersed in 750-1000 ppm IBA solution and planted in the rooting medium. In rooting studies, perlite was the most unsuccessful medium in terms of rooting rate and seedling development.

Table 1. Effects of different IBA concentrations and growing medium on rooting of *D. orientalis* cuttings in the nursery conditions.
Çizelge 1. Anaçlık koşullarında *D. orientalis* çeliklerinin köklenmesi üzerine farklı IBA konsantrasyonları ve yetiştirme ortamlarının etkileri.

IBA (mg/l)	Rooting rate / Çelik köklenme oranı (%)			Mean Ortalama
	Growing medium			
	Peat	P+P	Perlite	
0	2 c[1]	5 b	0 b	2 C[x]
50	7 c	2 b	5 ab	5 C
100	3 c	8 b	3 ab	5 C
500	7 c	15 ab	2 ab	8 BC
1000	25 ab	10 b	3 ab	13 AB
2000	28 a	13 ab	7 ab	16 A
3000	13 bc	25 a	8 a	15A
Mean	12 A	11 A	4 B	

F values: Media: ****, IBA: ****, Media x IBA: ***.
Means in the same columns and lines followed by the different letters are significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).
, *, **** significant at 0.05, 0.01, 0,001 respectively. CV (%): 72,39.
F değerleri: Ortam: ***, IBA:****, Ortam x IBA***
Aynı satır veya sütunda farklı harfle gösterilen ortalamalar Duncan Testine gore istatistiksel olarak ($p \leq 0,05$) farklıdır.
, *, **** istatistiksel olarak sırasıyla 0,05, 0,01 ve 0,001 düzeyinde önemli. VK (%): 72,39.

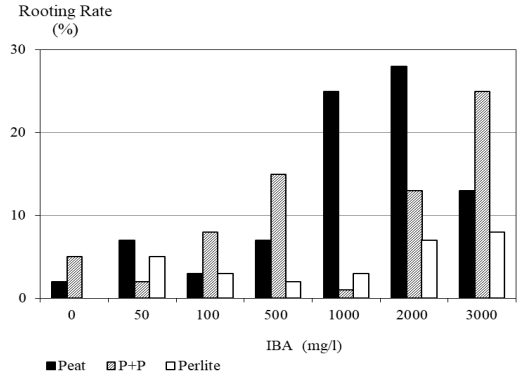

Figure 1. Effects on rooting of *D. orientalis* cuttings of the interactions between IBA and medium.
Şekil 1. IBA ve ortamlar arasındaki interaksiyonların *D. orientalis* çeliklerinin köklenmesi üzerine etkileri.

Greenhouse Seed Germination

The difference between GA_3 concentrations was found to be significant ($P<0.05$). On the other hand, the effects of media and media x GA_3 interaction on germination were not significant (Table 2).

2000 mg/l GA_3 applied seeds had the highest germination rate (30%) compared to other GA_3 applications and control. Germination rate was increased with increasing concentrations of GA_3, except for 250 mg/l GA_3 concentration, in the peat medium. The seeds which were applied with 2000 mg/l giberellic acid and sown in peat medium gave the best result with 40% germination rate (Figure 2). Similarly, pre-sowing treatment with GA_3 stimulated seed germination of Cleopatra mandarin and Rangpur lime rootstocks in comparison to control and the most successful treatment was 2000 ppm (Sharaf *et al.*, 2016).

Table 2. Effects of different GA_3 pre-sowing treatments and growing medium on germination of *D. orientalis* seeds in the greenhouse conditions.
Çizelge 2. Sera koşullarında *D. orientalis* tohumlarının çimlenmesi üzerine GA_3 ön uygulamaları ve yetiştirme ortamlarının etkileri.

GA_3 (mg/l)	Germination / Çimlenme (%)			Mean
	Growing medium			
	Peat	P+P	Perlite	
0	0.10 b[1]	0.23 a	0.20 a	0.18 AB[x]
10	0.10 b	0.20 ab	0.20 a	0.17 B
50	0.17 b	0.10 b	0.30 a	0.19 AB
100	0.17 b	0.17 ab	0.10 a	0.15 B
250	0.07 b	0.17 ab	0.13 a	0.12 B
500	0.17 b	0.03 b	0.17 a	0.12 B
1000	0.17 b	0.13 ab	0.23 a	0.18 AB
2000	0.40 a	0.37 a	0.13 a	0.30 A
Mean	0.17 A	0.17 A	0.18 A	

F values : GA_3:**, Media: NS, Media x GA_3: NS.
Means in the same columns and lines followed by the different letters are significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).
**, NS: Significant at 0.05 and non-significant, respectively. CV (%): 70.95
F değerleri: GA_3: **, Ortam: ÖD, Ortam x GA_3: ÖD
Aynı satır veya sütunda farklı harfle gösterilen ortalamalar Duncan Testine gore istatistiksel olarak ($p \leq 0,05$) farklıdır.
**, ÖD: Sırasıyla istatistiksel olarak 0,05 düzeyinde önemli ve önemli değil. VK (%): 70,95

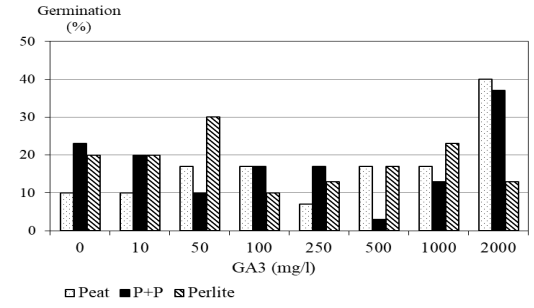

Figure 2. Effects on germination of *D. orientalis* seeds of the interactions between GA_3 and medium.
Şekil 2. GA_3 ve ortamlar arasındaki interaksiyonların *D. orientalis* tohumlarının çimlenmesi üzerine etkileri.

Ikinci (2014) also reported that in parallel with the increase in GA_3 dose, germination rate of argan seeds increased and 2000 ppm GA_3 was the best treatment. Also, positive effect of gibberellic acid

on germination of guava seeds has been reported by Kumar *et al.* (1991). The germination of GA_3 applied seeds in the other two media did not followed a regular course. When compared to the control, the germination rate in the mixture of peat and perlite decreased at lower doses of GA_3, and then increased at 2000 mg/l GA_3. However, there was a very irregular germination in the perlite. The control and GA_3 applied seeds of *D. orientalis* had the same average germination percentage in three media (17% for peat and peat+perlite, 18% for perlite).

The effects on germination of hot water treatments and the interactions between media and hot water treatments weren't found to have significant (Table 3). However, hot water treatment at 50°C for 2 minutes slightly increased germination rate of the seeds compared to the control, higher temperatures decreased the germination rate (Figure 3). In this study, many seeds treated with hot water rotted and died, indicating that they were very sensitive to high temperature.

Table 3. Effects of different hot water pre-sowing treatments and growing medium on germination of *D. orientalis* seeds in the greenhouse conditions.
Çizelge 3. Sera koşullarında *D. orientalis* tohumlarının çimlenmesi üzerine sıcak su ön uygulamaları ve yetiştirme ortamlarının etkileri.

Hot Water (HW) (°C)	Germination / Çimlenme (%)			Mean
	Growing medium			
	Peat	P+P	Perlite	
Control	23 a[1]	37 a	20 b	27 A[x]
50	17 a	33 a	43 a	31 A
60	13 a	30 a	0 b	14 A
70	27 a	33 a	6 b	22 A
Mean	20 AB	33 A	17 B	

F values: Media **, HW: NS, Media x HW: NS.
Means in the same columns and lines followed by the different letters are significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).
**, NS: Significant at 0.05 and non-significant, respectively. CV (%): 73.36
F değerleri: Ortam: **, Sıcak su: ÖD, Ortam x Sıcak su: ÖD
Aynı satır veya sütunda farklı harfle gösterilen ortalamalar Duncan Testine gore istatistiksel olarak ($p \leq 0,05$) farklıdır.
**, ÖD: sırasıyla istatistiksel olarak 0,05 düzeyinde önemli ve önemli değil.
VK (%): 73,36.

Sujatha and Manjappa (2015) reported that similarly hot water treatment (80°C for 10 minutes) was reduced the germination percentage of *Melia azedarach* significantly when compared to all other treatments. This might be due to lethal effect of higher temperature of water on embryo. Similar results reporting lethal effect of hot water was reported by Khantwal *et al.* (2008) who reported as low as 3% germination of *Bauhinia variegate* L. seeds in hot water treatment. Also, *Vitex doniana* seeds have a lower germination rate at high temperatures in hot water (Salisu and Jiya, 2016). Media were significantly ($P<0.05$) effective on seed germination rates. The tested seeds had the highest germination rates with 33% in the mixture of peat and perlite.

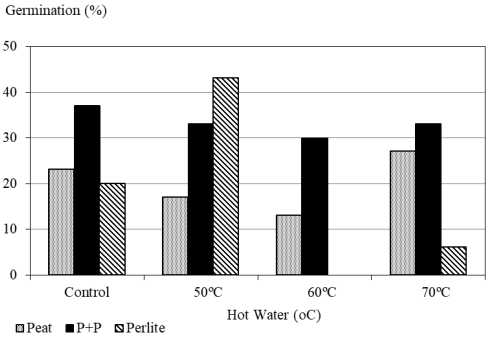

Figure 3. Effects on germination of *D. orientalis* seeds of the interactions between hot water and medium.
Şekil 3. Sıcak su ve ortamlar arasındaki interaksiyonların *D. orientalis* tohumlarının çimlenmesi üzerine etkileri.

Low temperature treatments ($P<0.01$) and its periods ($P<0.05$) had significant effect on germination. In this study, the control seeds (50%) had the highest germination rate (Table 4).

Low temperature treatments reduced germination rate of the seeds compared to the control seeds (Figure 4). This may be related to the growth of the species in coastal line and 20 m in height. Leo (2013) reported that similarly the cold stratification reduced the rate of seed germination in only one out of 28 species examined when they compared the control seeds.

Germination rate of the seeds were higher in the mixture of peat and perlite than that in the others. But there was no significant difference between the media. There was no significant difference in germination rates of the interactions between media, low temperatures and durations. The best

result of the experiment was obtained with 67% germination for the control seeds sown in the mixture of peat and perlite.

Table 4. Effects of different low temperature pre-sowing treatments and growing medium on germination of *D. orientalis* seeds in the greenhouse conditions.
Çizelge 4. Sera koşullarında *D. orientalis* tohumlarının çimlenmesi üzerine düşük sıcaklık ön uygulamaları ve yetiştirme ortamlarının etkileri.

Low Temp (LT) (°C)	Period (Days)	Germination/ Çimlenme (%) Growing medium Peat	P+P	Perlite	Mean
C	0	0.47 a[1]	0.67 a	0.37 a	0.50 A[x]
5	20	0.23 ab	0.23 b	0.30 a	0.23 B
	40	0.20 b	0.27 b	0.17 a	
10	20	0.36 ab	0.40 b	0.20 a	0.25 B
	40	0.23 b	0.17 b	0.13 a	
Mean		0.30 A	0.35 A	0.23 A	

F values: Period: **, LT: ***, Media: NS, Media x LT x Period: NS.
Means in the same collums and lines followed by the different letters are significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).
, *, NS; Significant at 0.05, 0.01 and non-significant, respectively. CV (%): 56.82.
F değerleri: Süre: **, Düşük sıcaklık: ***, Ortam x Düşük sıcaklık x Süre: ÖD.
Aynı satır veya sütunda farklı harfle gösterilen ortalamalar Duncan Testine gore istatistiksel olarak ($p \leq 0.05$) farklıdır.
, *, ÖD; sırasıyla istatistiksel olarak 0.05, 0.01 düzeyinde önemli ve ÖD
VK (%): 56,82.

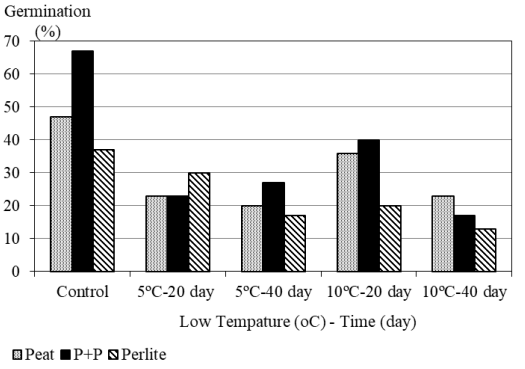

Figure 4. Effects on germination of *D. orientalis* seeds of the interactions between low temperature and medium.
Şekil 4. Düşük sıcaklık ve ortamlar arasındaki interaksiyonların *D. orientalis* tohumlarının çimlenmesi üzerine etkileri.

In almost all greenhouse seed germination studies, the lowest germination rate was obtained from the perlite medium. Seedling development was also poor in perlite. Peat and the mixture of perlite and peat were better than perlite in experiments.

In vitro Seed Germination

In vitro germination of seeds subjected to GA_3, hot water and low temperature pre-treatments were not statistically significant.

In vitro gibberellic acid pre-treatments gave a similar result to the nursery GA_3 treatments. When 10, 50 and 100 mg/l GA_3 concentrations were compared to the control, GA_3 prevented seed germination and caused a lower germination rate (Figure 5).

However the germination rate at 250 mg/l (50%), which was the highest dose of *in vitro* GA_3 treatments, was better than control and other GA_3 treatments. Srivastava *et al.* (2011) reported that GA_3 applications completely prevented seed germination in *Aconitum heterophyllum* and could not improve the seed germination even at low temperature. It is known that treatment of *Prunus mahaleb* seeds with gibberellic acid has been reported to overcome dormancy and ensure uniform germination (Al-Absi, 2010).

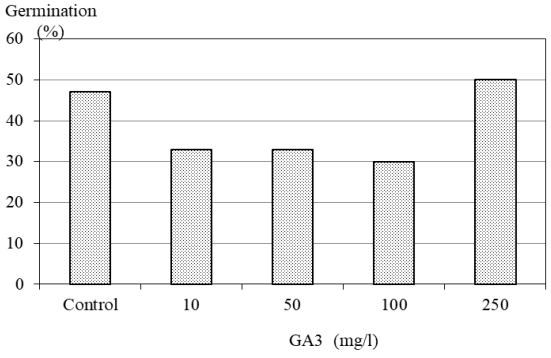

Figure 5. Effects of different GA_3 pre-sowing treatments on germination of *D. orientalis* seeds under *in vitro* conditions.
Şekil 5. *In vitro* koşullar altında *D. orientalis* tohumlarının çimlenmesi üzerine farklı GA_3 ön uygulamalarının etkileri.

After hot water pre-sowing treatment, *in vitro* germination graph of the seeds was similar to in vivo culture. Germination rate of seeds soaked in 50°C hot water was 40% and found to be higher than control (Figure 6). Increasing the application temperature caused reduction in the rate of germination. Hot water treatment at 70°C completely prevented seed germination. Sakhanokho (2009) reported that germination rates of 54% (*ex vitro*) and 95% (*in vitro*) were achieved when *Hibiscus dasycalyx* seeds were treated with hot water for 5 min, but exposing the seeds for 10, 15, or 20 min produced poor results in *H. acetosella* and *H. dasycalyx* as hot water

scarification appeared to result in severe injury or death of the embryos.

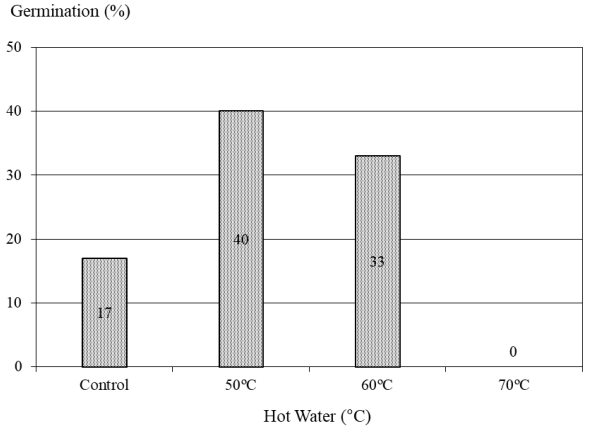

Figure 6. Effects of different hot water pre-sowing treatments on germination of *D. orientalis* seeds under *in vitro* conditions.
Şekil 6. *In vitro* koşullar altında *D. orientalis* tohumlarının çimlenmesi üzerine farklı sıcak su ön uygulamalarının etkileri.

In vitro low temperature pre-sowing treatments gave a different result from the nursery low temperature treatments. The highest germination rate with 47% was determined from seeds stored at 5^{o}C for 30 days (Figure 7).

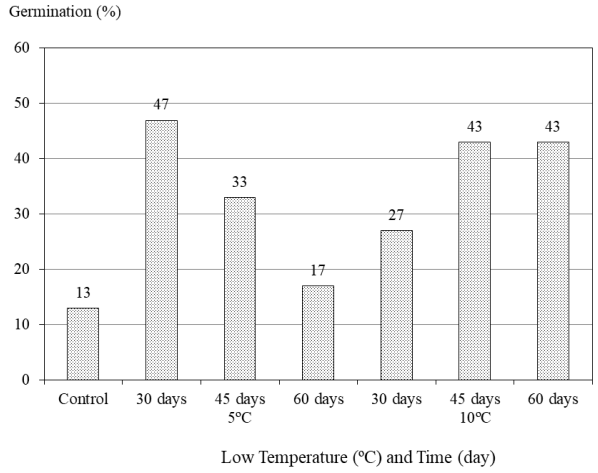

Figure 7. Effects of different low temperature pre-sowing treatments on germination of *D. orientalis* seeds under *in vitro* conditions.
Şekil 7. *In vitro* koşullar altında *D. orientalis* tohumlarının çimlenmesi üzerine farklı düşük sıcaklık ön uygulamalarının etkileri.

It was more successful than that seeds stored at 10^{o}C and the control. Similarly, Srivastava *et al.* (2011) reported that different low temperature applications of *A. heterophyllum* seeds had a higher germination rate than the control and that the highest germination rates were taken from the seeds that were kept at 15 ° C for 8 weeks.

In the study, it was determined that the germination rate increased at 5^{o}C as the storage period decreased, while at 10^{o}C, the germination rate of the seeds increased as the storage period increased.

CONCLUSION

This study was carried out in order to determine the propagation method of *D. orientalis* which is not studied much yet. For this purpose, the possibility of propagation by seed and cutting was investigated. Propagation by cutting was more unsuccessful than by seed. However, 28% rooting success could be achieved by cutting. This may be due to the thinness of cutting structure. The propagation by seed was carried out both in the nursery and *in vitro* conditions. Before sowing the seeds of *D. orientalis*, some pre-sowing treatments (GA_3, hot water and low temperature) were treated in both greenhouse and *in vitro*. Seeds showed mostly a maximum germination rate of 40% to 50% in all experiments in both of them.

In this study, perlite medium was unsuccessful in terms of both rooting rate and germination rate. Similarly, seedling development was also poor in perlite medium. This is due to the fact that perlite does not retain water as much as peat. For this reason, perlite must be watered frequently. Peat is known to work well with other components to provide better physical properties which are necessary for optimum plant growth. Therefore, peat or perlite and peat mixture as medium in the experiments was better than perlite.

As a result, *D. orientalis* can be propagated both by seed and by cutting. But it seems more appropriate to propagate it with seed. Along with planning new studies to increase the germination rate of seeds of this species, alternative production methods should be also tried.

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ANADOLU, J. of AARI
ISSN: 1300 - 0225
28 (1) 2018, 45 - 51
MFAL

Determination of Some Characteristics of Cocksfoot (Dactylis glomerata L.) Populations Collected from Natural Areas of Eskisehir for Breeding Purposes

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Received (Geliş tarihi): 13.04.2017 Accepted (Kabul tarihi): 23.01.2018

ABSTRACT: *The aim of the research is obtaining material and information for breeding of new varieties which can be used as pasture and forage plant in Central Anatolia and resembling regions. Some of the cocksfoot (**Dactylis glomerata** L.) seeds, collected from natural areas in 2010-2011, and were sent with passport information to the Gene Banks in Ankara and Izmir. The rest of the seeds were grown in greenhouse and then transfered to the field. In 2012, 32 populations were identified by observing (according to 1-9 and 1-5 scale) and measuring some characteristics. Then, mean and standard error values of the obtained data were determined. The mean values of the populations in main stem length, main stem thickness, flag leaf length, flag leaf width, number of nodes, internode length, growth pattern, rust resistance, winter resistance and tillering capacity changed between 43.9-72.4 cm, 2.0-3.3 mm, 7.8-16.0 cm, 2.9-5.4 mm, 2.3-4.5, 7.5-16.0 cm, 6.4-8.8, 6.6-8.0, 4.7-8.3 and 5.4-8.1, respectively. The relations between the characteristics were examined by correlation analysis. Furthermore, ploidy levels of some single plants selected from the natural populations for breeding purposes were determined by flow cytometry analysis and all of these plants studied were found tetrapolid.*

Key words: *Cocksfoot, **Dactylis glomerata** L., collection, breeding, correlation, ploidy.*

Eskişehir'de Doğal Alanlardan Toplanan Domuz Ayrığı (Dactylis glomerata L.) Populasyonlarında Islah Yönünden Önem Taşıyan Bazı Özelliklerin Belirlenmesi

ÖZET: *Araştırmanın amacı, Orta Anadolu ve benzeri ekolojilerde yürütülen mera ıslah çalışmalarında kullanılabilecek yeni çeşitlerin geliştirilmesi çalışmalarına materyal ve bilgi üretmektir. 2010 ve 2011 yıllarında doğal alanlardan toplanan domuz ayrığı (**Dactylis glomerata** L.) tohumlarının bir kısmı durak bilgileri ile birlikte Ankara ve İzmir'deki Gen Bankalarına gönderilmiştir. Tohumların kalan kısmı serada fide haline getirildikten sonra araziye aktarılmıştır. 2012 yılında toplam 32 populasyon gözlem (1-9 ve 1-5 skalaları) ve ölçümlere tabi tutularak bazı özellikleri belirlenmiştir. Daha sonra verilerin ortalama ve standart hata değerleri saptanmıştır. Populasyon ortalama değerleri ana sap uzunluğunda 43,9-72,4 cm, ana sap kalınlığında 2,0-3,3 mm, bayrak yaprak boyunda 7,8-16,0 cm, bayrak yaprak eninde 2,9 -5,4 mm, ana saptaki boğum sayısında 2,3-4,5 adet, ana saptaki boğum arası uzunluğunda 7,5-16,0 cm, büyüme şeklinde 6,4-8,8, pasa dayanıklılıkta 6,6-8,0, kışa dayanıklılıkta 4,7-8.3 ve kardeşlenme potansiyelinde 5,4-8,1 arasında değişmiştir. Özellikler arasındaki ilişkiler korelasyon analizi ile incelenmiştir. Ayrıca ıslah çalışmaları için seçilen tek bitkilerin ploidi düzeylerinin saptanması amacıyla flow sitometri yöntemi ile analiz gerçekleştirilmiş ve çalışılan tüm bitkilerin tetraploid olduğu belirlenmiştir.*

Anahtar sözcükler: *Domuz ayrığı, **Dactylis glomerata** L., toplama, ıslah, korelasyon, ploidi.*

INTRODUCTION

Although flora of Turkey is quite rich in terms of pasture grasses, imported varieties are used in pasture establishment, generally. There have been difficulties in adapting these varieties to different ecological conditions of the country (Oral and Acikgoz, 2002). Use of imported varieties is an important problem for the country's economy, as well. For these reasons domestic varieties are needed which are adaptable to different ecological conditions of the country. Collection and characterization of ecotypes widely found in natural areas of the country is an important resource for the development of ecologically adaptable varieties. It is reported that these natural ecotypes could be used in variety development studies using selection method (Tosun, 1973).

Coksfoot (*Dactylis glomerata* L.) is a perennial cool season grass used as pastures and forage plant. The species, which is resistant to drought and cold, can also tolerate shadow to a certain extent. The species show a rapid spring development (Manga *et al*., 2002). It is an economically important species with high yield as pasture and forage crop. When it is grazed or harvested in vegetative period, its feed quality and taste is high. Even when cultivation conditions are not ideal, it is easy to establish and grow rapidly. Cocksfoot is very resistant to cutting and grazing and is also used in erosion control (Alizadeh and Jafari, 2011).

MATERIALS AND METHODS

In this study, there have been presented some results of observations and measurements conducted in the ecotypes collected from natural areas to provide sources for breeding studies of pasture and forage type cocksfoot varieties for Eskisehir and similar ecologies.

The collected materials were characterized in terms of some properties and some single plants were selected for breeding purposes. Then, polidy levels of the collected material were determined using flow cytometer method. It has been reported that determination of ploidy levels of ecotypes collected from natural areas is important when these plants are used in plant breeding studies (Hatipoglu *et al*., 1994). Rapid and reliable results are obtained with flow cytometry in cases where the ploidy level of a large number of plants, such as plant genetic resources, need to be determined. The method is widely used in determining DNA content and ploidy level of many grass species (Wang *et al*., 2009).

The collection and characterization studies were carried out in 2010, 2011 and 2012 in Eskisehir Province and at the Central Field of Transitional Zone Agricultural Research Institute. Climate data were given in the table below (Table 1). According to long term climate data, the area receives 347 mm precipitation and experiences an average monthly temperature 10.8 °C and 77.7% relative humidity. In the years our research was conducted, precipitation and average relative humidity were higher than long term data.

According to the analysis results of the soil taken from the germplasm area, experimental area is slightly alkaline, clayey, with medium limy, slightly salty, rich in potassium and rich in phosphorus (Table 2).

In the collection study in 2010, single plant seeds were collected from the stations determined for each 10 km considering altitude and direction in Eskisehir Province (Alan, 1986). In addition, they were collected in sheltered areas such as village cemeteries where these plants are concentrated. In the study, the factors such as soil type, soil slope, soil appearance, and their relation with vegetation were considered. Selections also were made according to the characteristics such as general appearance of plant (upright, decumbent etc.), color, and plant height. During the collection, information such as coordinates, altitude, region or local name and distance to the nearest settlement were recorded (Table 3).

The collected seeds divided into 3 parts and one part was sent to the Gene Banks in Izmir and

Ankara with the station information data. One part has been preserved in the Institute. The rest of the seeds were grown in greenhouse and the seedling transferred to field separately for each single plant. There were 20 plants for each single plant (population) collected from natural areas but some were lost due to winter and diseases.

These populations were subjected to the observations and measurements:

Main stem length (cm), main stem thickness (mm), flag leaf length (cm), flag leaf width (mm), number of nodes, internode length (cm), growth pattern (1: oblique – 9: upright), rust resistance (1: susceptible – 9: resistant), winter resistance (1: susceptible - 9: resistant), and tillering capacity (1: poor – 9: abundant) (Tosun, 1973; Tokluoglu, 1979; Demiroglu *et al*., 2008; Anonymous, 2016).

The obtained data were subjected to statistical analysis and population averages and standard errors were determined. Standard error values were determined to indicate whether there is a variation between the single plants in population. In addition, a correlation analysis was conducted to determine the relations between the characteristics (Kackar and Harville, 1984; Pearson, 1920).

The selected plants for breeding purposes according to the observations and measurements were subjected to a ploidy analysis using flow cytometer method in 2015. In the method, ploidy levels were determined using the core DNA content of plants. Flow Cytometer device in Trakya University, Agricultural Faculty, Field Crops Department was used in the analysis which determines DNA content rapidly and accurately (Wang *et al*., 2009).

Table 1. Monthly precipitation, mean temperature and relative humidity in Eskisehir, Turkey.
Çizelge 1. Eskişehir İli aylık toplam yağış, ortalama sıcaklık ve oransal nem değerleri, Türkiye.

Month Aylar	Precipitation (mm) Toplam yağış (mm)			Temperature (^{0}C) Ortalama sıcaklık (C^0)			Relative humidity (%) Ortalama oransal nem (%)		
	2010	2011	2012	2010	2011	2012	2010	2011	2012
January / Ocak	36.0	26.6	58.0	1.5	0.3	-3.6	95.2	97.0	96.8
February / Şubat	42.6	8.9	42.1	4.9	0.1	-5.5	90.5	93.2	97.3
March / Mart	32.6	20.0	56.4	5.9	3.7	1.5	85.5	88.0	87.7
April / Nisan	23.9	56.9	22.1	9.2	7.2	12.0	84.3	91.0	72.6
May / Mayıs	20.7	145.8	80.9	15.2	0.5	14.4	70.4	87.7	83.3
June / Haziran	79.0	9.4	0.0	18.1	16.6	20.0	82.8	84.6	71.6
July / Temmuz	7.4	8.5	5.5	22.0	21.6	22.8	75.4	70.8	68.1
August / Ağustos	0.9	0.0	3.5	24.4	20.0	20.8	66.2	73.5	65.1
September / Eylül	22.5	2.1	0.0	18.2	17.4	18.7	75.8	68.5	66.1
November / Ekim	77.1	57.9	16.1	10.0	8.5	14.2	92.5	83.6	78.1
October / Kasım	7.5	0.0	14.5	9.3	0.8	7.3	81.0	86.8	92.3
December / Aralık	60.4	46.1	73.2	4.2	0.9	2.2	94.1	92.1	95.1
Total / Toplam	410.6	382.2	372.3						
Mean / Ortalama				11.9	8.1	11.0	82.8	84.7	81.2

*Meteorological Station of Transitional Zone Agricultural Research Institute.
*Geçit Kuşağı Tarımsal Araştırma Enstitüsü Meteoroloji İstasyonu.

Table 2. Germplasm area soil analysis.
Çizelge 2. Gözlem bahçesi toprak analizi.

Soil Structure Toprak yapısı	pH	Total Salt (%) Toplam tuz (%)	Lime (CaCO3) (%) Kireç (%)	Organic matter (%) Organik Madde (%)	Phosphorus (P_2O_5 - kg /da) Alınabilir fosfor (P_2O_5 - kg /da)	Potassium (K_2O - kg /da) Alınabilir potasyum (K_2O - kg /)
clayey killi	7.7	0.153	6.6	1.28	13.0	199.5

*Soil and Water Laboratory of Transitional Zone Agricultural Research Institute.
*Geçit Kuşağı Tarımsal Araştırma Enstitüsü Toprak ve Su Laboratuvarı.

Table 3. Station Information.
Çizelge 3. Durak bilgileri.

Population Number Populasyon Numarası	District İlçe	Collection site Toplama yapılan yöre	Latitude Enlem	Longitude Boylam	Altitude Yükseklik (m)
18	Sarıcakaya	Hamamlar-8 km to Taskopru	39.96086 °N	30.60667 °E	895
19	Sarıcakaya	Between Kuplu and Mayıslar-Village Grassland	40.00984 °N	30.66778 °E	784
20	Sarıcakaya	Between Kuplu and Mayıslar- Village Grassland	40.00984 °N	30.66778 °E	841
21	Sarıcakaya	Lacin Gobet-Village Grassland	40.02741 °N	30.80047 °E	1110
22	Sarıcakaya	Hamamlar-8 km to Taşköprü	39.96086 °N	30.60667 °E	895
23	Sarıcakaya	Lacin Gobet-Village Grassland	40.02741 °N	30.80047 °E	784
24	Mihalgazi	The Thermal Springs-500 m to Hamamlar	39.99404 °N	30.57988 °E	1022
25	Mihalıççık	Nallıhan Road 6. km-In Forest	39.90051 °N	31.43271 °E	1009
26	Sarıcakaya	3 km from Hekimdagı	39.90996 °N	30.62989 °E	1110
27	Mihalgazi	The Thermal Springs-500 m to Hamamlar	39.99404 °N	30.57988 °E	859
28	Mihalıççık	Nallıhan Road 6. km-In Forest	39.90051 °N	31.43271 °E	895
29	Seyitgazi	Han Road 10. km-Village Grassland	39.37383 °N	30.71631 °E	1036
30	Sarıcakaya	Lacin Gobet-Village Grassland	40.02741 °N	30.80047 °E	1224
31	Mihalıççık	The Road between Hamitoglu and Mihalıccık road 10. km-Grassland	39.82811 °N	31.47028 °E	1036
32	Mihalıççık	The Road between Hamitoglu and Mihalıccık road 10. km-Grassland	39.82811 °N	31.47028 °E	908
33	Sarıcakaya	Catacık Forest-Bride Fountain	40.02518 °N	30.78618 °E	784
34	Han	Afyon Road 4. km-Grassland	39.12894 °N	30.83604 °E	784
35	Seyitgazi	Han Road 10. km-Village Grassland	39.37383 °N	30.71631 °E	793
36	Inonu	Cemetery-Side of Aviation Facilities	39.81391 °N	30.12539 °E	356
37	Sarıcakaya	200 m from Eldem Houses-Village Cemetery	39.96145 °N	30.67784 °E	356
38	Sarıcakaya	200 m from Eldem Houses-Village Cemetery	39.96145 °N	30.67784 °E	552
39	Mihalıççık	The Road between Hamitoglu and Mihalıccık yolu 10. km-Grassland	39.82811 °N	31.47028 °E	793
40	Mihalıççık	The Road between Hamitoglu and Mihalıccık yolu 10. km-Grassland	39.82811°N	31.47028 °E	552
41	Merkez	3 km to Yahnikapan Village-Village Grassland	39.63041 °N	30.80647 °E	341
101	Merkez	Uludere-Village Cemetery	39.91566 °N	30.33850 °E	1025
102	Seyitgazi	Yazılıkaya-In front of the Monument	39.20018 °N	30.71504 °E	1292
103	Tepebaşı	Beklese Village-Village Grassland	39.99625 °N	31.00135 °E	1240
104	Mihalıççık	Gurleyik Village-Village Grassland	39.98858 °N	31.35383 °E	737
105	Mihalıççık	Karacaoren-Forestry Directorate Campus	30.00086 °N	31.09726 °E	1200
106	Mihalıççık	Otluk Village-Village Cemetery	40.01382 °N	31.12761 °E	1250
107	Merkez	Uludere-Village Cemetery	39.91566 °N	30.33850 °E	1025
109	Sivrihisar	Dumluca Village-Village Cemetery	39.38353 °N	31.25529 °E	1136

RESULTS

The mean values and standard errors of the characteristics of 32 cocksfoot populations are given below (Tabel 4). As stated before, the number of the plants in each population which initially is 20 decreased due to winter and disease damage. At the end of the observations and measurements, the mean values of the populations in main stem length, main stem thickness, flag leaf length, flag leaf width, number of nodes, internode length, growth pattern, rust resistance, winter resistance and tillering capacity changed between 43.9-72.4 cm, 2.0-3.3 mm, 7.8-16.0 cm, 2.9-5.4 mm, 2.3-4.5, 7.5-16.0 cm, 6.4-8.8, 6.6-8.0, 4.7-8.3 and 5.4-8.1, respectively.

Table 4. The mean values and standard errors of the cocksfoot populations.
Çizelge 4. Domuz ayrığı populasyonlarına ait ortalama ve standart hata değerleri.

pop. no pop. num.	plan. num. bit. say.	fll byb	flw bye	msl asu	mst ask	nn asbs	il asbau	gp bş	rr phd	wr kd	tc kp
18	11	13.6±1.1	4.4±0.2	69.5±2.6	2.5±0.2	4.5±0.2	9.6±0.4	8.6±0.2	7.0±0.0	8.0±0.0	7.5±0.3
19	14	14.0±1.4	5.1±0.3	71.4±3.7	2.9±0.1	3.2±0.2	13.5±0.6	8.4±0.4	6.7±0.2	8.0±0.0	7.2±0.3
20	13	14.8±1.6	4.2±0.3	68.8±3.2	2.8±0.1	3.3±0.2	13.6±0.5	7.4±0.4	7.0±0.0	8.0±0.0	7.4±0.2
21	15	10.2±0.7	3.5±0.3	63.4±3.1	2.5±0.1	3.4±0.1	12.0±0.7	7.7±0.3	6.7±0.1	8.0±0.0	7.5±0.3
22	11	11.0±1.3	3.8±0.3	65.3±2.3	2.5±0.2	3.5±0.2	12.1±0.7	7.5±0.3	6.8±0.2	7.8±0.1	7.3±0.2
23	13	14.1±1.0	4.4±0.3	69.9±2.5	2.6±0.1	3.5±0.1	12.5±0.8	7.6±0.2	7.0±0.0	7.9±0.1	7.4±0.2
24	17	13.2±1.2	4.0±0.3	72.4±2.4	2.4±0.1	3.6±0.2	13.7±0.9	6.4±0.3	6.9±0.1	8.2±0.1	7.5±0.2
25	16	9.0±0.8	3.1±0.3	50.0±4.3	2.2±0.1	2.9±0.1	12.0±0.6	7.8±0.3	7.0±0.0	7.9±0.1	7.9±0.1
26	13	11.4±1.4	4.3±0.3	69.2±3.2	2.8±0.2	3.2±0.2	13.1±0.8	8.8±0.2	7.0±0.0	8.0±0.0	7.9±0.2
27	18	14.0±1.2	3.8±0.3	71.5±2.3	2.6±0.1	3.5±0.2	12.0±0.6	7.4±0.3	6.6±0.1	8.0±0.0	7.4±0.2
28	18	7.8±0.7	2.9±0.2	45.3±2.0	2.2±0.1	2.9±0.2	9.1±0.7	7.8±0.2	7.0±0.0	8.3±0.1	7.8±0.2
29	16	10.9±0.8	4.3±0.3	59.4±2.6	2.8±0.1	2.4±0.1	14.5±0.8	8.2±0.3	6.9±0.1	8.3±0.1	7.0±0.0
30	17	16.0±0.2	5.3±0.3	72.4±1.4	3.3±0.2	3.2±0.1	14.0±0.8	7.5±0.2	6.9±0.1	8.0±0.0	7.9±0.2
31	6	9.8±0.9	3.5±0.3	49.2±2.6	2.2±0.4	3.8±0.1	7.5±0.7	8.3±0.3	7.0±0.0	8.0±0.0	7.0±0.4
32	14	10.3±1.2	3.4±0.3	60.0±2.9	2.6±0.1	3.5±0.2	9.9±0.6	8.5±0.2	7.0±0.0	8.0±0.0	7.0±0.0
33	9	8.4±0.9	3.2±0.4	56.7±3.8	2.3±0.2	3.2±0.2	11.0±1.3	8.0±0.3	8.0±0.0	8.0±0.0	8.1±0.1
34	13	13.8±1.2	3.6±0.4	66.0±2.4	2.4±0.1	2.9±0.2	14.7±1.0	7.5±0.3	6.9±0.1	6.8±0.2	6.6±0.1
35	16	10.1±0.7	3.6±0.3	63.3±2.7	2.4±0.1	2.9±0.1	16.0±0.8	6.9±0.3	7.0±0.0	8.0±0.0	8.0±0.0
36	14	8.3±1.0	3.0±0.3	66.5±2.1	2.6±0.1	3.7±0.2	11.6±1.0	8.0±0.3	7.0±0.0	8.0±0.0	7.9±0.1
37	14	11.4±1.1	4.4±0.3	64.7±4.1	2.9±0.2	3.9±0.2	12.9±1.1	6.7±0.3	7.0±0.0	8.0±0.0	7.2±0.2
38	19	12.3±1.1	3.8±0.2	64.2±1.8	2.7±0.1	3.4±0.2	12.1±0.6	6.8±0.3	8.0±0.0	8.0±0.0	8.0±0.2
39	19	11.1±0.1	3.5±0.2	60.8±1.8	2.7±0.2	3.1±0.2	13.2±0.6	7.4±0.2	7.1±0.1	8.0±0.0	6.8±0.2
40	15	9.4±0.8	3.0±0.1	56.1±1.3	2.5±0.2	3.1±0.2	12.5±0.7	8.0±0.1	6.9±0.1	7.7±0.1	7.0±0.0
41	10	9.4±1.1	3.6±0.3	43.9±1.7	2.3±0.2	2.3±0.2	10.0±0.7	7.3±0.2	7.0±0.0	8.0±0.0	6.7±0.2
101	16	10.9±0.9	5.4±0.3	64.2±1.5	3.1±0.2	2.8±0.1	13.6±0.8	7.6±0.1	7.0±0.1	5.6±0.2	6.0±0.2
102	19	11.0±0.9	4.8±0.3	61.9±2.1	2.5±0.1	2.5±0.1	14.1±0.8	7.1±0.1	7.0±0.0	5.7±0.2	5.9±0.2
103	19	9.6±0.7	4.6±0.2	53.7±1.5	2.3±0.1	2.4±0.2	11.9±0.9	6.8±0.1	7.0±0.0	5.9±0.2	5.9±0.1
104	20	12.3±1.0	4.7±0.2	64.4±1.7	2.7±0.2	3.3±0.2	13.0±1.1	7.0±0.1	6.7±0.1	5.8±0.2	6.1±0.1
105	18	9.2±0.4	4.4±0.2	55.9±1.5	2.6±0.1	2.8±0.2	11.6±0.8	6.9±0.1	6.9±0.1	5.8±0.1	6.3±0.1
106	20	10.5±0.6	4.4±0.2	64.6±2.3	2.4±0.1	3.0±0.2	13.8±0.9	7.2±0.1	6.9±0.1	5.9±0.1	6.3±0.1
107	20	8.1±0.8	4.0±0.2	53.9±1.4	2.3±0.1	2.6±0.2	13.1±1.0	6.9±0.1	7.0±0.0	5.7±0.1	5.9±0.1
109	11	8.3±0.9	4.2±0.1	46.9±1.6	2.0±0.0	2.4±0.2	11.5±0.3	6.6±0.2	7.0±0.0	4.7±0.4	5.4±0.3

*pop no.=populations, plan. num.=the plant number in populations, fll=flag leaf length, flw=flag leaf width, msl=main stem length, mst=main stem thickness, nn=number of nodes, il=internode length, gp=growth pattern, rr=rust resistance, wr=winter resistance, tc=tillering capacity.
* pop. num.= populasyon numarası, bit. say.= populasyon içindeki bitki sayısı, byb= bayrak yaprak boyu, bye= bayrak yaprak eni, asu= ana sap uzunluğu, ask= ana sap kalınlığı, asbs= ana sapta boğum sayısı, asbau= ana sapta boğum arası uzunluğu, bş= büyüme şekli, phd= pas hastalıklarına dayanıklılık, kd= kışa dayanıklılık, kp= kardeşlenme potansiyeli.

According to the results of the correlation analysis, there were some remarkable relationships between tillering capacity, which is important for pasture establishment and some other characteristics (Table 5). While there are significant ($p<0.01$) and positive correlations between tillering capacity and number of nodes (0.537**), tillering capacity and winter resistance (0.876**), there is a significant ($p<0.05$) and negative correlation between tillering capacity and flag leaf width (-0.401*). Some correlations between winter resistance and some other properties are also notable. There were significant ($p<0.01$)

and positive correlations between winter resistance and number of nodes (0.496**), winter resistance and growth pattern (0.477**).

As a result of the observations and measurements, some single plants were selected from populations for use in variety development studies. These plant were subjected to a ploidy analysis and they all were found tetraploid (Table 6).

Table 5. Correlation Coefficients-r (n=32).
Çizelge 5. Korelasyon katsayıları-r (n=32).

Character Karakter	msl asu	mst ask	fll byb	flw bye	il asbau	nn asbs	gp bş	wr kd	rr phd	tc kp
msl	-	0.667**	0.797**	0.436*	0.521**	0.533**	0.088	0.241	-0.179	0.323
mst			0.619**	0.595**	0.429*	0.155	0.167	0.192	-0.115	0.183
fll				0.523**	0.375*	0.404*	0.040	0.260	-0.213	0.216
flw					0.411*	-0.148	-0.181	-0.438*	-0.240	-0.401*
il						-0.332	-0.345	-0.177	-0.164	-0.081
nn							0.414*	0.496**	0.012	0.537**
gp								0.477**	-0.048	0.396*
wr									0.124	0.876**
rr										0.293
tc										-

*= p≤0.05, **=p≤0.01

Table 6. Ploidy analysis results.
Çizelge 6. Ploidi analizi sonuçları.

Population- single plant no Populasyon-tek bitki numarası	DNA content (pg) DNA içeriği (pg)	Ploidy level Ploidi düzeyi
105-3	9.19	tetraploid
105-7	8.99	tetraploid
102-18	8.64	tetraploid
104-1	8.82	tetraploid
104-3	9.00	tetraploid
104-15	8.66	tetraploid
102-9	9.03	tetraploid
102-13	9.13	tetraploid
101-5	8.94	tetraploid
101-8	9.08	tetraploid
102-11	8.95	tetraploid
102-19	8.84	tetraploid
104-5	8.75	tetraploid
105-2	8.86	tetraploid
105-3	8.90	tetraploid
105-19	8.90	tetraploid
106-1	9.09	tetraploid
106-4	9.07	tetraploid
107-2	9.00	tetraploid
18-20	9.15	tetraploid
19-3	8.88	tetraploid
20-7	8.90	tetraploid
20-4	9.15	tetraploid
20-18	9.18	tetraploid
21-16	9.00	tetraploid
24-8	8.80	tetraploid
24-20	9.10	tetraploid
25-11	8.88	tetraploid
26-4	9.16	tetraploid
27-20	8.90	tetraploid
37-1	9.19	tetraploid
38-20	8.80	tetraploid

CONCLUSION

In this collection and characterization study, when the mean and standard error values in the populations are examined, it appears that there is sufficient variation for the breeding studies. In addition, according to the results of the correlation analysis, some significant relationships were identified in terms of tillering capacity and winter resistance. Positive correlations between tillering capacity and number of nodes (0.537**), tillering capacity and winter resistance (0.876**) and negative correlation between tillering capacity and flag leaf width (-0.401*) were statistically significant. Positive correlations between winter resistance and number of nodes (0.496**), winter resistance and growth pattern (0.477**) were also notable.

The material and information obtained from the study have been used in variety development studies of pasture and forage type cocksfoot (*Dactylis glomerata* L.) in Transitional Zone Agricultural Research Institute.

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ANADOLU, J. of AARI
ISSN: 1300 - 0225
28 (1) 2018, 52 - 55
MFAL

Reproduction of Ficus pumila L. (Climbing Fig) with Tissue Culture

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Received (Geliş tarihi): 23.06.2017 *Accepted (Kabul tarihi): 14.02.2018*

***ABSTRACT: Ficus pumila** L., which provides a green wall appearance with its very close texture, is not commonly grown in Turkey despite its significance. Among the studies published so far, there is no record of successful micropropagation of **Ficus pumila** species. Thus, the objective of the present study was to propagate **Ficus pumila** with tissue culture, and to expand the use of the species in Turkey. Various growth regulators and doses (IAA, BA, BA + Kinetin) were tested in studies where Murashige and Skoog (MS) compounds are used commonly as nutrient media. Plant leafs and leaf stems were used as explant. It was possible to obtain plants with different explant and nutrient media combinations. Cultures were kept in climate chamber at 23 ° C temperature and 70% humidity and in 16 hours light and 8 hours dark photoperiodic conditions. In the present study, it was observed that the combination of BA dose and cytokinin in BA and MS were successful in the findings. The highest shoot formation was obtained in MS medium supplemented with 1 mg / l BA doses and combinations.*

***Keywords: Ficus pumila** L., **in vitro**, climbing fig, Creeping fig, tissue culture.*

Ficus pumila L. (Tırmanıcı Kauçuk)'nın Doku Kültürü ile Çoğaltılması Üzerinde Çalışmalar

***ÖZ:** Çok sık olan dokusuyla tamamen yeşil bir duvar görüntüsü veren **Ficus pumila** L., bu özelliği ile önem kazanasına rağmen ülkemizde bu türün kullanımı pek yaygın değildir. Bugüne kadar yayınlanan bilgiler arasında **Ficus pumila** türlerinde mikro çoğaltımın çok başarılı sonuçlar taşıdığına ilişkin bir kayıt bulunmamaktadır. Bu sebeple çalışmanın amacı **Ficus pumila**'nın doku kültürü ile çoğaltılmasını sağlamak, bununla birlikte ülkemizde kullanımını yaygın hale getirmek. Besin ortamı olarak Murashige ve Skoog (MS) bileşiminin yaygın olarak kullanıldığı çalışmalarda değişik büyüme düzenleyicileri ve dozları (IAA, BA, BA+Kinetin) denenmiştir. Eksplant olarak da bitkinin yaprak ve yaprak sapları kullanılmıştır. Farklı eksplant ve besin ortamı kombinasyonlarından bitki elde etmek mümkün olmuştur. Kültürler 16 saat aydınlık 8 saat karanlık fotoperyodik koşullarda, 23 °C sıcaklıkta ve % 70 nem ile iklim dolabında tutulmuştur. Bu çalışmada da ortaya konulan bulgular ışığında BA dozunun ve BA ile sitokinin kombinasyonlarında MS ortamında başarılı olduğu gözlemlenmiştir. En yüksek sürgün oluşumu 1 mg/l BA doz ve kombinasyonlarının eklendiği MS ortamında elde edilmiştir.*

***Anahtar Kelimeler: Ficus pumila** L., **in vitro**, sürünücü, tırmanıcı kauçuk, sürünücü, doku kültürü.*

INTRODUCTION

Creeping and climbing plants have fast-growing shoots and are easily used in several spaces due to this feature. They decorate walls or estrades very effectively. They also climb on enclosure elements such as wood and iron fences, covering their unwanted appearance and providing privacy. They provide shade by partially or completely covering architectural facilities such as pergolas, alcoves and arches.

The creeping and climbing plants are quite complacent compared to other ornamental plants. Their rapid growths even in adverse conditions are the reason for the selection of creeping plants (Güçlü, 1999, Tanrıverdi, 2001).

Ficus pumila L. (Climbing fig / Creeping fig) is a species with Chinese origins. This is a climber or creeping plant that grows on air roots. It is evergreen, and has small leaves in the form of a heart and they are 2.5 cm in length and 1.2 cm wide. It is a suitable species for sheltered places. The plant is resistant to drought and can also grow under extreme shadow. Fruit-bearing branches have broad leaves, but no air roots. It can also be grown indoors. In this case, the winter temperature should vary between 5º-8ºC. For propagation, head cuttings are used. Production; in hot, head steels. Vegetative cutting is used mainly in propagation; this imposes limitations for the breeding of high quality varieties. Sun-dried mature seeds which were traditionally used for propagation do not easily germinate. All of which are based on tissue culture as well as in this study. The *Ficus pumila* is very popular in Chinese but plantings are limited and do not meet the needs of growing markets (Hu and Liu, 1985; Hu *et al*., 1986; Chen, 1987; Pamay, 1993; Var, 2010).

MATERIALS AND METHODS

The study material *Ficus pumila* was procured from the Karadeniz Technical University, Faculty of Forestry breeding greenhouse.

The plant leaves and leave stems were used as explants. On the other hand, shoots of plantlets obtained from in vitro shoots were also considered as test material.

In the first experiment, leaves and leaf stalk explants (~ 1 cm in size) that were washed three times with distilled water were sterilized for 20 seconds in 70% ethanol and 30 minutes in 3% sodium hypochlorite. Thus, they were further sterilized for 2 minutes in 70% ethanol and 30 minutes in 3% sodium hypochlorite. In the last stage, they were washed in sterile distilled water and dried in sterile medium.

The experimental material and media were sterilized in the autoclave at 121 °C and under 1.05 kg/cm² pressure for 20 minutes, with the lids closed.

Murashige-Skoog (MS) medium (Murashige and Skoog, 1962) which is the basic medium for in vitro shoot studies, was used based on the soil structure and material requirements in the natural plant habitat (Table 1).

Table 1. Basic nutrition media used in the study.
Çizelge 1. Çalışmada kullanılan temel besi ortamı.

Compound Bileşim	MS medium (mg/l) MS besi ortamı (mg/l)
Macro nutrient element Makrobesin elementleri	
KNO_3	1900
NH_4NO_3	1650
$CaCl_2 . 2 H_2O$	440
$MgSO_4 . 7 H_2O$	370
KH_2PO_4	170
Micronutrient elements Mikrobesin elementleri	
H_3BO_3	6.200
$MnSO_4 . 4 H_2O$	22.300
$ZnSO_4 . 7 H_2O$	8.600
KI	0.830
$Na_2MoO_4 . 2 H_2O$	0.250
$CuSO_4 . 5 H_2O$	0.025
$CoCl_2 . 6 H_2O$	0.025
$FeSO_4 . 7 H_2O$	27.840
$Na_2EDTA . 2 H_2O$	37.240
Vitamins Vitaminler	
Nikotinik Asit	0.5
Thiamin HCl	0.5
Pridoksin HCI	0.5
İnositol	100.0
Glisin	2.0

For callus formation, 2 mg / l IAA, 1 mg / l BA, 3 mg / l BA + 1 mg / l Kinetin supplemented MS basic nutrient medium was used. The obtained calluses were renewed and placed in the same medium and the development of the shoots was observed. Callus became multiple shoots after 4 weeks and was rooted in the same medium after the fifth subculture.

6 g / l agar and 30 g / l sucrose were added to all media and the pH was adjusted to 5.7.

Germination experiments were conducted in a climatic chamber at a temperature of 25 ± 2 ° C for a 16/8 hour long day period.

Each experiment was set up in 3 replicates and 35 explants were used in total. At the end of the experiments, the obtained data were evaluated as percentage.

RESULTS AND DISCUSSION

Sterilized explants were taken to propagation experiments in a laminar flow sterile study cabinet.

Experiments were conducted in tubes and 50x93 mm culture jars. A total of 315 explants were tested in the MS medium. In the 2 mg / l IAA medium where 105 explants were tested, shoot formation was 67.6 ± 3.5%. In 1 mg / l BA medium where 105 explants were tested, shoot formation was 85.7 ± 2%. Furthermore, shoot formation in the 3 mg / l BA + 1 mg / l Kinetin medium with 105 implants was 83.8 ± 2.1% (Table 2).

Table 2. Shooting outcomes in different media.
Çizelge 2. Farklı ortamlarda görülen sürgün sonuçları.

Medium Ortam	Number of explants Eksplant sayısı	Shoot % Sürgün %
2 mg/l IAA	105	67.6 ± 3.5
1 mg/l BA	105	85.7 ± 2.0
3 mg/l BA+ 1 mg/l Kinetin	105	83.8 ± 2.1

The findings indicated that the best medium for *Ficus pumila* to develop shoots was MS medium that contained 1 mg / l BA (Figure 1). Kumar, *et al*. (1998), achieved 90% success in MS medium supplemented with 2 mg / l BA and 0.2 mg / l NAA on *Ficus carica*. These results support our work.

Healthy calli were formed and regeneration was observed in the subculture process in the same medium. The regenerated rootless plantlets that were cultivated in the subculture developed shoots in the same medium. Repeated subculturing may change the physiological state and gradually rejuvenate the shoot, which in turn promotes better rooting (Economou and Read, 1986). However, when these plantlets were kept in the subculture for more than 2 weeks, they faded out.

In the present study, callus and root formation was more common in cytokine-containing medium when compared to the auxin-containing medium. The initial explant hormone levels might have increased to desired levels during the subculture cultivation period. Thus, if the cytokine content in the culture medium is excessive, subculture cultivation without changing the media components might result in callus formation, regeneration and rooting, consecutively (Figure 2).

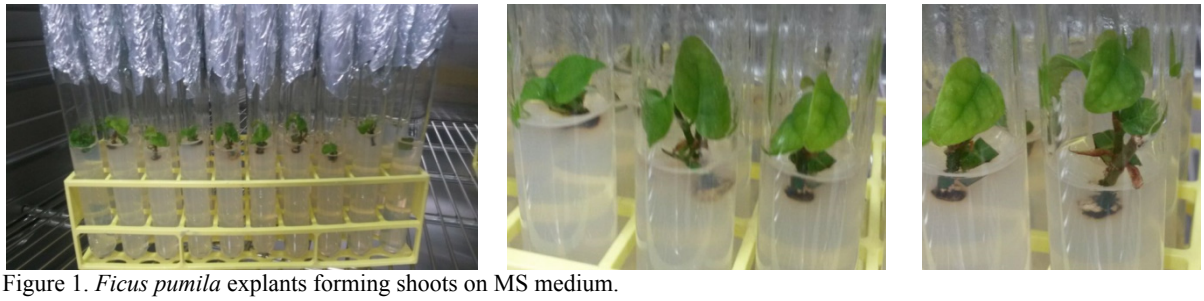

Figure 1. *Ficus pumila* explants forming shoots on MS medium.
Resim 1. *Ficus pumila* eksplantlarının MS ortamında oluşturduğu sürgün oluşumu.

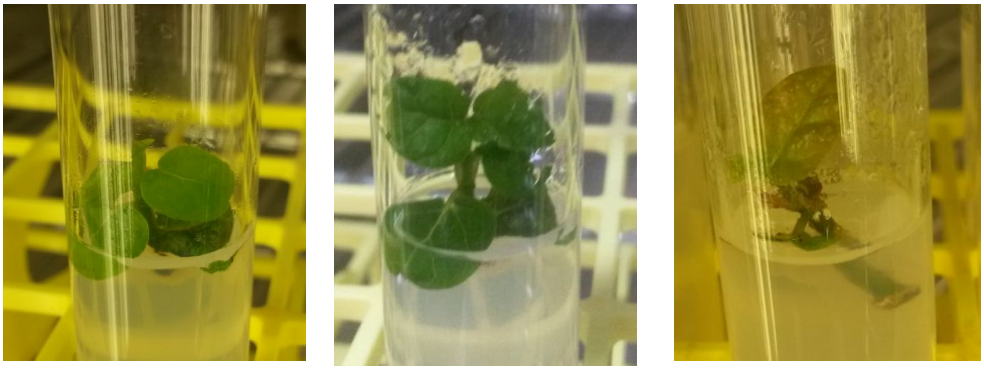

Figure 2. Shoot growth occurred in medium containing BA.
Resim 2. BA hormonu içeren besi ortamında gözlemlenen sürgün gelişimi.

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ANADOLU, J. of AARI
ISSN: 1300 - 0225
28 (1) 2018, 56 - 61
MFAL

Türlerarası Melezleme Yoluyla Lahana Kök Uru (Plasmodiophora brassicae Woronin)'na Karşı Dayanıklı Hatların Geliştirilmesinde Embriyo Kültür Tekniğinin Kullanım İmkanı

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Received (Geliş tarihi): 28.06.2017 Accepted (Kabul tarihi): 26.01.2018

ÖZ: *Bu araştırma Ondokuz Mayıs Üniversitesi'nde 2010 ve 2014 yılları arasında yürütülmüştür. Lahana kök ur hastalığına (**Plasmodiophora brassicae**) karşı dayanıklı hatların **B. oleraceae** L. ve **B. rapa** L arasında türlerarası melezleme yoluyla geliştirilmesi anacıyla yapılan bu çalışmada; **B. oreacea** L. var. Dürme ve ECD-1, ECD-2, ECD-3 ve ECD-4 olmak üzere **B. rapa** L. hatları arasında 831 çiçek melezlenmiştir. Bu melezlemeden oluşan 2663 primitif embriyo besi ortamına aktarılmıştır. Besi ortamına aktarılan ebriyolardan toplam 813 adet haploid ve 348 adet double haploid melez bitki elde edilmiştir. Elde edilen double haploid bitkilerden vernalizasyon sonrası toplam 162 bitki sera koşullarına aktarılmış ve çiçeklenme periyodunda kendileme yapılmıştır. DurmexECD-4 melez kombinasyonuna ait 8 melez bitkiden toplam 293 tohum elde edilmiştir. Kök ur hastalığına dayanıklılık durumunu test etmek amacıyla, elde edilen tohumlar ekilerek 95 melez double haploid bitki yetiştirilmiştir. Testleme sonrası lahana kök ur hastalığına dayanıklılık gösteren 33 bitkiden oluşan bir gen havuzu oluşturulmuştur.*

Anahtar Kelimeler: *Lahana kök uru hastalığı, **Plasmodiophora brassicae**, türlerarası melezleme, embriyo kültürü.*

Possibility of the Use of Embryo Culture Technique to Improve Resistant Lines Against Cabbage Clubroot (Plasmodiophora brassicae Woronin) via Interspesific Hybridization

ABSTRCT: *This research was carried out at Ondokuz Mayıs University between 2010 and 2014. Breeding of resistant lines against to clubroot (**Plasmodiophora brassicae**) via interspesific hybridisation between **B. oleraceae** L. and **B. rapa** L. In these research; 831 flowers were hybridisated between **B. oreacea** L. var. Dürme and **B. rapa** L. Lines ECD-1, ECD-2, ECD-3 and ECD-4. 2663 primitif embrios were transfered to medium and produced 813 haploid and 348 double haploid plants. 162 double haploid plants trasfered green condition after vernalisation and self crossing was done in flowering condition. 293 seeds were harvested from 8 hybrid plants from DurmexECD-4 hybrid combination. In order to test the endurance status of cabbage clubroot disease, 95 hybrid double-haploid plants were cultivated by seeding the obtained seeds. After the test, a gene pool was formed, consisting of 33 plants that tolerate cabbage root disease*

Keywords: *Cabbage clubroot disease, **Plasmodiophora brassicae**, interspecific hybridization, embrio culture.*

GİRİŞ

Brassicaceae familyası kültür bitkileri, süs bitkileri ve yabancı otlardan oluşan yaklaşık 375 cins ve 3200 türü kapsayan çok geniş bir familyadır. *Brassica* cinsi içerisinde yaklaşık 159 tür olmasına karşın ekonomik öneme sahip tür sayısı sadece 13 (Zhou and Zhang, 2001)'tür. Bu türler; *B. carinata* (Habeş hardalı), *B. Elongata* (uzun şalgam),

B. fruticulosa (Akdeniz lahanası), *B. juncea* (Hint hardalı), *B. napus* (kolza, kanola, İsveç şalgamı), *B. narinosa* (kaşık hardalı), *B. nigra* (siyah-kara hardal), *B. perviridis* (ıspanaklı hardal), *B. rupestris* (kahverengi hardal), *B. septiceps* (yeditop şalgam), *B. tournefortii* (Asya hardalı), *B. oleracea* (lahana, brokoli, karnabahar, Brüksel lahanası) ve *B. rapa syn B. campestris* (Çin lahanası, şalgam)'dır (Anonymous, 2011).

Dünya lahana üretimi yaklaşık 71 milyon tondur (Anonymous, 2013). Türkiye beyaz baş lahana üretimi ise yaklaşık 493 bin tondur. Karadeniz Bölgesi, 115 bin ton üretim ile Türkiye beyaz baş lahana üretiminin yaklaşık % 23,3'üne sahiptir (Anonim, 2014a). Karadeniz bölgesinde Samsun ili 92.68 bin ton üretim ile Türkiye beyaz baş lahana üretiminin % 18,8'ine sahiptir (Anonymous, 2013; Anonim, 2014a). Ancak Karadeniz Bölgesi'nde lahana üretim potansiyeli daha fazlaladır. Bu potansiyele ulaşılmasını kısıtlayan etkenlerden birisi de lahana kök-uru hastalığıdır (Anonim, 1995; 2005; 2014b).

Lahana kök uru drenaj suyu, hareket halindeki hayvanlar, bulaşık toprak-alet-ekipman, hastalıklı fide, bitki ve bitki atıkları ile yayılmaktadır (Walker, 1952). Yayılım kaynaklarının çeşitliliği dikkate alındığında, bu hastalığın, yakın bir gelecekte, Karadeniz Bölgesi'nde beyaz baş lahana yetiştiriciliğini sınırlandıracak önemli bir tehlike olacağını söylemek mümkündür.

Hastalıklarla etkili mücadele açısından en sağlıklı ve etkili yöntem, mukavim çeşit geliştirmektir. Mukavim çeşit geliştirmede yapılacak ilk iş; mukavemet genlerini taşıyan gen havuzundan, hassasiyetin bulunduğu gen havuzuna, geleneksel ya da biyoteknolojik yöntemler kullanılarak gen ya da genlerin aktarmasıdır (Kurt, 2015).

Beyaz baş lahananın içinde yer aldığı *B. oleracea* L. türünün gen havuzunda, lahana kök-uru hastalığına dayanıklılık sağlayan gen/genler yoktur. *B. oleracea* L. türünün akrabası olan, *B. rapa* L. (şalgam) gen havuzunda ise lahana kök-uru hastalığına dayanıklılık arz eden genler bulunmaktadır. Dolayısıyla bu iki tür arasında yapılacak türler arası melezlemeler ile lahana kök-uru hastalığına (*Plasmodiophora brassicae* Woronin) dayanıklılık genlerinin şalgam hatlarından, beyaz baş lahana hatlarına/çeşitlerine aktarılması mümkün olabilir.

Farklı kromozom sayısı ve farklı genoma sahip bu iki tür arasında yapılacak türlerarası melezlemeler sonucu oluşacak F1 dölleri normal olarak haploid ve kısırdırlar. Ancak Fı generasyonunda oluşacak primitif embriyolar, belirli bir fizyolojik olgunluk döneminde (yaklaşık 8-15 günlük), embriyo kurtarma tekniği ile besi ortamında kültüre alınmaları halinde, düşük frekansta da olsa fertil döller oluşturabilirler. Brassica türleri arasında yapılan melezlemelerden sağlıklı double haploid döllerin elde edilebilmesine ilişkin protokol Kurt ve ark. (2010) tarafından ortaya konmuştur.

Bu çalışmanın amacıyla; Lahana kök-uru hastalığının Karadeniz Bölgesi'nde tespit edilen "ECD 16/31/31" ırkına karşı dayanıklı olduğu tespit edilen bazı şalgam türleri (*Brassica rapa* var rapifera line aaBBCC, line AabbCC, line AABBcc ve line AABBCC) ile kök-uru hastalığına hassas olduğu bilinen beyaz baş lahana çeşidi "Dürme" arasında melezleme yaptıktan sonra oluşacak primitif embriyolardan double haploid melez bitkilerin elde edilmesinde embriyo kültürünün (embriyo kurtarma tekniği) kullanım potansiyelini belirlemektir. Nihai hedef olarak da embriyo kültür tekniğinin başarılı olması durumunda lahana kök uruna karşı muhavemet özelliği taşıyan genleri bünyesinde barındıran double haploid türler arası melez genotiplerden bitkilerden bir gen havuzu oluşturmaktır.

MATERYAL VE METOT

MATERYAL

Araştırmada; bitki materyali olarak Brassica familyasında yer alan lahana kök-uru hastalığına (*Plasmodiophora brassicae* Woronin) mukavim 4 şalgam hattı (ECD-01-02-03-04) ve lahana kök-uru hastalığına karşı aşırı hassas 1 adet beyazbaş lahana çeşidi (Dürme) kullanılmıştır. Testlemede;

DXECD-04 melez kombinasyonun 8 farklı melez bitkisinden 210 tohum testlemede kullanılmıştır. Araştırmada; çimlendirme, yetiştirme ve tarla koşulları olmak üzere farklı nitelikte toprak kullanılmıştır. *İn vitro* kuşullarda embriyo kültüründe MS5519 (Murashige and Skoog, 1962), sürgün gelişimi ortamı (0,1 mg/l IAA ve 0,5 mg/l BAP ihtiva eden MSD4 besi ortamı), kök gelişim ortamı (0,1 mg/l IAA ihtiva eden MSD4 besi ortamı), testlemede; steril bitkilerin yetiştirilmesinde 0,1 mg/l IAA ihtiva eden MSD4 ortamı, testlemede 10 ml içerisinde; 10^{+7} spor yoğunluğuna sahip Ordu-Kabadüz spor kültürü kullanılmıştır.

METOT

Laboratuvar koşullarında, her bir ebeveyn genotip, 3 hafta ara ile olmak üzere, altı farklı ekim zamanında, viollere, her violde 1 bitki ve toplam her genotip 12 bitki olacak şekilde ekilmiştir. Çıkıştan 1 hafta sonra, sağlıklı fideler 4 numaralı saksılara şaşırtıldıktan sonra sera koşullarına aktarılmışlardır. Sera koşullarında 10-12 yapraklı döneme ulaşan bitkiler vernalizasyon ihtiyaçlarının karşılanması amacıyla iklimlendirme odasına aktarılıp, 3 ay süre ile vernalize edildikten sonra tekrar sera aktarılmıştır.

Sera koşullarında melezleme olgunluğuna gelen çiçek tomurcuklarında, ana ebeveyn olarak dürme çeşidi ve baba ebeveyn olarak şalgam hatları olmak üzere melezlemeler yapılmıştır. Melezlemeden 10-15 gün sonra tohum tutan harnuplar izole edilerek laboratuvar koşullarına aktarılmış ve strilize edilen harnuplardan tohum taslakları, steril kabinde, binoküler mikroskop atında çıkarılıp, besi ortamına aktarılıp, 25 ^{o}C sıcaklık, 16 saat aydınlık 8 saat karanlık ihtiva eden iklim odasına aktarılmışlardır. Besi ortamında embriyolardan farklılaşmanın gözlenmesinden birkaç gün sonra oluşan primitif bitkicikler sürgün ortamına aktarılarak gelişmeleri teşvik edilmiştir. Besi ortamında belirli bir büyüklüğe ulaşan primitif sürgünler, kök gelişim ortamına aktarılmışlardır. Köklendirme ortamında 3-4 yapraklı ve saçak kök oluşumunu tamamlayan haploid bitkiler, steril suya alınarak köklerindeki agar ve diğer kimyasal maddeler temizlendikten sonra % 0,05'lik Kolcisin çözeltisinde 1 gece, karanlık ortamda, bekletilerek katlanmışlardır. Katlama sonrası sera koşullarına aktarılan double haploid bitkiler, 10-12 yaprak ihtiva eden büyüklüğe ulaştıklarında +4^{o}C'de 3 aylık bir süre ile vernalizasyon odasına alınmışlardır. Vernalize olan bitkiler, gelişmelerinin diğer bölümünü tamamlamaları ve çiçeklenme döneminde kendilenmelerini sağlamak amacıyla tekrar sera koşullarına aktarılmışlardır. İzolasyon poşeti içerisinde açan çiçeklerde kendilemeler yapılmış ve tohum bağlayan harnuplar olgunlaştıklarında hasat ve harman edildikten sonra tohumlar testlemede kullanılmak üzere +4^{o}C'de buzdolabında saklanmıştır.

Hastalık testlemesinde elde edilen DXECD-04 melez kombinasyonuna ait 8 melez bitkinin tohumları, 5 farklı zamanda, besi ortamına ekilmiştir. Çimlenen bitkicikler, testlemeye kadar gelişimlerini tamamlamaları için iklim odasına aktarılmışlardır. Köklenmesini tamamlamış, doku kültüründen gelen double haploid melez bitkiler steril kabinde çıkarılıp, steril suyla kökleri iyice yıkıp, kurulandıktan sonra spor aşılaması yapılmış saksılara şaşırtılmıştır. Şaşırtma sonra bitkiler iki hafta laboratuvar ortamında bekletildikten sonra açık alana alınmışlardır. Toprağa fidelerin şaşırtılmasından yaklaşık 70 gün sonra lahana kökleri incelenerek, hastalık okumaları Port ve ark. (2003)'a göre yapılmıştır. Yapılan hastalık okumalarına göre double haploid bitkiler ve kontrol çeşiti için 0=dayanıklı, 1=hassas, 2=hassas, 3=hassas şekilde reaksiyon kategorisi oluşturulmuştur.

BULGULAR

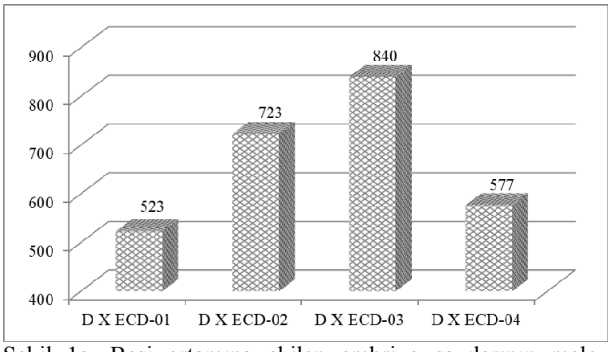
Melezlenen tomurcuk sayısı

Araştırmalarda ebeveyn bitkiler arasında yapılan melezlemelerde kullanılan çiçek tomurcuğu sayılarına ilişkin veriler Çizelge 1'de verilmiştir. Çizelge 1'in incelenmesinden de anlaşılacağı gibi bütün kombinasyonlarda toplam 831 çiçek tomurcuğu melezlenmiştir. Kombinasyon bazında değerlendirildiğinde; DXECD-01 kombinasyo-

nundan 242, DXECD-02 kombinasyonundan 234, DXECD-03 kombinasyonundan 237 ve DXECD-04 kombinasyonundan 118 çiçek tomurcuğu melezlenmiştir.

Besi ortamına ekilen embriyo sayısı

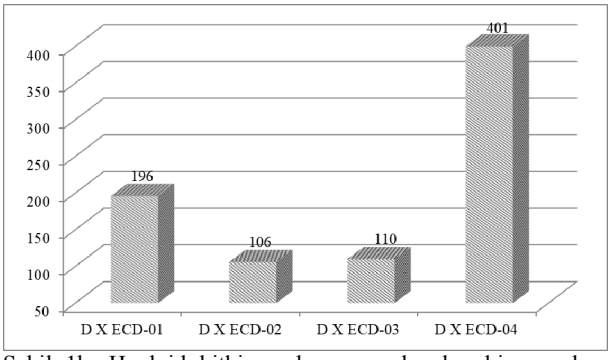
Besi ortamına ekilen embriyo sayılarına ilişkin veriler Çizelge 1'de, besi ortamına ekilen embriyoların kombinasyonlara göre dağılımları ise Şekil 1a'da verilmiştir. Çizelge 1'ın incelenmesinden de anlaşılacağı gibi toplam 2.663 embriyo besi ortamına ekilmiştir. Besi ortamına ekilen embriyolar kombinasyonlar bazında değerlendirldiğinde; DxECD-01 kombinasyonundan 523, DxECD-02 kombinasyonundan 723, DxECD-03 kombinasyonundan 840 ve DxECD-04 kombinasyonundan 577 embriyo besi ortamına ekilmiştir (Çizelge 1; Şekil 1a).



Şekil 1a. Besi ortamına ekilen embriyo sayılarının melez kombinasyonlara göre dağılımı.
Figure 1a. Distribution of embryo numbers of hybrid combinations transferred to medium.

Haploid bitki sayısı

Araştırma sonucu elde edilen haploid bitki sayılarına ilişkin veriler Çizelge 1'de ve haploid bitki sayılarının melez kombinasyonlara göre dağılımı Şekil 1b'de verilmiştir. Çizelge 1 ve Şekil 1b'nin incelenmesinden de anlaşılacağı gibi toplam 813 haploid bitki elde edilmiştir. Elde edilen haploid bitkilerin kombinasyonlar bazında değerlendrildiğinde; DXECD-01 kombinasyonundan 196, DXECD-02 kombinasyonundan 106, DXECD-03 kombinasyonundan 110 ve DXECD-04 kombinasyonundan 401 haploid bitki elde edilmiştir (Çizelge 1; Şekil 1b).



Şekil 1b. Haploid bitki sayılarının melez kombinasyonlara göre dağılımı.
Figure 1b. Distribution of haploid plant numbers of hybrid combinations.

Çizelge 1. Melezlenen tomurcak sayısı, kültüre alınan embriyo sayısı, elde edilen haploid bitki sayısı, hasat edilen double haploid tohum sayısı, testlemeye alınan F2 bitki sayısı ve dayanıklı bitki sayısına ilişkin veriler.
Table 1. The number of hybridized flowers, the number of embryos cultured, the number of haploid plants obtained, the number of double haploid seeds harvested and the number of F2 plants tested and the test results.

Melezleme kombinasyonları Hybridization combinations	Melezlenen tomurcuk sayısı (adet) Number of hybridized flowers (number)	Kültüre alınan embriyo sayısı (adet) Number of embryos cultured (number)	Oluşan haploid bitki sayısı (adet) Number of haploid plants obtained (number)	Hasat edilen double haploid tohum sayısı (adet) Number of double haploid seeds harvested (number)	Test edilen F2 bitki sayısı (adet) Number of F2 Plants tested (number)	Dayanıklı bitki sayısı (adet) Number of resistant plants (number)
D X ECD-01	242	523	196	-	-	-
D X ECD-02	234	723	106	-	-	-
D X ECD-03	237	840	110	-	-	-
D X ECD-04	118	577	401	293	33	33
Toplam	831	2663	813	293	33	33

Double haploid tohum sayısı ve mukavim bitki sayısı

Hasat edilen double haploid melez kombinasyonlara ait tohum sayılarına ilişkin veriler Çizelge 1'de verilmiştir. Çizelge 1'in incelenmesinden de anlaşılacağı gibi kendileme sonrası fizyolojik olgunluğunu tamamlamış olan DXECD-04 melez kombinasyonuna ait 8 melez bitkisinden toplam 293 adet tohum elde edilmiştir. Elde edilen bu tohumların *in vitro* koşullarda ekilmesi sonucu elde edilen fidelerde, yapılan testleme soncu, 33 adet fidenin lahana kök-uru hastalığına karşı toleransa sahip olduğu belirlenmiştir (Çizelge 1).

TARTIŞMA

B. oleracea ve *B. rapa* türleri arasında melezleme yapıldığında fertil döllerin elde edilme şansı, kromozom sayılarının ve genomlarnın farklı olması sebebiyle genetik olarak sıfırdır. Dolayısıyla hücre bölünmesi esnasında gametlerin eşlerini bularak eş oluşturma şansı olmadığından oluşacak döller kısırdır. Türler arasındaki melezlemelerde fertil döl elde etme şansının; dokusal uyumun ve akrabalık seviyesinin artması ile artmakta, azalması ile de azalmaktadır (Kurt, 2015). Araştırmada; 4 şalgam hattı ve 1 lahana çeşidi arasında 4 kombinasyonu (DxECD-01, DxECD-02, DxECD-03 ve DxECD-04) kapsamayacak biçimde melezleme yapmak amacıyla 831 adet çiçek tozlanmıştır. Bitkilerin melezlenmesinde başarı oranı; yetiştirme tekniği paketi uygulamalarına, çevresel faktörlere ve genotipe bağlı olarak değişir. Bu nedenle bu araştırmada kullanılan hatların genetik olarak sahip oldukları zorluklar göz önünde bulundurularak, çevre faktörleri optimal düzeyde tutulması için bitkiler, sıcaklık ve fotoperiyot bakımından kontrollü sera koşullarında yetiştirilerek, çevre koşullarının etkisi minimize edilmeye çalışılmıştır.

Araştırmada embriyo kültürü yapmak üzere fizyolojik yaşı 10-12 gün olan tohum taslakları besi ortamında kültüre alınmıştır. Besi ortamına ekilen tohum taslaklarının şalgam ve lahana melez kombinasyonuna ait olması, melezin bu iki türün özelliklerini bünyesinde barındırıyor olması, bitkicik oluşumundaki başarı üzerine hem olumlu hem de olumsuz etki etmiş olabilir. Embriyo kültüründe ana hedef maksimum sayıda sağlıklı ve fertil bitkiler elde etmektir. Şalgam ve lahana hatları arasında yapılan melezlemelerden oluşan melez harnuplardaki tohum taslaklarından toplam 813 adet hapoid melez bitki elde edilmiştir. Besi ortamına ekilen embriyo başına bitki oluşum oranı % 30.5 gibi oldukça yüksek bir oranda gerçekleşmiştir. Elde edilen bu sonuç üzerinde birinci derecede genotip faktörü rol oynamıştır. Kültüre alınan embriyoların rejenerasyon yeteneği üzerine, genotipin etkili olduğunu daha önce yapılan çeşitli çalışmalarda ortaya konmuştur (Hanzel ve ark., 1985; Can ve ark., 2000), genotiplerin somatik embriyo üretim kapasitesi açısından farklılık arz ettikleri ve yüksek embriyojenik kapasiteli genotiplerde, bitkiye dönüşüm oranının da yüksek olduğu saptanmıştır (Brown ve Atanasov, 1985).

B. oleracea ile *B. rapa* arasında yapılan melezlemelerden oluşan hapoid bitkilerin *in vitro* koşullardan çıktıktan sonra kolchisin ile muamele edilmeleri sayesinde AC şeklinde (amphihaploid) olan genomlarının AACC (amphidiploid) şekline ulaşması sayesinde tohum bağladıkları dolayısıyla bu tohumlar bünyelerinde şalgam ve lahana genomlarını birlikte taşırlar. Nitekim melez bitkilerin morfolojik yapıları incelendiğinde; lahana gibi uzun ve dik saplı ve seyrek boğum arasına, şalgam gibi yaprak kenarlarının dişli, yaprak yüzeyinin tüylü, yaprakların sert olduğu gözlenmiştir. Birçok seleksiyon ıslah çalışmasında bu tip karakterlerin göz önünde bulundurulması sayesinde bitki çeşitleri geliştirilmiştr. Dolayısıyla geleneksel ıslah yöntemleri ile kombine edilen embriyo kültür tekniği sayesinde, lahana kök uru hastalığına karşı dayanıklı melez bitkilerden oluşan bir gen havuzu elde edilmiştir.

Oluşturulan gen havuzundaki tohumların, kök uru hastalığına karşı dayanıklılık geni taşıyıp-taşımadıklarını belirlemek amacıyla, kontrollü koşullarda ters yapılmış ve bu tohumların

ekilmesinden elde edilen 33 double haploid kendilenmiş fidenin, kök uru hastalığına karşı dayanıklılık gen/genleri taşıdıkları belirlenmiştir. Melezlemelerde Mendel kuralları dikkate alındığında bu sonuç, teorik olarak da beklenen bir sonuçtur. Diğer taraftan şalgam ve lahana türlerinin genomlarının ve kromozom sayılarının farklı olmasına rağmen fertil döllerin elde edilmiş olması, gen havuzundaki bitkilerin lahana kök uru hastalığına karşı dayanıklılık genlerini taşıyabileceği ön görüsünü de birkez daha teyid etmektedir.

Sonuç olarak; embriyo kültür tekniği kullanılarak lahana kök uru hastalığına karşı dayanıklı topbaş lahana hatlarının geliştirilmesinde embriyo kültür tekniğinin kullanım potansiyelinin belirlenmesi amacıyla yapılan bu çalışma sonucu arzu edilen hedef gerçekleştirilmiştir. Böylece lahana kök uru hastalığına karşı dayanıklılık geni taşıyan bir gen havuzu oluşturulmuştur. Geliştirilen yöntemin, brassica türleri arasında, farklı amaçlarla yapılacak melezlemelerde de başarılı bir şekilde kullanılabileceğini de ortaya konmuştur.

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ANADOLU, J. of AARI
ISSN: 1300 - 0225
28 (1) 2018, 62 - 68
MFAL

Effect of NaCl and PEG-Induced Osmotic Stress on Germination and Seedling Growth Properties in Wild Mustard (Sinapis arvensis L.)

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Received (Geliş tarihi): 07.07.2017 Accepted (Kabul tarihi): 12.02.2018

ABSTRACT: *This study was conducted to evaluate the effect of NaCl and peg-induced osmotic stress on the germination and early seedling growth of wild mustard (**Sinapis arvensis** L.) in 2016. The seeds of mustard used in this study were obtained from plants growing under wild conditions in the Konya, Turkey. Trials were carried out to study the effects of osmotic potential of polyethylene glycol (PEG-6000) solutions at seven levels (0, -2, -4, -6, -8, -10 and -12 bars) and salt concentrations at eight levels (0, 2, 5, 10, 15, 20, 25, 30 and 35 dSm^{-1}) on seedling growth of wild mustard. Under PEG and salt stress the radicula length (cm), plumula length (cm), seedling fresh weight (mg), seedling dry weight (mg), final germination percentage (%), germination index (%) and seedling vigor index (%) were measured in the study. Final germination percentage and seedling growth parameters decreased by concentration of -4 to -10 bar for PEG stress; final germination percentage decreased by 10 dSm^{-1} (-4.4 bar) and seedling growth parameters decreased by 5 dSm^{-1} (-2.2 bar) for NaCl stress. Both NaCl and PEG treatments decreased final germination percentage and seedling growth properties in wild mustard, but the effects of NaCl compared to PEG were less on germination. Seed germination was completely inhibited at -12 bar for PEG stress, 35 dSm^{-1} (-15.6 bar) for NaCl stress.*

Keywords: ***Sinapis arvensis*** *L.,* *germination, mustard, salt and drought stress.*

NaCl ve PEG'e Bağlı Osmotik Stresin Yabani Hardal (Sinapis arvensis L.)'ın Çimlenme ve Fide Gelişimi Özellikleri Üzerine Etkisi

ÖZ: *Bu çalışma farklı NaCl ve PEG uygulamalarının yabani hardalın (**Sinapis arvensis** L.) çimlenme ve erken vejetatif gelişme üzerine etkisini belirlemek amacıyla 2016 yılında yürütüldü. Çalışmada kullanılan yabani hardal tohumları Konya'da yabani koşullarda yetişen bitkilerden alınmıştır. Denemede yedi farklı polietilen glikol (PEG-6000) solüsyonu (0, -2, -4, -6, -8, -10 ve -12 bar) ve dokuz farklı NaCI solüsyonu (0, 2, 5, 10, 15, 20, 25, 30 ve 35 dSm^{-1}) kullanılmıştır. Farklı PEG ve NaCl uygulamalarında kök uzunluğu (cm), sürgün uzunluğu (cm), fide yaş ağırlığı (mg), fide kuru ağırlığı (mg), final çimlenme oranı (%), çimlenme indeksi (%) ve fide canlılık indeksi ölçümleri yapılmıştır. Çalışma sonucunda, final çimlenme oranının ve fide büyüme parametrelerinin artan PEG konsantrasyonlarına (-4 / -10 bar) bağlı olarak azaldığı; final çimlenme oranının 10 dSm^{-1} (-4,4 bar)'den itibaren azalmaya başladığı ve fide büyüme parametrelerinin 5 dSm^{-1} (-2,2 bar)'den itibaren azalmaya başladığı belirlenmiştir. Ayrıca, NaCl ve PEG uygulamalarının çimlenme ve fide gelişimini düşürdüğü; fakat PEG'e kıyasla NaCl'ün etkisinin çimlenmede daha az olduğu; çimlenmenin -12 bar PEG ve 35 dSm^{-1} (-15,6 bar) NaCl dozlarında tamamen durduğu tespit edilmiştir.*

Anahtar kelimeler: ***Sinapis arvensis*** *L., çimlenme, hardal, tuz ve kuraklık stresi.*

INTRODUCTION

Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threat to agriculture and result in the deterioration of the environment (Odabas *et al.,* 2014; Temizel *et al.,* 2014; Toosi *et al.,* 2014).

Brassica occupy third place among the various oilseed species due to its considerable economic

and nutritional value. However, their growth, yield, and oil production are markedly reduced due to salinity. In particular, seed germination and early seedling growth have been reported to be relatively more sensitive towards salinity (Ashraf and McNeilly, 2004).

Other than the economically important Brassica species, various wild relatives belonging to the genera serve as a repository of vast gene pool (Prakash *et al.,* 1999). Wild relatives, such as *Sinapis arvensis* L., also possess a number of useful agronomic traits which could be incorporated into breeding programs, including: cytoplasmic and nuclear malesterility; resistance to disease and insect and nematode pests; intermediate C3-C4 photosynthetic activity; and tolerance for cold, salt and drought conditions (Rollins, 1981; Warwick and Black 1991; Bing *et al.,* 1996).

Seed germination and seedling establishment are the most sensitive stages of plant growth in pastures and crops under environmental stresses (drought, salinity, heat and cold conditions) (Taherkhani, 2013). Salinity and drought may delay the onset, reduce the rate, and increase the dispersion of germination events, leading to reductions in plant growth and final crop yield.

Salinity and drought affect the plants in a similar way. Reduced water potential is a common consequence of both salinity and drought. Water stress acts by decreasing the percentage and rate of germination and seedling growth (Macar, 2008). The deleterious effects of salinity on plant growth are attributed to a decrease in osmotic potential of the growing medium, specific ion toxicity and nutrition deficiency (Greenway and Munns, 1980). Germination rates decrease with an increase in NaCl concentration (Murillo Amador *et al*., 2002).

Drought plays an important role not only in determining germination rate, but also influences seedling development (Macar, 2008). This stress type is one of the most important environmental stresses affecting agricultural productivity worldwide and can result in considerable yield reductions (Mohammadkhani and Heidari, 2008). It is one of the main causes for crop yield reduction in the majority of agricultural and natural regions of the world.

Mustard is sensitive to soil salinity at early stage of growth but fairly tolerant at later developmental stages. Germination and seedling growth of mustard is critical one and thus becomes a major limiting factor for full potential production of crop (Singh, 2011).

Salinity and drought stress are important environmental factors that affect different development stages of crops, especially germination stage. The objective of the present investigation was to evaluate the effect of NaCl and PEG-induced osmotic stress on the germination and early seedling growth of wild mustard (*Sinapis arvensis* L.), a commercially important weed for providing raw material for biodiesel energy (Blackshaw *et al*., 2011; Eryilmaz and Ogut, 2011; Kayacetin *et al*., 2016).

MATERIALS AND METHODS

This study was carried out at the Central Research Institute for Field Crops; oil seed crop unit, Turkey. The seeds of mustard used in this study were obtained from plants growing under wild conditions in the Konya, Turkey. They were multiplied and selected for use in the study.

Each experiment was a two-factor factorial in a Completely Randomized Design with four replication. The first factor was solutions (PEG and NaCl) and the second factor was solution levels (0, -2, -4, -6, -8, -10 and -12 bars; 0, 2, 5, 10, 15, 20, 25, 30 and 35 dSm^{-1}) (electrical conductivities of the solutions). Drought stresses were induced by polyethylene glycol (PEG-6000) treatment. Salt stresses were induced by NaCl using a conductivity meter (Model WTW Cond. 314i, Germany). Distilled water served as a control. For all investigated parameters, analysis of variance was performed using the MSTAT-C software package. Significant differences among the mean values were compared by LSD test ($P < 0.01$).

Four replications of 25 seeds of each treatment were germinated in two rolled whatman filter papers with 5 ml of respective test solutions in glass petri dishes. Then petri-dishes containing two layer filter paper were moistened by respective prepared solutions. Seeds were allowed to germinate at 22 ±1 °C (Fallah-Toosi and Baki, 2013) in the dark for 10 days. Daily germination rate was measured and filter papers were replaced, when needed. Similarly, respective NaCl and PEG solutions were added when required. Germination percentage was measured by ISTA (Anonymous, 1996) standard method. Germinated percentage was recorded on daily until a constants count was achieved (every 24 h for 10 days). Seed was considered to be germinated when radicle length exceeded 2 mm (Huang and Redmann, 1995). Tentht day, 10 seedlings were randomly selected from each replicate at the end of tenth day of standard germination test and measured parameters including radicula length (RL), plumula length (PL), seedling fresh weight (SFW), final germination percentage (FGP), germination index (GI) and seedling vigor index (SVI) were evaluated (Kandil *et al*., 2012). Seedling dry weights (SDW) were measured after drying samples at 70 °C for 48 h in an oven (Böhm, 1979).

Parameters were calculated as follows;

SFW: Radicula and plumula fresh weight of averages 10 normal seedlings at random/replicate, were determined, expressed as mg.

SDW: Radicula and plumula fresh weight of averages 10 normal seedlings at random/replicate, dry weight recorded, expressed as mg.

FGP: (number of germinated seeds/number of total seeds) x 100.

GI: (germination percentage in each treatment/ germination percentage in the control) x 100.

SVI: (average radicula length+average plumula length) x final germination percentage.

RESULTS

Effect of PEG on seed germinability and seedling growth in mustard are presented in Table 1; effect of NaCl on seed germinability and seedling growth in mustard are presented in Table 2.

Growth Parameters for Drought Stress Treatments

Statistically significant differences were found between the seven PEG treatments in mustard. PEG treatments caused significant decrease in terms of radicula length, plumula length, seedling fresh weight, seedling dry weight and final germination percentage for all treatments PEG treatment compared with control (Table 1). Radicula length was changed between 3.62-12.34 cm. The greatest decrease in radicula length was observed in -10 bar PEG treatment by 69.93%. In -8, -6 and -4 bar PEG treatments showed a decrease by 50.66%, 41.20% and 19.60% respectively. Plumula length was changed between 0.66-6.27 cm. The greatest decrease in plumula length was observed in -10 bar PEG treatment by 89.47%. In -8, -6, -4 and -2 bar PEG treatments showed a decrease by 74.80%, 52.79%, 39.08% and 13.24% respectively. Seedling fresh weight was changed between 13.83-46.10 mg. The greatest decrease in seedling fresh weight was observed in -10 bar PEG treatment by 70.00%. In -8, -6, -4 and -2 bar PEG treatments showed a decrease by 64.21%, 47.55%, 36.12% and 4.06% respectively. Seedling dry weight was changed between 0.42-3.03 mg. The greatest decrease in seedling dry weight was observed in -10 bar PEG treatment by 86.14%. In -8, -6, -4 and -2 bar PEG treatments showed a decrease by 75.58 %, 64.69%, 42.57% and 22.44% respectively. Final germination percentage was changed between 45.83-83.33%. The greatest decrease in final germination percentage was observed in -10 bar PEG treatment by 45.00%. In -8, -6, -4 and -2 bar PEG treatments showed a decrease by 34.00%, 28.99%, 13.00% and 7.00% respectively. Germination index was changed between 55-100. The greatest decrease in germination index was observed in -10 bar PEG treatment by 45. In -8, -6, -4 and -2 bar PEG

treatments showed a decrease by 34, 24, 13 and 7 respectively. Seedling vigor index was changed between 196.43-1526.14. The greatest decrease in seedling vigor index was observed in -10 bar PEG treatment by 87.13. In -8, -6, -4 and -2 bar PEG treatments showed a decrease by 72.67, 61.33, 35.80 and 9.78 respectively. -12 bar PEG treatments were excluded from this study since seed germination completely inhibited.

Table 1. Effect of PEG on germination and seedling growth in wild mustard.
Çizelge 1. Yabani hardalda çimlenme ve fide gelişimi üzerine PEG'in etkisi.

Treatment		Radicula length (cm)	Plumula length (cm)	Seedling fresh weight (mg)	Seedling dry weight (mg)	Final germination percentage (%)	Germination index (%)	Seedling vigor index (%)
Uygulama		Kök uzunluğu (cm)	Sürgün uzunluğu (cm)	Fide yaş ağırlığı (mg)	Fide kuru ağırlığı (mg)	Final çimlenme oranı (%)	Çimlenme indeksi (%)	Fide canlılık indeksi (%)
PEG (bar)	0	12.04 a	6.27 a	46.10 a	3.03 a	83.33 a	100 a	1526.14 a
	2	12.34 a	5.44 a	44.23 a	2.35 b	77.50 a	93 a	1376.84 a
	4	9.68 b	3.82 b	29.45 b	1.74 c	72.50 ab	87 ab	979.73 b
	6	7.08 c	2.96 b	24.18 bc	1.07 d	59.17 bc	76 bc	590.09 c
	8	5.94 c	1.58 c	16.50 cd	0.74 de	55.00 c	66 cd	417.05 cd
	10	3.62 d	0.66 d	13.83 d	0.42 e	45.83 c	55 d	196.43 d
	12	0	0	0	0	0	0	0
F value		58.15	97.65	43.13	66.36	16.17	29.10	99.33
LSD value		1.38	0.66	6.27	0.37	10.85	9.53	161.95
CV (%)		10.84	12.73	14.33	15.82	10.98	9.95	12.68

Aynı harfle gösterilen ortalamalar arasında önemli fark ($P \leq 0,05$) yoktur.
Same letters in a column are not significantly difference at the 0,05 probability levels.

Growth Parameters for Salt Stress Treatments

Statistically significant differences were found among the seven NaCl treatments in mustard. NaCl treatments caused significant decrease in terms of radicula and plumula length, seedling fresh weight, seedling dry weight and final germination percentage for almost all treatments NaCl treatment compared with control (Table 2). Radicula length was changed between 0.79-12.46 cm. The greatest decrease in radicula length was observed in 30 dSm^{-1} NaCl treatment by 93.66%. In 25, 20, 15, 10, 5 and 2 dSm^{-1} NaCl treatments showed a decrease by 89.25%, 69.66%, 45.91%, 38.52%, 35.39% and 8.91% respectively. Plumula length was changed between 0.34-6.88 cm. The greatest decrease in plumula length was observed in 30 dSm^{-1} NaCl treatment by 95.06%. In 25, 20, 15, 10, 5 and 2 dSm^{-1} NaCl treatments showed a decrease by 82.56%, 66.13%, 34.30%, 30.96%, 31.25% and 5.96% respectively. Seedling fresh weight was changed between 4.85-67.63 mg. The greatest decrease in seedling fresh weight was observed in 30 dSm^{-1} NaCl treatment by 92.83%. In 25, 20, 15, 10, 5 and 2 dSm^{-1} NaCl treatments showed a decrease by 72.28%, 58.04%, 24.07%, 8.58%, 8.21% and 2.85% respectively. Seedling dry weight was changed between 0.12-4.60 mg. The greatest decrease in seedling dry weight was observed in 30 dSm^{-1} NaCl treatment by 97.39%. In 25, 20, 15, 10, 5 and 2 dSm^{-1} NaCl treatments showed a decrease by 88.26%, 73.48%, 48.26%, 29.78%, 21.96% and 8.48% respectively. Final germination percentage was changed between 29-77%. The greatest decrease in final germination percentage was observed in 30 dSm^{-1} NaCl treatment by 62.34%. In 25, 20, 15, 10, 5 and 2 dSm^{-1} NaCl treatments showed a decrease by 27.27%, 19.48%, 11.69%, 10.39%, 5.19% and 5.52% respectively. Germination index was changed between 37.66-100. The greatest decrease in germination index was observed in 30 dSm^{-1} NaCl treatment by 62.34. In 25, 20, 15, 10, 5 and 2 dSm^{-1} NaCl treatments showed a decrease by 27.27, 19.48, 11.69, 11.90, 5.19 and 5.52 respectively. Seedling vigor index was changer between 34.68-1451.70. The greatest decrease in seedling vigor index was observed in 30 dSm^{-1} NaCl treatment by 97.61. In 25, 20, 15, 5, 10 and 2

dSm^{-1} NaCl treatments showed a decrease by 88.76%, 80.35%, 43.54%, 32.23%, 35.10% and 12.44% respectively. 35 dSm^{-1} NaCl treatments were excluded from this study since seed germination completely inhibited.

Table 2. Effect of NaCl on germination and seedling growth in wild mustard.
Çizelge 2. Yabani hardalda çimlenme ve fide gelişimi üzerine NaCl'nin etkisi.

Treatment		Radicula length (cm)	Plumula length (cm)	Seedling fresh weight (mg)	Seedling dry weight (mg)	Final germination percentage (%)	Germination index (%)	Seedling vigor index (%)
Uygulama		Kök uzunluğu (cm)	Sürgün uzunluğu (cm)	Fide yaş ağırlığı (mg)	Fide kuru ağırlığı (mg)	Final çimlenme oranı (%)	Çimlenme indeksi (%)	Fide canlılık indeksi (%)
NaCl (dSm^{-1})	0	12.46 a	6.88 a	67.63 a	4.60 a	77.00 a	100.00 a	1451.70 a
	2	11.35 a	6.47 a	65.70 a	4.21 a	72.75 a	94.48 ab	1271.12 a
	5	8.05 b	4.73 b	62.08 a	3.59 ab	73.00 a	94.81 ab	942.11 b
	10	7.66 b	4.75 b	61.83 a	3.23 abc	69.00 ab	88.10 bc	983.80 b
	15	6.74 b	4.52 b	51.35 b	2.38 bc	68.00 ab	88.31 bc	819.67 b
	20	3.78 c	2.33 c	28.38 c	1.22 c	62.00 bc	80.52 cd	285.27 c
	25	1.34 d	1.20 d	18.75 d	0.54 c	56.00 c	72.73 d	163.12 cd
	30	0.79 d	0.34 e	4.85 e	0.12 d	29.00 d	37.66 e	34.68 d
	35	0	0	0	0	0	0	0
F value		97.90	284.58	179.40	180.06	41.65	51.85	72.90
LSD value		1.28	0.42	5.34	0.37	7.01	8.13	180.97
CV (%)		13.33	7.24	8.05	10.19	7.53	6.74	16.54

Aynı harfle gösterilen ortalamalar arasında önemli fark ($P \leq 0,05$) yoktur.
Same letters in a column are not significantly difference at the 0,05 probability levels.

DISCUSSION

PEG and NaCl treatments had a significant effect on all investigated characters ($P<0.01$). Germination parameters were decreased by increasing osmotic potential of PEG 6000. In distilled water, percentage of seed germination was the highest. The higher amount of PEG 6000 concentration in this research (-12 bar) completely inhibited seed germination. The first physiological disorder, which takes place during germination, is the reduction in imbibitions of water by seeds which leads to a series of metabolic changes, including general reduction in hydrolysis and utilization of the seed reserve. Upon imbibition, the quiescent dry seeds rapidly resume oxygen uptake and oxidative phosphorylation, processes required for supporting the high energy cost of germination (Baranova *et al*., 2006; Toosi *et al*., 2014).

Seed germination decreased with the increase in NaCl concentration. According to Ayaz *et al*. (2000), decrease of seed germination under conditions of salt stress is due to occur of some metabolically disorders. It seems that, decrease of germination percentage is related to reduction in water absorption into the seeds at imbibitions and seed turgoscence stages. It is reported that, radicula and shoot length are important traits in salt stress sensitivity evaluation (Jamil *et al*., 2006). Decrease of growth in root and stem can be related to NaCl toxicity and disproportion in nutrient absorption by seedlings. According to results of Werner and Finkelstein (1995) salinity decreases water absorption and growth of root and shoot. It's reported that, salinity decreases significantly nutrient absorption and root growth speed (Khan and Gulzar, 2003). Srivastava *et al*. (2004) have been reported that, proteins especially PR10 protein increases salt resistance in canola varieties at germination stages. In other research, increase of salinity from 6 to 11 dSm^{-1} decreased canola seed germination by 50% (Francois, 1996). Our result showed that seed germination was completely inhibited at 35 dSm^{-1} (-15.6 bar) for NaCl stress. Gulzar and Khan (2001) reported that, NaCl salinity prevents water absorption by seeds and decreases significantly seed germination

percentage and germination pace (Bybordi and Tabatabaei, 2009). The greatest decrease in germination percentage in this study was observed in 30 dSm^{-1} treatment by 62.3% which might implicate that wild mustard seed is tolerant to salt stress.

Both NaCl and PEG treatments inhibited seed germination and seedling growth properties in wild mustard. However, seed germination parameters were better in NaCl than in PEG with the earlier observation made for alfaalfa by Tilaki *et al*. (2009); soybean by Khajeh-Hosseini *et al*. (2003); sunflower by Luan *et al*. (2014). This may be due to the uptake of Na^+ and Cl^- ions by the seed, maintaining a water potential gradient allowing water uptake during seed germination (Heshmat *et al*., 2011). Our findings showed that NaCl had greater inhibitory effects on seedling growth than on germination with respect to PEG. This might be explained by more rapid water uptake in NaCl solutions and achivement of a moisture content that allowed germination. This result confirms the findings of Khajeh-Hosseini *et al*., 2003 in soybean; Okcu *et al*. (2005) in pea; Saeedipour (2009) in canola; Heasmat (2011) in canola; Asmare (2013) in harricot bean.

While seed germination and seedling growth parameters decreased by concentration of -4 to -10 bar for PEG stress; seed germination decreased by 10 dSm^{-1} (-4.4 bar) and seedling growth parameters decreased by 5 dSm^{-1} (-2.2 bar) for NaCl stress. Seed germination was completely inhibited at -12 bar for PEG stress, 35 dSm^{-1} (-15.6 bar) for NaCl stress. The marked differences in germination percentages observed with NaCl and PEG at the same osmotic potentials indicate specific ionic effects and point that germination is solely controlled by the osmotic potential.

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ANADOLU, J. of AARI
ISSN: 1300 - 0225
28 (1) 2018, 69 - 76
MFAL

Yaş İncir (Mor Güz - Sarı Lop) Çekirdek ve Çekirdek Yağlarının Fiziko-Kimyasal Özellikleri

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Received (Geliş tarihi): 03.07.2017 Accepted (Kabul tarihi): 29.01.2018

ÖZ: *Bu araştırmada, Türkiye de Aydın ilinde farklı lokasyonlardan toplanan (Gencelli, Feslek ve Kurtuluş ilçeleri) yaş incirlerin (Mor Güz ve Sarı Lop) çekirdekleri kullanılmıştır. Araştırma sonucuna göre, taze Mor Güz cinsine ait çekirdek ve çekirdek yağlarının fiziko-kimyasal açıdan, lokasyon ortalamaları çekirdek sayısı, nem , kül, bindane ağırlığı, yabancı madde, en, boy ve yağ içerikleri sırasıyla 947 adet, % 9,70, %4,12, 3,75 gr, % 0,40, 1,23 mm, 1,40 mm ve % 23,53 bulunmuştur. Çekirdek yağlarında önemli yağ asitleri palmitik, oleik, linoleik ve linolenik yağ asitleri olarak sırasıyla % 7,06 , % 15,78 , % 31,87 ve % 40,88 tespit edilmiştir. Diğer taraftan, taze Sarı Lop cinsine ait çekirdek ve çekirdek yağlarının fiziko-kimyasal açıdan, lokasyon ortalamaları çekirdek sayısı, nem , kül, bindane ağırlığı, yabancı madde, en, boy, ve yağ içerikleri ise sırasıyla 957 adet, % 11,81, % 3,31 , 3,90 gr, % 0,43, 1,40 mm, 1,55 mm ve % 20,54 bulunmuştur. Çekirdek yağlarında önemli yağ asitleri palmitik, oleik, linoleik ve linolenik yağ asitleri olarak sırasıyla% 6,96, % 15,98, % 30,33 ve % 42,11 tespit edilmiştir. Bu yönüyle incir çekirdek yağlarının serbest yağ asitliği düşürüldükten sonra insan gıdası olarak tüketiminin uygun olduğu ve incir çekirdek yağının, keten tohumu yağı gibi iyi bir linolenik asit kaynağı olduğu belirlenmiştir.*

Anahtar Kelimeler: Ficus carica *L., incir çekirdeği, Sarı Lop, Mor Güz, fiziko-kimyasal, yağ asitleri kompozisyonu.*

Physico-Chemical Properties of Fresh Fig (Mor Güz - Sarı Lop) Seed and Seed Oil

ABSTRACT: *In this study, we used to fresh fig seeds (Mor Güz and Sarı Lop) collected from Gencelli, Feslek and Kurtuluş town different location in Aydın Province in Turkey. Due to this study, Genus of fresh Mor Güz fig's seeds and oils' have found some physicochemical characteristics of average values; seeds number, moisture content, ash, 1000 seed weight, foreing matter, wıdth, length and oil content respectively, 947 pieces, 9.70 %, 4.12 %, 3.75 gr, 0.40 %, 1.23 mm, 1.40 mm and 23.53 %. Important fatty acids found palmitic, oleic, linoleic and linolenic respectively 7.06 %, 15.78 %, 31.87 % and 40.88 %. On the other hand, genus of Sarı Lop fig's dry seeds and oils' have found some physicochemical characteristics of average values diffrerent locaton seeds number, moisture content, ash, 1000 seed weight, foreing matter, wıdth, length and oil content respectively, 957 pieces, 11.81 %, 3.31 %, 3.90 gr, 0.43 %, 1.40 mm, 1.55 mm ve 20.54 %. Important fatty acids found palmitic, oleic, linoleic and linolenic respectively% 6.96 %, 15.98 %, 30.33 % and 42.11 %. In this respect, fig seed oils' has been determined to suitable for consumption as human food after reduce free fatty acid and also its determined to good linolenic acid source as flaxseed oil.*

Keywords: Ficus carica *L., fig seed, Sarı Loop, Mor Güz, physicochemical, fatty acids chomposition.*

GİRİŞ

Akdeniz diyetinin önemli bir parçasını oluşturan sağlıklı ve uzun yaşamın simgesi incir (Trichopoulou ve ark., 2006), son yıllarda yetiştiriciliği yapılamayan Batı ve Kuzey Avrupa ülkelerinde egzotik meyve olarak büyük ilgi görmektedir (Polat ve Çalışkan, 2008). Bu ilginin artmasında incirin kutsal meyve olarak görülmesi, besin içeriğinin diğer birçok meyve türüne göre yüksek olması (Aksoy ve ark., 2007) özellikle ham ve indirgen lif, mineral ve polifenol içeriğince zengin

olması, yağ ve kolesterol içermemesi (Vinson, 1999) farklı değerlendirme şekillerine sahip ticari bir meyve olması gibi faktörler sayılabilmektedir.

İncir (*Ficus carica* L.*),* kışın yaprağını döken bir bitkidir. İncir kültürü Anadolu'da insanlık tarihi kadar eski dönemlere dayanır ve kültür meyveleri içinde en eski gelişme tarihine sahip meyvelerden biridir. Arap Yarımadası'nda ve Ortadoğu'da kültüre alındığı bilinmektedir (Aksoy, 1991). İncirin anavatanı Türkiye olup, buradan Suriye, Filistin ve daha sonrada Ortadoğu üzerinden Çin ve Hindistan'a yayılmıştır. Amerika, Güney ve Güney-Batı Afrika ve Doğu Avustralya incirin pek yeni kültür merkezlerini teşkil etmekte ve Anadolu'dan sonra Kaliforniya'da incir, kültür tarihinin ikinci bir gelişme evrimini yaşamaktadır (Özbek, 1978).

Ülkemiz kurutmalık ve sofralık incir yetiştiriciliği ve ticaretinde dünyanın ilk sırasında yer almaktadır (Çalışkan ve Polat, 2012). Kurutmalık incir çeşidi olarak Sarı Lop, sofralık incir çeşidi olarak da Mor Güz ve Bursa Siyahı incir ihracatımızın yaklaşık tamamını oluşturmaktadır. Sarı lop incir çeşidi yetiştiriciliği Aydın ve İzmir yörelerinde yoğunlaşmış olup, 2002-2011 yılları arasında toplam incir üretimin % 65' ini karşılarken, sofralık incir yetiştiriciliği ise başta Bursa yöresi olmak üzere, son yıllarda Aydın, İzmir, Mersin gibi sahil yörelerinde de yaygınlaşmaya başladığı bildirilmiştir (Anonim, 2012a).

Subtropik ve ılıman kuşağın sıcak kesimlerinde yetişme alanı bulmuş olan incirin meyveleri sofralık (taze) ve kurutmalık olarak değerlendirilmektedir. Yüksek kalori değeri, mineral maddeler ve besin maddeleri içeriğiyle gıda maddeleri arasında özel bir yeri olan kuru incirin çok çeşitli tüketim alanları mevcuttur. Kuru incir, uluslararası pazarlarda, çerezlik olarak tüketildiği gibi pasta imalatında, çeşitli yemeklerin yapımında, dilimlenmiş olarak ekmek imalatında, şekerli mamuller imalatında ve meyve karışımlarında kullanılmaktadır. II. kalitedeki olanlardan, pekmez; III. ve diğer düşük kalitedeki incirlerden de etil alkol üretilmektedir. Etil alkolün üretimi esnasında ortaya çıkan incir çekirdekleri de boya, kozmetik ve ilaç sanayinde değerlendirilmektedir (Tuğ, 2002).

Sofralık incir kalite kriterleri olarak; ortalama meyve ağırlığı (g), ortalama hacim (cm^3), maksimum en (mm), maksimum boy (mm), boyun uzunluğu (mm), ostiol açıklığı (mm), tabla(mm), pH, asitlik (%), meyve iç boşluğu, meyve iç rengi ve suda çözünür kuru madde (%) yer almaktadır (Aksoy ve ark., 2001).

Kuru incir, yüksek enerji değeri, içerdiği mineral, vitamin ve diğer besin maddeleri ile insan sağlığı ve beslenmesinde önemli bir yere sahiptir. Kuru incir, diğer meyveler arasında en yüksek mineral içeriğine sahip meyvedir. Kalsiyum, bakır, magnezyum, potasyum ve kükürt içeriği açısından diğer meyvelerle kıyaslandığında birinci sırada yer alan kuru incir, enerji, pantotenik asit, riboflavin, tiamin ve piridoksin bakımından ikinci sırada bulunmaktadır. Kolay sindirilebilen fruktoz ve glikoz içeriğine sahip kuru incirin protein miktarı ise diğer birçok kuru meyvenin iki katından daha fazladır (Anonim, 2012a).

Kuru incirin anti-kanserojenik etkileri üzerine yapılan çalışmalar bulunmaktadır. Kuru incirler, pek çok tahıl, sebze ve meyve ile karşılaştırıldığında oldukça yüksek oranda polifenol içeriğine sahiptir. 100 gr incirde 1090-1100 mg polifenol bulunduğu belirlenmiştir. Antioksidan özelliğine sahip polifenoller, kanser oluşumunu ve kardiyovasküler hastalık riskini düşürücü özelliğe sahip olduğundan incir tüketimi sağlık açısından önem taşımaktadır.

Polifenollerin yanında anti-kanserojenik aktivite gösteren diğer bileşikler de kuru incirin yapısında yer almaktadır. Benzaldehit ve kumarinler bu bileşiklerdendir.

Benzaldehit, ölümcül kanser vakalarının tedavisinde başarıyla kullanılabilmektedir. İncirlerin uçucu ekstraktından izole edilen kumarinler ise prostat kanserinin tedavisinde kullanılmaktadır. Kumarinlerin deri kanserinin tedavisinde de kullanımı üzerine araştırmalar sürdürülmektedir. Kuru incirlerin kolesterol düşürücü özellikteki

fitosterol, lanosterol ve stigmasterol içerdiği de tespit edilmiştir (Vinson, 1999).

MATERYAL VE METOT

MATERYAL

Çalışma materyalinin temini

Araştırmada kullanılan incir çekirdekleri, 2014 yılının ağustos ayında Aydın İlinin Kurtuluş, Feslek ve Gencelli lokasyonlarında toplanmış incir meyvelerinden elde edilmiştir. Bu amaçla her bölgeden, 10'ar kg'lık toplanan incir meyveleri terlemeyi ve küflenmeyi engelleyecek, hava geçiren karton kutulara koyularak muhafaza edilmiştir. Toplanan incirler yaş halde muhafaza edilerek hemen analizlere tabi tutulmuştur. Ayrıca, Aydın İncir Araştırma Enstitüsü tarafından toplanan incirlerin çeşitleri sarı renkli olanlar Sarı Lop, mor renkli olanlar ise Mor Güz çeşidi olarak tespit edilmiştir.

İncir çekirdeği örneklerinin hazırlanması

Araştırmada laboratuvar şartlarında önce meyve ile ilgili analizleri gerçekleştirmek için her bölgeye ait incir meyvelerinden rastgele seçilerek ağırlıkları tartılmıştır. Daha sonra incir meyveleri spatula yardımıyla açılarak kabuk ve iç kısmı birbirinden ayrılmıştır. Elde edilen tohumların dışındaki yapışkan kısım ise içilebilir su içerisinde yıkanmış ve süzülerek tohumdan uzaklaştırılmış ve belirtilen analizler yapılmıştır.

METOT

İncir çekirdeklerinde yapılan analizler

Nem miktarı

Yaklaşık 10 g örnek, daha önce 105 ^{o}C' de kurutulmuş ve darası alınmış kaplarda tartılmıştır. Tartım işleminden sonra 105 ^{o}C' deki etüvde sabit ağırlığa gelinceye kadar kurutulmuştur. Rutubet miktarı ağırlık kaybından % olarak hesaplanmıştır (Anonim, 1987).

Kül miktarı

Her numuneden yaklaşık 3 g örnek daha önce 105^{o}C' de kurutulup, soğutulan ve darası alınan kül krozesine tartılmıştır. Daha sonra 700^{o}C' deki kül fırınına konulup ve numuneler tamamen yanıncaya kadar (kül tamamen beyaz renge dönünceye kadar) beklenmiştir. Numunelerin tamamen yanması için geçen süre 5-6 saat arasında değişmiştir. Kül miktarı % olarak hesaplanmıştır (Anonim, 1990).

Yabancı madde

İncirden başka gözle görülebilir her türlü madde için değerlendirme yapılmıştır.

Çekirdek bin tane ağırlığı

5 adet meyvenin çekirdekleri çıkartılıp karıştırılarak ve içinden 1000 adet çekirdek sayılıp 0.01 g hassasiyetindeki terazide tartılmak suretiyle belirlenmiştir (Aksoy, 1991).

İrilik tayini

Büyük, orta ve küçük irilikte seçilen danelerde kumpasla en ve boy ölçümü yapılmıştır.

Tohum sayısı analizi

Belirlenen lokasyonlardan toplanan incir meyvelerinin belirlenen türlerinden belirli sayıda alınarak içleri spatula yardımıyla açılmış ve tohum kısımları alüminyum folyo üzerine alınarak tek tek sayılarak meyvelerdeki tohum sayısı miktarları hesaplanmıştır.

Yağ tayini

5-10g kurutulmuş, öğütülmüş çekirdek süzgeç kâğıdıyla kartuş içine ve kartuş da ekstraktöre yerleştirilmiştir. Balon ve ekstraktörü birbirine bağlayarak petrol eterini devir daim yapmasını sağlayacak kadar kartuşun üzerinden konulmuş ve 6 saat sonra çıkarılmıştır. Petrol eteri damıtılarak geri alınmıştır. Cam balonu etüvde 103°C' de eterin fazlalığı uçuncaya kadar bekletilmiş ve desikatörde soğutulup tartılarak % yağ değeri kuru madde üzerinden hesaplanmıştır (Uylaşer ve Başoğlu, 2000).

İncir çekirdek yağlarında yapılan analizler

Serbest yağ asitliği (SYA) tayini

5 veya 10 g örnek tartılıp 50 veya 150 ml etil alkol- dietil eter karışımında çözülmüş ve elde

edilen çözelti fenol fitalein indikatörü eşliğinde 0,1 N KOH çözeltisin titre edilmiştir. SYA değeri mg KOH/g yağ cinsinden hesaplanmıştır (Nas, 2001).

Renk tayini

Araştırmamızda elde edilen incir yağları numunelerinin renk ölçümleri, (L^*, a^* ve b^* değerleri) D_{65} aydınlatmalı, 2° gözlemciye sahip Diffuse/ O modundaki 8 mm'lik aydınlatma aralığına sahip Kromametre (CR-400 model, Konica Minolta, Osaka, Japonya) kullanılarak gerçekleştirilmiştir. L^* (parlaklık), a^* (+60 kırmızı; -60 yeşil) ve b^* (+60 sarı; -60 mavi) renk koordinatları CIE L^*, a^*, b^* renk koordinat sistemine göre belirlenmiştir. Ölçümler, her bir tür ve varyetedeki incir yağı örneklerinde 3 farklı okuma yapılarak gerçekleştirilmiştir (Anonymous, 1976).

Peroksit sayısı analizi

AOCS'nin Cd 8-53 standart metodu kullanılmıştır. 5'er g yağ örneği üzerine 30 mL asetik asit-kloroform (3:2 v/v) ve 0,5 mL doymuş KI (Potasyum iyodür) ilave edildi. Bir dakika karıştırma işleminden sonra üzerine 30 mL H_2O 0,5 mL nişasta çözeltisi eklendi ve karışım 0,01 N sodyum tiyosülfat ile sarı renk görülene kadar titre edildi. Aynı işlem şahit deney içinde numune kullanılmadan da yapıldı ve peroksit sayısı metottaki formüle göre, meq/kg yağ olarak hesaplanmıştır (Anonymous, 1989).

Kırılma indisi

İncir yağı numunelerinin kırılma indisleri Abbe refraktometresi ile tespit edilmiştir. Bu amaçla spatula yardımıyla alınan incir yağı numuneleri, refraktometrenin prizması üzerine dökülmüş ve 20 °C okuma yapılmıştır (Anonim, 2004).

Yağ asitleri kompozisyonu

Gökalp (2001), tarafından belirtilen metod uygulanmış ve yağ numunelerinin yağ asidi kompozisyonu teşhisinin yapılması için 1 µl gaz kromotografisine injekte edilmiştir (Gökalp, 2001). Analizde SHIMADZU GC-14B markalı gaz kromotografisi kullanılmıştır. Cihaz üzerinde FID (flammenionisation detektor / alev iyonizasyon detektörü) detektörü kullanılmıştır. Kolon ise RTX-2330 marka olup, 60 m uzunluğunda, 0,25 mm çapında ve 0,20 µm film kalınlığına sahiptir.

İstatiksel analizler

Araştırmada 2 farklı yaş incir çeşidi (Sarı Lop ve Mor Güz), Aydın ilinde üç farklı lokasyondan (Kurtuluş, Gencelli ve Feslek) 2014 yılında toplanarak gerçekleştirilmiş ve analizler her bir tekerrürde üç paralel olarak yürütülmüştür. Böylece her bir parametre, faktöriyel deneme modeline göre 2 x 3 x 2 şeklinde düzenlenmiştir.

Araştırma sonucunda elde edilen veriler, deneme desenlerine uygun olarak varyans analizlerine tabi tutulmuşlardır. Varyans analizleri, SPSS 17.0 for Windows isimli paket programı kullanılarak gerçekleştirilmiştir. Araştırma sonuçlarının varyans analizinde one-way Anova ve önem dereceleri için Duncan çoklu karşılaştırma testi kullanılmıştır.

BULGULAR

Sarı Lop cinsi incir çekirdeği ve yağının bazı fizikokimyasal özelliklerinin ortalama değerleri şu şekilde tespit edilmiştir. Çekirdek sayısı, 1000 tane ağırlığı, kuru madde ile meyve ve çekirdek nem içeriği sırasıyla 957 adet, 3,911 g, % 91,61 ve % 11,81 olarak belirlenmiştir. Sarı Lop cinsi incir çekirdeği ortalama % 20,54 yağ içermektedir (Çizelge 1 ve 2).

Mor Güz cinsi incir çekirdeği ve yağının bazı fizikokimyasal özelliklerinin ortalama değerleri şu şekilde tespit edilmiştir. Çekirdek sayısı, 1000 tane ağırlığı, kuru madde ile meyve ve çekirdek nem içeriği sırasıyla 946 adet, 32,36 g, 3,761 g, % 92,02 ve % 9,71 olarak belirlenmiştir. Mor Güz cinsi incir çekirdeği % 23.53 yağ içermektedir. İstatistiki olarak Çizelge 1 ve 2' de görüldüğü gibi Mor Güz ve Sarı Lop cinsi incirlerde lokasyona göre belirtilen değerlerin $p<0,05$ düzeyinde değişebildiği belirlenmiştir.

Çizelge 1. Taze incir çekirdeklerinin fiziksel özellikleri.
Table 1. Physical properties of fresh fig seeds.

Çeşit Variety	Lokasyon Location	Nem Moisture (%)	Kül Ash (%)	1000 tane ağırlığı 1000 seed weight (g)	Yabancı Madde Foreign matter (%)	Yağ içeriği Oil content (%)	Ortalama büyüklüğü Average size	
							En Width (mm)	Boy Length (mm)
Mor Güz	Feslek	$8,08^{e} \pm 0,14$	$4,68^{a} \pm 0,05$	$2,52^{d} \pm 0,02$	$0,18^{d} \pm 0,04$	$26,01^{c} \pm 0,11$	$1,2^{c} \pm 0,05$	$1,3^{d} \pm 0,17$
	Gencelli	$11,62^{b} \pm 0,07$	$4,46^{a} \pm 0,08$	$3,25^{cd} \pm 0,01$	$0,39^{cd} \pm 0,08$	$16,14^{f} \pm 0,14$	$1,3^{b} \pm 0,16$	$1,4^{c} \pm 0,11$
	Kurtuluş	$9,42^{d} \pm 0,05$	$3,22^{b} \pm 0,14$	$5,50^{a} \pm 0,01$	$0,65^{e} \pm 0,05$	$28,44^{a} \pm 0,27$	$1,2^{c} \pm 0,05$	$1,5^{b} \pm 0,09$
	Ortalama	9,70	4,12	3,75	0,40	23,53	1,23	1,40
Sarı Lop	Feslek	$10,71^{c} \pm 0,10$	$3,11^{b} \pm 0,09$	$3,47^{c} \pm 0,02$	$0,72^{a} \pm 0,02$	$26,74^{b} \pm 0,10$	$1,4^{a} \pm 0,18$	$1,5^{b} \pm 0,06$
	Gencelli	$12,62^{a} \pm 0,05$	$3,48^{b} \pm 0,09$	$4,28^{b} \pm 0,03$	$0,42^{b} \pm 0,01$	$17,77^{d} \pm 0,16$	$1,4^{a} \pm 0,06$	$1,5^{b} \pm 0,10$
	Kurtuluş	$12,10^{a} \pm 0,06$	$3,34^{b} \pm 0,05$	$3,97^{bc} \pm 0,04$	$0,17^{a} \pm 0,01$	$17,11^{e} \pm 0,07$	$1,4^{a} \pm 0,09$	$1,6^{a} \pm 0,09$
	Ortalama	11,81	3,31	3,90	0,43	20,54	1,40	1,55

Aynı harfle gösterilen ortalamalar arasında önemli fark ($P \leq 0,05$) yoktur.
Same letters in a column are not significantly difference at the 0.05 probability levels.

Çizelge 2. Taze incir çekirdek ve yağının fiziko-kimyasal özellikleri.
Table 2. Physicochemical properties of fresh fig seeds and oil.

Çeşit Variety	Lokasyon Location	Asitlik Acidity (%)	Peroksit Sayısı Peroxide Number (meq/kg)	Kırılma indisi Refractive index (nD)	Renk Değeri Color Value		Çekirdek sayısı (Adet) Seed number (number)
					Kırmızı (R) Red	Sarı (Y) Yellow	
Mor Güz	Feslek	$1,1^{b} \pm 0,16$	$1,9^{b} \pm 0,12$	$1,4438^{c} \pm 0,0002$	$7,1^{b} \pm 0,12$	$6,5 \pm 0,23$	$801^{c} \pm 32,53$
	Gencelli	$1,2^{a} \pm 0,09$	$6,0^{a} \pm 0,06$	$1,4795^{a} \pm 0,0002$	$7,4^{a} \pm 0,04$	$3,4 \pm 0,02$	$936^{bc} \pm 30,56$
	Kurtuluş	$1,0^{c} \pm 0,20$	$0.0^{f} \pm 0,08$	$1,4699^{b} \pm 0,0003$	$4,4^{f} \pm 0,10$	$2,2 \pm 0,04$	$1103^{a} \pm 16,56$
	Ortalama	1,1	3,95	1,4644	6,3	4,03	947
Sarı Lop	Feslek	$0,7^{e} \pm 0,26$	$0,70^{e} \pm 0,14$	$1,4792^{a} \pm 0,0004$	$5,6^{d} \pm 0,03$	$2,4 \pm 0,08$	$948^{bc} \pm 16,53$
	Gencelli	$0,6^{f} \pm 0,09$	$1,46^{c} \pm 0,20$	$1,4791^{a} \pm 0,0003$	$5,8^{c} \pm 0,11$	$1,8 \pm 0,11$	$970^{b} \pm 20,69$
	Kurtuluş	$0,8^{d} \pm 0,06$	$1,04^{d} \pm 0,10$	$1,4780^{a} \pm 0,0003$	$5,4^{e} \pm 0,16$	$7,0 \pm 0,11$	$952^{bc} \pm 17,25$
	Ortalama	0,7	1,06	1,4788	5,6	3,73	957

Aynı harfle gösterilen ortalamalar arasında önemli fark ($P \leq 0,05$) yoktur.
Same letters in a column are not significantly difference at the 0.05 probability levels.

Mor Güz çeşidinde en yüksek çekirdek sayısı 1103 adet çekirdekle Kurtuluş lokasyonundan elde edilmiş iken, en düşük tohum sayısı 801 adet çekirdek ile Feslek lokasyonundan elde edilmiştir. Sarı lop çeşidinde ise en yüksek çekirdek sayısı 970 adet çekirdekle Gencelli lokasyonundan elde edilmiş iken, en düşük tohum sayısı 948 adet çekirdek ile Kurtuluş lokasyonundan elde edilmiştir.

Türk Gıda Kodeksi'nde belirtilen tebliğe göre; çekirdek içeren hammadde kullanıldığında çekirdek veya çekirdek parçası en fazla 1 adet/100 g yabancı madde bulunabileceği bildirilmiştir (Anonim, 2002). Yapılan araştırma sonucunda incir çekirdeği yabancı madde miktarı belirtilen değeri aşmamıştır. Belirtilen çeşit ve lokasyonlarda % 0,17-0,72 arasında belirlenmiştir. Bitkisel yağ teknolojisinde, bazı tohumların % yağ miktarları şu şekilde belirtilmiştir; ayçiçeği tohumu için % 22-36, aspir için % 25-37 ve kolza tohumu % 22-49 olduğu belirtilmiştir (Gökalp, 2001). Buna göre, incir çekirdekleri % yağ değerleri % 17-28 ile ayçiçeği ve aspir yağ değerleri arasında tespit edilmiştir. En düşük yağ değerine sahip incir çekirdekleri %16,14 ile Mor Güz çeşidi, Gencelli lokasyonundan elde edilir iken en yüksek yağ içeriği % 28,44 ile Mor Güz çeşidi, Kurtuluş lokasyonundan elde edilmiş ve ortalama yağ içeikleri incelendiğinde Mor Güz çeşidinin Sarı lop çeşidinden daha yağlı olduğu belirlenmiştir.

Türk Gıda Kodeksinde bir yağın, yemeklik yağ olarak tüketilebilmesi için serbest yağ asitliği değerinin mak. % 0,6 değerinde olmalıdır ibaresi bulunmaktadır. Bu doğrultuda, Çizelge 2'de görüldüğü gibi, elde ettiğimiz sonuçlar incir çekirdeği yağının, diğer kullanılabilir yağların serbest yağ asitliği değerlerinden yüksek olduğu

belirlenmiştir. Kayahan (1975), peroksit sayısı 5 meq/kg'dan sonra yağların acılaşmaya başladığını, 10 meq/kg' dan sonra ise yağın kullanılamaz hale geldiğini belirtmiştir. Buna göre incir çekirdeği yağı, peroksit sayısı açısından kullanılabilir düzeyde tespit edilmiştir.

Yağların kırılma indislerine genellikle, yağların kaynağını belirlemek amacıyla bakılmaktadır (Anonim, 2012b). Ayçiçek, mısır, kanola, fındık gibi bitkisel sıvı yağların kırılma indisleri 1,463 ile 1,476 arasında değişmektedir. Tespit ettiğimiz sonuçlar da, incir yağının kırılma indisinin, diğer bitkisel sıvı yağların kırılma indisleri ile paralellik gösterdiği görülmektedir.

Bitkisel yağ teknolojisinde, sağlam ve iyi temizlenmiş çeşitli tohumların kritik nem düzeyleri tespit edilmiş ve buna göre keten tohumu için % 11,8-12,8, ayçiçeği için % 9,9-10,8 ve genel olarak %12'nin altında olması gerektiği belirtilmiştir (Gökalp, 2001). Buna göre, incir çekirdekleri nem değerleri açısından; Sarı Lop çeşidinin çekirdek nem değerleri Gencelli (%12,62) ve Kurtuluş (%12,10) lokasyonlarında kritik nem düzeyini geçerken diğer çeşidin nem değerleri tüm lokasyonlarda kritik nem düzeyinin altında bulunmuştur.

Literatür bilgilerine göre, yaygın olarak kullanılan ayçiçeği yağı ile Çizelge 3' de görüldüğü gibi incir çekirdeği yağını, yağ asitleri kompozisyonu bakımından kıyasladığımızda, ayçiçeği yağının palmitik asit (% 7,9-12,0), oleik asit (% 34,4-45,5), linoleik asit oranı (% 36,9-47,9) iken (Yazıcıoğlu ve Karaali, 1983), tarafımızdan yapılan araştırma da incir çekirdeği yağlarının palmitik asit (% 6-7), oleik asit (%15-16), linoleik asit (%29-31) ve linolenik asit (%41-42) saptanmıştır. Bu karşılaştırma incir çekirdeği yağı ile ayçiçeği yağının yağ asidi kompozisyonunun birbirinden farklı olduğu ve incir çekirdeği yağının linolenik asit bakımından zengin olduğu tespit edilmiştir. Bu yönüyle keten tohumu linolenik asit (%55) içeriğine yakınlık göstermektedir (İşleroğlu ve ark., 2005). İncir çekirdek yağının yağ asidi kompozisyonu birden fazla çekirdek yağlarının önceki çalışmalardan elde edilen değerleri ile farklılık göstermiştir. Jeong ve Lachance (2001), tarafından kurutulmuş incir meyvesi çekirdek yağları üzerine yapılan çalışmada en baskın yağ asidi (% 53,1) olan linolenik asiti, linoleik asit (% 21,1), palmitik asit (% 13,8), ve oleik asit (% 9,8) olarak takip etmektedir. Doymamış yağ asitleri, toplam yağ asitlerinin % 89,87'sini oluşturmaktadır. Linolenik asit içeriği sonuçlarımız daha önce rapor edilen (% 53,1) değerden kısmen daha düşük seviyede (% 41,85) saptanmıştır (Jeong ve Lachance, 2001). Genel olarak, yağ asit kompozisyonu, doymuş ve doymamış yağ asitleri oranı literatür ile benzerdir. İnsan beslenmesinde en önemli çoklu doymamış yağ asitlerinden linoleik asit, belirgin kalp damar hastalıklarının önlenmesinde önemlidir (Boelhouwer, 1983). Bir başka çalışmada, *Opuntia ficus indica* tohumu yağı; % 9,32 palmitik, % 3,11 stearik,% 16,8 oleik, % 70,3 linoleik asit içerdiği belirtilmektedir (Ennouri ve ark., 2005).

Çizelge 3. Taze incir çekirdek yağlarının yağ asitleri lokasyon ortalama kompozisyonu (%).
Table 3. Fresh fig seed oils location average fatty acid composition (%).

Yağ asidi (Y. A.) Fatty acid	Taze incir çekirdeği Fresh fig seeds	
	Sarı Lop	Mor Güz
Miristik	0,06	0,01
Palmitik	6,96	7,06
Araşidik	0,39	0,22
Behenik	0,08	0,08
Lignoserik	0,03	0,03
Toplam doymuş Y. A.	7,52	7,40
Palmitoleik	0,05	0,05
Heptadesenoik	0,03	0,03
Cis-10-heptadesenoik	3,12	3,074
Oleik Asit	15,98	15,78
Elaidik Asit	0,75	0,79
Eikosenoik Asit	0,03	0,03
Toplam tekli doymamış Y. A.	19,96	19,75
Linoleik Asit (C18:02)	30,33	31,87
Linolenik Asit	42,11	40,88
Araşidonik Asit	0,02	0,021
Toplam çoklu doymamış Y. A.	72,46	72,77
Toplam doymamış Y. A.	92,42	92,52

SONUÇ VE ÖNERİLER

Sonuç olarak; Türkiye'de geniş bir alanda bol miktarda bulunan, *Sarı Lop* ve *Mor Güz* türlerine ait incir çekirdeği yağlarının, insan sağlığı açısından besleyici öneme sahip besin maddeleri özellikle de balık yağı, keten tohumu gibi hayvansal ve bitkisel yağlara paralel olarak linolenik yağ asidi yüksek oranda içerdiği tespit edilmiştir. Bu yönüyle linolenik asit bakımından iyi bir kaynaktır. Çekirdek yağlarının da Türk Gıda Kodeksi Bitki Adı ile Anılan Yemeklik Yağlar Tebliği' ne uygun olduğu ve yemeklik yağ olarak kullanılabileceği belirlenmiştir. Yüksek serbest yağ asitliği ve renk gibi değerlerinden dolayı, ayçiçeği, mısır ve kanola yağlarında olduğu gibi rafinasyon işlemine tabi tutulması da önerilmektedir. Yapılan literatür incelemeleri doğrultusunda incir ile ilgili belli konularda araştırma yapılmış, incir çekirdekleri ve yağı ile ilgili gerek sağlık gerekse besleyicilik açısından kapsamlı bir çalışma yapılmadığı görülmüştür. Bu yönüyle, yapılan araştırma literatüre yeni bilgiler sunmakta ve özgünlük taşımaktadır. Ayrıca, bu konu ile ilgili çalışmaların devam ettirilmesi önerilmektedir.

TEŞEKKÜR

Yazarlar desteklerinden dolayı T.C. Gıda Tarım ve Hayvancılık Bakanlığı'na bağlı Aydın İncir Araştırma Enstitüsü ve 15. Fen. Bil. 05 nolu projeye verilen destekten dolayı Afyon Kocatepe Üniversitesi Bilimsel Araştırma Projeleri birimine teşekkür etmektedir.

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ANADOLU, J. of AARI
ISSN: 1300 - 0225
28 (1) 2018, 77 - 82
MFAL

Determination of Some Characteristics of Perennial Ryegrass (Lolium perenne L.) Populations Collected from Natural Areas of Eskisehir for Breeding Purposes

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Received (Geliş tarihi): 13.04.2017 Accepted (Kabul tarihi): 23.01.2018

ABSTRACT: *The aim of the research is obtaining material and information for breeding of new varieties which can be used as turf and pasture plant in Central Anatolia and resembling regions. Some of the perennial ryegrass (**Lolium perenne** L.) seeds, collected from natural areas in 2010-2011, send with passport information to the Gene Banks in Ankara and Izmir. The rest of the seeds were grown in greenhouse and then transferred into field. In 2012, 17 populations were identified by observing (according to 1-9 and 1-5 scale) and measuring some characteristics. Then, mean and standard error values of the obtained data were determined. The mean values of the populations in main stem length, main stem thickness, flag leaf length, flag leaf width, number of nodes, internode length, growth pattern, rust resistance, winter resistance, tillering capacity, and leaf color changed between 30.2-48.7 cm, 1.9-2.8 mm, 5.7-10.8 cm, 3.3-4.9 mm, 2.6-3.7, 2.7-6.7 cm, 3.8-8.0, 3.8-7.9, 5.2-8.8, 5.1-9.0, and 3.9-5.0, respectively. The relations between the characteristics were examined by correlation analysis. Furthermore, ploidy levels of some single plants selected from the natural populations for breeding purposes were determined by flow cytometry analysis and all of these plants studied were found diploid.*

Key words: *Perennial ryegrass, **Lolium perenne** L. collection, breeding, correlation, ploidy.*

Eskişehir'de Doğal Alanlardan Toplanan Çok Yıllık Çim (Lolium perenne L.) Populasyonlarında Islah Yönünden Önem Taşıyan Bazı Özelliklerin Belirlenmesi

ÖZET: *Araştırmanın amacı, Orta Anadolu ve benzeri ekolojilerde yürütülen yeşil alan tesisi ve mera ıslah çalışmalarında kullanılabilecek yeni çeşitlerin geliştirilmesi çalışmalarına materyal ve bilgi üretmektir. 2010 ve 2011 yıllarında doğal alanlardan toplanan çok yıllık çim (**Lolium perenne L.**) tohumlarının bir kısmı durak bilgileri ile birlikte Ankara ve İzmir'deki Gen Bankalarına gönderilmiştir. Tohumların kalan kısmı serada fide haline getirildikten sonra araziye aktarılmıştır. 2012 yılında toplam 17 populasyon gözlem (1-9 ve 1-5 skalaları) ve ölçümlere tabi tutularak bazı özellikleri belirlenmiştir. Daha sonra verilerin ortalama ve standart hata değerleri saptanmıştır. Populasyon ortalama değerleri ana sap uzunluğunda 30,2-48,7 cm, ana sap kalınlığında 1.9-2.8 mm, bayrak yaprak boyunda 5,7-10,8 cm, bayrak yaprak eninde 3,3-4,9 mm, ana saptaki boğum sayısında 2,6-3,7 adet, ana saptaki boğum arası uzunluğunda 2,7-6,7 cm, büyüme şeklinde 3,8-8,0, pasa dayanıklılıkta 3,8-7,9, kışa dayanıklılıkta 5,2-8,8, kardeşlenme potansiyelinde 5,1-9,0 ve renkte 3,9-5,0 arasında değişmiştir. Özellikler arasındaki ilişkiler korelasyon analizi ile incelenmiştir. Ayrıca ıslah çalışmaları için seçilen tek bitkilerin ploidi düzeylerinin saptanması amacıyla flow sitometri yöntemi ile analiz gerçekleştirilmiş ve çalışılan tüm bitkilerin diploid olduğu belirlenmiştir.*

Anahtar sözcükler: *Çok yıllık çim, **Lolium perenne** L., toplama, ıslah, korelasyon, ploidi.*

INTRODUCTION

Although flora of Turkey is quite rich in terms of grasses used as turf and pasture plant, imported varieties are used for these purposes in general. There have been difficulties in adapting these varieties to different ecological conditions in the country (Oral and Acıkgoz, 2002). Perennial ryegrass (*Lolium perenne*) is a perennial cool season grass used as turf, pasture and forage plant. As a turf plant, it is widely used in establishing turf areas on sport and golf areas, parks, and gardens. The species germinates easily and covers the planting area quickly. Especially, the genotypes short and with thin leaves are extremely resistant to crushing. Origin of the species is Europe, Temperate Asia and North Africa. The use of imported varieties in turf area establishment in Turkey is an important problem for the country's agriculture and economy. Because turf areas are established at one time with intensive effort and expenditure, these are expected to benefit for a long time (Acıkgoz, 2001). Even in the pasture development studies in Turkey, foreign varieties are being used.

For these reasons domestic varieties are needed which are adaptable to different ecological conditions of the country. Collection and characterization of ecotypes widely found in natural areas of the country is an important resource for the development of ecologically adaptable varieties. It is reported that these natural ecotypes could be used in variety development studies using selection method (Tosun, 1973).

MATERIALS AND METHODS

In this research, there have been presented some observation and measurement data conducted in the ecotypes collected from natural areas to provide sources for breeding studies on turf and pasture type perennial ryegrass for Eskisehir and similar ecologies.

The collected materials were characterized in terms of some properties and then some single plants were selected for breeding purposes. Because ploidy levels should be known in breeding studies with the material collected from natural areas, these plants were subjected to ploidy analysis using flow cytometer method. Although perennial ryegrass is diploid naturally, tetraploid ones are found because of autopoliploidy in natural areas. Rapid and reliable results are obtained with flow cytometer in cases where the ploidy level of a large number of plants, such as plant genetic resources, need to be determined. The method is widely used in determining the DNA content and ploidy level of many turf grass species (Wang *et al*., 2009).

The collection and characterization studies were carried out in 2010, 2011 and 2012 in Eskisehir Province and at the Central Field of Transitional Zone Agricultural Research Institute. Climate data were given in the table below (Table 1). According to long term climate data, the area receives 347 mm precipitation and experiences an average monthly temperature 10.8 °C and 77.7% relative humidity. In the years our research was conducted, precipitation and average relative humidity were higher than long term data.

According to the analysis results of the soil taken from the germplasm area, experimental area is slightly alkaline, clayey, with medium limy, slightly salty, rich in potassium and rich in phosphorus (Table 2).

In the collection study in 2010, single plant seeds were collected from the stations determined for each 10 km considering altitude and direction in Eskisehir Province (Alan, 1986). In addition, they were collected in sheltered areas such as village cemeteries where these plants are concentrated. In the study, the factors such as soil type, soil slope, soil appearance, and their relation with vegetation were considered. Selections also were made according to the characteristics such as general appearance of plant (upright, decumbent etc.), color, and plant height. During the collection, information such as coordinates, altitude, region or local name and distance to the nearest settlement were recorded (Table 3).

The collected seeds divided into 3 parts and one part was sent to the Gene Banks in Izmir and Ankara with the station information data. One part has been preserved in the Institute. The rest of the seeds were grown in greenhouse and the seedlings transferred to field separately for each single plant. There were 20 plants for each single plant (population) collected from natural areas but some were lost due to winter and diseases.

These populations were subjected to the observations and measurements:

Main stem length (cm), main stem thickness (mm), flag leaf length (cm), flag leaf width (mm), number of nodes, internode length (cm), growth pattern (1 oblique - 9 upright), rust resistance (1 susceptible - 9 resistant), winter resistance (1 susceptible -9 resistant), tillering capacity (1 poor - 9 abundant), and leaf color (1 light green - 5 dark green) (Tosun, 1973; Sagsoz, 1974; Tokluoglu, 1979; Kornerup and Wanscher, 1978;Anonymous, 2016).

The obtained data were subjected to statistical analysis and population averages and standard errors were determined. Standard error values were determined to indicate whether there is variation between single plants in population. In addition, a correlation analysis was conducted to determine the relations between the characteristics (Kackar and Harville, 1984; Pearson, 1920).

Table 1. Monthly precipitation, mean temperature and relative humidity in Eskisehir, Turkey.
Çizelge 1. Eskişehir İli aylık toplam yağış, ortalama sıcaklık ve oransal nem değerleri, Türkiye.

Month Aylar	Precipitation (mm) Toplam yağış (mm)			Temperature (^{0}C) Ortalama sıcaklık (C^0)			Relative humidity (%) Ortalama oransal nem (%)		
	2010	2011	2012	2010	2011	2012	2010	2011	2012
January / Ocak	36.0	26.6	58.0	1.5	0.3	-3.6	95.2	97.0	96.8
February / Şubat	42.6	8.9	42.1	4.9	0.1	-5.5	90.5	93.2	97.3
March / Mart	32.6	20.0	56.4	5.9	3.7	1.5	85.5	88.0	87.7
April / Nisan	23.9	56.9	22.1	9.2	7.2	12.0	84.3	91.0	72.6
May / Mayıs	20.7	145.8	80.9	15.2	0.5	14.4	70.4	87.7	83.3
June / Haziran	79.0	9.4	0.0	18.1	16.6	20.0	82.8	84.6	71.6
July / Temmuz	7.4	8.5	5.5	22.0	21.6	22.8	75.4	70.8	68.1
August / Ağustos	0.9	0.0	3.5	24.4	20.0	20.8	66.2	73.5	65.1
September / Eylül	22.5	2.1	0.0	18.2	17.4	18.7	75.8	68.5	66.1
November / Ekim	77.1	57.9	16.1	10.0	8.5	14.2	92.5	83.6	78.1
October / Kasım	7.5	0.0	14.5	9.3	0.8	7.3	81.0	86.8	92.3
December / Aralık	60.4	46.1	73.2	4.2	0.9	2.2	94.1	92.1	95.1
Total / Toplam	410.6	382.2	372.3						
Mean / Ortalama				11.9	8.1	11.0	82.8	84.7	81.2

*Meteorological Station of Transitional Zone Agricultural Research Institute.
*Geçit Kuşağı Tarımsal Araştırma Enstitüsü Meteoroloji İstasyonu.

Table 2. Germplasm area soil analysis.
Çizelge 2. Gözlem bahçesi toprak analizi.

Soil Structure Toprak yapısı	pH	Total Salt (%) Toplam tuz (%)	Lime (CaCO3) (%) Kireç (%)	Organic matter (%) Organik Madde (%)	Phosphorus (kg P_2O_5/da) Alınabilir fosfor (kg P_2O_5/da)	Potassium (kg K_2O/da) Alınabilir potasyum (kg K_2O/da)
clayey killi	7.7	0.153	6.6	1.28	13.0	199.5

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Table 3. Collection site information.
Çizelge 3. Toplama yapılan yöre bilgileri.

Population number Populasyon numarası	District İlçe	Collection site Toplanan yöre	Latitude Enlem	Longitude Boylam	Altitude Yükseklik (m)
42	Sarıcakaya	3 km from Hekimdagı	39.90996 °N	30.62989 °E	1230
43	Han	Center-Cemetery	39.15439 °N	30.86392 °E	1224
44	Sivrihisar	Selimiye Village-Grassland	39.36983 °N	31.29889 °E	843
45	İnonu	Between Asagı Kuzfındık and Yukarı Kuzfındık	39.67896 °N	30.06833 °E	1066
46	İnonu	Between Asağı Kuzfındık and Yukarı Kuzfındık-Village Center	39.67738 °N	30.07262 °E	1075
47	İnonu	Oklubalı Village-Cemetery	39.81717 °N	30.24116 °E	831
48	Sivrihisar	Gerenli-Grassland	39.38882 °N	31.24430 °E	843
49	İnonu	Cemetery	39.81391 °N	30.12539 °E	842
50	Han	Gokcekuyu Village-Cemetery	39.24546 °N	30.83020 °E	1110
51	İnonu	Between Aşagı Kuzfındık and Yukarı Kuzfındık	39.67896 °N	30.06833 °E	1066
110	Merkez	Kargın-Cemetery	39.58781 °N	30.22781 °E	887
111	Gunyuzu	Ertugrul-Cemetery	39.29004 °N	31.59472 °E	863
112	Seyitgazi	Yazılıkaya-In front of the Monument	39.20018 °N	30.71504 °E	1292
113	Merkez	Avdan Village-Cemetery	39.56622 °N	30.49832 °E	1109
114	Tepebası	Taycılar-Cemetery	39.98288 °N	30.91326 °E	1104
115	Merkez	Uludere-Cemetery	39.91566 °N	30.33850 °E	1025
116	Seyitgazi	Bessaray Village-Cemetery	39.43322 °N	30.53410 °E	1028

The selected plants for breeding purposes according to the observations and measurements were subjected to a ploidy analysis using flow cytometer method in 2015. In the method, ploidy levels are determined using the core DNA content of plants (Wang *et al*., 2009). Flow cytometry device in Trakya University, Agricultural Faculty, Field Crops Department was used in the analysis which determines DNA content accurately.

RESULTS

The mean values and standard errors of the characteristics of 17 perennial ryegrass populations are given below (Table 4). As stated before, the number of plants in each population which initially were 20 decreased due to winter and disease damage. At the end of the observations and measurements, the population mean values in main stem length, main stem thickness, flag leaf length, flag leaf width, number of nodes, internode length, growth pattern, rust resistance, winter resistance, tillering capacity, and leaf color changed between 30.2-48.7 cm, 1.9-2.8 mm, 5.7-10.8 cm, 3.3-4.9 mm, 2.6-3.7, 2.7-6.7 cm, 3.8-8.0, 3.8-7.9, 5.2-8.8, 5.1-9.0, and 3.9-5.0, respectively (Table 4).

According to the results of the correlation analysis, there were some remarkable relationships between tillering capacity which is important for turf area and pasture establishment and some other characteristics (Table 5). While there are significant ($p<0.01$) and positive correlations between tillering capacity and internode length (0.642^{**}), tillering capacity and growth pattern (0.789^{**}), tillering capacity and winter resistance (0.980^{**}), there are significant ($p<0.01$) and negative correlations between tillering capacity and main stem length (-0.665^{**}), tillering capacity and number of nodes (-0.841^{**}). Some correlations between winter resistance and some other properties are also notable. While there are significant ($p<0.01$) and positive correlations between winter resistance

and internode length (0.636**), winter resistance and growth pattern (0.731**), there are significant (p<0.01) and negative correlations between winter resistance and number of nodes (-0.883**), winter resistance and main stem length (-0.636**)

As a result of the observations and measurements, some single plants were selected from populations for use in variety development studies. These plants were subjected to a ploidy analysis and they all were found diploid (Table 6).

Table 4. The mean values and standard errors of the perennial ryegrass populations.
Çizelge 4. Çok yıllık çim populasyonlarına ait ortalama ve standart hata değerleri.

*pop. no *pop. num.	plan. num. bit. say.	fll byb	flw bye	msl asu	mst ask	nn asbs	il asbau	c renk	gp bş	rr phd	wr kd	tc kp
42	8	8.0±0.7	3.9±0.4	37.1±2.8	1.9±0.2	2.9±0.2	5.8±0.8	4.4±0.2	6.1±0.3	6.3±0.2	8.8±0.2	8.9±0.1
43	13	8.9±0.7	3.4±0.2	38.3±1.6	2.5±0.1	2.8±0.1	5.6±0.3	3.9±0.1	7.8±0.1	4.8±0.6	8.4±0.1	9.0±0.0
44	4	10.5±0.7	4.8±0.3	36.1±2.5	3.0±0.0	3.0±0.0	5.9±0.7	4.0±0.0	7.8±0.3	6.5±0.3	8.0±0.4	8.5±0.5
45	5	8.0±0.5	3.7±0.2	37.1±4.3	2.2±0.2	3.0±0.0	4.7±0.6	4.0±0.0	7.6±0.2	6.0±0.0	8.0±0.0	9.0±0.0
46	8	7.8±0.7	3.9±0.3	31.8±2.5	2.1±0.1	2.6±0.3	4.3±1.0	5.0±0.0	5.5±0.3	6.0±0.0	8.5±0.2	9.0±0.0
47	7	10.8±1.0	4.6±0.4	37.6±1.8	2.4±0.2	2.9±0.1	4.9±0.4	4.0±0.0	7.1±0.1	5.9±0.3	8.3±0.3	8.7±0.3
48	7	8.4±0.9	3.9±0.3	30.2±2.3	2.1±0.1	2.7±0.2	3.9±0.5	3.9±0.1	6.4±0.4	5.0±0.0	8.3±0.2	9.0±0.0
49	9	7.8±0.8	3.7±0.3	33.4±1.8	2.0±0.0	2.8±0.2	4.8±0.7	4.0±0.0	7.3±0.2	5.8±0.3	8.0±0.0	9.0±0.0
50	5	10.3±1.4	4.0±0.6	41.0±3.3	2.2±0.2	2.6±0.2	6.7±0.4	4.2±0.2	6.2±0.6	3.8±0.5	8.2±0.2	8.8±0.2
51	4	7.9±1.9	3.3±0.3	32.5±2.7	2.8±0.3	2.8±0.3	5.4±0.6	4.0±0.0	8.0±0.0	6.0±0.0	8.0±0.0	9.0±0.0
110	17	8.0±0.7	4.5±0.7	41.9±2.0	2.4±0.1	3.4±0.1	4.3±0.4	3.9±0.1	3.9±0.1	7.9±0.2	5.6±0.2	5.5±0.1
111	14	8.8±0.9	3.4±0.3	39.1±1.6	1.9±0.2	3.1±0.1	3.6±0.3	3.9±0.1	3.9±0.3	7.1±0.1	5.2±0.2	5.1±0.2
112	15	8.5±0.6	4.3±0.1	43.4±1.1	2.1±0.1	3.7±0.1	2.9±0.3	3.9±0.1	3.9±0.2	7.0±0.0	5.4±0.3	5.5±0.2
113	18	5.7±0.4	3.8±0.2	36.5±1.8	2.1±0.1	3.3±0.1	2.7±0.2	4.1±0.1	5.6±0.1	7.0±0.0	5.6±0.2	5.4±0.3
114	20	7.8±0.5	4.9±0.2	48.7±2.1	2.7±0.1	3.6±0.1	4.4±0.5	4.2±0.1	3.8±0.1	7.1±0.1	6.3±0.2	6.0±0.2
115	20	6.8±0.4	4.2±0.2	44.1±1.5	2.3±0.1	3.4±0.2	5.2±0.3	4.2±0.1	5.1±0.1	7.9±0.1	5.8±0.2	6.5±0.1
116	20	8.7±0.2	4.1±0.1	45.1±1.7	2.2±0.1	3.2±0.1	4.5±0.6	4.1±0.1	6.5±0.1	7.0±0.0	5.8±0.2	5.9±0.2

*pop. no: population numbers, plan. num.: the plant number in population, fll: flag leaf length, flw: flag leaf width, msl: main stem length, mst: main stem thickness, nn: number of nodes, il: internode length, c: leaf color, gp: growth pattern, rr: rust resistance, wr: winter resistance, tc: tillering capacity.

* pop. num.: populasyon numarası, bit. say.: populasyon içindeki bitki sayısı, byb: bayrak yaprak boyu, bye: bayrak yaprak eni, asu: ana sap uzunluğu, ask: ana sap kalınlığı, asbs: ana sapta boğum sayısı, asbau: ana sapta boğum arası uzunluğu, renk: yaprak rengi, bş: büyüme şekli, phd: pas hastalıklarına dayanıklılık, kd: kışa dayanıklılık, kp: kardeşlenme potansiyeli.

Table 5. Correlation coefficients-r (n=17).
Çizelge 5. Korelasyon katsayıları-r (n=17).

Character Karakter	msl asu	mst ask	fll byb	flw bye	il asbau	nn asbs	gp bş	wr kd	rr phd	tc kp	c renk
msl	-	0.123	0.001	0.489*	-0.042	0.754**	-0.564*	-0.636**	0.469	-0.665**	-0.142
mst			0.302	0.398	0.383	0.095	0.315	0.130	0.039	0.138	-0.192
fll				0.294	0.507*	-0.348	0.306	0.386	-0.465	0.352	-0.201
flw					0.018	0.460	-0.367	-0.210	0.346	-0.293	0.058
il						-0.528*	0.547*	0.636**	-0.495*	0.642**	0.133
nn							-0.668**	-0.833**	0.802**	-0.841**	-0.286
gp								0.731**	-0.580*	0.789**	-0.103
wr									-0.764**	0.980**	0.294
rr										-0.761**	-0.045
tc											0.232
c											-

**(p<0.01), *(p<0.05).

Table 6. Ploidy analysis results.
Çizelge 6. Ploidi analizi sonuçları.

Population-single plant no Populasyon-tek bitki no	DNA content (pg) DNA içeriği (pg)	Ploidy level Ploidi düzeyi
113-4	5.46	diploid
114-2	5.51	diploid
114-4	5.43	diploid
114-17	5.42	diploid
114-15	5.48	diploid
113-18	5.44	diploid
113-9	5.51	diploid
113-4	5.46	diploid
42-12	5.48	diploid
42-17	5.43	diploid
43-1	5.44	diploid
43-2	5.47	diploid
43-15	5.39	diploid
45-5	5.43	diploid
45-6	5.46	diploid
46-12	5.49	diploid
46-18	5.49	diploid
47-1	5.58	diploid
47-5	5.57	diploid
47-6	5.49	diploid
48-14	5.48	diploid
49-1	5.39	diploid
49-16	5.39	diploid
50-7	5.55	diploid
51-1	5.53	diploid
51-2	5.56	diploid

CONCLUSION

In this collection and characterization study, when the mean and standard error values in the populations are examined, it appears that there is sufficient variation for breeding studies. In addition, according to the results of the correlation analysis, some important relationships were identified in terms of tillering capacity and winter resistance which are important for turf area and pasture establishment. Positive correlations between tillering capacity and internode length (0.642^{**}), tillering capacity and growth pattern (0.789^{**}), tillering capacity and winter resistance (0.980^{**}) and negative correlations between tillering capacity and main stem length (-0.665^{**}), tillering capacity and number of nodes (-0.841^{**}) were statistically significant. Positive correlations between winter resistance and internode length (0.636^{**}), winter resistance and growth pattern (0.731^{**}) and negative correlations between winter resistance and number of nodes (-0.883^{**}), winter resistance and main stem length (-0.636^{**}) were also notable.

The material and information obtained from the collection and characterization studies will be used in variety development studies of turf, pasture and forage type perennial ryegrass (*Lolium perenne* L.) in Transitional Zone Agricultural Research Institute.

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ANADOLU, J. of AARI
ISSN: 1300 - 0225
28 (1) 2018, 83 - 88
MFAL

The Effects of Different Doses of Organic Chicken Fertilizer on the Element Analysis of Sweet Basil (Ocimum basilicum L.)

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Received (Geliş tarihi): 29.05.2017 Accepted (Kabul tarihi): 29.01.2018

ABSTRACT: *Sweet basil (**Ocimum basilicum** L.) genus belonging to the Lamiaceae family is an important aromatic plant cultivated in many parts of the world for its essential oil The present study was performed to determine the effects of different organic chicken fertilizer doses (0,750, 1000, 1250 and 1500 kg/da) on some elements of sweet basil. The experiments were established in Bolu location (40°41' 28" N, 31°32' 38"E, 760 m elevation), during the years 2015-2016. The ions in aerosol samples were determined by using Dionex ICS 1100 Series ion chromatography. In leaves, the highest K^+ and Cl^- contents were observed in the control (0 kg/da) application (41.50 mg/g and 11.90 mg/g), the highest PO_4^{-3} and Mg^{+2} contents were detected in 750 kg/da organic chicken fertilizer (12.3 mg/g and 1.99 mg/g), the highest Ca^{+2} content was detected in 1000 kg/da organic chicken fertilizer (22.6 mg/g), the highest SO_4^{-2} content was detected in 1500 kg/da organic chicken fertilizer (84 mg/g) and the lowest Ca content was observed in 1500 kg/da organic chicken fertilizer (6.22 mg/g) and the lowest PO_4^{-3} and Cl^- contents were detected in 1250 kg/da organic chicken fertilizer (8.14 mg/g, 8.34 mg/g). As a result of this research, 750 and 1000 kg/da application of organic chicken fertilizer dose had significant effect on some elements of sweet basil.*

Keywords*: Sweet basil, **Ocimum basilicum** L., organic chicken fertilizer, element content.*

Organik Tavuk Gübresinin Farklı Dozlarının Fesleğen (Ocimum basilicum L.)'in Element Analizine Etkileri

ÖZ: *Lamiaceae familyasına ait fesleğen (**Ocimum basilicum** L.), dünyanın birçok yerinde uçucu yağı için yetiştirilen önemli bir aromatik bitkidir. Bu çalışma, farklı organik tavuk gübresi dozlarının (0,750, 1000, 1250 ve 1500 kg/da) fesleğen bitkisinin bazı elementleri üzerine etkilerini belirlemek amacıyla, 2015-2016 yıllarında Bolu lokasyonunda (40°41' 28" K, 31°32' 38"D, 760 m rakım) yürütülmüştür. Aerosol numunelerindeki iyonlar, Dionex ICS 1100 Seri iyon kromatografisi kullanılarak belirlenmiştir. Bitki yapraklarında en yüksek element içeriklerine, K^+ (41.50 mg/g) ve Cl (1.90 mg/g) kontrol şartlarında, Ca^{+2} (22.6 mg/g) 1000 kg/da organik tavuk gübre uygulamasında, PO_4^{-3} (12.3 mg/g) ve Mg^{+2} (1.99 mg/g) 750 kg/da organik tavuk gübre uygulamasında, SO_4^{-2} (4.84 mg/g) 1500 kg/da organik tavuk gübre uygulamasında ulaşılırken, en düşük element içeriklerine Ca^{+2}(6.22 mg/g) 1500 kg/da organik tavuk gübre uygulamasında, PO_4^{-3} (8.14 mg/g) ve Cl^- (8.34 mg/g) 1250 kg/da organik tavuk gübre uygulamasında ulaşılmıştır. Bu araştırmanın sonucunda, 750 ve 1000 kg/da organik tavuk gübre dozlarının fesleğenin bazı elementleri üzerine olumlu etkileri olduğu saptanmıştır.*

Anahtar Kelimeler: *Fesleğen, **Ocimum basilicum** L., organik tavuk gübresi, element içeriği.*

INTRODUCTION

Sweet basil (*Ocimum basilicum* L.), a member of the Lamiaceae family is an annual herb which grows in several regions around the world. Among more than 150 species of the genus *Ocimum,* sweet basil is the major essential oil crop which is cultivated commercially in many countries (Sajjadi, 2006). Sweet basil does not show natural

distribution in Turkey, but they are cultivated as medicinal, seasoning or oil plants especially in the western and southern Anatolia. Its leaves contain 0.5-2 % essential oil and methyl chavicol (estragol), eugenol, linalool, methyl cinnamate and camphor are the important components of essential oil of basil leaves. Due to the chemicals contained in essential oils, essential oils of sweet basil are used for treatment of dry mouth and dental complaints, diarrhea and chronic dysentery, respiratory disorders, and effective in the treatment of fungal diseases and stomach discomfort in addition, the influential antitussive, diuretic, anthelminthic, tranquilizer and expectorant roles in medicinal approach. Moreover, ceasing nasal-bleeding and preventing constipation, good for fatigue and insomnia, and uses for healing migraine headaches and incomplete paraplegia were reported (Telci *et al*., 2006). However, recently the potential uses of sweet basil essential oil, particularly as antimicrobial and antioxidant agents have also been investigated (Suppakul *et al*., 2003; Sartoratotto *et al*., 2004; Lee *et al*., 2005; Wannissorn *et al*., 2005; Politeo *et al*., 2007). Minerals are important in human nutrition. It is well known that enzymatic activities as well as electrolyte balance of the blood fluid are related to adequacy of Na, K and Mg. Potassium is very important in maintaining the body fluid volume and osmotic equilibrium. Metal deficiency syndrome like rickets and calcification of bones is caused by calcium deficiency. Several studies on nutrition in developing countries have shown that adequate nutrient intake (daily calories, daily protein, daily fat, minerals and vitamins) is an essential ingredient for improved well-being, economic growth and development, since a healthy body enhances the capacity to learn which in turn determines productivity and economic growth (Flores, 2001; Smith and Haddad, 2001; Diao *et al*., 2007). The objective of this work is to estimate the concentration of some nutrient elements of different doses of organic chicken fertilizer for sweet basil.

MATERIALS AND METHODS

The present study was performed to determine the effects of organic chicken fertilizer application of different doses on some elements of sweet basil during 2015-216 years in Bolu location (40°41′ 28″ N, 31°32′ 38″E, 760 m elevation). In this context, five different doses (0, 750, 1000, 1250 and 1500 kg/da) were applied. The experiments were arranged in the Completely Randomized Blocks Design with three replications in May in 2015 and 2016 in open-field conditions. Each experimental plot consisted of five rows that were a row-to-row distance of 0.3 m and plant-to-plant distance of 0.2 m. Sweet basil was regularly irrigated to demonstrate good progress in its period vegetation since irrigation is a very important factor for cultivation of basil. Soil properties of experimental fields were as follows: rich in phosphorus (12.19 ppm), potassium (51.04 ppm) and organic matter (3.1%), clay-loam and slightly alkaline (pH=7.6). Climatic data during the vegetation period of the experimental years (may-september) was 19.1 ^{o}C (average temperature), 259.1 mm (total rainfall) and 71.2% (average humidity) (Anonim, 2016). The properties of organic chicken fertilizer is given in Table 1 (Anonymous, 2017).

Table 1. Properties of the organic chicken fertilizer.
Çizelge 1. Organik tavuk gübresinin özellikleri.

Analysis Parameters	Unit	Analysis results (W/W)
EC	dS/m	2.6
Organic matter	%	72.6
Beneficial ratio	%	60-65
Total Humic and Fluvic Acid	%	61.5
pH	-	6.2
Moisture	%	19.8
Total N	%	2.2
Total P_2O_5	%	1.76
Water soluble K_2O	%	1.62
Organic N	%	1.4
C/N	-	17.4
Organic Carbon	%	38.4

Estimation of element content

To prepare the samples for the element content determination, 5 g of samples were extracted with

50 mL deionized water, in ultrasonic water bath during 30 minutes. Then, extracts were filtered with 0.22 µm cellulose acetate filter and prepared for the analysis. Before sample analysis, the standard Dionex anion mix and Dionex cation mix were used for calibration. The ions in aerosol samples were determined by using Dionex ICS 1100 Series ion chromatography (Table 2). The results were checked by using the ERM-CA408 simulated rainwater (low contents). Operation conditions of the instrument are given in Table 3.

The results were checked by using the ERM-CA408 simulated rainwater (low contents). The percent error was shown in Table 3. The analysis of the element contents were the average of three replicates of field experiment.

RESULTS

The present study was conducted for the evaluation of elements such as Calcium (Ca^{+2}), Magnesium (Mg^{+2}), Lithium (Li^{+}), Amonyum (NH_4^{+}), Potassium (K^{+}), Sodium (Na^{+}), Fluoride (F^{-}), Chloride (Cl^{-}), Nitrite (NO_2^{-}), Nitrate (NO_3^{-}), Sulfat (SO_4^{-2}), and Phosphorus (PO_4^{-3}) in the leaves of sweet basil. The results indicated that the leaves contain highest concentration of K^{+}, Ca^{+2}, PO_4^{-3} and Cl 41.50, 22.6, 12.30 and 11.90 mg/g and lowest concentration of Mg (1.39 mg/g) and SO_4^{-2} (2.73 mg/g), respectively (Table 4).

Table 2. Optimum operation conditions for Dionex ICS 1100 Ion Chromatography.
Çizelge 2. Dionex ICS 1100 İyon Kromatografisi için optimum çalışma koşulları.

Operation conditions Çalışma koşulları	Anion Anyon	Cation Katyon
Mobile phase (Mobil aşama)	9 mM Na_2CO_3	20 mM Metansulfonic acid
Column (Sütun)	Ionpac AS9-HC (250 x 4 mm)	Ionpac CS12-A (250 x 4 mm)
Guard column (Koruma sütunu)	Ionpac AG9-HC (50 x 4 mm)	Ionpac CG12-A (50 x 4 mm)
Supressor (Süpressör)	ASRS-4 mm	CSRS-4mm
Supressor current (Süpressör akımı)	45 mA	65 mA
Detector (Dedektör)	Conductance detector	Conductance detector
Pressure (psi) (Basınç)	2000-3000	2000-3000
Oven temperature (Fırın sıcaklığı)	30 ^{o}C	30 ^{o}C
Background conductance (Arkaplan iletkenliği)	< 30 µS	0.5-2 µS
Flow rate (Akış hızı)	1.00 mL/min	1.00 mL/min
Injection volume (Enjeksiyon hacmi)	500 µL	1000 µL
Rate of data transfer (Veri aktarım hızı)	5.0 Hz	5.0 Hz
Duration (Süre)	30 min	15 min

Table 3. ERM-CA408 simulated rainwater (low contents) results.
Çizelge 3. ERM-CA408 yağmur suyu (düşük içerikler) benzeri sonuçlar.

Element Element	Certified value (mg/L) Belirlenmiş değer	Uncertanity Belirsizlik	Aritmetic mean (mg/L) Aritmetik ortalama	Standard deviation Standart sapma	Error (%) Hata
NH_4^{+}	0.910	0.028	0.789	0.03870	-13.300
Mg^{2+}	0.145	0.022	0.113	0.01220	-21.700
F^{-}	0.194	0.008	0.187	0.00481	-3.150
Cl^{-}	1.960	0.070	1.940	0.01350	-0.730
NO_3^{-}	2.010	0.090	1.970	0.02110	-1.700
SO_4^{2-}	1.460	0.040	1.490	0.02910	2.390

Table 4. Concentration of elements in *Ocimum basilicum* (mg/g) by different doses of organic chicken fertilizer.
Çizelge 4. Organik tavuk gübresinin farklı dozlarına bağlı olarak *Ocimum basilicum* (mg/g) bitkisindeki element konsantrasyonları.

Organic chicken fertilizer doses Organik tavuk gübre dozları	K^{+}	Mg^{+2}	Ca^{+2}	Cl^{-}	PO_4^{-3}	SO_4^{-2}
Control (Kontrol)	41.50	1.71	7.66	11.90	10.60	3.06
750 kg/da	35.70	1.99	13.50	10.6	12.30	4.10
1000 kg/da	35.04	1.94	22.60	10.4	9.60	2.73
1250 kg/da	37.0	1.94	14.80	8.34	8.14	3.18
1500 kg/da	37.60	1.39	6.22	8.55	10.09	4.84

All tested extracts did not include NH_4^{+}, F^{-}, Na^{+}, NO_2^{-}, NO_3^{-}, Li^{+} elements. In this study, the concentration of Mg ranged from 1.39 to 1.99 mg/g. The highest value was determined from 750 kg/da of organic chicken fertilizer application in sweet basil leaves, and the lowest value was determined from 1500 kg/da of organic chicken fertilizer application in sweet basil leaves (Table 4). Mg has got prime role in the maintenance of normal physiology in all living organisms. Mg prevents cardiac arrhythmia disorders, high blood pressure (Witte et al., 2008; Soetan et al., 2010). K concentrations of sweet basil varied between 35.04 and 41.50 mg/g. Control (0 kg/da) application demonstrated highest K concentration (41.50 mg/g) compared to other organic chicken fertilizer leaves extracts applied. The importance of K is speculated from its participation in large number of biological processes, such as acid base balance, movement of muscles, nerve impulse conduction, and regulation of osmotic pressure (Hajjar et al., 2001). Ca concentrations of sweet basil varied between 6.22 and 22.60 mg/g (Table 4). While the highest values were obtained from 1000 kg/da of organic chicken fertilizer application in sweet basil leaves, the lowest values were obtained from 1500 kg/da of organic chicken fertilizer application in sweet basil leaves. Ca is an extremely important element in human body. Ca plays a significant role in building strong bones teeth and heart functions (Brody, 1994). Ca may result in tetany and convulsions due to impetuous discharges of nerve impulses. The recommended daily Ca intake required for normal biochemical activities of the body is 1500 mg (Hassan et al. 2015). Cl was present in the range of 8.34-11.90 mg/g. The highest concentration was present in control application followed by 750 kg/da (10.60 mg/g). SO_4^{-2} concentrations of sweet basil ranged from 2.73 to 4.84 mg/g. While its maximum content (4.84 mg/g) was presented in 1500 kg/da organic chicken fertilizer application, and its minimum content (2.73 mg/g) was presented in 1000 kg/da organic chicken fertilizer extracts. In the present study, the concentration range of PO_4^{-3} was 8.14-12.30 mg/g, as shown in (Table 4). The highest level of that form of PO_4^{-3} was found in 750 kg/da of organic chicken fertilizer application followed by control doses of organic chicken fertilizer application (10.60 mg/g).

DISCUSSION

According to the results obtained from this study, the highest mineral values were found in organic chicken fertilizer application at 750-1000 kg/da (Table 4). According to the earlier scientific studies conducted on nutritive composition of wild plants, high quantities of minerals can be found especially in K, Na, Ca, P and Mg (Guil Guerrero et al., 1998; Agrahar-Murugkar and Subbulakshmi, 2005). The metal ions including Fe^{3+}, Zn^{2+}, $Mg^{2+,}$ K^{+}, Ca^{2+} and some other micronutrients are cofactor for nearly 100 enzymes, which are involved in cell division, nucleic acid metabolism and protein synthesis. The researches have shown that application of micronutrients reduces the effects of environmental stresses (Cakmak and Hors, 1991). Ozcan (2002) determined the mineral contents of 32 plants used as condiments in Turkey and as a result Al, Ba, Ca, Fe, K, Mg, P and S contents were high in all plants analyzed. B, Mg, S, Sr, Zn contents of basil were found as 31.75 ppm, 5737.8 ppm, 1923 ppm, 141.97 ppm, 13.71 ppm, respectively. Lavilla *et al*., (1999) indicated that *O. basilicum* contented Mg (7458 mg/g) and

Ca (21500 mg/g). Yamawaki *et al*., (1993) reported that one hundred grams of fresh basil leaves contained 250 mg of calcium, 37 mg of phosphorus, 5.5 mg of iron, and 11 mg of magnesium. Daniel *et al*., (2011) reported that *O. basillicum* exhibited high potassium content (28.770 mg/kg), calcium (17.460 mg/kg) and appreciable quantity of sodium (290 mg/kg) and magnesium (266 mg/kg). Tarchonue *et al*., (2012) reported that the concentration of the accompanying anions had an important role in *O. basilicum* in response to salinity. They indicated a marked selectivity for K^+ and Ca^{2+} over Na^+ with values of selectivity ratio significantly increasing with both Na_2SO_4 and NaCl salinity treatments. They also indicated Mg^{+2} and Sulphate content was decreased significantly by both treatments as compared to the controls, but Cl^- and SO_4^{-2} anions were found at high concentration in basil affected by NaCl and Na_2SO_4 treatments, respectively. Tewari *et al*., (2012) indicated that *O. basilicum* contented Na (85.9±1.29), K (397.57±4.12), Ca (1133.4±0.04), Li (6.66±1.15), Fe (59.83±1.5), Cu (1.26±0.03), Mn (5.73±0.12), Co (0.16±0.21), Zn (16.06±0.84) mg/100g. The contents of Ca and K obtained from the present study are higher compared to the results of researches. The differences between our results and earlier studies may be due to the applications of different organic chicken fertilizer, use of different extracts for analysis, different environmental and genetic factors.

CONCLUSION

In this study, among all the extracts analyse, 750 and 1000 kg/da organic chicken fertilizer applications had higher element content than the control and other organic chicken fertilizer applications. It was also shown that sweet basil leaves are rather rich sources of K^+, Ca^{+2}, PO_4^{-3}, NO^{3-}, NO^{2-} and Cl^- and potentially bioavailable for human consumption.

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ANADOLU, J. of AARI
ISSN: 1300 - 0225
28 (1) 2018, 89 - 93
MFAL

Evaluation of Yield and Quality Characteristics of Dill (Anethum graveolans L.) in Turkey and the World

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Received (Geliş tarihi): 29.05.2017 Accepted (Kabul tarihi): 24.01.2018

ABSTRACT: *In recent years, the volume of use of Medicinal and Aromatic Plants (MAPs) increases with the formation of new usage areas and increasing the demand for natural products day by day. One of these plants is dill (**Anethum graveolans** L.), which is usually an annual herb in Umbelliferae (Apiaceae) family. Its origin is Asia and it is seen commonly in our country due to grow naturally in the mediterranean basin. India and Pakistani are the most producer countries of this plant. Moreover, USA, UK, Mexico, Germany, Hungary and Netherlands produce high amounts of dill. In addition to growing naturally, it also is grown in gardens in our country frequently. In Turkey dill had cultivated in 7387 da areas and it was produced 7208 tons in 2017. 111 tons of dill was produced in 84 da areas under protective cover (low tunnel, plastic greenhouse and high tunnel) greenhouse conditions in 2017. Dill was most growth in Mediterranean region with 4113 da cultivation areas and 4061 tones production and followed Marmara, Aegean and Internal Anatolia in 2017 year in Turkey. In the present review, the chemistry, yield and quality characteristics, and economic value of dill and its components were discussed.*

Keywords: Anethum graveolans *L., dill, cultivation area, economic value, quality characteristics, yield and componenet.*

Dünya'da ve Türkiye'de Dereotu (Anethum graveolans L.) Bitkisinin Verim ve Kalite Özelliklerinin Değerlendirilmesi

ÖZ: *Son yıllarda, tıbbi ve aromatik bitkilerin yeni kullanım alanlarının oluşması ve doğal ürünlere olan talebin artmasına bağlı olarak, bu bitkilerin kullanım hacmi her geçen gün artmaktadır. Bu bitkilerden birisi de Apiaceae (Umbelliferae) familyasından genellikle tek yıllık bir bitki olan dereotu (**Anethum graveolans** L.)'dur. Anavatanı Asya olup, Akdeniz havzasında da doğal olarak yetiştiğinden, ülkemizde yaygın bir şekilde görülmektedir. En önemli üreticileri Hindistan ve Pakistan'dır. Aynı zamanda, ABD, İngiltere, Macaristan, Almanya, Meksika ve Hollanda gibi ülkelerde de yüksek miktarlarda üretilmektedir. Ülkemizde doğal olarak yetişmesinin yanı sıra, bahçelerde de sıklıkla yetiştirilen bir bitki türüdür. Türkiye'de 2017 yılında dereotu 7387 da alanda yetiştirilmiş ve 7208 ton üretilmiştir. 2017 yılında 84 da alan örtü altı yetiştiriciliğinde (alçak tünel, plastic sera ve yüksek tünel) 111 ton üretilmiştir. Türkiye'de en fazla 4113 da alan ve 4061 ton üretimi ile Akdeniz bölgesinde, yetiştirilirken, bu bölgeyi Marmara, Ege ve İç Anadolu bölgeleri takip etmiştir. Bu derleme de, dereotu bitkisinin kimyasal yapısı, verim ve kalite kriterleri, ekonomik değeri ve bileşenleri tartışılmıştır.*

Anahtar Kelimeler: Anethum graveolans *L., dereotu, ekim alanı, ekonomik değer, kalite kriterleri, verim ve bileşenleri.*

INTRODUCTION

Apiaceae (Umbelliferae) is one of the largest plant family and it is called "maydanozgiller" in Turkish and "carrot family" in English. Apiceae family contains annual and perennial plants which are generally herbaceous and sometimes growing in bush form (Pulur, 2012). It is also rich in terms of essential oil. This family has a widespread in the nothern hemipshere and also rare spread in the tropical regions. Turkey is the one of the countries included these plants. Apiaceae consists of approximately 300 genera and more than 3000 species (Stace, 1999). One of these species is dill (*Anethum graveolans*) which is called "dill" in English and called "dereotu" in Turkey, but it is named "tereotu, turakotu, durakotu, tarhanaotu, darakotu" different local names of Turkey (Baytop, 1994). Dill is an annual herb from Apiaceae family and it's native to south west Asia or south east Europe and it has been noted that it has cultivated throught the history (Bailer et al., 2001) and it has grown in Mediterranean region. Today, dill is cultivated almost all of Europe, United States (US) and many other countries, including the Mediterranean countries. Dill is grown in moist areas and also has not soil selectivity (Ceylan, 1997). Therefore, cultivation of dill is widespread in different places of Turkey. Some researchers reported that its medicinal uses are as a vegetable, a carminative, an aromatic, an antispasmodic, diuretic, stimulant and stomachic (Simon *et al*., 1984; Hornok, 1992; Sharma, 2004). Dill contains a large variety of antioxidant photochemical, bioactive molecules or antimicrobial activity against *Saccharomyces cerevisia* and *Listeria monocytogenes* (Pascal *et al*., 2002) and it can neutralize the free radicals thanks to retarding the progression of many chronic diseases associated with oxidative stress and reactive oxygen species (Sun *et al*., 2002; Liu, 2003). Dill has been used against digestive system disorders as natural drugs for centuries. It has been reported that dill included flavonoids, phenolics and essential oil (Delaquis *et al*., 2002). Dill has also aromatic smell and flavor depending on carrying high volatile oil.

The objective of this review is to determine the chemistry, yield and quality characteristics, and economic value of dill and its components in Turkey and the world.

Situation of dill in the World

Dill is one of the most traded medicinal and aromatic plants in the world. It is reported that dill was firstly cultivated in Europe in 1500 year, but it met with America continent in the 19th century (Small, 2006). India and Pakistan are the most important producer countries. In terms of its essential oil, the most producer countries are Hungary, the highest quality dill plants are cultivated in Egypt, Fiji, Mexico, Netherlands, United States, United Kingdom, Hungary and Germany. Pioneer producing essential oil countries are Hungary, The United States and Bulgaria (20 tons). In USA, dill is the third essential oil plants after *Mentha spicata* L. (spearmint) and *Mentha piperita* L. (peppermint). In addition, 10-15% of the world's annual essential oil production was obtained from Canada (Small, 2006; Pulur, 2012).

Situation of dill in the Turkey

While dill had the largest cultivation areas in Mediterranean Regions (4113 da), it had the smallest cultivation areas in Southeastern Anatolia (10 da) in Turkey (Table 1). In terms of production of dill, the main producers of dill plants were Mediterranean Region (4061 tones) and Marmara Region (1639 da) and followed by Aegean (671 tones) and Internal Anatolia (495 tones). The least production regions were East Anatolia and Southeastern Anatolia with 25 and 3 tones in Turkey (Table 1) (Anonymous, 2018).

Among the provinces, 32 provinces had cultivation area and production of dill in Turkey. Hatay had the most cultivation area (4035 da) and production (4027 tones) in Mediterranean region and followed by Balıkesir from Marmara in terms of cultivation area with 841 da, yet Bursa had the second production after Hatay with 882 tones. The lowest production was found as 56 tones in Ankara and

followed by Çanakkale, Muğla, Samsun with 63, 66, 75 tones among the first 10 provinces in Turkey, respectively (Figure 1) (Anonymous, 2018).

In Turkey, the Mediterranean Region has the largest cultivation area (4113 da) and the Southeastern Anatolia has the smallest (3 da) cultivation area of dill (Table 1).

Cultivation of dill in Turkey changed between 3259-7387 da between 2012-2017 years (Table 2). According to 2012 year, it was significantly getting increase both cultivation area (7387 da) and production (7208 tones) in 2017. Between 2012-2014 years, in Turkey, cultivation area and production values getting increase every year. After 2014 year, cultivation area and production values remained partly stable. In 2017 year, cultivation area and production values of dill had the highest level among 2012-2017 years. It is also seen that there were very few fluctuations in cultivation areas and productions. Dill was also grown under protective cover as low tunnel, plastic greenhouse, and high tunnel. Among the 2014-2017 years, cultivation area and production of dill remained as a stable with 3 da and 3 tones in low tunnel. The cultivation areas of dill increased in plastic greenhouse (26 da) and high tunnel (55 da); its production increased in plastic greenhouse (33 tones) and in high tunnel (75 tones) in 2017 data compared with 2014 (Table 3) (Anonymous, 2018).

As a shown Table 3, the maximum production was observed in high tunnel conditions and followed by plastic greenhouse and low tunnel between 2014-2017 years.

Totally, dill was produced 7319 tones and cultivated 7471 da with under protective cover in 2017 (Table 1, 3).

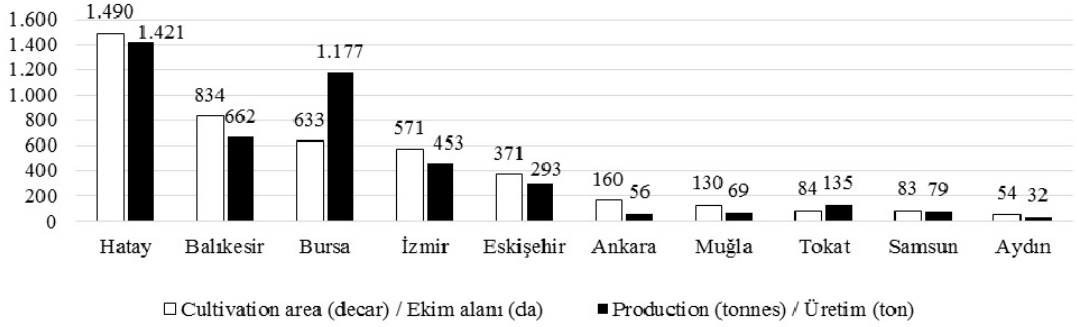

Figure 1. Cultivation area (decar) and production (tones) of dill among the first 10 provinces in Turkey (2017).
Şekil 1. Türkiye'de dereotu üretiminde ilk 10 ilin ekim alanı (da) ve üretim miktarları (ton).

Table 1. Cultivation areas and production of dill in Turkey (2017).
Çizelge 1. Türkiye'de dereotunun ekim alanları ve üretim miktarı (2017).

Regions Bölgeler	Cultivation area (decar) Ekim alanı (da)	Production (tonnes) Üretim miktarı (ton)
Mediterranean Region (Akdeniz Bölgesi)	4113	4061
Marmara Region (Marmara Bölgesi)	1439	1639
Aegean Region (Ege Bölgesi)	849	671
Black Sea Region (Karadeniz Bölgesi)	283	314
Internal Anatolia Region (İç Anadolu Bölgesi)	674	495
East Anatolia Region (Doğu Anadolu Bölgesi)	19	25
Southeastern Anatolia Region (Güneydoğu Anadolu Bölgesi)	10	3
Total (Toplam)	7387	7208

Table 2. Cultivation area and production of dill among the 2012-2017 years.
Çizelge 2. 2012-2017 yılları arasında dereotunun ekim alanı ve üretimi.

Years Yıllar	Cultivation areas (decar) Ekim alanı (da)	Production (tones) Üretim (ton)
2012	3259	2901
2013	4167	3806
2014	4824	4603
2015	4777	4488
2016	4763	4589
2017	7387	7208

Table 3. Cultivation areas and production of dill in greenhouse conditions in Turkey.
Çizelge 3. Türkiye'de sera koşullarında dereotunun ekim alanı ve üretimi.

Growing conditions Yetiştirme koşulları	Years Yıllar	Cultivation areas (decar) Ekim alanı (da)	Production (tones) Üretim (ton)
Low tunnel Alçak tünel	2014	3	3
	2015	3	3
	2016	3	3
	2017	3	3
Plastic greenhouse Plastik sera	2014	16	22
	2015	16	22
	2016	15	21
	2017	26	33
High tunnel Yüksek tünel	2014	45	49
	2015	42	50
	2016	38	51
	2017	55	75

Some studies about yield and quality characteristics of dill (*Anethum graveolans* L.) in Turkey and the World

There are several studies about dill in Turkey and the world. Elik *et al*. (2013) reported that in different sowing times, the yield components of dill is ranged; plant height (64.1-79.3 cm), branch number (3.2-6.3 per plant), umbel number (4.4-9.8 per plant), umbel diameter (4.6-7.3 cm) and they also indicated that fruit number per plant (172.5-210.5), fruit yield (35.6-73.2 kg/da) and fruit essential oil content (1.3-1.55%), fruit yield (35.6-73.2 kg/da), herb yield (1270.5 kg/da). Darzi and Seyed Hadi (2012) observed that plant height (72.7-77.8 cm), umbel number per plant (11.1-15.1), weight of 1000 seeds (1.48-1.58), biomass yield (2671.7-6169.7 kg/ha), fruit yield (1280.5-2196.8 kg/ha) of dill yield in Iran conditions. Khamssi (2014) have found height (90.80-97.67 cm) umbel/plant (62.6-99.3), umbellate/umbel (32-37), biomass (93.2-173.8 g/m^2), grain yield (65.67-84.71 g/m^2), oil essence (235.7-370.3 mg/50g DW) of dill. Agarwal (2008) reported that ratios of essential oil changed between 0.1-5.0%. The highest essential oil was obtained from fruits and the lowest was observed from herb of dill.

When the carried out studies examined in Turkey, they showed that yield of dill changed between 1000-2000 kg/da as fresh herb and between 200-400 kg/da as a dry herb (Ceylan, 1997). It has been reported that dill contains 2.5% essential oil, 10.0% water, 305 kcal energy, 16.0 g/100 g protein, 14.5 g/100 g raw oil, 55.2 g/100 g carbohydrate and 6.6 g/100 g ash according to ASTA chemical standard and USDA food composition (Elik, 2010). In addition to this, the quality of dill essential oil depends on essential oil rates such as carvone and α-phellandren.

CONCLUSION

Dill has been used since ancient times in ayurvedic medicines such as carminative, stomachic and diuretic. It is also used as a spice and aromatic smell and flavor because of essential oil. According to data, production quantities and cultivation areas of dill have increased in recent years. The Mediterranean Region has the largest cultivation area, and the highest production in Turkey.

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ANADOLU, J. of AARI
ISSN: 1300 - 0225
28 (1) 2018, 94 - 99
MFAL

Preliminary Study on Edible Insect Species Cybister limbatus (Fabricius 1775) and Its Heavy Element Contents

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Received (Geliş tarihi): 20.04.2017 Accepted (Kabul tarihi): 07.02.2018

ABSTRACT: *The objective of the study was to determine the concentration of heavy elements in edible insect pack and estimate the potential health risks of elements to humans via consumption of the insect pack. Aquatic edible insect Diving Beetle pack,* ***Cybister limbatus*** *(Fabricius 1775) (Coleoptera: Dytiscidae) was chosen for the study. Sixteen heavy elements (Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br, Rb, Sr, Pb) were determined in the edible beetle. Energy Dispersive X-ray Fluorescence (EDXRF) was used to determine the concentration of the elements. Among the sixteen studied elements Ca (0.98 ppm), Ti (0.49 ppm) and V (0.38 ppm) had the highest concentration, whereas Rb (0.05 ppm), Sr (0.04 ppm), As (0.32 ppm), Br (0.06 ppm) and Pb (0.06 ppm) had the lowest concentration. The results compared with US institute of medicine (IOM) panel on micronutrient guidelines, the levels of elements in this study were found safe for consumption. But especially residues of Pb and As, which are thought as the potential hazardous elements, in this edible insect may pose health problem in the future.*

Keywords: *Edible insects,* ***Cybister limbatus*** *(Fabricius 1775), entomophagy, EDXRF, Dytiscidae, heavy element.*

Yenilebilir Böcek Türü Cybister limbatus (Fabricius 1775) ve Ağır Element Seviyeleri Üzerine Bir Ön Çalışma

ÖZ :*Bu çalışmanın amacı yenilebilir böcek paketlerindeki ağır element miktarını değerlendirmek ve bu böcek paketinin tüketimi yoluyla insanlar için sağlık riskini değerlendirmektir. Çalışma için sucul yenilebilir böcek* ***Cybister limbatus*** *(Fabricius 1775) (Coleoptera: Dytiscidae) seçilmiştir. Bu türde on altı ağır element (Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br, Rb, Sr, Pb) tespit edilmiştir. Çalışma için EDXRF (Enerji Dağılımlı X-Işını Floresans) spektrometresi kullanılmıştır. Çalışılan 16 element arasında Ca (0,98 ppm), Ti (0,49 ppm) ve V (0,38 ppm) en yüksek konsantrasyonda, Rb (0,05 ppm), Sr (0,04 ppm), As (0,32 ppm), Br (0,06 ppm) ve Pb (0,06 ppm) ise en düşük konsantrasyondadır. Sonuçlar Birleşmiş Millletler İlaç Enstitüsü mikrobesin esasları hakkındaki panel ile karşılaştırıldığında, bu çalışmadaki değerler tüketim için uygun görülmüştür. Fakat, özellikle potansiyel zararlı elementlerden olduğu düşünülen Pb ve As miktarı gelecekte sağlık problemleri oluşturabilir.*

Anahtar Sözcükler: *Yenilebilir böcek,* ***Cybister limbatus*** *(Fabricius 1775), entomofaji, EDXRF, Dytiscidae, ağır element.*

INTRODUCTION

Entomophagy is a term that describes eating insects as a food source. Besides insects play an important role in survival of human kind like to help plant reproduction and waste biodegradation, using their product in technology and engineering etc., also insects serve as a source of food for human. Contrary to many societies, entomophagy or consumption of insects is part of human nutrition in some countries including China, Thailand, India, Korea, Japan, Mexico, New

Zealand, Australia (Bodenheimer, 1951). Hundreds of insect species have been used as human food in these countries. Some of the popular edible insects are grasshoppers, caterpillars, winged termites, bee, wasp and ant brood, winged ants, cicadas, and a variety of aquatic insects (Raubenheimer and Rothman, 2013).

As a food source, insects are highly nutritious, and nowadays entomophagy is a major entomological research interest with focus on its future prospect for food and feed security. In many country insects are clearly a "food of choice" rather than necessity, but in some poorest of society insects are consumed to combat hunger and malnutrition and scientist recommends to people eat more insects to these societies because edible insects have low-fat, high-protein, mineral and vitamin for people (Adeoye *et al*., 2014; Anankware *et al*., 2015).

The members of the Dytiscidae (Predacious diving beetles) under the order Coleoptera represented in most of the freshwater and feed vigorously on almost all invertebrates as well as fish eggs. They are active swimmers and swift divers (Nilson and Holmen, 1995). Dytiscidae is highly diverse and estimated to include about 4,000 species in over 160 genera. The genus *Cybister* is the most well-known groups (Deepa, 2009). *Cybister limbatus* (Fabricius 1775) belongs to Dytiscidae and mostly eaten in China, in Africa (by Bantu people), Japan, Senegal, Sierra, Leone, USA, Mexico, Indonesia, Madagascar, Sri Lanka, Thailand, Korea, Myanmar, Vietnam, India, Cameroon, Congo and Laos (Ramos-Elorduy *et al*., 2009; Mitsuhashi, 2016). Generally, insects have been collected from nature by hand picking but they can also collect with the aid of sweep net, dead raphia or oil palm (Adeoye *et al*., 2014). Instead of this, insects are now increasingly sold in local markets or online shopping.

Heavy element pollution has expanded many parts of the world, especially developing countries. These elements are quite reactive and generally toxic even at low concentration. When enter abiotic environment they bioaccumulate in food chain and affect biota negatively (Gall *et al*., 2015). Heavy metal accumulation level can vary with species and kind of element. Some species are intolerant to environmental disturbance and they change their habitat or getting extinct while others can tolerate the pollution and continue to living in a polluted or naturally high-metal environment. These hyper-accumulator organisms are using in mostly bioremediation studies (Dixit *et al*., 2015).

These days, contamination of different kind of food products by heavy elements is becoming an unavoidable problem. Studies showed that long time exposure to lead (Pb) and Arsenic (As) via food or air may affect brain development in children and fetus negatively, in adults affect generally kidney and other organs. The aim of the study is to quantify the accumulation of heavy elements in this edible insect pack and to determine whether these levels pose a human health concern. The results obtained from this study would provide information for background levels of elements in the edible insect Diving Beetle pack.

MATERIALS AND METHODS

In this study heavy element concentration of aquatic edible insect Diving Beetle, *Cybister limbatus* (Coleoptera: Dytiscidae) pack was evaluated. The insect sample measured by Energy Dispersive X-Ray Fluorescence (EDXRF) spectrometry at Atatürk University Professor Dr. H. C Wolf Weyrich High Energy Spectrometer Research Laboratory. Different kind of samples such as liquid, gas, soil or powder can be analyzed easily and wide range of elements can be measured simultaneously with EDXRF. The edible insect pack was taken from online. Only one insect was enough to measure heavy element content with EDXRF and one insect weight was 0.098 g. Firstly, the sample dried in an oven at 80˚C for 36 hours to extract moisture then pulverized in mortar and cellulose was added as a binder. Five tons of pressure applied to make 13 mm diameter pellet. 5 Ci ^{241}Am radioactive source and an HPGe detector

with resolution ~180 eV at 5.9 keV was used to determine the heavy elements in 13 mm diameter pellet of *Cybister limbatus*. The sample was excited by using 59.5 keV photons which emitted from ^{241}Am radioactive source. The measurement was carried out under vacuum. The concentration of elements in the sample was determined by WinAXIL software.

RESULTS

The current study describes the dietary exposure to heavy elements in consuming the edible insect and heavy element concentration of this edible insect pack was evaluated. Results of measured sixteen elements were given in Table 1, and photo of the studied insect *Cybister limbatus* (Fabricius 1775) were given in Figure 1.

Figure 1. *Cybister limbatus* (Photo by: Zeynep Aydogan).
Şekil 1. *Cybister limbatus* (Fotoğraf: Zeynep Aydoğan).

Among the sixteen studied elements Ca (0.98 ppm), Ti (0.49 ppm) and V (0.38 ppm) had the highest concentration, whereas Rb (0.05 ppm), Sr (0.04 ppm), As (0.32 ppm), Br (0.06 ppm) and Pb (0.06 ppm) had the lowest concentration.

Bio-accumulation of the elements in *Cybister limbatus* showed a trend in the Ca>Ti>V>Cr>As>Mn>Co>Ni>Fe>Cu>Zn>Se>Br =Pb>Rb>Sr.

Table 1. Heavy element concentrations in *Cybister limbatus*.
Çizelge 1. *Cybister limbatus*'un ağır element konsantrasyonları.

Heavy Element Ağır element	*Cybister limbatus* (ppm)
Ca	0.98
Ti	0.49
V	0.38
Cr	0.34
Mn	0.30
Fe	0.17
Co	0.25
Ni	0.19
Cu	0.15
Zn	0.13
As	0.32
Se	0.08
Br	0.06
Rb	0.05
Sr	0.04
Pb	0.06

DISCUSSION AND CONCLUSIONS

Insects are eaten traditionally in most cultures and are playing an important role in human nutrition and provide many nutrients to the consumer especially the people who suffer from malnutrition. Eating edible insect at sufficient levels can promote the level of essential elements and also vitamins to human body. Even though insects are not used in Turkish food culture, it is a growing industry as an alternative source of unprocessed raw materials in the world. Potential of insect to bio-accumulate chemical substances it is probable to accumulate in the consumers' body which later may reach toxic concentration. It is therefore necessary to control the levels of these toxic elements in food in order to protect human health. Besides to insects' nutritional value there is no standard that only refer to use of insects as food.

Permissible daily intakes of the measured elements were given in below;

Ca has an essential role in nervous system, muscles, bone and tooth. Recommended daily intake for male/female is 1000 mg/d (Anonymous, 2001). In this study Ca level is found within the normal level (0.98 ppm).

Cr predominantly found in the body as trivalent form (Cr^3) and helps to metabolism the fats and carbohydrates. Recommended daily intakes for male/female are 35 and 25 µg/d respectively (Anonymous, 2001). In this study Cr level is found as 0.34 ppm.

Mn is necessary for the brain and nerve function, required for metabolism and component of some enzyme. Recommended daily intakes for male/ female are 2.3 and 1.8 mg/d respectively (Anonymous, 2001). In this study the insect Mn level is found as 0.30 ppm.

Fe is a component of hemoglobin and many enzyme systems. Recommended daily intakes for male/female are 8 and 18 mg/d respectively (Anonymous, 2001). In this study the insect Fe level is found as 0.17 ppm.

Co is a part of cobalamin or vitamin B_{12} and help to produce red blood cell. In the literature recommended daily intake of Co is variable and may be as much as 1 mg. In this study the insect Co level is found as 0.25 ppm.

There has been no clear identified biological function of Ni in human but it can be component of some metabolic enzymes and therefore performs vital functions in metabolism. There is no reference daily intake for Ni in IOM list (Anonymous, 2001) but Anke *et al*. (1984) gave Ni value for human requirements less than 500 µg/kg. In this study the insect Ni level is found as 0.19 ppm.

Cu is a cofactor of many redox enzymes IOM recommended daily intake for man and woman is 900 µg/d (Anonymous, 2001), and in this study measured level is 0.15 ppm.

According to Marger *et al*. (2014) divalent form of Zn is the second most abundant element in the human body and modulates the activity of protein folding and function. IOM recommended daily intake for man and woman is 11 and 8 mg/d respectively (Anonymous, 2001), in this study measured level is 0.13 ppm.

Se studies showed that it has possible protective effects against cancer and other chronic disease (Fairweather-Tait *et al*., 2011). IOM recommended daily intake for man and woman is 55 µg/d, in this study measured level is 0.08 ppm (Anonymous, 2001).

There is no clear statement of biological function of Br and it has no toxicological concern in nutrition. In the WHO report (Anonymous, 2009) it is stated that some hemodialysis patients, who has insomnia, is related to Br deficiency. In the same report express that 320 mg/l plasma Br level may sometimes fatal. In this study measured Br level in the insect is 0.06 ppm.

Ti, V, As, Rb, Sr and Pb has no biological function in human body. In this study, levels of these elements in the insect total body are 0.49, 0.38, 0.32, 0.05, 0.04 and 0.06 ppm respectively. Elemental Ti is not reactive but the researchers from UCLA indicated that Ti damages to DNA and chromosome (Trouiller *et al.,* 2009). According to IOM maximum level of daily V intake in both man and woman is 1.8 mg/d and is not likely to pose a risk of adverse effects (Anonymous, 2001). US drinking water standards stated that >50 µg/l As lead to cancer, cardiovascular and neural disease (Anonymous, 1980). According to ATSDR Sr is found everywhere in small degree and can be exposed to low levels via food, breathing and water (Anonymous, 2004). Stable form of Sr is not harmful but strontium chromate is hazardous due to toxic form of chromium not Sr itself. When it enters to body, acts like calcium and accumulate in the bone. This may lead to weakened of growing bone. Radioactive Sr may cause cancer but there is no any data indicate that stable Sr cause cancer.

Residues of Pb, As etc. in this edible insect may pose health problem in the future. Toxicity of As depend on mostly its chemical form i.e. arsenide and its compounds are toxic for human health, its dose and duration of exposure are also important. Exposure or intake inorganic As via food, medications, work places or environment leads to multi-organ and system dysfunction such as skin,

cardiovascular, nervous system, gastrointestinal system (Mazumder, 2008). Like As, Pb level in organisms is a sign of contamination because they are not essential for biota. According to WHO report in the general population source of lead uptake predominantly based on food (Tong *et al*., 2000). Lead generally stored in bone but this storage is an age dependent process. While lead deposits in spongy bones of children, in adults leads is deposited in spongy cortical bone and teeth. Iron and Calcium supplementation impair lead uptake and decrease the absorption of lead. In literature low level exposure of lead (blood level below 10 µg/dl) cause neurological damage, cognitive disorders and renal dysfunction (Tong *et al*., 2000; Patrick, 2006). In the present study lead level was measured 0.06 ppm.

As it seen the Table 1 all the heavy elements are far below the tolerable limits for consumption. However, the whole values of heavy elements are acceptable for human consumption; adequate check should be maintained against the risk of these elements in the *Cybister limbatus* pack. Because we do not ensure that adequate actions were taken of its production, manufacture, preparation and packaging to reduce contamination of insect pack. Although our study did not show any clear relationship between heavy element concentration in the selected insect and its environment, our data still suggest that consuming this insect should be monitored for heavy element level. This contamination may be via naturally or anthropogenically. More comprehensive study should be done to clarify such comparison. Industrialized edible insects may include less heavy element than the collected from nature. The study needs to more research to understand if industrialized edible insects more safe for consumption than the collect from nature.

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ANADOLU, J. of AARI
ISSN: 1300 - 0225
28 (1) 2018, 100 - 107
MFAL

Patates Böceği [Leptinotarsa decemlineata Say.(Coleoptera: Chrysomelidae)]'nin Nevşehir İlinde Yaşamsal Etkileşim ve Çeşitliliği Üzerine Bir Ön Çalışma

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Received (Geliş tarihi): 03.07.2017 Accepted (Kabul tarihi): 16.02.2018

ÖZ: *Patates böceği (**Leptinotarsa decemlineata** Say.) patates bitkisinde önemli zararlar oluşturan bir böcek türüdür. Patates bitkisi, bu böcekler için tercih edilen en temel konukçudur. Bununla birlikte; Solanacaea familyasına ait farklı bitki türleri üzerinde de beslenebilmekte ve yaşamını sürdürebilmektedir. **L. decemlineata** popülasyonunu kontrol etmek amacı ile, insektisitler yaygın olarak kullanılmakta fakat bu ilaçlara karşı **L. decemlineata** tarafından çok hızlı direnç geliştirilmektedir. **L. decemlineata** 'nın birçok doğal düşmanı olmasına rağmen; bunlar, **L. decemlineata** populayonunu kontrol etmede yeterli olamamaktadır. Bu bağlamda bu ön çalışma ile; patates üretimi ile öne çıkan Nevşehir ili ve çevresinde patates zararlısı **L. decemlineata**'nın patates ile yaşamsal etkileşimini; biyolojik ve morfolojik gelişim sürecini belirlemek ve bu zararlı ile mücadelede yapılacak çalışmalara katkı sağlamak amaçlanmıştır. Patates böceğinin yaşam döngüsü kışlama dönemi sonrasında ergin bir birey olarak başlar ve 30 gün kadar sürer. 10-12 mm uzunluğunda olan **L. decemlineata**, sarımsı turuncu renklerdeki sert ve uzun her iki kanadında beş siyah çizgi bulundurmaktadır. **L. decemlineata'** nın larvaları küçük, kırmızımsıdır ve olgunlaştığında yaklaşık 10 mm uzunluğa ulaşmaktadır. Olgun larva toprağın 4-8 cm kadar içine girmekte ve yaklaşık 2. günden sonra pupa haline gelmeye başlamaktadır. Gelişim süreçleri 5-8 gün aralığında olan **L. decemlineata** pupaları oval ve turuncumsudur. Yumurtalar; parlak turuncu renkte, futbol topu şeklinde, 1.5-2.0 mm uzunluğunda ve 0.7 mm genişliğindedir. Dişi ergin bireyler yumurtalarını, 9-49'lu kümeler halinde bitki yapraklarının alt yüzeyine bırakmakta ve yumurtaları tutturmak için sarımsı bir yapışkan madde kullanmaktadır. **L. decemlineata** larvası yumurtadan çıkmadan önceki son 12 saatte gözle görülür hale gelebilmektedir. Doğal arazi şartları altında dişiler 200-500 tane yumurta bırakabilmektedir.*

Anahtar kelimeler*: Patates böceği, **Leptinotarsa decemlineata** Say., çevre, biyolojik çeşitlilik, sürdürülebilir tarım, Nevşehir.*

Preliminary Study on Life Effects and Diversity of the Colorado Potato Beetle [Leptinotarsa decemlineata Say. (Coleoptera: Chrysomelidae)] in Nevsehir Province

ABSTRACT: *The Colorado Potato Beetle (**Leptinotarsa decemlineata** Say.) is an insect that causes significant damage to potato plants, Potato plant is the most preferred host for these insects. However, it can feed and survive on various plant species of the Solanacaea family. With the aim of controlling the **L. decemlineata** population, insecticides are widely used but very rapid resistance is developed by **L. decemlineata** against these insecticides. Even though many natural enemies have been identified, they are usually not able to control **L. decemlineata** populations below the necessary levels. In this context, with this preliminary study; it is intended to determine the interaction between **L. decemlineata** with potatoes and the work to be done with this pest by determining the biological and morphological development process as, **L. decemlineata** is the main potato pest in and around Nevşehir Province which is leading in potato production. The life cycle of this potato beetle starts with the adult at the overwintering stage and can be as short as 30 days. The adults of **L. decemlineata** measure about 10-12 mm long and are yellowish-orange with five black stripes per elytron. The small, reddish larvae of the **L. decemlineata** are 10 mm long when mature. Mature larvae burrow 4–8 cm into the soil and after about two days begin to pupate. **L. decemlineata** pupae are oval and orangish in color. The mean development time is about 5-8 days. The eggs are bright orange and football-shaped, about 1.5–2.0 mm long and 0.7 mm wide. Females use a yellowish adhesive to deposit eggs on the lower surface of the foliage in clusters of 9 to 49. The larva of **L. decemlineata** becomes visible in the last 12 hours before hatching. Under field conditions, females can lay 200–500 eggs.*

Keywords: *Colorado Potato Beetle, **Leptinotarsa decemlineata** Say., environment, biodiversity, sustainable agriculture, Nevsehir.*

GİRİŞ

Dünya ülkelerinin %79'unda patates yetiştirilmekte, üretilen miktar olarak buğday, mısır ve pirinçten sonra 4. sırada yer almaktadır. Temelde çeşitli yöntemlerle hazırlanıp tüketilen patates, gelişmiş ülkelerde sanayi alanında konserve, dondurulmuş gıda, cips, püre, granül ve toz formlarda işlenip pazarlanmaktadır. Bunun dışında yan ürün olarak alkol, nişasta ve hayvan yemi yapımında kullanılmaktadır (Onaran ve ark., 2000).

Patates üretiminde Çin, Hindistan, ABD, Rusya önemli ülkelere arasında bulunmakta ve Türkiye 12. sırada yer almaktadır. Türkiye de 2012 yılında 174 bin hektar alanda, 4 milyon 822 bin ton patates üretimi gerçekleşmiştir (Anonim, 2014). Ülkemizde patates üretimine baktığımızda, hemen her ilde patates üretimi yapılmaktadır. Nevşehir ülkemiz için önemli olmak üzere üretimin yoğun olarak yapıldığı iller sırası ile; Niğde, Nevşehir, İzmir, Bolu ve Afyonkarahisar'dır. Ülkemizde üretimin % 57,9'u bu illerimiz tarafından yapılmaktadır (Anonim, 1998). Niğde 716 bin 849 ton üretimle ilk sırada yer alırken, Nevşehir 430 bin 650, İzmir 407 bin 247, Afyon 342 bin 459 ve Bolu 310 bin 542 ton üretim ile Niğde'yi izlemektedir (Anonim, 2017).

Patates böceği *L. decemlineata* (Coleoptera: Chrysomelidae) başta olmak üzere patates üretimini azaltan diğer böcek türleri patates yaprakbiti, *Aphis nasturtii* Kalt. *Aphis gossypii* Glov. (Homoptera: Aphididae), *Macrosiphum euphorbia* Thomas., *Myzus persicae* Sulz. ve *Hyalesthes obsoletus* Sign. (Homoptera: Cixiidae), patates güvesi *Scrobipalpa operculella* Zell. (Lepidoptera: Gelechiidae), *Thrips tabaci* Lind. (Thysanoptera: Thripidae), kırmızı örümcek *Tetranychus cinnabarinus* Boisd. (Acarina: Tetranychidae), pis kokulu yeşil böcek *Nezara viridula* L. (Heteroptera: Pentatomidae) ve yaprak pireleri (Homoptera: Cicadellidae) olarak bilinmektedir (Kayapınar ve Kornoşor, 1990).

L. decemlineata'nın ana vatanı Güneybatı Amerika ve Meksika olup, ilk olarak 1811 yılında Thomas Nuttall tarafından toplanmış, 1824 yılında Thomas Say tarafından adlandırılmış ve *Solanum rostratum* Dunal. üzerinde beslendiği tespit edilmiştir (Alyokhin, 2009; Piper, 2011). *Leptinotarsa decemlineata* türünün 1859 yılına kadar patates bitkisine zarar verdiği bilinmemektedir (Alyokhin, 2009; Piper, 2011; Telli, 2012).

L. decemlineata Say. (Coleoptera: Chrysomelidae) (Patates böceği), Türkiye'de ilk kez 1963 yılında Edirne'nin Yunanistan ile sınırı olan Bosna ve Karaağaç köylerinde saptanmıştır. Daha sonra zararlı, Trakya Bölgesinden başlayarak yurdumuzun iç bölgelerine doğru ilerlemiştir (Atak, 1973; Telli, 2012).

Patates böceğinin en uygun konukçusu patates (*Solanum tuberosum* L.) olmakla birlikte zararlı bu bitkiyi bulamadığı zaman Solanaceae familyasından domates (*Lycopersicum esculentum* Mill.), patlıcan (*Solanum melongena* L.), tütün (*Nicotiana tabacum* L.) ve biber (*Capsicum annuum* L.) bitkileriyle de beslenmektedir. Bu bitkilerden başka yaban yasemini (*Solanum dulcamara* L.), köpek üzümü (*Solanum nigrum* L.), marul (*Lacuca sativa* L.), soğan (*Allium cepa* L.), *Hyoscymus niger* L ., *Solanum angustifolium* Mill., *Solanum carolinense* L., *Solanum sarrachoiides* Sendtner ve *Solanum elaeagnifolium* Cav.'un zararlının konukçuları arasında yer aldığı belirtilmektir (Metcalf ve Flint, 1962; Gürkan ve Boşgelmez, 1984; Hare, 1990; Has, 1992; Anonim, 2008).

Leptinotarsa decemlineata ergin ve larvaları, konukçularının yapraklarında beslenmekte, gerek ergin, gerekse larva döneminde konukçularının yapraklarını genellikle dıştan başlayarak içe doğru kemirmekte ya da yaprakta bir delik açarak bu deliği genişletmek suretiyle beslenmektedir. Önce yaprakların ana damarlarını bırakarak beslenmekte, sonra onu da yiyerek bitkileri sadece gövdeden ibaret bir hale getirmektedir (Çakıllar, 1960; Has, 1992; Anonim, 2008). Araştırmalarda zararlının patateste %70-%80'lere varan ürün kaybına neden olduğu belirlenmiştir (Oerke ve ark., 1994).

Patates böceği yaprakla beslenerek doğrudan yaptığı zararın yanı sıra patatesin önemli hastalıklarından olan patates kahverengi çürüklüğü, iğ yumru viroidi ve patates halkalı çürüklüğü hastalıklarının

yayılmasına da taşıyıcı olarak neden olmaktadır (Yüceer, 2011).

Bu ön çalışmanın ana amacı; patates üretimi ile öne çıkan Nevşehir ili ve çevresinde patates zararlısı *L. decemlineata* Say.'ın patates ile yaşamsal etkileşimini; biyolojik ve morfolojik gelişim sürecini belirlemek olmuştur.

MATERYAL VE METOT

Arazi çalışmaları

Bu çalışma Nisan-Eylül 2016 ayları arasında Kapadokya bölgesi-Nevşehir İli, Ürgüp İlçesi, Mazı lokalitesinde yer alan patates tarlaları ve çevresinde yapılmıştır.

Toplanan larva ve ergin örnekler cam kavanozlara konulmuştur. Toplanan larva ve ergin örneklerinin konulduğu kavanoz kapların üzeri ince gözenekli bir bezle kapatılarak uygulamanın yapılacağı laboratuvar ortamına getirilmiştir. Toplanan yumurta örnekleri ise yaprakları ile birlikte petri kaplarına alınarak laboratuvar ortamına getirilmiştir.

Laboratuvar çalışmaları

Nevşehir il merkezindeki Nevşehir Hacı Bektaş Veli Üniversitesi'nde Entomoloji Araştırma Laboratuvar ortamına tarladan toplanan 80 ergin ve 200 larva örneği getirilmiştir.

Laboratuvarda 654 yumurta, 468 larva, 127 ergin ve 58 pupa birey incelenmiştir.

Getirilen ergin ve larvalar dönemlerine göre kavanozlara ayrılmıştır. Toplanan yumurtalar laboratuvarda içinde nemlendirilmiş kurutma kağıdı bulunan petri kaplarına yerleştirilmiş ve numaralandırılmıştır.

Araştırmada kullanılan örnekler 24 ^{o}C oda sıcaklığında % 43,6 oranında neme sahip, yaklaşık 12 saat, gün ışığı alan ortamda muhafaza edilmiştir. Günlük bakım kontrolleri günde en az iki kez yapılmıştır.

Yumurtadan çıkarak 4. döneme ulaşan larvalarının ise içerisinde 10 cm patates yetiştirilen tarla toprağı bulunan plastik kovalara kapağından hava alacak şekilde delikler açılarak pupa evresine girmesi sağlanmıştır.

Getirilen örnekleri gözlem ve ölçüm yapmak amacı ile ergin bireyleri % 70'lik etil alkol ile oluşturulan öldürme şişelerinde, larvaları ise % 9'luk etil alkol içerisine bırakılarak inceleme yapılmıştır.

Ölçü almak ve gözlem yapmak için stereo özellikte VWR marka mikroskop kullanılmıştır. Ölçüm yapmak için mm'lik ayrımı olan cetvel kullanılmıştır.

BULGULAR

Yumurta

Yumurtaları koni biçiminde uzun ve oval şekildedir. Yumurtalar sarı ya da turuncu renklerde, eni 0,7 mm ve boyu 1,5-2 mm ölçülerindedir.

Yumurtalar genellikle 9-49 adet arası değişmekle beraber, ortalama 26±9,673'lü gruplar halinde yaprağın alt yüzeyine dikey biçimde bırakılmaktadır. Ayrıca yumurta kümelerini bir arada ve dikey konumda tutmak için ipliksi bir yapı bulunmaktadır. Doğal arazi şartları altında dişiler toplamda 200-500 yumurta bırakabilmektedir (Capinera, 2001).

İncelenen 654 yumurtaya göre yumurtadan çıkma süresi laboratuvar ortamında 23,3 ^{o}C sıcaklık ve %43,6 nemde 4- 6 gün iken, yumurta açılma oranı %47,876 olarak bulunmuştur.

Birinci dönem larvaları

Yumurtadan çıkan 1. dönem larvaları yumurta ile aynı renk ve boyutlardadır. *L. decemlineata* larvası yumurtadan çıkmadan önceki, son 12 saatte gözle görülür hale gelmiştir. Açık sarı tonlarında yumurtadan çıkan larvanın üzerinde küçük siyah noktaları bulunduğu saptanmıştır.

Yumurtadan çıkan larvalar yumurta kabukları ile beslenirler ve beslendikçe baş tamamen siyah ve ayakları ise uç kısımları siyah renge, gövde ise vişne rengine dönüşmeye başlamıştır.

Birinci dönem larvasının boyu ilk yumurtadan çıkınca 2 mm kadar olmasına rağmen 1. dönemin sonlarında 3,5-4 mm, eni ise 2 mm'yi bulmuştur. 1. dönem larvalar yaklaşık 3-10 günde 2. larva halini

almaktadır. Gömlek değiştirerek bir sonraki larva dönemine geçmiştir.

İkinci dönem larvaları

Gömlek değiştiren 1. dönem larvaları gömleği çıkardıktan sonra parlak turuncu bir renk almakta ve *Solanum sp*. yaprakları ile beslendikçe 2. dönemin özelliklerini kazanmıştır. İkinci dönem larvaları 1. dönem larvalarına göre daha açık tonlarda olan havuç rengi görünümünde olduğu belirlenmiştir. 1.dönemde tamamen siyah olan baştaki siyahlık azalırken 9 boğumlu olan abdomen boğumları netleşmeye başlamıştır.

İkinci dönem patates böceği larvalarının boyu yaklaşık 8 mm, eni ise 4,5 mm'dir. 2. dönem larvalarının gelişim süresi 2-5 gün arası sıcaklık, nem ve yaprak tazeliğine göre değişmiş olduğu saptanmıştır.

Üçüncü dönem larvaları

Gömlek değiştiren 2. dönem patates böceği larvalarının baş ve gövde rengi 2. dönem larvaya göre daha açık renkte ve portakal kabuğu renginde, 3. dönem larvaları kamburumsu bir duruş halini almaya başladığı tespit edilmiştir. Gövdenin yan kısmındaki siyah noktalar oldukça belirgin halde olup, ağız parçalarının gelişmiş olduğu görülmüştür.

3. dönem larvalarının gelişim süresi ortalama 3-4 gün arasında değişim göstermektedir. Bu dönem larvalarının boyu yaklaşık 8,5 mm iken eni 6 mm uzunluğunda olduğu saptanmıştır.

Dördüncü dönem larvaları

L. decemlineata'nın son dönem larvaları iri, kamburumsu bir duruşta, yavaş hareketlere sahiptir. Gövde açık sarı ya da soluk turuncu rengini almıştır. 9 segmentli karın bölgesinin yanlarındaki siyah noktalar oldukça belirgin ve çift sıra halinde olduğu saptanmıştır. Dördüncü dönem larvalarının boyu 9,5 mm, eni 6,5 mm olarak belirlenmiştir.

Pupa devresi

İncelenen 58 pupaya göre holometabola (tam başkalaşım) tipi başkalaşım gösteren patates böceğinin 4. dönem larvaları 2- 3 gün aktif beslenmenin ardından bir süre toprak yüzeyinde prepupa dönemi geçirdikten sonra toprağın 4- 8 cm altına girerek yaklaşık 2. günden sonra pupa dönemine geçmiştir. Gelişim süreçleri 5-8 gün aralığında olan *L. decemlineata* pupaları oval ve turuncumsu renktedir.

Patates böceğinin pupası serbest pupa tipi olup, koni şeklindedir. Pupanın boyu yaklaşık 8-9 mm ve eni 5-6 mm ölçülerindedir. Pupanın rengi parlak sarı ya da açık turuncu tonlarında olduğu saptanmıştır. Bacaklarını içine çeken pupanın abdomen, thorax ve baş kısmı hemen hemen birbirine kaynaşmış durumda, kanat belirginleşmeye başlayarak thoraxtan itibaren yan kısımdan alt karına doğru uzanmakta olduğu görülmüştür.

Ergin dönemi

Pupa döneminden çıkan erginler yapraklar ile beslenip, gelişerek ergin döneme ulaşmışlardır.

Ergin bireyin gövdesi kubbe şeklinde bombelidir ve kanatları sarı ya da portakal kabuğu tonlarda, her iki kanadında 5'er uzun siyah çizgi bulunan bir yapıya sahiptir ve kanat arka kısımlara doğru incelmekte olduğu saptanmıştır. Dişi ve erkek ergin bireyleri arasında bazı renk boyut ve şekil farklılıkları bulunmaktadır. Dişi bireyler görüntü olarak erkek bireyden daha iri yapıda ve daha açık renklerde olduğu görülmüştür.

Ergin bir bireyin boyunun 10-12 mm, eninin ise 5-7 mm olduğu saptanmıştır.

Patates böceklerinde ayırt edici özellik olarak elytra denilen dış kanat bulunmaktadır. Dişi bireyler üst kanat rengi, erkek bireylerden daha açık sarı tonlarındadır. Elytra boyu 9 mm iken eni 4,5-5 mm ölçülerinde olduğu saptanmıştır.

Sert yapıdaki üst kanatların (elytra) alt kısmında uçuşları sırasında gözlenebilen alt kanatları vardır. Alt kanat mercan rengi tonlarında fakat sarı damarlarla ayrılmış ve uç kısımlara doğru grileşen bir renktedir. Dişi bireyde alt kanat erkek bireye göre daha açık tonlarda olduğu görülmüştür.

Kışlama döneminde bulunan patates böceği (*L. decemlineata*) Mayıs 2016 ayının ilk haftasından

toprak yüzeyine çıkarak yaklaşık 5 ay kadar toprak yüzeyinde bulunmaktadır.

Eylül ayının son haftası itibari ile yeniden kışlama dönemine girdiği belirlenmektedir. Çiftleşme sırasında erkek birey dişinin arka kısmında, abdomenden tutunarak çiftleşmektedir. Çiftleşme esnasında erkeğin spermaları kopulasyon organı aracılığıyla dişi bireyin genitalyasına iletildiği, çiftleşmeyi takip eden gün içerisinde yumurta bırakma davranışı olduğu saptanmıştır.

Araştırmada laboratuvarda incelenen 127 ergin bireye göre çiftleşmeden sonra aynı gün içerisinde ya da ertesi gün yumurta bıraktığı gözlemlenmiştir. Yumurtalar genellikle yaprağın alt yüzeyine, 9 - 49 arasında değişen sayı aralığında, çoğunlukla 25-26'lı kümeler halinde bırakıldığı belirlenmiştir.

Patates böceği 4 gömlek değiştirerek 4 larva dönemi geçirmektedir. Gömlek çıkaran larva parlak turuncu renkte olmakta ve beslendikçe diğer larva dönemi özelliklerini almıştır. Gömlek çıkarırken önce kafa kısmını sonra arka kısımları çıkarmaktadır ve çıkan gömlek koyu gri-siyah tonlarında olduğu saptanmıştır. Elytra ve baş kısmı parlak olan ergin birey yaşlandıkça dış kabuğunun rengi parlaklığını yitirmekte ve yaşamını yitiren ergin birey bacaklarını içine çekerek ölmekte ve kısa süre içinde kararmakta olduğu saptanmıştır.

TARTIŞMA VE SONUÇ

Leptinotarsa decemlineata ile ilgili yapılan bu ön çalışmada; öncelikle patates böceğinin doğal arazi koşullarında, hava sıcaklığının 15 ^{o}C olduğu mayıs ayının ilk haftasında kışlamadan çıktığı gözlemlenmiştir. Bu gözlem verilerinin literatür bulguları ile de örtüştüğü görülmektedir (Çizelge 1) (Small, 1947; Przybylskı, 1970; Atak, 1973; Gürkan ve Boşgelmez, 1984; Şahin, 1997).

Yumurtaların laboratuvar ortamında ortalama 23,3 °C sıcaklıkta 3-6 gün aralığında olmak üzere ortalama 4,263±1,045 günde açıldığı görülmüştür. Elde edilen bulguların yapılan araştırmalar ile paralellik gösterdiği görülmekte fakat gün aralıklarının değiştiği görülmektedir (Çizelge 2). Bu da çalışılan bölge, sıcaklık ve nem gibi faktörlerin farklı olmasından kaynaklanmaktadır. Yapılan araştırmadaki açılma oranının % 47,876 olması laboratuvar ortamında yumurtanın bulunduğu yaprağın tazeliğinin birkaç gün içinde yitirmeye başlamasından kaynaklandığı tespit edilmiştir. Tazeliğini daha uzun süre koruyan yapraklarda açılma oranının daha yüksek olduğu gözlemlenmiştir (Kozlovsky, 1937; Rivnay, 1962; Atak, 1973; Gürkan ve Boşgelmez, 1984; Has, 1992; Şahin, 1997).

Çizelge 1. *L. decemlianata* bireylerinin farklı kaynaklara göre kışlamadan çıkma zamanları.
Table 1. Departure dates of wintering of *L. decemlianata* individuals according to different sources.

Kaynak Source	Hava sıcaklığı (^{o}C) Air temperature (^{o}C)	Toprak sıcaklığı (^{o}C) Soil temperature (^{o}C)	Kışlamadan çıkma tarihleri Departure dates of wintering	Bölge Area
Bulgularımız Our findings	15,26 °C	24,308 ^{o}C	Mayıs ayının ilk haftasında	Nevşehir
Uluslararası Komitenin III. Konferans kayıtları III. Conference records of International Committee	14 °C - 15 °C	>10 °C	-	-
Small (1948)	-*	-	Mayıs ayı sonunda	Jersey
Przybylski (1970)	-	-	Nisan sonu Mayıs başı	Polonya
Atak(1973)	17 °C	-	Nisan sonu ile Mayısın ilk haftaları	Trakya
Gürkan ve Boşgelmez (1984)	-	13,4 °C - 14 °C	Mayıs'ın ilk haftasında	Ankara
Şahin (1997)			Mayıs'ın son haftası	Erzurum ve Pasinler
Şahin (1997)			Mayıs'ın 2. ve 3. haftası	Oltu/Erzurum

* - : Belirtilmemiş (Not specified).

Çizelge 2. Farklı kaynaklara göre *L. decemlianata* yumurtalarının açılma süresi ve oranı.
Table 2. Duration and rate of *L. decemlianata* eggs according to different sources.

Kaynak Source	Sıcaklık (°C) Temperature (°C)	Yumurta açılma süresi (gün) Egg opening periods(days)	Yumurta açılma yüzdesi (%) Egg opening percentage (%)
Bulgular (laboratuar) Findings (laboratory)	23,3 °C	Ort. 4,263 ± 1,045) Min. 3 –Mak. 6	Ort. % 47,876 Min. % 3,846 - Mak. % 83,3)
Kozlovsky (1937)	-	6 - 10	-
Sorauer(1954)	-	3 - 12	-
Rivnay(1962)	-	6 - 8	-
Atak (1973)	21,5 °C - 14,7 °C	6 - 10	-
Gürkan ve Boşgelmez (1984)	27,5 ± 0.52 °C	5,90 ± 0,27	% 87,3
Has (1992)	-*	4 - 8	(% 44- 100)
Şahin(1997)	-	4 - 13	% 85 - %79

* - : Belirtilmemiş (Not specified).

L. decemlineata'nın yapılan sayımlarda bir yumurta kümesinde 9 - 49 adet olmak üzere (26 ± 9,673) farklı sayılarda yumurta bıraktığı gözlemlenmiştir. Literatürdeki araştırmalarda elde edilen verilerin bu rakamlardan çok farklı olmadığı görülmektedir (Çizelge 3) (Atak, 1973; Rivnay, 1962; Has, 1992).

Çizelge 3.Farklı kaynaklara göre *L.decemlianata* yumurta bırakma sayıları.
Table 3. Number of layed eggs in *L.decemlianata* according to different sources.

Kaynak Source	Yumurta adeti Number of eggs
Bulgularımız Our findings	9 - 49 (26 ± 9,673)
Atak (1973)	20 - 60
Rivnay (1962)	4 - 80
Has (1992)	2 - 57

Bu araştırmada larva gelişim süreleri ve pupa süresi takip edildiğinde 1.dönem larvanın 3-10 gün (6,294 ± 1,794 gün), 2. dönem larvanın 2-5 gün (3,833 ± 0,937 gün), 3.dönem larvanın 3-4 gün (3,5 ± 0,547 gün) ve 4. dönem larvanın 4-9 günde (6,384 ± 1,445 gün) geliştiğini ayrıca pupa evresinde de 8-13 gün (10,5 ± 1,51658 gün) kalarak ergin hale geldikleri gözlemlenmiştir. Söz konusu çalışma ile elde edilen gelişim sürelerine ait veriler; mevcut literatürle karşılaştırıldığında; genellikle uyumlu olduğu görülmektedir. Bununla birlikte; bazı larval gelişim sürelerinde farklılıklar da tespit edilebilmektedir (Çizelge 4) (Şahin ve Kozlovsky, 1937; Rivnay, 1962; Atak, 1973; Gürkan ve Boşgelmez, 1984; Has ve Kansu, 1987; Ionnidis ve ark., 1991; Has, 1992). Tespit edilen bu farklılıkların da; araştırma başlangıcında tarla ortamından toplanan örneklerin doğal olarak biyolojik gelişim sürecine etki edebilecek; çalışma alanının; coğrafi, ekolojik ve iklimsel farklılıklarından kaynaklandığı düşünülmektedir.

Laboratuvar ortamında yaptığımız gözlemlerde toprak altına giren 4. dönem larvalarının pupa olma oranı % 64,285 olarak saptanmış olup diğer bazı araştırmalardan elde edilen değerlere yakın olduğu görülmektedir. Nitekim bu değeri Has (1992) %73, Ushatinskaya (1976) ise %53 olarak bildirmiştir.

TEŞEKKÜR

Bu ön çalışma; Nevşehir Hacı Bektaş Veli Üniversitesi Fen Bilimleri Enstitüsü Biyoloji ABD kapsamında yürütülen "Kapadokya Bölgesi: Nevşehir İli- Mazı Lokalitesi *Leptinotarsa decemlineata* (Insecta: Hymenoptera: Coleoptera) Türünün Biyoekolojisi ve Morfolojisinin İncelenmesi" başlıklı yüksek lisans tez çalışmasının bir kısmıdır. Bu nedenle, katkı ve desteklerinden dolayı, Nevşehir Hacı Bektaş Veli Üniversitesi Fen Bilimleri Enstitüsü Biyoloji ABD'ye teşekkür ederiz.

Çizelge 4. Farklı kaynaklara göre *L.decemlianata* larva ve pupa gelişim süreleri ile toplam larva süresi.
Table 4. Developmental periods of Larvae and pupae with total larval duration of *L. decemlineata* according to different sources.

Kaynak	Sıcaklık (°C)	1. Larva (gün)	2.Larva (gün)	3. Larva (gün)	4. Larva (gün)	Pupa (gün)	Toplam Larva süresi (gün)
Source	Temperature (°C)	1st Larva (day)	2nd Larva (day)	3rd Larva (day)	4th Larva (day)	Pupa (day)	Total larval periods (day)
Laboratuar bulguları	24 °C	3 - 10	2 - 5	3 - 4	4 - 9	8 - 13	12 - 28
Laboratory findings		(6,294±1,794)	(3,833±0.937)	(3,5±0,547)	(6,384±1,445)	(10,5±1,51658)	
Balachowsky (1936)	-*	-	-	-	-	8 - 10	-
Kovlovsky (1937)	14 - 24 °C	3 - 5	2 - 4	2 - 5	9 - 18	5 - 19	-
Soruer (1954)	-	-	-	-	-	14	10 - 30
Rivnay (1962)	20 - 24 °C	-	-	-	-	10	16
Grisson (1963)	-	-	-	-	-	10 - 20	-
Atak (1973)	19 °C	4 - 5	3 - 5	4 - 5	8 - 9	5 - 14	-
Gürkan ve Boşgelmez (1984)	27,5 °C	3 - 5	2 - 4	3 - 4	8- 17	5 - 12	17 - 27
Has (1992)	-	3 - 8	2 - 4	3 - 8	3 - 7	7 - 32	-
Şahin (1997)	-	4 - 8	3 - 7	3- 7	4 - 11	9 - 13	16 - 23

* - : Belirtilmemiş (Not specified)

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ANADOLU, J. of AARI
ISSN: 1300 - 0225
28 (1) 2018, 108 - 112
MFAL

Carob Bean (Ceratonia siliqua L.) and Its Products

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Received (Geliş tarihi): 28.06.2017 Accepted (Kabul tarihi): 30.11.2017

ABSTRACT: *Carob bean (**Ceratonia siliqua** L.) can be seen in Mediterranean climate regions. Carob fruit has high amounts of nutrients such as sugars, minerals and phenolic compounds. Main idea of consuming carob is to take energy from a natural source. Furthermore, carob is also used for producing some commercial products. Flour (powder), syrup, locust bean gum and d-pinitol are the examples. Carob flour produced from deseeded carob by roasting and grinding. It is a substituent of cacao in food industry. In Turkey, carob bean consumed as carob syrup the most. It is a traditional product obtained by extraction and evaporation respectively. Locust bean gum is a food additive and produced from seeds. D-pinitol is a bioactive compound and there are many researches about the effect of D-pinitol on diabetes and some cancer types. In the world, there is an increase in some nutrition originated diseases. For preventing this, nutritional habits should include unrefined energy sources. Carob is an option for taking unrefined sugar, minerals and phenolic compounds at once. The aim of this review is to gather different information about carob and its products and form a source for further researches.*

Keywords: *Carob bean, **Ceratonia siliqua** L., carob powder, carob syrup, locust bean gum, dietary fiber, D-pinitol.*

Keçiboynuzu (Ceratonia siliqua L.) ve Ürünleri

ÖZ: *Keçiboynuzu (**Ceratonia siliqua** L.) Akdeniz ikliminin görüldüğü bölgelerde yetişen şeker içeriği yüksek bir meyvedir. Zengin şeker içeriğinden kaynaklanan doğal olarak enerji verici olması özelliğinin yanı sıra mineral ve fenolik maddelerce zengin olma özelliğiyle yetişkin ve çocuk beslenmesinde önemli bir yere sahiptir. Keçiboynuzu aynı zamanda çeşitli ticari ürünlerin üretiminde de hammadde olarak kullanılmaktadır. Keçiboynuzu unu, pekmez, gam ve d-pinitol bu ürünlere örnektir. Keçiboynuzu unu, çekirdekleri alınmış keçiboynuzunun fırınlanması ve öğütülmesi ile elde edilmekte olup gıda endüstrisinde kakao ikamesi olarak kullanılmaktadır. Ülkemizde keçiboynuzunun en yaygın tüketim şekli pekmezdir. Geleneksel bir ürün olan pekmez keçiboynuzunun su ile ekstraksiyonu ardından yoğunlaştırılması ile elde edilmektedir. Keçiboynuzu gamı çekirdeklerden üretilir ve gıda katkı maddesi olarak kullanılmaktadır. D-pinitol biyoaktif bir bileşendir. Günümüzde d-pinitol'ün diyabet ve çeşitli kanser tipleri üzerine olan etkilerinin araştırıldığı çalışmalar mevcuttur. Dünyada beslenme kaynaklı hastalıkların görülme sıklığı artmaktadır. Beslenme alışkanlıklarının işlenmemiş gıdaları tüketme yönünde değişmesi bu tip hastalıkların önlenmesinde önem taşımaktadır. Keçiboynuzu işlenmemiş gıdalar arasında hem enerji verici olması hem de mineral ve fenolik maddelerce zengin olması nedeniyle iyi bir seçenektir. Derlememizin amacı keçiboynuzu ile ilgili çeşitli bilgileri bir araya toplayarak gelecekteki çalışmalara kaynak oluşturmaktır.*

Anahtar Sözcükler: *Keçiboynuzu, **Ceratonia siliqua** L., keçiboynuzu unu, keçiboynuzu pekmezi, keçiboynuzu gamı, diyet lifi, D-pinitol.*

INTRODUCTION

Carob (*Ceratonia siliqua* L.) is an evergreen tree belongs to *Leguminoseae* (*Fabaceae*) family and *Caesalpinaceae* sub-family. It has wild and cultivated types. Turkey has a wide area for both types of carob bean. Carob tree is grown since antiquity in most countries of Mediterranean basin and it has an important value from economic and environmental point of view (Battle and Tous, 1997).

Carob bean is a rich source of valuable compounds such as phenolic compounds, minerals, dietary fiber and d-pinitol. Chemical composition of carob varies with genetic, environmental, climatic factors and harvesting time (Nasar-Abbas *et al*., 2016). Carob bean consist of 90% eatable part and 10% seed. The unripe pod is green and acrid, ripe one is brown and sweet.

Carob fruit has high amounts and varieties of nutrients (Karkacier and Artik, 1995; Owen *et al*., 2003; Anonim, 2017) such as sugar, dietary fiber, minerals, and phenolics. It has 62-67 % total sugar, 4-6 % protein, 23-27% dietary fiber (Table 1). 100 g deseeded carob fruit gives 293 kcal energy (Anonim, 2017).

Table 1. Composition of carob bean.
Çizelge 1. Keçiboynuzu meyvesinin kompozisyonu.

Constituent Bileşen	Amount (%) Miktar (%)
Total dry matter Toplam kuru madde	91 - 92
Total sugar Toplam şeker	62 - 67
Saccharose Sakkaroz	34 - 42
Glucose Glikoz	7 - 10
Fructose Fruktoz	10 - 12
Protein Protein	4 - 6
Dietary fiber Diyet lifi	23 - 27
Fat Yağ	0,2 - 0,4
Total mineral matter Toplam mineral madde	2,2 - 2,4
Pectic matter Pektik madde	0,03 - 0,05
D-Pinitol D-Pinitol	7 - 10
Total phenolic matter Toplam fenolik madde	3944,7 mg/kg

Carob has several kinds of minerals such as potassium (843-1215 mg/100 g), calcium (251-361mg/100 g), magnesium (63-326 mg/ 100 g), phosphorous (85-681 mg/100 g) (Table 2), and also it has 3944.7 mg/kg total phenolic matter. It has been detected that carob fruit has 24 different phenolic compounds and also gallic acid is the most commonly found (Owen *et al*., 2003).

Table 2. Mineral content of carob bean (Anonim, 2017).
Çizelge 2. Keçiboynuzu meyvesinin mineral madde içeriği (Anonim, 2017).

Minerals (Mineral)	mg /100 g
Potassium (Potasyum)	843 - 1215
Calcium (Kalsiyum)	251 - 361
Phosphorus (Fosfor)	85 - 681
Magnesium (Magnezyum)	63 - 326
Sodium (Sodyum)	4 - 7
Selenium (Selenyum)	0 - 5,9
Iron (Demir)	1,25 - 5,44
Zinc (Çinko)	0,61 - 4,27

CAROB BEAN PRODUCTS

Carob bean is also used for producing some commercial products. Powder (flour), syrup, locust bean gum and D-pinitol are the main examples of these products.

Carob powder (flour)

Carob powder is produced by crashing, roasting and grinding of deseeded carob respectively (Yousif and Alghzawi, 2000). It can be named as 'functional ingredient' and promotes nutritional value of foods prepared with (Seczyk *et al*., 2016). Carob flour can be used as fortification agent for products such as tarhana, pasta and some diet products (Tsatsaragkou *et al*., 2012; Tsatsaragkou *et al*., 2014; Seczyk *et al*., 2016; Çağlar *et al*., 2013).

Carob flour is used as cacao substituent. Unlike cacao, carob flour does not contain caffeine and theobromine (Ayaz *et al*., 2009). Rosa *et al*. (2015), have researched on replacing cacao powder with carob flour in different ratios for producing gluten free cakes. Final product described as rich in protein, low in calorie, pleasant sensory characteristics and suitable for people with celiac disease.

Carob syrup

Carob syrup is called as 'pekmez' in Turkey. Pekmez is a traditional product, obtained by extraction and evaporation of deseeded carob bean.

Carob syrup is rich in polyphenols, vitamins and minerals. Also it provides high energy to people.

Locust bean gum

Carob seed has three parts; Husk- Endosperm-Germ. Isolated endosperms are subjected to grinding, sifting, grading and packaging. It is a creamy white powder obtained after milling of carob seed endosperm. It is also known as locust bean gum. Locust bean gum is widely used as an additive in food industry. The main property of this gum is performing high, viscosity gel structure at wide pH range. It is used in several kinds of foods as stabilizer and thickener (Barak and Mudgil, 2014).

Dietary fiber

Carob fiber is the main by-product of carob syrup production. Mostly consists of insoluble fibers. Its glycemic index is very low. Their digestion occurs slowly. That means, blood sugar increases slowly when consumed (Anderson *et al*., 2009). Nutrition with fiber rich foods such as carob provides colon health. There are some different researches about colon health and carob (Ferguson, 2005; Klenow *et al*., 2008; Klenow *et al*., 2009; Klenow and Glei, 2009). Carob fiber has a great potential for producing supplements and functional foods (Santos *et al*., 2015).

D- Pinitol

Carob fruit is a source of a bioactive component called 'D-Pinitol'. It can obtain by several methods such as using ion exchange resins and supercritical fluids (Chul-Shin *et al*., 2003; Karhan *et al*., 2010; Alper, 2016).

D-pinitol's name comes from *Pinus lambertiana* where it has extracted first (Anderson, 1952). In addition to carob, soy bean, carnation, pine tree are other example of d-pinitol sources (Ichimura *et al*., 1998; Do, 2007).

Diabetes is a disease related with insulin and blood sugar regulation. D-Pinitol can mimic the ability of insulin, for lowering and balancing the blood sugar, in diabetes type 2 patients (Camero and Merino, 2004). Diabetes and d-pinitol relation is an important topic for global world and there are different researches about this subject (Ortmeyer *et al*. 1992; Ostlund and Sherman, 1996; Nestler *et al*. 1999; Davis *et al*., 2000; Kim *et al*., 2005).

Benefits of Carob and Its Products

The main idea of consuming this fruit is taking energy from a natural source. In our world, this kind of human diets, which includes unrefined energy sources are more beneficial for human health (Pazır and Alper, 2016).

In many developed countries, there is an increase in deaths from cardiovascular diseases. Nutritional habits should include rich dietary fiber intake for decreasing cholesterol level which is an important factor for cardiovascular diseases (Köksel and Özboy, 1993). Also dietary fiber rich nutrition provides positive effects on colon health. Hassanein *et al*. (2015) have researched the effect of carob powder on lipid profile of rats. Results show that, carob powder improved lipid profile parameters such as total cholesterol and LDL cholesterol. Carob has positive effects on cardiovascular health (Kumazawa *et al*. 2002; Ruis-Roso *et al*. 2010).

In human metabolism, d-pinitol can act like insulin and helps to decrease and balance glucose level in blood (Camero and Merino, 2004).

CONCLUSION

In the world, there is an increase in some nutrition originated diseases. For preventing this, nutritional habits should include unrefined energy sources. Carob is a suitable option for taking unrefined sugar, minerals and phenolic compounds at the same time.

In addition, carob tree can prevent soil degradation. For poor soils, carob tree is valuable from agricultural point of view.

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ANADOLU, J. of AARI
ISSN: 1300 - 0225
28 (1) 2018, 113 - 118
MFAL

Pıtrak (Xanthium strumarium L.) Bitkisinin Farklı Açılardan Değerlendirilmesi

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Received (Geliş tarihi): 24.04.2017 Accepted (Kabul tarihi): 25.01.2018

ÖZ: *Biyoçeşitlilik hayatın sürdürülebilirliği bakımından çok önemlidir. Bu zenginliğin kıymetinin bilinememesi birçok çevre felaketinin yaşanmasının sebebidir. İnsanlar tarımsal faaliyetlerinde tamamen kendi kârlılıklarını düşündükleri için uzun yıllar tabiatın zarar görmesini ciddiye almamışlardır. Tarımsal faaliyetlerinde tamamen üretmiş oldukları bitkilerin yetiştiriciliğini dikkate alarak çevrede yetişen canlıların hayatiyetlerinin devamlılığını önemsemedikleri için çok büyük sahalarda biyolojik canlılık ve çeşitlilik iyice sığlaşmıştır. Tabiatta insan gıdası olarak kullanılmayan çok sayıda bitki mevcuttur. Bu bitkilerin bitkisel üretimde kullanılmamasının değişik sebepleri olsa da içeriklerine bakıldığında insan gıdası olarak kullanımı mümkün olabilecek özelliklere sahip olduğu görülmektedir. Bazı bitkiler ise insan gıdası olarak kullanılacak kalitede olmasa bile bu bitkilerin yağları ya da biyokütleleri enerji kaynağı olarak kullanılmakta bu da diğer bitkilerin üzerinde oluşan tüketim baskısını düzenlemeye fayda sağlamaktadır. Tohumlarında yaklaşık %25-42 oranında yağ ve %35 oranında protein olduğu tespit edilen pıtrak (**Xanthium stramarium** L.) bitkisi bu özelliklere sahip bir bitkidir. Yabancı ot olarak bilinen ve bitkisel üretimde mücadelesi yapılmadığında tarımsal verimliliğe çok büyük zararlar oluşturabilen bu bitkinin tohumlarında yüksek oranda yağ bulunmasının yanısıra kurak alanlarda yetişebilmesi ve olumsuz şartlara dayanabilmesi bakımından bitkisel üretime kazandırıldığında oldukça önemli faydalar sağlayabileceği düşünülmektedir.*

Anahtar Kelimeler: *Pıtrak, **Xanthium strumarium** L., biyoçeşitlilik, biyodizel, bitkisel yağ.*

Evaluation of Cocklebur (Xanthium strumarium L.) from Different Viewpoint

ABSTRACT: *Biodiversity is very important for the sustainability of life. Because of less attach value on a biodiversity, people are face to environmental disaster. People's first goal is profitability in agriculture and they didn't care about destruction of environment. Thus far, people just focus on cultivated plants and its cause to loose biodiversity and biological viability in massive lands. Nature have many plant which isn't use directly for human food. When looked their content, these plant can use for human food but some reasons block to cultivate it. If plant isn't suitable for human consumption, it can use for biomass energy or biodiesel industry because of its oil content. Cocklebur (**Xanthium strumarium** L.) plant is seen as weed but it contains 25% to 42% crude oil and %35 protein. It is thought that this plant, which is known as weed and can cause great harm to agricultural productivity when there is no struggle in vegetable production, can provide considerable benefits when it comes to plant production in terms of being able to grow in arid areas and adverse conditions in addition to high oil content in seeds of this plant.*

Keywords: *Cocklebur, **Xanthium strumarium** L., biodiversity, biodiesel, crude oil.*

GİRİŞ

Karbonhidratlar ve proteinler gibi temel yapı ve besin maddelerinden olan yağlar insan ve hayvanlar için önemli bir besin kaynağıdır. Tohum ve meyvelerinde yüksek miktarda yağ depolayan ve bu yağlardan ekonomik fayda sağlanabilen bitkiler yağlı tohumlu bitkiler ya da yağ bitkileri diye isimlendirilir. Yağlar besin maddelerini meydana getiren çeşitli bileşikler içerisinde enerji bakımından en yoğun kaynağı teşkil etmeleri, esansiyel niteliğe sahip çeşitli yağ asitlerini içermeleri, yemeklerden sonra tokluk hissine

katkıda bulunmaları, gıdaların daha lezzetli olmalarını sağlamaları ve aynı zamanda yağda çözünen vitaminler içinde taşıyıcı fonksiyona sahip olmaları gibi birçok görevi ifa eder. (Nas ve ark., 2001) Yağ, üç değerli bir alkol olan gliserol ve üç adet yağ asidinin ester bağları ile birleşmesi sonucunda oluşan bir trigliserit esteridir. Yağların temel yapı taşı olan yağ asidi, karboksil grubu (-COOH) ile sonlanan düz bir hidrokarbon zinciridir. Bu zincirde yer alan karbon ve çift bağ sayısı yağın fiziksel ve kimyasal özelliklerini belirler (Baydar ve Erbaş, 2014). Yağların kullanım alanları da fiziksel ve kimyasal özelliklerine göre değişir. Bitkisel yağlar hem gıda kaynağı olarak hem de endüstriyel amaçlı kullanılabilirler (Ardabili ve ark., 2011).

Türkiye'nin tarımsal üretim alanlarına bakıldığında ihtiyacı olan yağı üretebilecek genişliğe sahip olduğu görülmektedir ancak yeterli su kaynağına sahip olamadığı için bu üretim yapılamamaktadır. Bu sahalar sulanamadığı için de ekseri tahıl ve hububata dayalı bir üretim yapılabilmektedir. Bu hal ekonomik, sosyal ve çevre düzensizliği gibi birçok yetersizliklere sebep olmaktadır. Bu yetersizlikleri giderebilmek için bu kurak ve kıraç alanları daha verimli değerlendirebilecek bitkisel üretim desenlerine ihtiyaç vardır. Geleneksel tıbbi tedavi usullerinden, modern farmakolojiye kadar birçok alanda kullanılabilen pıtrak (*Xanthium strumarium* L.) bitkisi böyle bir potansiyele sahiptir (Hsu ve ark., 2000; Farooq ve ark., 2014). Aynı zamanda zor şartlarda yetişebilen, sıcağa, soğuğa, kurak ve kıraç şartlarda verim elde edilebilen pıtrak bitkisinin tohumlarında bulunan yağ bu bitkinin önemli bir biyoyakıt kaynağı olacağını da göstermektedir (Ruan ve ark., 2012).

Özellikle fosil yakıtların çevreye verdiği zararlar nedeniyle, yenilenebilir enerji kaynakları içerisinde bitkisel üretimden elde edilen enerji miktarı her geçen gün daha da önemli hale gelmektedir (Zhu ve ark., 2006). Fosil yakıtların bir gün tükeneceğinin fark edilmesi de yenilenebilir enerji kaynakları üzerindeki ilginin gelişmesine etkili olmuştur (Karmakar ve ark., 2010). Biyodizel üretmek için kullanılan mısır, aspir, soya, kanola gibi yağlar aynı zamanda insan gıdası olarak da kullanılmaktadır. Bu yağların biyodizel hammaddesi olarak kullanılması insan gıdası olarak kullanılacak yağ tüketiminin arz ve talep dengesinde düzensizlikler oluşturmaktadır. Günümüzde insan gıdası olarak ihtiyaç duyulan yağ pazarında özellikle bazı ülkelerde ciddi yetersizlikler söz konusudur (Chang ve ark., 2013). Bu durumun aşılabilmesi için, insan gıdası olarak tüketilmesi sakıncalı olan yağların üretim desenine alınarak biyodizel ve diğer sanayi ürünleri için gerekli yağ hammaddesi olarak kullanılmaları yenilebilir yağların üretimi üzerindeki baskıyı azaltacaktır (Hasheminejad ve ark., 2011).

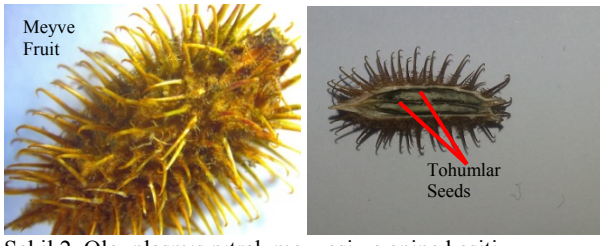
Pıtrak Bitkisinin Bitkisel Özellikleri ve Kullanım Sahaları

Pıtrak (*Xanthium* L.) bitkisi, Asteraceae familyasında yer alan tek yıllık otsu yapıda bir bitki olup (Şekil 1) kimyasal ve biyolojik birçok çalışmaya konu olmaktadır (Romero ve ark., 2015). Dünya'da yaklaşık 30 türüyle bütün orta kuşak içerisinde yaygın görülen *Xanthium* cinsi Türkiye'de üç tür (*X. orientale* L., *X. spinosum* L. ve *X. strumarium* L.) ve üç alttür (*X. orientale* L. subsp. italicum-domuz pıtrağı, *X. strumarium* L. subsp. brasilicum-yitik pıtrak, *X. strumarium* L. subsp. strumarium- koca pıtrak) ile temsil edilmektedir (Caius, 1986; Lee ve Owen, 2003; Güner ve ark., 2012). 1m'ye kadar boylanan bitki haziran - temmuz aylarında, çiçeklenir ve eylül - ekim aylarında olgunlaşır. 1-3,5 cm uzunluğunda, yumurta şeklinde, üzerinde iğnemsi çıkıntılar bulunan meyvelerin her birinde 2 tohum bulunur (Şekil 2). Bitkinin gövdesi küçük tüylerle kaplı olup, alacalı mor renklidir. Beyaz ya da soluk yeşil renkli çiçekleri vardır (Eymirli ve Torun, 2015). Marjinal alanlarda yetişen ve yabancı ot kapsamında olan bitkinin, bitkisel üretim yapılan sahalardan uzaklaştırılması için emek ve kaynak harcanmaktadır. Pıtrak bitkisinin tohumlarında % 25-45 oranında ham yağ bulunabilmektedir (Chang ve ark., 2013). Bu yağın da yaklaşık % 77'sini linoleik asit oluşturmaktadır. Linoleik asit insan vücudunun sentezleyemediği bir kaç nadir yağ asidinden biridir. Bu yağ asidinin kalp damar sağlığı ve kolesterol dengesinin sağlanmasında büyük önemi vardır (Arslan, 2007). Bu yönüyle

içeriğinde yüksek linoleik asit bulunduran yağların insan gıdası olarak kullanılma ihtimali olabilir (Nagaraj, 1993; Bowles ve ark., 2010; Baydar ve Erbaş, 2014). Pıtrak tohumlarındaki protein oranı ise %35 olarak tespit edilmiştir.

Geleneksel ve modern tıpta kullanılan pıtrağın, antitümör ve antibakteriyel gibi çeşitli biyololojik aktivitelere sahip olmasından dolayı alerjik, diyabetik, apandis, sinüzit, kanser, ishal, kuduz, sıtma ve yüksek tansiyon gibi rahatsızlıkların tedavisinde kullanılmaktadır (Ciulei ve ark., 1993; Ansari ve Dubey, 2000; Torres, 2009; Peng ve ark., 2014; Chen ve ark., 2015). Aynı zamanda bitki özlerinden hazırlanan formülasyonların bitki koruma ilacı olarak kullanımının da mümkün olabileceği söylenebilir. Bu özelliği ile biyolojik mücadele için önemli bir materyaldir.

Şekil 1. Pıtrak bitkisinin deneme alanındaki genel görünümü.
Figure 1. General appearance of Cocklebur plant in experimental area.



Şekil 2. Olgunlaşmış pıtrak meyvesi ve enine kesiti.
Figure 2. Ripened cocklebur fruit and cross section of fruit.

Pıtrak Bitkisinin Ham Yağ Oranı ve Yağ Asiti Kompozisyonu Bakımından bazı Önemli Yağ Bitkileri ile Karşılaştırılması

Soya, kolza, ayçiçeği, keten ve pamuk gibi önemli yağlı tohumlu bitkilerin tohumunda bulunan ham yağ oranları ile mukayese edildiğinde pıtrak bitkisinin tabiattan toplanan tohumlarında ki % 24,19 ham yağ içeriğinin önemli ekonomik fayda temin edilebilecek bir oranda olduğu söylenebilir. Çizelge 1. incelendiğinde, yağ oranının kolzada % 36-50, ketende % 35-50, ayçiçeğinde % 33-50 ve pamukta % 21 olduğu görülmektedir. Bu bitkilerin bir kültür bitkisi olarak değişik yetiştiricilik teknikleri ile desteklendiği için ham yağ oranlarının bu miktarlarda olduğunu söylemekte zor olmasa gerektir. Bu şartlar göz önüne alındığında, genel sistematikte bir yabancı ot olarak görülen pıtrak bitkisinin de tarımsal üretim desenlerine bir yağ bitkisi olarak eklenmesi hem biyoçeşitliliğin gelişmesi bakımından hem de tarımsal çeşitlilik bakımdan elverişli bir bitki olacağı söylenebilir.

Pıtrak bitkisinin yağ asitleri dağılımı bakımından da önemli potansiyele sahip olduğu söylenebilir (Çizelge 2). İnsan gıdası bakımından, kaliteli yenilebilir yağlar sınıfından ve oleik $C_{18:1}$ asit bakımından zengin olan zeytin, yerfıstığı, koza ve susam gibi yağlarla karşılaştırıldığında; pıtrak yağında oleik $C_{18:1}$ asidin daha düşük olduğu görülmektedir. Ancak, bitkisel yağlarda bir başka kalite unsuru olarak bilinen linolenik ($C_{18:3}$) asid miktarına bakıldığında durum tam tersi bir hal arz etmektedir. En yüksek linoleik ($C_{18:2}$) asid miktarı (% 76,97) pıtrak bitkisinde olduğu anlaşılmaktadır. Bu oranlar sırasıyla zeytinde % 18,51, yerfıstığında % 33,06, kolzada % 19,49, susamda % 41,30, ayçiçeğinde % 58,83, aspirde % 53, ketende % 12,23 ve ketencikde % 14,31 olduğu görülmektedir (Çizelge 2). Oleik asit zengini yağlar, linoleik asit zengini yağlarla birlikte, yemeklik sıvı yağ olarak en fazla tüketilen yağlardır (Baydar ve Erbaş, 2014). Bu duruma göre oleik asit ve linoleik asit toplam oranı pıtrak bitkisinde % 88,34'dir. Bu oran yemeklik olarak en fazla kullanılan zeytinyağı ve ayçiçek yağlarında sırasıyla % 82,66 ve % 88,82 olduğu görülmektedir. Diğer birçok yağlı tohumlu bitkinin oleik ve linoleik asit toplamları bu değerlerin altında kalmaktadır (Çizelge 2).

Çizelge 1. Pıtrak bitkisinin ham yağ oranının bazı önemli yağlı bitkilerle mukayesesi.
Table 1. Cocklebur crude oil ratio compared with some important oil plants.

Bitki Plant	Ham Yağ (%) Crude oil content (%)	Kaynak References
Soya (*Glycine max* L.) Soybean	20	Wilson, 2004
Kolza (*Brassica napus* ssp.) Rapeseed	36 - 50	Salunkhe, 1992
Ayçiçeği (*Helianthus annuus* L.) Sunflower	33 - 50	Panchenco, 1966
Keten (*Linum usitatissimum* L.) Flaxseed	35 - 50	Anonymous, 2017
Pamuk (*Gossypium* spp.) Cotton	21	Lukonge et al., 2007
Pıtrak (*X. strumarium* subsp. *strumarium*) Cocklebur	25	Collected From Nature Cesur ve Coşge Şenkal, 2016
	42	Chang ve ark., 2013
	33	Cesur ve ark., 2017

Zeytin, yerfıstığı susam, aspir, ayçiçeği ve soya bitkilerinin muhteviyatında linolenik ($C_{18:3}$) asit bulunmazken, bu oran ketende % 64,25, ketencikte % 52,47, kolzada % 7,97 olarak belirlenmiştir. Pıtrakta ise bu oran %0,74 olarak tespit edilmiştir (Çizelge 2). Linolenik asit her ne kadar en önemli üç yağ asidinden birisi olmakla beraber hızlı oksitlendikleri için raf ömürleri ve dayanıklılıkları daha düşüktür. Bu sebeple bünyesinde linolenik asit bulunan yağlar hızlı kururlar ve bu sebeple endüstriyel alanlarda kullanıma daha uygundur. Vernik, boya, cila üretiminde yaygın olarak kullanılır. Yine son yıllarda önemi gittikçe artan yenilenebilir enerji kaynaklarının içinde önemli bir unsur olan biyodizel üretimi içinde linolenik asit nispeti yüksek olan bitkiler kullanılmaktadır.

Çizelge 2. Bazı önemli yağ bitkilerinin yağ asitleri dağılımı.
Table 2. Fatty acid composition of some important oil plants.

	Yağ asitleri dağılımı (%) / Fatty acids distribution (%)										
	$C_{14:0}$[a]	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$	$C_{22:0}$	$C_{22:1}$
Oleik ($C_{18:1}$) asitçe zengin bitkiler / Oleic ($C_{18:1}$) acid rich plants											
Zeytin (Olive) *	0,21	8,10	-	1,99	64,15	18,51	-	0,08	-	-	-
Yerfıstığı (Peanut) *	-	13,82	-	9,29	48,40	33,06	-	0,12	0,05	1,28	-
Kolza (Rapeseed) *	0,11	5,42	-	1,40	63,49	19,49	7,97	-	-	-	2,10
Susam (Sesame) *	-	9,40	-	4,60	43,90	41,30	-	-	-	-	-
Linoleik ($C_{18:2}$) asitçe zengin bitkiler / Linoleic ($C_{18:2}$) acid rich plants											
Ayçiçeği (Sunflower) *	-	8,86	-	1,86	29,99	58,83	-	-	-	0,43	-
Aspir (Safflower) *	0,24	6,50	-	1,74	38,50	53,00	-	-	-	-	-
Soya (Soybean) *	-	14,13	-	4,34	28,36	49,50	-	-	-	-	-
Pıtrak (Cocklebur) **	-	6,51	0,08	3,80	11,37	76,97	0,74	0,19	0,31	-	-
Linolenik ($C_{18:3}$) asitçe zengin bitkiler / Linolenic ($C_{18:3}$) acid rich plants											
Keten (Linseed) *	0,14	5,68	0,09	3,69	13,76	12,23	64,25	0,04	-	-	-
Ketencik (Camelina) *	0,03	6,50	0,06	5,27	20,92	14,31	52,47	0,10	0,02	0,27	-

[a] $C_{14:0}$ (Miristik asit), $C_{16:0}$ (Palmitik asit), $C_{16:1}$(Palmitoleik asit), $C_{18:0}$ (Stearik asit), $C_{18:1}$(Oleik asit), $C_{18:2}$ (Linoleik asit), $C_{18:3}$ (α-Linolenik asit), $C_{20:0}$ (Araşidik asit), $C_{20:1}$ (Gadoleik asit), $C_{22:0}$ (Behenik asit), $C_{22:1}$ (Erüsik asit).
* Baydar ve Erbaş. 2014 ** 2016 yılında hasat edilen bitkilerden elde edilen yağın yağ asitleri kompozisyu.
[a] $C_{14:0}$ (Myristic acid), $C_{16:0}$ (Palmitic acid), $C_{16:1}$ (Palmitoleic acid), $C_{18:0}$ (Stearic acid), $C_{18:1}$ (Oleic acid), $C_{18:2}$ (Linoleic acid), $C_{18:3}$ (alpha-linolenic acid), $C_{20:1}$ (Gadoleic acid), $C_{22:0}$ (Behenic acid), $C_{22:1}$ (Erucic acid).
* Baydar and Erbaş 2014 ** Composition of fatty acids obtained from harvested plants in 2016.

Bitkisel yağlar öncelikle yenilebilir yağ olarak kullanılmak üzere önemli bir bitkisel ürün olmasının yanında, gıda kalitesi yeterli olmayanlarda neredeyse aynı değerde olmak üzere sanayide ihtiyaç duyulan bir maddedir.

Pıtrak bitkisinin yağ asidi dağılımı içerisinde yenilebilir yağ bakımından zararlı olan erusik asit ($C_{22:1}$) görülmemektedir. Erusik asitin kaslarda, kalpte ve hayvanların büyüme hızlarında istenmeyen etkiler yaptığının anlaşılması üzerine 1960'lı yıllarda zararlı olduğu anlaşılmıştır. Dünya Sağlık Örgütü (WHO) 1982 yılına kadar erusik asitin yemeklik yağlarda bulunma oranını %10 olarak belirlerken, 1982 yılından sonra bu oranın bulunması gerekli olan miktarını %5 olarak belirlemiştir. Dolayısıyla pıtırak yağında erusik asit olmaması bu bitkiden elde edilecek olan yağ için elverişli bir değerdir (Tosun ve Özkal, 2000).

Çizelge 2 incelendiğinde, pıtrak bitkisi ile zeytin, yerfıstığı, kolza ve susam gibi ekonomik anlamda önemli olan bazı yağ bitkileri arasında iz yağ asitleri bağlamından herhangi bir fark görülmemekte, buna karşın, bariz fark oleik ve linoleik arasındaki değişimde görülmektedir. Zeytin, susam, yerfıstığı ve kolzanın oleik asit oranları pıtrak yağına göre daha yüksek seyrederken, linoleik asit oranında ise tam tersi bir durumla pıtrak yağının değeri çok yüksek görünmektedir.

SONUÇ VE ÖNERİLER

Bitkilerden elde edilen ham yağ oranı ve yağ asitleri kompozisyonu bitkinin ekonomikliğinin ve faydalanma sahalarının belirlenmesi bakımından çok önemlidir. Tohumlardan elde edilen bitkisel yağların oranı yüksek ve elde edilmesi kolay ise bu yağ ekonomik bir yağ olarak düşünülürken, muhteviyatında bulunan yağ asitleri de yağın faydalanma sahasını (gıda ya da endüstriyel) belirlemede önemli bir kalite parametredir. Pıtrak bitkisinin tohumlarından elde edilen ham yağ oranı ekonomik bir değer arz ederken, yağ asiti kompozisyonu ise sanayi, tıb, eczacılık ve bitkisel ilaç gibi sahalarda kullanımının yanında yenilebilir yağ olarak da kullanılabileceği ihtimali üzerinde çalışmaya değer bir bitki olduğunu düşündürmektedir.

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ANADOLU (ISSN 1300 - 0225) DERGİSİ
YAYIN İLKELERİ

1. ANADOLU dergisinde, tarım bilimleri alanında hazırlanan ve daha önce yayımlanmamış orijinal araştırma, derleme ve bitki tescil makaleleri yayımlanır.
2. ANADOLU, hakemli bir dergi olup, yılda 2 sayı yayımlanır.
3. Makale Türkçe veya İngilizce dilinde, 25 sayfayı geçmeyecek şekilde, çift aralıklı olarak yazılmalı, başlangıç sayfası dahil tüm sayfalar numaralandırılmalıdır.
4. Makale, Microsoft Word yazılım programıyla bir basılı kopya ve CD ile birlikte yayın komisyonuna posta ile gönderilir. Ayrıca, elektronik ortamda e-posta ile "etae@tarim.gov.tr" adresine gönderilmesi de önemle önerilir.
5. Makalenin işleme konulduğu, makale numarası ile birlikte üç gün içinde yazara e-posta yoluyla bildirilir.
6. Yazarlar, posta ile gönderilen başvuru dilekçelerinde ekli makalelerinin orijinal olduğunu, daha önce başka bir yerde yayımlanmadığını ve sorumlu yazarın iletişim bilgilerini (tam adres, telefon, faks, e-posta) belirtmelidirler. Anadolu'da yayımlanmayan makaleler iade edilmez.
7. **Makalenin ana bölümleri aşağıdaki sıraya uygun olmalıdır**

   Makale; Başlık, Öz, Anahtar Sözcükler, Abstract, Keywords, Giriş, Materyal ve Metot, Bulgular ve Tartışma, Sonuç ve Öneriler (isteğe bağlı), Teşekkür (isteğe bağlı) ve Literatür Listesi ana başlıkları altında hazırlanmalıdır. Tüm başlıklar büyük harflerle koyu punto olmalıdır.

   BAŞLIK: Metne uygun, kısa ve açık olmalı; yazar ad (adlarını) ve adresini kapsamalıdır.

   ÖZ (ABSTRACT): 200 kelimeyi geçmemeli, literatür bildirişi ve şekil içermemeli, Türkçe ve İngilizce olarak yazılmalı, makalenin içeriğini yansıtan anahtar sözcükleri kapsamalıdır. İngilizce Abstract'ın başına, eserin İngilizce başlığı yazılmalıdır. Özet ve Abstract'tan sonra 3-10 anahtar sözcük ve keywords yer almalıdır.

   GİRİŞ

   MATERYAL VE METOT

   BULGULAR VE TARTIŞMA

   SONUÇ VE ÖNERİLER (isteğe bağlı)

   TEŞEKKÜR (isteğe bağlı)

   LİTERATÜR LİSTESİ

8. Makalenin yazı tipi Times New Roman olmalıdır. Öz, Abstract başlığı 1,25 cm içten, metin içindeki diğer başlıklar ise girinti verilmeden yazılmalıdır. Makale başlığı koyu, 14 punto, bölüm başlıkları koyu, 11 punto olmalıdır. Giriş, materyal ve metot, araştırma bulguları, tartışma ve sonuç bölümleri 11; özet, anahtar sözcükler, abstract, keywords, çizelgeler, grafikler, resimler ile bunların başlıkları, şekiller ve alt yazıları, dipnot ile literatür listesi 9 punto yazılmalıdır.
9. Yazar isimleri, makale başlığının altında bir satır boşluktan sonra unvan belirtilmeden, koyu ve 11 punto ile verilmelidir. Yazarın ön ismi açık olarak ve küçük harfle, soyadı ise büyük harfle yazılmalıdır. Birden fazla yazar varsa onlar da aynı şekilde araya virgül vb. işaret konulmadan verilmelidir.
10. Yazarlarla ilgili açıklayıcı bilgiler ve diğer dipnotlar rakamla belirtilmeli, yazarlarla ilgili dipnotta, adres öncesi unvan verilmelidir. Ayrıca dipnotla sorumlu yazarın e-posta adresi de eklenmelidir.
11. Makale A4 kağıdına yazılmalı, marjin olarak; üst: 4,0 cm, alt: 3,35 cm, sağ: 2,25 cm, sol: 2,25 cm, üst bilgi: 2,55 cm, alt bilgi: 2,35 cm boşluk bırakılmalıdır. Paragraflar girinti verilmeden satır başından başlamalı ve her iki yana dayalı olmalıdır.
12. Makalede yer alan cins ve türlerin bilimsel isimleri ile Latince kelimeler italik olmalıdır.
13. Literatür listesi makalenin en sonunda yer alır. Listedeki literatürler alfabetik sırada "yazar-tarih" sistemine göre verilmelidir. Numaralama kullanılmamalıdır. Aynı yazarla başlayan tek yazarlı makale çok yazarlı makaleden önce yer almalıdır. Aynı yazarların yer aldığı makaleler metinde ve literatür listesinde tarih sırasına göre, aynı yazarların aynı yılda yaptığı birden fazla makale için ise yılın yanına "a", "b" gibi harf konur. Makale metninde ikiden fazla yazarlı literatürlerde sadece ilk yazar ismi belirtilir ve bunu "ve ark." ile "tarihi" takip etmelidir. Bilimsel kitap adının tüm kelimelerinin baş harfleri, kitap bölümünün adı veya literatür bir makaleden alıntı ise; sadece ilk kelimesi büyük harf olmalıdır. Bir kuruluşun yayını, yayın numarasıyla yazılmalı, diğer kitaplar için basıldığı matbaa adı ve şehri belirtilmelidir. Literatür listesinde her literatürün ilk satırını izleyen satırlar 1 cm içeri çekilmelidir. Makale içindeki atıflarda da "yazar-tarih" sistemi kullanılmalıdır. Birden çok kaynağa aynı anda atıf yapılacaksa yayınlar noktalı virgül ile ayrılmalı ve kronolojik sıra ile verilmelidir. Dergi adları ve kısaltmalar Science & Engineering Journal Abbreviations (http://scieng.library.ubc.ca/)'a göre yapılmalıdır. Yazarlar referansların ya da literatürlerin doğruluğundan sorumludur.

    Makalede yer alan literatür bildirişleri aşağıdaki örneklere uygun olmalıdır:

Kongre, sempozyum veya seminer

Yang, S. M. 1988. Report of the ad hoc committee on sunflower rust. p. 250-255. *In* Proc. 12th Int. Sunflower Conf., Vol. II. Novi Sad, Yugoslavia. 25-29 July. Int. Sunflower Assoc. Paris, France.

Arslanoğlu, F. ve İ. Atakişi. 1997. Bazı patates çeşitlerinde farklı yumru iriliklerinin ve dikim şekillerinin yumru verimi ve verim kriterleri üzerine etkisi. Türkiye II. Tarla Bitkileri Kongresi. 22-25 Eylül 1997. Samsun. s. 648-651.

Kitap

Demir, İ. 1975. Genel Bitki Islahı. Ege Üniv. Zir. Fak. Yay. No: 212. Bornova, İzmir.

Hallauer, A. R., and J. B. Miranda. 1981. Ouantitative Genetics in Maize Breeding lowa State Univ. Press Ames. IA, USA.

Kitaptan bir bölüm

Miller, J. F., and G. N. Fick. 1977. The genetics of sunflower. pp. 441-495. *In*: A. A. Schneiter (Ed.). Sunflower Technology and Production. Argon. Monogr. 35. ASA, CSSA, and SSSA, Madison, Wl, USA.

Tosun, M. 2005. Kalıtım derecesi. s. 10-32. A. Ş. Tan (Ed.). Bitki Islahında İstatistik ve Genetik Metotlar. Ege Tarımsal Araştırma Enst. No: 121. Menemen, İzmir.

Bilimsel dergiden makale

Tan, A. S., C. C. Jan., and T. J. Gulya. 1993. Inheritance of resistance to race 4 of sunflower downy mildew in wild sunflower accessions. Crop Sci. 32: 949-952.

Kıtıkı, A., T. Kesercioğlu, A. Tan, M. Nakiboğlu, H. Otan, A. O. Sarı ve B. Oğuz. 1997. Ege ve Batı Akdeniz Bölgelerinde yayılış gösteren bazı *Origanum* L. türlerinde biyosistematik araştırmalar. Anadolu 7 (2): 26-40.

Doktora ve yüksek lisans tezi

Tan, A. Ş. 1993. Ayçiçeğinde (*Heliaıthus aıvıuus* L.) melez varyete (F1) ıslahında kendilenmiş hatların çoklu dizi (Line x Tester) analiz yöntemine göre kombinasyon yeteneklerinin saptanması üzerine araştırmalar. Doktora tezi. E. Ü. Zir. Fak. Fen Bil. Ens. Tarla Bitkileri Ana Bilim Dalı Bornova - İzmir.

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İnternet sitesinden alıntı

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USDA-ARS National Genetic Resources Program. 2005. Germplasm Resources Information Network (GRIN) database. Available at http://www.ars-grin.gov/ npgs/acc/ acc_queries. html. National Germplasm Resources Laboratory, Beltsville, MD, USA.

Anonim yayın

Resmi yayınlara ve yazarı olmayan kaynaklara "Anonim" veya "Anonymous" olarak atıfta bulunulmalıdır.

Anonim. 1996. İmla kılavuzu. Türk Dil Kurumu yayınları. No: 525. Ankara.

Anonymous. 1970. *Septoria helianthi.* CMI distribution maps of plan diseases. No: 468. Commonwealth Mycol. Inst., Kew, England.

14. Grafik, harita, fotoğraf, resim ve benzeri sunuşlar "Şekil", sayısal değerler ise "Çizelge" olarak isimlendirilmelidir.

15. Çizelge ve grafikler MS Word ve MS Excel ile yapılmalıdır. Çizelge ve grafik rengi siyah-beyaz ve çizgi kalınlığı ¼ pt olmalıdır. Çizelgelerde her rakam veya öge ayrı bir hücrede yer almalıdır. Kısaltmalar başlıkta veya dipnotta açıklanmalıdır.

16. Çizelgeler, grafikler ve bunların başlıkları metinden ayrı sayfalarda, ayrıca grafikler elektronik ortamda "Excel" formunda teslim edilmelidir. Eğer gerekliyse, makalede yer alması planlanan resimler yüksek çözünürlükte, JPEG, GIF veya TIFF dosyası olarak teslim edilmelidir.

17. Çizelge ve grafiklerin Türkçe isimlerinin altına İngilizceleri ve ayrıca çizelgelerde tanımlayıcı nitelikteki ilk satır ve ilk sütundaki ifadeler ile grafiklerin apsis (x) ve ordinat (y) eksenindeki ifadelerin yanına veya altına İngilizceleri de yazılmalıdır.

18. Ondalık sayılar virgül ile ayrılmalıdır. İstatistik önemlilik; 0,05, 0,01 ve 0,001 olasılık düzeyinde sırasıyla tek, iki ve üç yıldız ile (*, ** ve ***) gösterilmelidir. Bu nedenle de bu simgeler diğer notlar için kullanılamaz. Eğer farklı seviyede bir önemlilik derecesi mevcutsa bu da ilave bir açıklama ile bildirilebilir. Önemlilik olmaması durumu ÖD (NS) ile belirtilmelidir. Tablo dipnotları için ise ‡, §, #, ∫ gibi semboller kullanılır.

19. Metin içinde yer alan kısaltmalar ilk yazıldığında tam açılımının yanında parantez içinde gösterilmelidir. DNA vb. standart kısaltmalar için böyle bir tanımlamaya gerek yoktur. Kısaltmalar için Türk Dil Kurumu (TDK) yazım kuralları dikkate alınmalıdır.

20. Yayının benimsenen bilimsel standartlara uymadığı veya anlaşılması zor ve gereksiz tekrarlamalarla dolu olduğu durumlarda, Anadolu Yayın Kurulu, yayımlanmak üzere sunulan yayınlarda değişiklik yapma hakkına sahiptir. Büyük ölçüde düzenlenme gerektiren yazılar düzeltme ve yeniden yazım için yazarına geri gönderilir. Bu gibi makalelerin, düzeltilerek iki ay içinde Anadolu Yayın Kurulu'na tekrar gönderilmesi gerekir.

21. Dergiye gönderilen yazıların Anadolu'da yayımlanıp, yayımlanamayacağı dört ay içerisinde yazara bildirilir.

22. Bir makalenin Anadolu'da yer alması, içeriğinin benimsendiği anlamını taşımaz ve bu konuda dergiye herhangi bir sorumluluk yüklemez. Makalelerin bilimsel sorumlulukları yazarlarına aittir.

23. Yazarlara telif hakkı olarak herhangi bir maddi ödeme yapılmaz. Makale yazarına bir adet ayrı basım gönderilir. Daha fazla ayrı basım ücrete tabidir.

24. Anadolu yazım kuralları Ege Tarımsal Araştırma Enstitü Müdürlüğü'nden veya web sitesinden temin edilebilir. (http://arastirma.tarim.gov.tr/etae/Menu/48/Anadolu-Dergisi).

INSTRUCTIONS TO AUTHORS OF MANUSCRIPTS FOR ANADOLU (ISSN 1300 - 0225)

1. Journal of ANADOLU aims to provide a medium of communication among scientists in the fields of agricultural science, by publishing original research articles, reviews, and crop registration papers.
2. Journal of Aegean Agricultural Research Institute, ANADOLU is biannually issued and published by the Aegean Agricultural Research Institute (AARI).
3. Manuscripts should not exceed 25 pages, must be typed double-spaced, all pages numbered starting from the title page and written in Turkish or English.
4. ANADOLU encourages authors to submit their articles prepared according to publication policy of ANADOLU. Three clearly legible copies of the manuscript prepared by using MS Word copied in a CD must be submitted to the directorate of AARI. Submission of manuscripts as e-mail attachment to the AARI directorate at "etae@tarim.gov.tr" is strongly encouraged.
5. A manuscript number will be mailed to the corresponding author within three days.
6. Authors should declare that the manuscript is original research or review and no similar paper has been published or submitted for publication elsewhere. The cover letter should include the corresponding author's full address, telephone/fax numbers and an e-mail address. The manuscripts are not sent back to the author if it is not published.
7. **Manuscripts should be arranged as follows**

   The manuscript should consist of the parts of Title, Abstract, Keywords, Introduction, Material and Methods, Results and Discussion, Conclusion (if necessary), Acknowledgement (if necessary) and References. All these headings should be written as bold capital letters.

   TITLE: Should be clear, concise but informative containing key words that reflect all important aspects of the article. The title should be followed by the author (s) name (s), and address (es).

   ABSTRACT: Should be complete in itself and informative without reference to text or figures, including keywords, and not exceeding 200 words. Following the abstract, about 3 to 10 keywords should be listed.

   INTRODUCTION

   MATERIALS AND METHODS

   RESULTS AND DISCUSSION

   CONCLUSIONS (If necessary)

   ACKNOWLEDGEMENT (If necessary)

   REFERENCES
8. The manuscript should be written in Times New Roman font. All headings should be written without indendation except heading of abstract that should be written with 1.25 cm indent. Size of headings and their styles should be written as follows: Title of manuscript should be bold and 14 size; Introduction, Material and Methods, Results and Discussion, Conclusion (if necessary), Acknowledgement (if necessary) and their headings 11 size; Abstract, Keywords, Tables, Graphics, Figures, Legends and References 9 size.
9. The Title Page should include the authors' full names. Following the title and 1 space line, authors' names should be written with 11 sizes and bold. First name of the authors are written miniscule and the last name capital letters.
10. The name of the corresponding author along with phone, fax and e-mail information and present addresses should be written as a footnote.
11. The page size and margins of manuscript are as follows: A4; top: 4.0 cm, bottom: 3.35 cm, right: 2.25 cm, left: 2.25 cm, header: 2.55 cm, footer: 2.35 cm. Each paragraph should start without indentation, and be aligned to both side.
12. Species, genus, and latin names should be written in italic.
13. References should be arranged alphabetically at the end of the paper. The author-year notation system is required; do not use numbered notation. All single-author entries precede multiple-author entries for the same first author. Use chronological order only within entries with identical authorship (alphabetizing by title for same-author, same-year entries). Add a lowercase letter a, b, c, etc. to the year to identify same-year entries for text citation. Do this also for any multiple-author entries. When there are more than two authors, only the first author's name should be mentioned, followed by "*et al*" and "year". In the References each book should be listed by their publisher name, publication number (if available). All words of the book title and only the first word of the book parts and manuscript title should start with a capital letter. Each reference should be written with 1 cm indent except for the first line. Journal names are abbreviated according to Science & Engineering Journal Abbreviations (http://scieng.l ibrary.ubc.ca/). Authors are fully responsible for the accuracy of the references. The author-year notation system is also required in the manuscript. More than one citation are placed chronologically in order and separated by semicolon ";".

Reference examples

Paper from a Symposium, Conference or Seminar:

Yang, S. M. 1988. Report of the ad hoc committee on sunflower rust. p. 250-255. *In*: Proc. 12th Int. Sunflower Conf., Vol. II. Novi Sad, Yugoslavia. 25-29 July. Int. Sunflower Assoc. Paris, France.

Arslanoğlu, F. ve İ. Atakişi. 1997. Bazı patates çeşitlerinde farklı yumru iriliklerinin ve dikim şekillerinin yumru verimi ve verim kriterleri üzerine etkisi. Türkiye II. Tarla Bitkileri Kongresi. 22-25 Eylül 1997. Samsun. s. 648-651.

Book

Demir, I 1975. Genel Bitki Islahı. Ege Üniv. Zir. Fak. Yay. No: 212. Bornova, Izmir.

Hallauer, A. R., and J. B. Miranda. 1981. Quantitative Genetics in Maize Breeding. Iowa State Univ. Press. Ames, IA.

Part of the book

Miller, J. F., and G. N. Fick. 1977. The genetics of sunflower. pp. 441-495. *In*: A. A. Schneiter (Ed.) Sunflower Technology and Production. Argon. Monogr. 35. ASA, CSSA, and SSSA, Madison, WI, USA.

Tosun, M. 2005. Kalıtım derecesi. s. 10-32. A. Ş. Tan (Ed.). Bitki Islahında İstatistik ve Genetik Metotlar. Ege Tarımsal Araştırma Ens. No: 121. Menemen, İzmir.

Paper from a scientific journal

Tan, A. S., C. C. Jan., and T. J. Gulya. 1993. Inheritance of resistance to race 4 of sunflovver downy mildew in wild sunflower accessions. Crop Sci. 32: 949-952.

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Tan, A. Ş. 1993. Ayçiçeğinde (*Heliauthus avvuus* L.) melez varyete (F1) ıslahında kendilenmiş hatların çoklu dizi (Line x Tester) analiz yöntemine göre kombinasyon yeteneklerinin saptanması üzerine araştırmalar. Doktora tezi. E. Ü. Zir. Fak. Fen Bil. Ens. Tarla Bitkileri Ana Bilim Dalı Bornova - İzmir.

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Anonymous

Official and collective documents without an author should be cited as "Anonymous" and "Anonim"

Anonim. 1996. İmla kılavuzu. Türk Dil Kurumu yayınları. No: 525. Ankara.

Anonymous. 1970. *Septoria helianthi*. CMI distribution maps of plan diseases. No: 468. Commonwealth Mycol. Inst., Kew, England.

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