e-ISSN: 2458-8377 http://sjafs.selcuk.edu.tr

Selcuk Journal of Agriculture and Food Sciences

Number:34 Volume: 2 Year: 2020
AUGUST

Selcuk Journal of Agriculture and Food Sciences

Editor-in-Chief	Dr. Kazım ÇARMAN, Selçuk University, Turkey
Vice Editor	Dr. Yusuf CUFADAR, Selçuk University, Turkey
Technical Editor	Agric. Eng. (M. Sc.) Hasan KIRILMAZ, Selçuk University, Turkey
Editorial Board	Dr. Adel Salah KHATTAB, Tanta University, Animal Science, Egypt, Dr. Ahmet Tuğrul POLAT, Selçuk University, Landscape Architecture, Turkey Dr. Ali KAHRAMAN, Selçuk University, Field Crops, Turkey Dr. Ali SABIR, Selçuk University, Horticulture, Turkey Dr. Anamika PANDEY, Selçuk University, Soil Science and Plant Nutrition, Turkey Dr. Azmi Dato YAHYA, Malaysia Putra University, Biological and Agricultural Engineering, Malaysia Dr. Carmen HUBBARD, Newcastle University, Agricultural Economy, United Kingdom Dr. Cemalettin SARIÇOBAN, Selçuk University, Food Engineering, Turkey Dr. Duran YAVUZ, Selçuk University, Agricultural Structures and Irrigation, Turkey Dr. Hamid EL-BİLALİ, Bari University, Food Engineering, Italy Dr. İbrahim AYTEKİN, Selçuk University, Animal Science, Turkey Dr. Kasem Zaki AHMED, Minia University, Genetics, Egypt Dr. Majeti Narasimha Vara PRASAD, Hyderabad University , Plant Sciences, India Dr. Mohd Kamran KHAN, Selçuk University, Soil Science and Plant Nutrition, Turkey Dr. Murat KARACA, Selçuk University, Plant Protection, Turkey Dr. Osman ÖZBEK, Selçuk University, Agricultural Machinery and Technologies Engineering, Turkey Dr. Pooja BOHRA, Central Island Agricultural Research Institute, Horticulture , India Dr. Ramakrishnan M. NAİR, International Crops Research Institute for the Semi-Arid Tropics, Crop Improvement and Biotechnology, India Dr. Shafiqur RAHMAN, North Dakota State University, Agricultural and Biosystems Engineering, Canada Dr. Ummuhan ÇETİN KARACA, Selçuk University, Soil Science and Plant Nutrition, Turkey Dr. Zuhal KARAKAYACI, Selçuk University, Agricultural Economics, Turkey
Advisory Board	Dr. Ali KAYGISIZ, Sütçü İmam University, Turkey Dr. Betül Zehra SARIÇİÇEK, Ankara University, Turkey Dr. Cengiz SAYIN, Akdeniz University, Turkey Dr. Ebubekir ALTUNTAŞ, Gaziosmanpaşa University, Turkey Dr. Ercan CEYHAN, Selçuk University, Turkey Dr. Filiz Hallaç TÜRK, Süleyman Demirel University, Turkey Dr. Mohammad Masood TARİQ, Balochistan University, Pakistan Dr. Muhammad Khalid BASHIR, Faisalabad Agriculture University, Pakistan Dr. Musa TÜRKER, Yildiz Technical University, Turkey Dr. Safder BAYAZİT, Mustafa Kemal University, Turkey

Aims and Scope	Selcuk Journal of Agriculture and Food Sciences is unique journal covering mostly theoretical and applied all disciplines of agriculture, food and energy sciences such as agronomy, crop sciences, animal and feed sciences, poultry sciences, field crops, horticulture, agricultural microbiology, soil science, plant nutrition, agricultural engineering and technology, irrigation, land scape, agricultural economics, plant pathology, entomology, herbology, energy, biofuels and biomass, food chemistry, aroma, microbiology, food science and technology, biotechnology, food biotechnology, agricultural production, nutrition and related subjects.

Selcuk Journal of Agriculture and Food Sciences

Product Information

Publisher	Selçuk University Agriculture Faculty
Owner (On Behalf of SUAF)	Prof. Dr. Zeki BAYRAMOĞLU Dean
Editor in Chief	Prof. Dr. Kazım ÇARMAN, Selcuk University, Turkey
Printing House	Selçuk University
Date of Publication	20.08.2020
Language	English
Frequency	Published three times a year
Type of Publication	Double-blind peer-reviewed, widely distributed periodical
Indexed and Abstracted in	GOOGLE SCHOLAR SCIENTIFIC INDEXING SERVICES (SIS) ARAŞTIRMAX CAB ABSTRACTS CROSSREF CAB DİRECT MIAR SCILIT ESJİ Dimensions OAJI.net
Web Address	http://sjafs.selcuk.edu.tr/
Address	Selçuk University, Agriculture Faculty, 42075, Konya, Turkey Telephone : +90 (332) 223 28 05 Fax : +90 (332) 241 01 08 E-mail: kcarman@selcuk.edu.tr

Selcuk Journal of Agriculture and Food Sciences

CONTENTS

Selcuk Journal of Agriculture and Food Sciences

Reviewers

Dr. Ahmet KUBAŞ, Tekirdağ Namık Kemal University, Turkey
Dr. Altuğ ÖZDEN, Adnan Menderes University, Turkey
Dr. Aydın UZUN, Erciyes University, Turkey
Dr. Ertan Sait KURTAR, Selçuk University, Turkey
Dr. Hale ÖZTÜRK, Republic of Turkey Ministry of Agriculture and Forestry, Turkey
Dr. Halit İMİK, Atatürk University, Turkey
Dr. Hatice BİLİR KESKİN, OrduUniversity, Turkey
Dr. İhsan CANAN, Bolu Abant İzzet Baysal University, Turkey
Dr. Murat MERCAN, Ege University, Turkey
Dr. Mustafa ÖNDER, Selçuk University, Turkey
Dr. Mustafa YILDIZ, Ankara University, Turkey
Dr. Ömer SÖZEN, Kırşehir Ahi Evran University, Turkey
Dr. Özge Can NİYAZ, Çanakkale Onsekiz Mart University, Turkey
Dr. Selçuk KODAZ, Atatürk University, Turkey
Dr. Sultan ARSLAN TONTUL, Selçuk University, Turkey
Dr. Şebnem KUŞVURAN, Çankırı Karatekin University, Turkey
Dr. Taki KARSLI, Akdeniz University, Turkey
Dr. Yasemin GEDİK, Ankara University, Turkey
Dr. Yusuf CUFADAR, Selçuk University, Turkey
Dr. Yusuf ÇELİK, Selçuk University, Turkey

Selcuk Journal of Agriculture and Food Sciences

http://sjafs.selcuk.edu.tr/sjafs/index

Research Article

SJAFS
(2020) 34 (2), 118-123
e-ISSN: 2458-8377
DOI:10.15316/SJAFS.2020.204

The Opinions and Expactations of the Farmers on Socio-Economic Impacts of Yortanlı Dam in Bergama District of Izmir Province

Betül Susam SEREZ[1], Sait ENGİNDENİZ[1*]

[1]Ege University, Faculty of Agriculture, Department of Agricultural Economics, İzmir, Turkey

ARTICLE INFO

Article history:
Received date: 19.03.2020
Accepted date: 04.05.2020

Edited by:
Zuhal KARAKAYACI ; *Selçuk University, Turkey*

Reviewed by:
Altuğ ÖZDEN; *Adnan Menderes University, Turkey*
Murat MERCAN; *Ege University, Turkey*
Yusuf ÇELİK; *Selçuk University, Turkey*

Keywords:
Agricultural irrigation
Land use
Rural development
Yortanlı dam

ABSTRACT

The aim of this study is to determine opinions and expectations of the farmers on socio-economic impacts of Yortanlı dam in Bergama district. For this purpose, nine settlements of Bergama district that will benefit from dam irrigation were included. In this research, data were collected from 87 farmers with proportional sampling and by the survey. In the analysis of the data, firstly the socio-economic characteristics of the farmers were examined. Then, the opinions and expectations of the farmers about the socio-economic impacts of the dam in various aspects were determined. Five-point Likert scale was used in this stage. According to the results of the research, 72.41% of the farmers stated that the dam had positive impacts on agricultural production. 34.48% of the farmers think that the dam increases agricultural income. 85.06% of the farmers believe that agricultural lands are used more effectively after the dam. On the other hand, 65.52% of the farmers think that the dam will not reduce the migration from the region. However, 51.72% of the farmes stated that the dam will affect the young farmers positively. As a result, with the development of irrigation opportunities with the dam, income level and employment opportunities in the region may increase. Therefore, the young population in the region should be encouraged to agricultural production and private sector investments should be encouraged for processing agricultural products.

1. Introduction

Today, irrigated agriculture makes important contributions to maintaining food security and plays a critical role in world food production (Le Visage et al, 2018). There are 1.52 billion hectares of cultivated land in the world and 20.1% of this land is irrigated. 41.3% of the irrigated lands are located in China and India. Turkey has 1.7% of irrigated land in the world (FAO, 2018).

Too many dams have been built for agricultural irrigation in different countries of the world. Dams provide socio-economic and environmental benefits in rural areas. In countries with water problems, dams are needed for efficient use of water in terms of resource sustainability and economic development (Engindeniz et al., 2014). On the other hand, the positive and negative effects of dams can emerge over time. Therefore, scientific research in this direction should be done after each dam and the results should be evaluated. Generally, the effects of dams on agricultural production and income level, population and employment are emphasized. While the effects of irrigation dams on agricultural production and income levels are evaluated as direct effects, the effect on employment is considered as an indirect effect.

So far about the effects of dams in Turkey have been numerous studies. In some of these studies, the dams' environmental impacts (Gümüş et al., 2006; Yıldırım, 2006; Tahmişçioğlu et al., 2007; Satılmiş, 2009; Akkaya et al., 2009; Üslü, 2011; Sönmez, 2012; Özdemir, 2015; Yıldırımer et al., 2015; Doğan et al., 2016), the dams' impacts on climate (Emiroğlu et al., 1996; Yeşilnacar and Gülşen, 1999; Bulut et al., 2006; Şengün, 2007; Bacanlı et al., 2015; Kum, 2016), the dams' impacts on fishes (Özkurt, 2000; Kırankaya and Ekmekçi, 2007; Berkün et al., 2008), the dams' impacts on cultural assets (Sarıyıldız et al., 2008), and the dams' social and economic impacts have been analyzed (Sarıyıldız et al., 2005; Ulaş, 2008; Engindeniz et al., 2010; Tumer and Aksoy, 2011; Engindeniz et al., 2014, Baskaya and Turk, 2015; Kurt, 2015; Kocyigit and Emiroglu, 2016; Özbey, 2017; Akgün, 2018). However, the dams' impacts in different regions should also be evaluated in terms of farmers.

Yortanlı Dam, the construction of which was completed in 2011 and opened to operation since 2013, is located 18 km the northeast of Bergama district center and on the Yortanlı Stream. The dam is expected to

* Corresponding author email: *sait.engindeniz@ege.edu.tr*

provide agricultural irrigation in an area of 6,990 hectares. The aim of this study is to determine opinions and expectations of the farmers on socio-economic impacts of Yortanlı dam in Bergama district.

2. Materials and Methods

This research covers the farmers in nine settlements consisting of Alibeyli, Ayaskent, Aziziye, Bölcek, Dagestan, Göçbeyli, Kadıköy, Sarıcalar and Zağnos located in the Yortanlı dam region (Map 1). According to the data of Directorate of the Ministry of Agriculture and Forestry of Bergama District, the number of farmers registered in the Farmer Registration System in nine settlements is 842 (Table 1).

Figure 1
The location of the Yortanlı dam

Table 1
Distribution of farmers by settlements

Settlements	Total number of farmers	%	Sample size
Alibeyli	104	12.35	11
Ayaskent	125	14.85	13
Aziziye	34	4.04	3
Bölcek	141	16.75	14
Dağıstan	74	8.78	8
Göçbeyli	222	26.36	23
Kadıköy	84	9.98	9
Sarıcalar	40	4.75	4
Zağnos	18	2.14	2
Total	842	100.00	87

In the research, it was decided that it would be appropriate to include of farmers with sampling and the following the proportional sampling formula was used (Newbold, 1995). This sampling method has been used in many previous studies (Özdemir et al., 2015, Tiryakioğlu and Artukoğlu, 2015; Çonoğlu et al., 2016; Kızıloğlu and Kızılaslan, 2017; Yüzbaşıoğlu, 2019; Bozdemir et al., 2019; Barlas et al., 2019).

The compost was produced by vertical silo method by the Kemerburgaz Organic Waste Compost Factory in Istanbul, which is one of a few compost producing organizations in Turkey. Relevant chemical properties of the compost are given in Table 2.

$$n = \frac{Np(1-p)}{(N-1)\sigma^{2}_{px} + p(1-p)}$$

In formula;

n = Sample size

N = Total number of farmers

p = Proportion of farmers that cultivate irrigable land (based on 0.5)

σ^{2}_{px} = Variance.

The calculations are based on a 95% confidence interval and a 10% error margin, and the sample size is 87. While determining the number of farmers to be surveyed in the settlement units, the calculation was made on the share of each settlement in the total number of farmers. Research data was collected in 2017.

In the analysis of the data, primarily the socio-economic characteristics of the farmers were examined. Then, the opinions and expectations of the farmers regarding the socio-economic impacts of the dam were determined. At this stage, the five-point Likert scale was used.

In the conversion of the population in farms to the unit of male labor force (EIB); the coefficients of 0.50 for males and females in the 7-14 age group, 1.00 for males in the 15-49 age group, 0.75 for males in the 50-64 age group, 0.50 for females were based on (Aras, 1988).

3. Results and Discussion

The socio-economic characteristics of the farmers are given in Table 2. The age of the farmers varies between 29-74, and the average age is 52.10. The average education period and agricultural experience of the farmers was determined as 8.18 years and as 14.31 years, respectively.

Table 2
Socio-economic characteristics of farmers

Age of farmers	52.10
Education periods of farmers (years)	8.18
Agricultural experience of farmers (year)	14.31
Household size (person)	3.96
Labor force potential of family (unit of male labor force)	2.85
Land size (decare)	72.15
Rate of equity capital (%)	62.08
Rate of being a cooperative member (%)	98.85
Sarıcalar	4

The household size of the farms is 3.96 person and 50.72% of them are male. The average family labor force potential in farms is 2.85 as a male labor unit (EIB) and 855 as a male labor day (EIG).

The average land size in the farms is 72.15 decares. The average number of parcels is 3.56 and the average parcel size is 20.27 decares. 49.70% of the lands in the farms are operated lands by the owner, 34.61% of the lands are rented land and 15.69% of the lands are operated lands by the partner. Cotton, wheat, corn and tomato are generally produced in the farms.

As an average of farms, 86.30% of total assets are land assets. When the distribution of the assets according to the items is examined; a large share of land assets (78.04%), followed by tool-machine assets (10.56%) and land reclamation (6.49%) respectively. However, equity capital constitutes 62.08% of passive assets. 86 of 87 farmers included in the research are partners to at least one agricultural cooperative.

The farmers in the study were asked how their agricultural production was affected after the dam was completed. 72.41% of the farmers stated that the dam had positive affects (Table 3).

Table 3
The farmers' answers to the question "how did the completion of the dam affect your agricultural production?"

Answers	Number of farmers	%
Positively affected	63	72.41
Negatively affected	0	0
No affected	8	9.20
No idea	16	18.39
Total	87	100.00

Dams can positively affect agricultural lands and usage patterns, as well as increase the irrigation opportunities and increase production. These expectations were also revealed in the studies conducted before the Yortanlı Dam was put into operation (Sarıyıldız et al., 2005; Engindeniz et al., 2010).

It is expected that cotton production will continue in the region after the dam, whereas other products will be preferred by partially giving up wheat production. It is thought that the most important of the products that can be an alternative to wheat may be corn, and also tomato and cotton farming can be preferred.

When the farmers were asked how the agricultural income levels changed after the dam was completed; 34.48% of farmers stated that their agricultural income increased and 33.33% did not change (Table 4).

Table 4
The farmers' answers to the question "how did your agricultural income level change after the dam was completed?"

Answers	Number of farmers	%
My income increased	30	34.48
My income decreased	1	1.15
My income has not changed	29	33.33
No idea	27	31.04
Total	87	100.00

When the farmers' opinons and expectations on the effects in the region after the dam is completed are examined; it was determined that they agree with the expresions 'irrigation opportunities increased' (4.03), 'fly increased' (3.78), 'environmental pollution has occurred' (3.76), 'land became fragmented' (3.74), 'air quality deteriorated' (3.50), 'land prices and rents increased' (3.49), 'marketing opportunities improved' (3.05). On the other hand, they do not agree with the expressions 'employment opportunities increased' (2.78), 'roads are extended' (2.75), 'transportation opportunities improved' (2.52), 'local population increased' (2.44) (Table 5).

When the opinions of the farmers about the frequency of land sales in the region after the dam was completed, 49.42% stated that they had no idea, 25.29% increased of sales frequency and 25.29% it has not change (Table 6).

Table 5
The farmers' answers to the question "what level do you participate in the local effects after the dam is completed?"

Effects of dam	Strongly disagree (1)		Disagree (2)		Undecided (3)		Agree (4)		Strongly agree (5)		Mean
	N	%	N	%	N	%	N	%	N	%	
Irrigation opportunities increased	0	0	0	0	1	1.15	82	94.25	4	4.60	4.03
Fly increased	0	0	6	6.90	12	13.79	64	73.56	5	5.75	3.78
Environmental pollution has occurred	0	0	9	10.34	9	10.34	63	72.42	6	6.90	3.76
Land became fragmented	0	0	10	11.49	8	9.20	64	73.56	5	5.75	3.74
Air quality deteriorated	0	0	21	24.14	8	9.20	51	58.62	7	8.04	3.50
Land prices and rents increased	1	1.15	17	19.54	13	14.94	50	57.47	6	6.90	3.49
Marketing opportunities improved	0	0	33	37.93	20	22.99	31	35.63	3	3.45	3.05
Employment opportunities increased	0	0	47	54.02	14	16.09	24	27.59	2	2.30	2.78
The roads are extended	0	0	43	49.42	24	27.59	19	21.84	1	1.15	2.75
Transportation opportunities improved	0	0	61	70.11	8	9.20	17	19.54	1	1.15	2.52
Local population increased	1	1.15	64	73.56	6	6.90	15	17.24	1	1.15	2.44

Table 6
The farmers' answers to the question "has the number of land sold after the dam completed?"

Answers	Number of farmers	%
Sales frequency increased	22	25.29
Sales frequency decreased	0	0
It has not changed	22	25.29
No idea	43	49.42
Total	87	100.00

41.38% of the farmers within the scope of the research stated that after the dam was completed, they had no idea about the change of land prices, 29.88% of the land prices did not change and 28.74% of the prices increased (Table 7).

When the farmers were asked whether the lands sold after the dam was completed were used for agricultural purposes, they all answered yes.

Table 7
The farmers' answers to the question "has the land purchase-sale prices changed after the dam was completed?"

Answers	Number of farmers	%
Prices increased	25	28.74
Prıces not changed	26	29.88
No idea	36	41.38
Total	87	100.00

85.06% of the farmers evaluated the dam in their region positively in terms of effective use of agricultural lands (Table 8).

When farmers were asked whether the dam would reduce migration in the region, 65.52% gave no reduce answer (Table 9).

In previous studies conducted in different regions, it has been revealed that dams cannot reduce migration (Tümer and Aksoy, 2011; Koçyiğit and Emiroğlu, 2016).

Table 8
The farmers' answers to the question "how do you evaluate the dam in your region area in terms of effective use of agricultural lands?"

Answers	Number of farmers	%
Positive	74	85.06
Negative	0	0
No idea	13	14.94
Total	87	100.00

Table 9
The farmers' answers to the question "does the dam in your region reduce migration?"

Answers	Number of farmers	%
Reduce	8	9.19
Not reduce	57	65.52
No idea	22	25.29
Total	87	100.00

When asked how the dam would affect younger farmers, it stated that it could affect 51.72% positively (Table 10).

Table 10
The farmers' answers to the question "how does a dam in your region affect the young farmers?"

Answers	Number of farmers	%
Positive	45	51.72
Negative	0	0
No effect	31	35.63
No idea	11	12.65
Total	87	100.00

According to the research results, the farmers believe that the dam is beneficial for the effective use of the local lands. They express that their agricultural production is positively affected by the increase of irrigation opportunities. However, they also emphasize that the dam may have some environmental and physical adverse effects.

With the development of irrigation opportunities with the dam, it is expected that the income level and employment opportunities in the region may increase, therefore, the population may continue to live in the region and consequently migration will decrease. Apart from this, it is estimated that there may be a population flow by immigration to the region from other regions. However, although some of the farmers within the scope of the research think that immigration will not decrease, they believe that the dam can positively affect young people. It is necessary to encourage the young population in the region to agricultural production and to encourage private sector investments in the processing of agricultural products.

After the dam is completed, it is expected that the corn will have the most important share in the product pattern in the region and that the tomato will follow. However, while determining the product pattern, farmers should also conduct market researches and make the most of the supports provided.

As a conclusion, dams provide socio-economic and environmental benefits in rural areas. However, the positive and negative effects of dams can emerge over time. Therefore, scientific research in this direction should be conducted after each dam and the results should be evaluated.

4. Acknowledgements

We would like to thank the Ege University Scientific Research Projects Commission which provided financial support for the project No 16-ZRF-020 and the local farmers who patiently responded to the survey questions.

5. References

Akgün Ç (2018). Cost Analysis of Environmental Impacts for Tuzluca Dam. Master Thesis, Institute of Science and Technology of Gümüşhane University, Gümüşhane.

Akkaya U, Gültekin AB, Dikmen ÇB, Durmuş G (2009). The Analysis of Environmental Impacts of

Dams and Hydroelectric Power Plants (HEPP): Sample of Ilısu Dam. 5th International Advanced Technologies Symposium, 13-15 May, 2009, Karabük.

Aras A (1988). Agricultural Accounting. Publication of Faculty of Agriculture of Ege University, No:486, Izmir.

Bacanlı ÜG, Tuğrul AT (2015). A climate impact of dam lake and Recep Yazıcıoğlu Gökpınar dam lake sample. *Pamukkale University Journal of Engineering Sciences* 22(3):154-159.

Barlas S, Örmeci Kart, MÇ, Kınıklı F, Işın Ş (2019). Factors affecting the production decisions of cut flowers producers in Menderes Region of Izmir province. *Atatürk University Journal of the Agricutural Faculty* 50(3): 231-238. (in Turkish)

Berkün M, Aras E, Koç T (2008). Effects of dams and hydroelectric power plants on river ecology. *Turkey Engineering News* 452:41-48.

Bozdemir M, Bayramoğlu Z, Ağızan K, Ağızan S (2019). Prudential expectation analysis in maize production. *Turkish Journal of Agriculture - Food Science and Technology* 7(3):390-400.

Bulut H, Yeşilata B, Yeşilnacar İ (2006). Deterination of the Impact of Atatürk Dam Lake on Regional Climate by Trend Analysis. Proceedings of the Fifth GAP Engineering Congress, 26-28 April, 2006, Şanlıurfa, p.79-86.

Çonoğlu S, Kaynak T, Demirbaş N, Tosun N (2016). Tendencies of farmers to soil analysis subsidy: a case study of Izmir province. *Ege Journal of Agricultural Research 53(4):441-449.*

Doğan E, Çeribaşı G, Akkaya U (2016). Investigation for effecting of dam to river flow regime by trend analysis method: case study of Sakarya River. *Karaelmas Science and Engineering Journal* 6(1):50-55.

Emiroğlu ME, Özkan F, Öztürk M (1996). A Research on the Impact of Keban Dam Reservoir on Climate Conditions of Elazig Province. Proceedings of First GAP Engineering Congress, 29 May 1996, Şanlıurfa, p.167-174.

Engindeniz S, Atış E, Saner G (2010). The Effects of Yortanlı Dam on the Socio-Economic Structure of the Region. Yediveren Printing House, Izmir.

Engindeniz S, Atış E, Saner G, Coşar, G (2014). Irrigation Dams in terms of Sustainability of Agriculture and Farmers' Expectations: Yortanlı Dam. 11th National Agricultural Economics Congress, 3-5 September, 2014, Samsun, p.54-63.

FAO (2018). Agricultural statistics, http://faostat.fao.org, Access: December 2018.

Gümüş B, Dalkılıç N, Toprak F (2006). Versatile Evaluation of Ilısu Dam and HEPP. Ilısu Dam and HEPP Research Commission Report, Dicle University Faculty of Engineering and Architecture, Diyarbakır.

Kırankaya ŞG, Ekmekçi FG (2007). Variations in growth properties of chub (*squalius cephalus, l.*, 1758) living in Gelingüllü Dam Lake. *Journal of Balıkesir University Institute of Science and Technology* 9(2): 125-134.

Kızıloğlu R, Kızılaslan N (2017). Fertilizer use the condition of cultivator province center in Kahramanmaras. *Turkish Journal of Agriculture - Food Science and Technology* 5(1): 18-23.

Koçyiğit N, Emiroğlu A (2016). An analysis of productive potential of ermenek reservoir formation and its impact on the reasons of socio-economic condition and migration of the regional population, *Academic Journal of History and Idea* 3(10):90-107.

Kum G (2016). The influence of dams on surrounding climate: the case of Keban Dam. *Gaziantep University Journal of Social Sciences* 15(1):193-204.

Kurt E (2015). Problems wıthin Dam Expropriation: Kayseri Gümüşören Dam Case. Master Thesis, Institute of Science and Technology of Aksaray University, Aksaray.

Le Visage S, Kuper M, Venot JP, Yercan M, Atış E (2018). Pursuing the state's hydraulic mission in a context of private groundwater use in the Izmir Province, Turkey. *Water Alternatives* 11(2): 421-438.

Newbold P (1995). Statistics for Business and Economics. Prentice Hall International Editions.

Özdemir B, Akbay C, Çelik A (2015). The current status and problems of apple growers in Gülnar District of Mersin City. *Turkish Journal of Scientific Reviews* 8 (2): 56-58.

Özdemir HY (2015). Determination of Land Use Change in Hasanlar Dam Basin. Master Thesis, Institute of Science and Technology of Düzce University, Düzce.

Özkurt Ş (2000). Boron accumulation in carp tissues (*Cyprinus carpio l.*, 1758) in Dam Lakes Çötören and Kunduzlar (Kırka-Eskişehir). *Turkish Journal of Biology* 24 (2000): 663-676.

Sarıyıldız A, Atış İ, Tomar A (2005). The evaluation of the Bakırçay Kınık project Yortanlı and Çaltıkoru dams in terms of local economy and social effects and some facts. *Journal of Izmir Commodity Exchange*, October.

Sarıyıldız A, Tahmiscioğlu S, Silay A, Tomar A (2008). Investigation of the Conservation of Cultural Heritage Approaches in the Development of Water Resources in the Example of Yortanlı Dam. The Union of Chambers of Turkish Engineers and Architects 2nd Water Policy Congress, 20-22 March, 2008, Ankara, p. 541-554.

Satılmış M (2009). Environmental Impact Assessment of Dams and Hydroelectric Power Plants. Forum-2009, Eastern Black Sea Region Hydroelectric Energy Potential and Its Place in Country Energy Policies, 13-15 November, 2009, Trabzon, p.151-154.

Sönmez ME (2012). The negative impacts of dams on envıronment and their samples in Turkey. *Gaziantep University Journal of Social Sciences* 11 (1) : 213 -231.

Şengün MT (2007). The effect of Keban Dam on Elazig climate in the light of recent evaluations. *Journal of East Anatolian Region Research* 5:116-121.

Tahmişçioğlu MS, Anul N, Ekmekçi F, Durmuş N (2007). Positive and Negative Impacts of Dams on the Environment. International Congress on River Basin Management, 22-24 March, 2007, Antalya-Turkey, pp:759-769.

Tiryakioğlu M, Artukoğlu MM (2015). A research on table olive production, marketing, problems and solutions: the case of Akhisar. *Ege Journal of Agricultural Research* 52(2):131-139.

Tümer Eİ, Aksoy A (2011). Attitudes towards construction of the Laleli Dam and socio-economical characteristics of the farmers in Laleli Basin. *Atatürk University Journal of Agricultural Faculty* 42(1): 41-47.

Ulaş H (2013). Irrigation Cost of Irrigable Agricultural Areas: The Case of Adıyaman Çamgazi Dam. Master Thesis, Institute of Social Sciences oF Hasan Kalyoncu University, Gaziantep.

Üslü A (2011). Environmental Impact Cost Analysis of Ilısu Dam. Master Thesis, Institute of Science and Technology of Karadeniz Technical University, Trabzon.

Yeşilnacar Mİ, Gülşen H (1999). Climatic Characteristics of Şanlıurfa and the Surrounding Region and the Effects of Atatürk Dam Lake on Regional Climate. Proceedings of the 52nd Geological Congress of Turkey, 10-12 May, 1999, Ankara, p.122-128.

Yıldırım A (2006). Karakaya Dams and effects of naturel environment. *Journal of Ziya Gökalp Education Faculty of Dicle University* 6:32-39.

Yıldırımer S, Özalp M, Erdoğan Yüksel E (2015). Determining loss and degradation of lands as a result of large dam projects and associated road constructions within the Coruh River Watershed. *Artvin Coruh University Journal of Forestry Faculty* 16(1):1-17.

Yüzbaşıoğlu R (2019). Tendencies of rural producer to soil analysis province center in Tokat. *Journal of Bahri Dagdas Crop Research* 8(1): 163-169.

Selcuk Journal of Agriculture and Food Sciences

SJAFS

http://sjafs.selcuk.edu.tr/sjafs/index

(2020) 34 (2), 124-131

e-ISSN: 2458-8377

Research Article

DOI:10.15316/SJAFS.2020.205

The Effect of Exopolysaccharide Producer *Pediococcus Damnosus* 2.6 and Yoghurt Starter Cultures on Ethanol Content, Some Physicochemical and Sensory Properties of Oat Boza

Çiğdem KONAK GÖKTEPE[1,*], Adem ELGÜN[2]

[1]Selçuk University, Karapınar Aydoğanlar Vocational School, Konya, Turkey
[2]Selcuk University, Faculty of Agriculture, Department of Food Engineering, Konya, Turkey

ARTICLE INFO

Article history:
Received date: 18.03.2020
Accepted date: 07.05.2020

Edited by:
Cemalettin SARIÇOBAN; *Selçuk University, Turkey*

Reviewed by:
Hale ÖZTÜRK; *Republic of Turkey Ministry of Agriculture and Forestry, Turkey*
Sultan ARSLAN TONTUL; *Selçuk University, Turkey*

Keywords:
Fermentation
Boza
Oat
Viscosity
Ethanol

ABSTRACT

Cereal-based fermented beverages like boza are known functional and probiotic foods. Boza is manufactured by yeast and lactic acid bacteria fermentation of only ones or mixture of various cereals. In this research, the effects of sugar, different microorganisms and inoculum ratios on physicochemical, nutritional and sensory characteristics of oat based boza were investigated. For this purpose, oat based boza production was carried out by 3 different of inoculation rates (0, 3 and 5%) of 3 starter cultures (*Pediococcus damnosus* 2.6, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus)*. The usage aim of *Pediococcus damnosus* 2.6 and yoghurt starter cultures was for the production of exopolysaccharide and lactic acid. As a result of using mixed culture and rising inoculation rates stimulated fermentation activity in formulation of boza. Thus, the amount of total solid matter was reduced and increased the contents of ash, protein and mineral matter of oat boza samples. Although the lowest viscosity was determined in *Pediococcus damnosus* 2.6 inoculation, the most uniform texture was provided. Raw oat had unpleasant odor and flavor but fermentation enhanced sensorial properties of oats. The highest overall acceptance score was observed in oat boza with 3% of yoghurt starter cultures.

1. Introduction

In recent years, increasing attention of consumers to minimum-treated, chemical preservatives-free and natural foods have necessitated the development of alternative food preservation methods. Among these, the biological protection method, in which the lactic acid bacteria play an important role, is of great importance (Wood & Hodge, 1985). Cereal-based fermented beverages are known functional and probiotic foods because they have nutrimental and health promoting components such as nutritional elements, fibers and phytochemicals. In cereal based fermented product, fermentation enhance protein digestibility, nutritional bioavailability and organoleptic properties (Lorenzo, Emanuele, & Elke, 2016).

Boza, which is one of the fermented cereal products, is prepared with only ones or mixture of various cereals (maize, rice, barley, oats, wheat or millet). Semolina or flour of this cereals cooked by adding water. After that, sugar is added to the mixture and this slurry is subjected to fermentation by yeasts and lactic acid bacteria (Anonymous, 1992).

Oat is a fundamental cereal crop and is used commonly for the feeding of farm animals. However, in recent years, it is utilized in human diets due to its various natritional components that have beneficial effects to human health. Oat is richer nutritional source than other cereals in terms of protein quality, lipid, minerals, vitamins and phytochemicals contents (Arendt & Zannini, 2013). The oat contains starch approximately up to 60% as dry basis. The lipid content with a high content of unsaturated fatty acids of oat kernel is higher as two-five times than other cereal grains. They have high protein content ratio of 9-15% with a high lysine concentration. Oats are a grateful antioxidant resource (Zhu, 2017). The health promoting effects of oats associated with β-glucan contents (2-8%) that had the ability to reduce blood cholesterol and glucose levels (Skendi, Biliaderis, Lazaridou, &

* Corresponding author email: *ckonak@selcuk.edu.tr*

Izydorczyk, 2003). Oat products like oat flakes, oatmeal and oat milk are used to prepare human diets such as ready-to-eat breakfast cereals, baby foods, bread, cookies, and snacks (Bryngelsson, Dimberg, & Kamal-Eldin, 2002).

In this study, it was aimed to improve nutritional and functional properties of oats that was fermented by probiotic bacteria *Lactobacillus acidophilus*, and EPS forming bacteria *Pediococcus damnosus* 2.6. The effects of various microorganisms, different sugar and different inoculation rates used in boza production were investigated on the technological, chemical and sensory properties of the oat boza.

2. Materials and Methods

2.1. Materials

Hulled oat grain (*Avena sativa*), sugar (saccharose) and baker's yeast (*Saccharomyces cerevisiae*, press form) (Pakmaya) were purchased from local market, in Konya. *Lactobacillus acidophilus*, yoghurt bacteria (*Lactobacillus delbrueckii* ssp. *bulgaricus*, *Streptococcus thermophilus*) and *Pediococcus damnosus* 2.6 were obtained from Refik Saydam National Type Culture Collection Laboratory (Refik Saydam National Public Health Agency) in Ankara, Turkey, from Şekersüt Dairy Plant in Konya, Turkey and from the Lund University Biotechnology Laboratory of Sweden in Lund, Sweden, respectively.

2.2. Methods

2.2.1. Preparation of inoculum suspensions

For the activation of *L. acidophilus* strains were inoculated into 5 mL of sterile MRS broth (Merck KGaA, Darmstadt, Germany) and incubated at 42 ˚C for 24 h (pH 4.80 ± 0.2). The active culture was inoculated into UHT milk at a rate of 2% and incubated for the second time at 42 ˚C for 24 h (Mårtensson, Öste, & Holst, 2002).

Yoghurt bacteria (*S. thermophilus* + *L. delbrueckii* ssp. *bulgaricus* 1:1) were obtained from milk factory as an active form. The active cultures were inoculated into UHT milk at a rate of 2% and incubated at 42 ˚C for 24 h (pH 4.50 ± 0.2). After the second activation, this active culture was used in the oat boza production (Anonymous, 2005).

Pediococcus damnosus 2.6 strains were inoculated into 5 mL of sterile MRS broth (Merck KGaA, Darmstadt, Germany) and incubated at 30 ˚C for 18-20 h. This culture was inoculated into sterile MRS broth at a rate of 2% for the second activation. This activated culture were inoculated into 2% UHT milk and incubated 30 ˚C for 24 h and this active culture was used in the oat boza production (Mårtensson, Dueñas-Chasco, Irastorza, Öste, & Holst, 2003).

2.2.2 Production of oat boza samples

Oat boza was produced with some modification according to describe method by Hayta, Alpaslan, and Köse (2001). First of all, oat groats were ground in a hammer mill (Falling Number-3100 Laboratory Mill, Perten Instruments AB, Huddinge, Sweden) equipped with 1 mm opening screen to obtain whole-grain oatmeal as raw materials in boza production. After milling, the oatmeal was stored at -18 ˚C to stop the enzyme activity until used. Oatmeal was mixed with water (1:5 w/v) and slurry was boiled by continuous stirring for 1 hour. The oat mash was cooled at 4-6 ˚C for 7-8 hr. After cooling, the mash diluted with water at levels of 10% and it was blended homogeneously for obtained oat milk. The oat milk was filled in sterile conical flasks with (5%) or without saccharose. 1% of yeast and 0.5 % of *Lactobacillus acidophilus* were also added all boza formulation as constant ratio. The oat milk was inoculated with *Pediococcus damnosus* 2.6, yoghurt bacteria (*S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*1:1) or mixed culture (*Pediococcus damnosus* 2.6+ yoghurt bacteria 1:1) in 3 different of inoculation rates (0, 3 and 5%). Inoculated oat milk was incubated at 30 ˚C for nearly 6 h. Boza samples were analyzed at the end of the fermentation within the same day and were stored at +4 ˚C. Before sensory evaluation, 10% saccharose was added in boza samples for sweetening. Boza samples were identified as follows: inoculated with *Pediococcus domnasus 2.6* (Pd), inoculated with yoghurt culture (YC) and inoculated with *Pediococcus domnasus 2.6* + yoghurt culture (Pd+YC).

2.2.3. Chemical analyses of oatmeal and oat boza samples

Total solid matter (method 44-19), crude ash (method 08-03), protein (AACC 46-12) contents of oatmeal and oat boza samples were measured according to the AACC methods (AACC, 1990). Mineral matter content of the oatmeal was performed according to the method described by Skujins (1998).

2.2.4. pH and titratable acidity of oat boza samples

pH measurements of the boza samples were performed by a digital type pH meter, WTW pH315 i/set model in compliance with TS 9778 (Anonymous, 1992). Potentiometric titration techniques were used for quantification of total titratable acidity of samples in lactic acid (Kentel, 2001).

2.2.5. Ethanol content of oat boza samples

The determination of ethanol in oatmeal boza samples was performed according to TS 1594 (Anonymous, 1998). Ethanol content was found in grams per 100 milliliters of the product.

2.2.6. Viscosity measurement of oat boza samples

A Brookfield viscometer which was equipped with a spindle 7 (Lab line, Model No 4535, Lab Line Instruments, Inc., Melrose Park, IL.,U.K.) was

used for measurements of viscosity of samples at 4˚C at 20 rpm.

2.2.7. Sensory evaluation of oat boza

Sensory properties were determined oat boza samples by five panelists who were members of the academicians of the Department of Food Engineering, Selcuk University, Konya, Turkey. The oat boza samples were evaluated with regards to product acceptability using 5 point hedonic scale with 1-2 dislike, 3 acceptable, 4-5 like extremely.

2.2.8. Statistical analysis

Statistical analysis was performed with General Linear Model ANOVA by Minitab 7.1 (Minitab, 1991). The means which were statistically different from each other were compared using Tukey's test at $p < 0.01$.

3. Results and Discussion

3.1. Chemical properties of oatmeal

Some chemical results of oatmeal are shown in Table 1.

Table 1
Some chemical properties of oatmeal

Total solid matter (%)	91.56
Crude ash (%)[a]	1.98
Protein (%)[b]	15.48
Mineral matter content(mg/100g)	
Ca	1977.50
Fe	45.80
Zn	28.96
K	5331.73
Mg	1895.41
P	5075.90

[a] in dry basis
[b]Protein = N x 6.25

The protein and crude ash contents of the hulled oat kernel were determined to be 15.48% and 1.98%, respectively (Table 1). Oat has highest amount of protein among other cereals, its content ranges from 12 to 24% in oat kernel (Lasztity, 1999). In previous study, Kirk and Sawyer (1999) reported that oat grain had about 3.1% ash and 13% protein content. Wholegrain oat has high amounts of valuable components therefore it is a good cereal for nutrition of human.

3.2. Determination of changes in pH value of oat boza samples during fermentation

The pH values of samples ranged from 6.28 to 5.80 at the beginning of the fermentation and there were no statistical differences in initial pH values of boza samples ($P>0.05$). The pH values of boza samples showed a continuous reduction during fermentation ($P<0.01$). At the end of the fermentation, pH values of samples ranged between 5.67-4.76. Similar findings with pH values were reported by Rathore, Salmerón, and Pandiella (2012) who researched the effect of two probiotic strains on fermentation of single and mixed cereal substrates. In this previous study, it was indicated that the pH value was determined to be below 3.5 in mixed and single cereal media at the end of fermentation.

Whereas the highest pH value was determined in control without sugar, the lowest pH value was observed in 5% YC samples with sugar (Figure 1-2). The addition of sugar into the boza formulation was accelerated fermentation and supported the formation of lactic acid, which caused quickly reduction in pH values. Similarly, Hancioğlu and Karapinar (1997) and Gotcheva, Pandiella, Angelov, Roshkova, and Webb (2001) reported that pH of boza samples decreased from 6.13 to 3.48 and from 5.4 to 3.1 during the fermentation time, respectively.

3.3. Some chemical properties of oat boza samples

Total solid matter contents of oat boza are given in Table 2. The total solid matter content ranged from 16.11 to 18.68% and increased significantly ($p<0.01$) by addition of sugar into boza formulation. Moreover, the highest total solid matter content was determined in boza sample prepared with 5% Pd+YC inoculation and sugar addition. Besides, statistically no significant difference was found between the other inoculation rates and cultures types in boza samples with and without sugar.

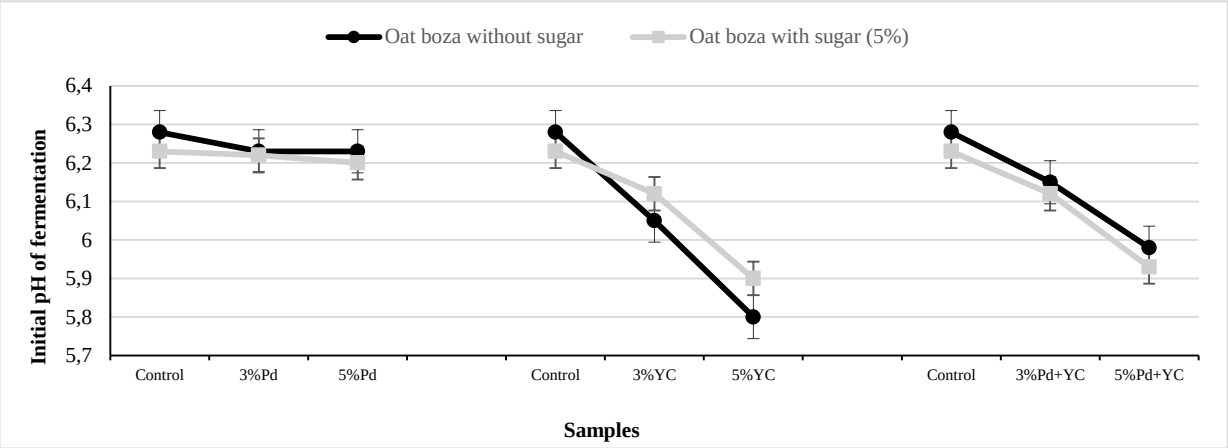

Figure 1
pH values of oat boza samples at the beginning of the fermentation

Pd: boza inoculated with *Pediococcus domnasus 2.6*; YC: boza inoculated with yoghurt culture (*S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*1:1); Pd+YC: boza inoculated with *Pediococcus domnasus 2.6* + yoghurt culture (1:1); 3-5%: inoculation rates of starter cultures

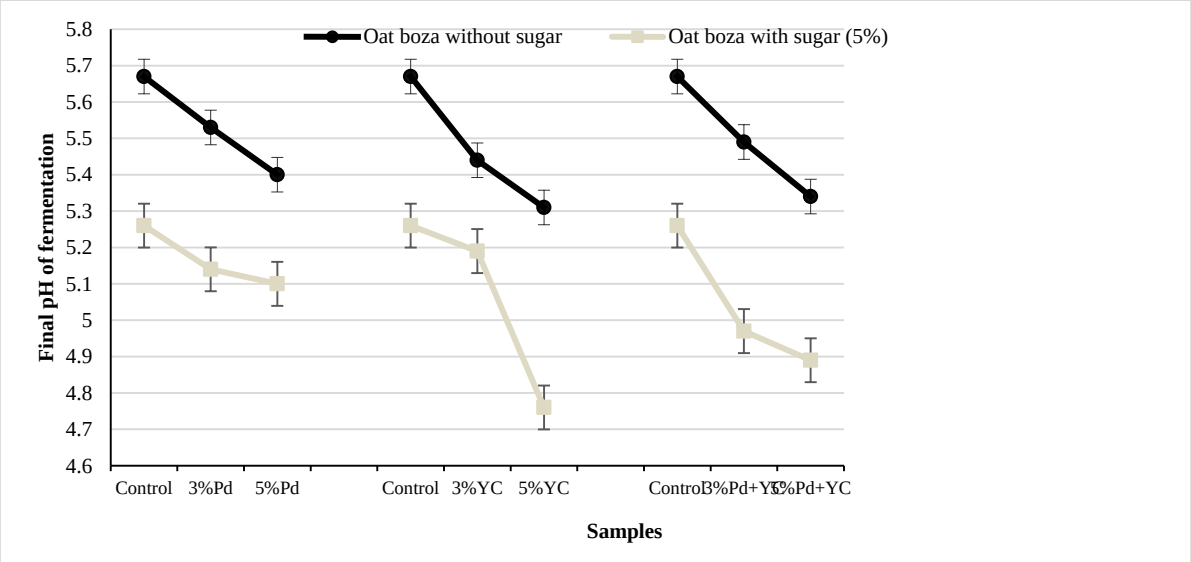

Figure 2
pH values of oat boza samples at the end of the fermentation

Pd: boza inoculated with *Pediococcus domnasus 2.6*; YC: boza inoculated with yoghurt culture (*S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*1:1); Pd+YC: boza inoculated with *Pediococcus domnasus 2.6* + yoghurt culture (1:1); 3-5%: inoculation rates of starter cultures

Crude ash content of oat boza are presented in Table 2. The highest crude ash value was determined in 5% Pd+YC sample to be 0.3944% ($P<0.01$). It was determined that adding sugar in boza formulation and increasing of amount of fermentation losses were proportionately decreased ash content of samples. These findings are in agreement with Aytekin (2001) who reported that as the sugar content increased in boza formulation, the ash contents of boza samples decreased. The protein contents of oat boza samples were determined to be between 3.32 and 3.81% (Table 2). While there were no statistical differences in protein contents between the boza samples which were inoculated different culture ($P>0.05$), the increasing inoculation rates and adding sugar were increased protein content of oat boza samples ($P<0.01$).

Table 2
Some chemical properties of oat boza

Sample	Titratable acidity (Lactic acid %)	Total solid matter (%)	Crude ash (%)[a]	Protein (%)[b]	Ethanol (g/100ml)
Control	0.28±0.01^{d}	16.50±0.15^{d}	0.3550±0.007^{de}	3.62±0.04^{bcd}	0.22± 0.01^{bc}
3% Pd	0.30±0.07^{cd}	16.44±0.15^{d}	0.3682±0.005^{cd}	3.68±0.04^{abc}	0.13±0.03^{de}
5% Pd	0.34±0.03^{bcd}	16.21±0.07^{d}	0.3934±0.006^{ab}	3.70±0.04 ^{ab}	0.24±0.03^{b}
3% YC	0.33±0.01^{bcd}	16.55±0.21^{d}	0.3722±0.006^{bcd}	3.73±0.02^{ab}	0.20±0.01^{bcd}
5% YC	0.37±0.03^{abcd}	16.11±0.11^{d}	0.3874±0.002^{abc}	3.81±0.01^{a}	0.10± 0.01^{e}
3% Pd+YC	0.33±0.04^{bcd}	16.3±0.21^{d}	0.3740±0.005^{abcd}	3.63±0.04^{bcd}	0.14±0.01^{cde}
5% Pd+YC	0.34±0.04^{bcd}	16.12±0.11^{d}	0.3945±0.006^{a}	3.70±0.04^{ab}	0.17±0.02^{bcde}
Control with sugar	0.38±0.01^{abcd}	18.41±0.10^{ab}	0.3250±0.007^{fg}	3.32±0.04^{f}	1.91± 0.01^{a}
3% Pd with sugar	0.38±0.03^{abcd}	17.90±0.04^{c}	0.3241±0.004^{g}	3.33±0.05^{f}	1.86±0.041^{a}
5% Pd with sugar	0.41±0.00^{abc}	18.14±0.09^{bc}	0.3422±0.004^{efg}	3.54±0.04^{cde}	1.86±0.01^{a}
3% YC with sugar	0.39±0.06^{abcd}	17.69±0.26^{c}	0.3461±0.006^{ef}	3.35±0.04^{f}	1.89±0.02^{a}
5% YC with sugar	0.43±0.04^{abc}	17.95±0.06^{bc}	0.3619±0.002^{de}	3.51±0.03^{de}	1.92±0.03^{a}
3% Pd+YC with sugar	0.44±0.01^{ab}	18.16±0.09^{bc}	0.3525±0.006^{de}	3.41±0.03^{ef}	1.91±0.02^{a}
5% Pd+YC with sugar	0.48±0.04^{a}	18.68±0.11^{a}	0.35845±0.006^{de}	3.60±0.04^{bcd}	1.92±0.02^{a}

[a]in dry basis; [b]Protein = N x 6.25

Different letters indicate significantly different at P< 0.01

Pd: boza sample inoculated with *Pediococcus domnasus 2.6*; YC: boza inoculated with yoghurt culture (*S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*1:1); Pd+YC: boza inoculated with *Pediococcus domnasus 2.6* + yoghurt culture (1:1); 3-5%: inoculation rates of starter cultures

It could be revealed that increasing amount of inoculum and adding sugar supported fermentation of boza. Similar results were obtained in previous studies and an increase in amount of protein was observed in fermented products compared with non-fermented substrates (Hamad & Fields, 1979; Morcos, Hegazi, & El-Damhougy, 1973). Furthermore, Odunfa (1985) reported that increasing in the amount of protein during the fermentation could be resulted from proteinase activity of the fermentative microorganisms. The oat grain has higher protein content than other cereals and therefore it constitutes a good potential for protein source products (Pomeranz, 1975).

3.4. Titratable acidity of oat boza samples

Titratable acidity values of oat boza samples are shown Table 2. Titratable acidity values (as lactic acid) of boza were expressed to might be in between 0.2-0.5% according to TS 9778 standard of boza. Similar results were observed in oat boza samples and total acidity (in terms of lactic acid) ranged between 0.28-0.48%. While there was no statistically significant effect of culture type on titratable acidity values, adding sugar in oat boza samples before fermentation and inoculation rates of cultures had a significant effect on the amount of acidity of samples (P< 0.01). These results could be related to microbial activity of using cultures in fermentation of boza samples. Adding sugar (5%) in boza formulation could be supported growth of microorganisms. In parallel with our results, Üstün and Evren (1998) determined the acidity values of boza samples in between 0.242-0.448%. Besides, Salmerón, Thomas, and Pandiella (2015) reported that the oat beverages inoculated with *L. plantarum* and *L. acidophilus* had lactic acid at a concentration of 0.52 and 0.98 g/L, respectively. 0.3-0.5% lactic acid and carbon dioxide produced during fermentation gives aroma and refreshing feature in boza (Topal & Yazıcıoğlu, 1986).

3.5. Ethanol content of oat boza samples

The ethanol content of boza samples with (5%) or without sugar were determined in between 1.86-1.92 and 0.10-0.22 g/100mL, respectively (Table 2). Ethanol content increased significantly by increasing inoculation ratio of cultures in boza samples produced without the addition of sugar. On the other hand, increased inoculation rate of yoghurt bacteria decreased ethanol amount of the samples without sugar. In previous study, Kedia, Wang, Patel, and Pandiella (2007) determined that the ethanol production in mixed culture containing *Lactobacillus reuteri* and yeast was higher than the pure culture of yeast in a medium of 5% (w/v) malt suspension. Although, the highest ethanol content was determined in oat boza samples prepared with sugar addition, there were no statistical differences in ethanol contents of the samples with sugar. Add-

ing sugar before fermentation supported metabolic activities of lactic acid bacteria and yeasts, so that the amount of ethanol in oat boza samples added sugar before fermentation was much higher than those without added sugar. This fact was also detected with an alcoholic odor and a bitter taste in boza samples with sugar. Salmerón et al. (2015) detected concentrations of the ethanol in fermented oat beverages, inoculated with *L. acidophilus*, *L. plantarum* or *L. reuteri* to be 0.67, 0.78 and 0.64 mg/L respectively. Likewise, Hancioğlu and Karapinar (1997) reported that alcohol content of boza samples increased from 0.02 to 0.79% during fermentation period (24h).

3.6. Viscosity of oat boza samples

Viscosity values of oat boza are shown in Figure 3. The viscosity values of boza samples with (5%) or without sugar were measured in between 14200-18150 and 18200-23150 Pa.s., respectively. The highest viscosity value (21150 Pa.s.) was observed in oat boza samples without sugar inoculated with yoghurt culture, however the lowest viscosity value (13900 Pa.s.) was determined in the oat boza prepared with 5% Pd inoculation with sugar addition. The fact that the adding sugar promoted growing of microorganisms during fermentation could be considered as the reason for the decrease in viscosity in the oat boza with sugar (Peyer, Zannini, & Arendt, 2016). While inoculation rates in boza samples increased, viscosity values of samples decreased significantly ($P< 0.01$). The use of gelatinize starch and derivatives in the medium by microorganisms as a nutrients could be shown as a reason for decreasing the viscosity values. The present results are consistent with the findings of Lambo, Öste, and Nyman (2005) who determined lower viscosity values in oat concentrate fermented by lactobacilli than non-fermented substrates.

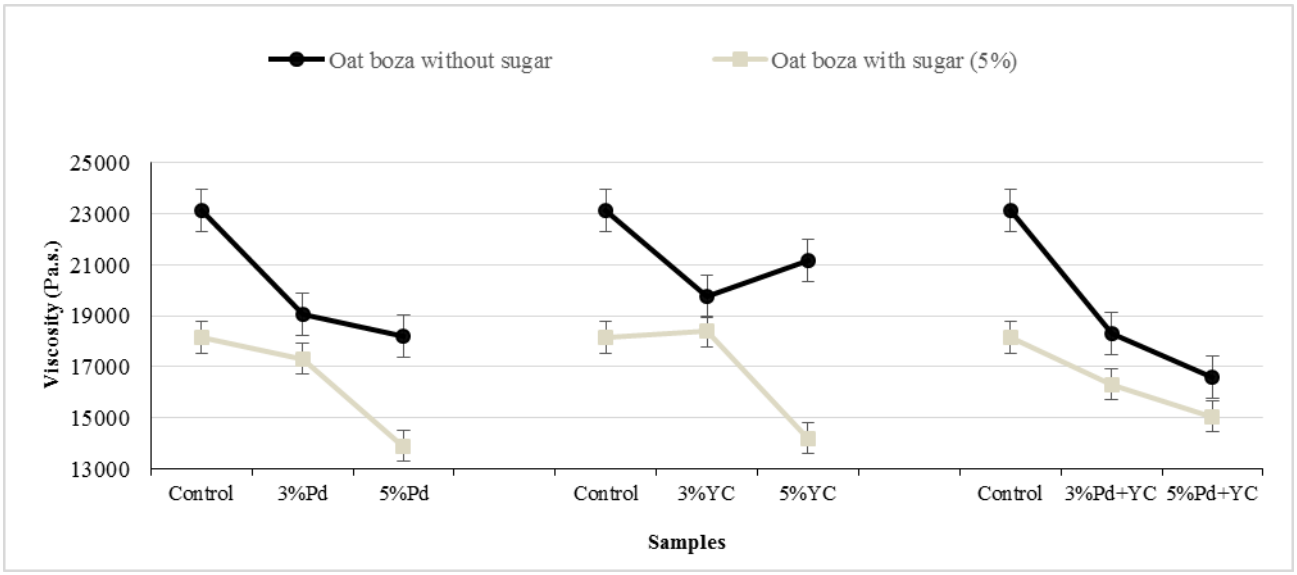

Figure 3
Viscosity values of oat boza samples

Pd: boza inoculated with *Pediococcus domnasus 2.6*; YC: boza inoculated with yoghurt culture (*S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*1:1); Pd+YC: boza inoculated with *Pediococcus domnasus 2.6* + yoghurt culture (1:1); 3-5%: inoculation rates of starter cultures

3.7. Sensory evaluation of oat boza samples

Sensory evaluation of oat boza that are presented in Figure 4. Sensory analysis was performed in oat boza samples that were 3% YC, 3% Pd and 3% Pd+YC without sugar addition before fermentation. Oat boza samples with adding sugar before fermentation presented bitter flavor and acid taste, so that these oat boza samples did not evaluated in terms of sensory characteristics. 3% Pd samples had the highest score in terms of mouthfeel ($p<0.05$). The odor scores observed for all boza samples were quite similar. However, the difference between color scores of 3% YC and 3% Pd+YC samples was statistically insignificant and their scores were higher than 3% Pd. The highest overall acceptability score was determined in the 3% YC samples. According to sensory evaluation score, 3% YC samples without sugar was the most preferred yoghurt samples for consumers.

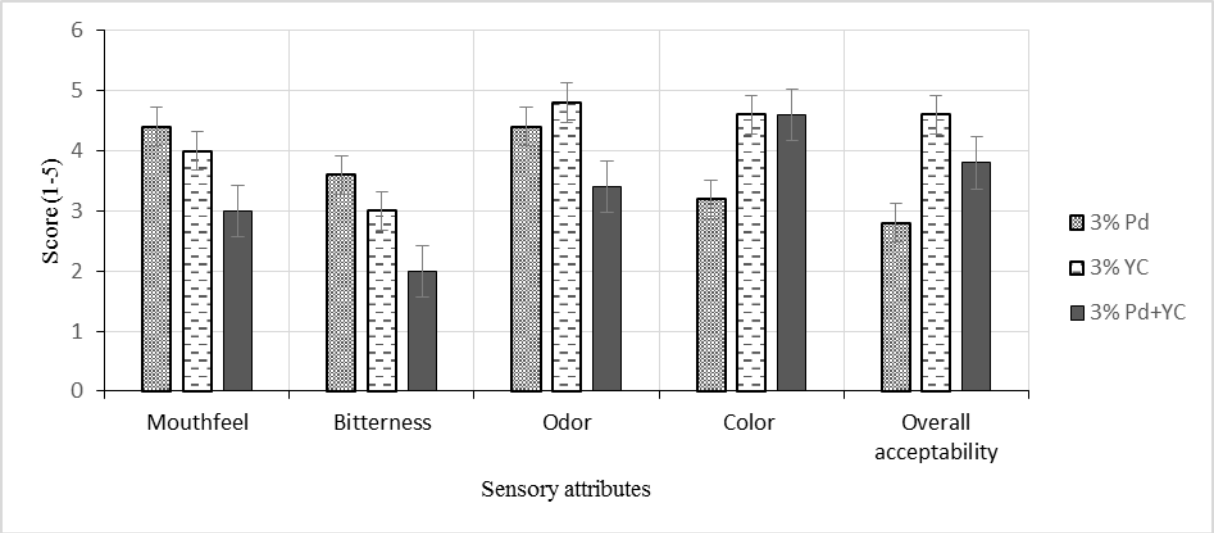

Figure 4
The sensory evaluation of oat boza samples

Pd: boza inoculated with *Pediococcus domnasus 2.6*; YC: boza inoculated with yoghurt culture (*S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*1:1); Pd+YC: boza inoculated with *Pediococcus domnasus 2.6* + yoghurt culture (1:1); 3-5%: inoculation rates of starter cultures

4. Conclusions

In this study, sugar addition into the boza before fermentation caused excessive alcohol production and bitter taste as a sensorial. *Pediococcus damnosus* 2.6, which is exopolysaccharide producer, obtained the highest mouthfeel score with smooth texture despite the low viscosity, but it did not give the desired palate in terms of overall acceptability. On the other hand, 3% YC samples gave optimal results and they got the highest overall acceptability scores by panelists. Raw oat has unpleasant odors and flavors, but fermentation improve the sensorial properties of oat product. In this context, fermentation of oat by lactic acid bacteria and yeast is thought to increase the consumption of oat as a human diet. Considering the scores of sensory and technological properties of produced oat boza samples, it can be proposed inoculation of yoghurt cultures at a rate of 3% without adding sugar for the best formulation of oat boza.

5. Acknowledgement

This experimental data was obtained from master dissertation and it was supported by Selcuk University Scientific Research Projects Coordinatorship (Grant number, 07201022).

6. References

AACC (1990). Approved Methods of the American Association of Cereal Chemists *8th Ed.* . St. Paul: MN.

Anonymous (1992). Boza Standardı *TS 9978* (pp. 6). Ankara: Türk Standartları Enstitüsü.

Anonymous (1998). Meyve ve Sebze Ürünleri - Etanol Muhtevası Tayini: TS 1594 Türk Standartları Enstitüsü, Ankara.

Anonymous (2005). *Merck Gıda Mikrobiyolojisi Uygulamaları* (A. K. Halkman Ed.). Başak Matbaacılık Ltd. Şti. Ankara.

Arendt EK, Zannini E (2013). Oats *Cereal Grains for the Food and Beverage Industries* (pp. 243-283). Cambridge, UK: Woodhead Publishing.

Aytekin S (2001). *Değişik hammaddelerden farklı oranlarda şeker katkısıyla üretilen bozaların kalite kriterleri üzerinde araştırmalar.* (Yüksek Lisans Tezi), Uludağ Üniversitesi Fen Bilimleri Enstitüsü, Bursa.

Bryngelsson S, Dimberg LH, Kamal-Eldin A (2002). Effects of Commercial Processing on Levels of Antioxidants in Oats (Avena sativa L.). *Journal of Agricultural and Food Chemistry, 50*(7): 1890-1896.

Gotcheva V, Pandiella SS, Angelov A, Roshkova Z, Webb C (2001). Monitoring the fermentation of the traditional Bulgarian beverage boza. *International Journal of Food Science & Technology, 36*(2): 129-134.

Hamad AM, Fields ML (1979). Evaluation of the protein quality and available lysine of germinated and fermented cereals. *Journal of Food Science, 44*(2): 456-459.

Hancioğlu Ö, Karapinar M (1997). Microflora of Boza, a traditional fermented Turkish beverage. *International Journal of Food Microbiology, 35*(3): 271-274.

Hayta M, Alpaslan M, Köse E (2001). The effect of fermentation on viscosity and protein solubility of Boza, a traditional cereal-based fermented

Turkish beverage. *European Food Research and Technology, 213*(4): 335-337.

Kedia G, Wang R, Patel H, Pandiella SS (2007). Use of mixed cultures for the fermentation of cereal-based substrates with potential probiotic properties. *Process Biochemistry, 42*(1): 65-70.

Kentel ZB (2001). *Bozanın raf ömrünün uzatılması üzerine çalışma.* (Yüksek Lisans Tezi), Ankara Üniversitesi, Fen Bilimleri Enstitüsü,Ankara.

Kirk RS, Sawyer R (1999). *Pearson's compostion and analysis of foods* (9th ed.). Addison-Wesley Longmam Inc.,Harlow, England.

Lambo A, Öste R, Nyman M (2005). Cereal Grains for the Food and Beverage Industries. *Food Chemistry, 89*(2): 283-293.

Lasztity R (1999). *The Chemistry of Cereal Proteins* (2nd ed.): CRC Press, Boca Raton, Florida.

Lorenzo CP, Emanuele Z, Elke KA (2016). Lactic acid bacteria as sensory biomodulators for fermented cereal-based beverages. *Trends in Food Science & Technology, 54*: 17-25.

Mårtensson O, Dueñas-Chasco M, Irastorza A, Öste R, Holst O (2003). Comparison of growth characteristics and exopolysaccharide formation of two lactic acid bacteria strains, Pediococcus damnosus 2.6 and Lactobacillus brevis G-77, in an oat-based, nondairy medium. *LWT - Food Science and Technology, 36*(3): 353-357.

Mårtensson O, Öste R, Holst O (2002). The effect of yoghurt culture on the survival of probiotic bacteria in oat-based, non-dairy products. *Food Research International, 35*(8): 775-784.

Minitab C (1991). Minitab reference manual (Release 7.1). State Coll., PA16801, USA.

Morcos SR, Hegazi SM, El-Damhougy ST (1973). *Fermented foods in common use in Egypt I. The nutritive value of kishk* (Vol. 24).

Odunfa SA (1985). Biochemical changes in fermenting African locust bean (Parkia biglobosa) during 'iru' fermentation. *International Journal of Food Science & Technology, 20*(3): 295-303.

Peyer LC, Zannini E, Arendt EK (2016). Lactic acid bacteria as sensory biomodulators for fermented cereal-based beverages. *Trends in Food Science & Technology, 54*: 17-25.

Pomeranz Y (1975). Proteins and amino acid of barley,oats and buckwheat. In M. Friedman (Ed.), *Protein nutritional quality of foods and feeds* (Vol. I, pp. 13-78). New York.

Rathore S, Salmerón I, Pandiella SS (2012). Production of potentially probiotic beverages using single and mixed cereal substrates fermented with lactic acid bacteria cultures. *Food Microbiology, 30*(1): 239-244.

Salmerón I, Thomas K, Pandiella SS (2015). Effect of potentially probiotic lactic acid bacteria on the physicochemical composition and acceptance of fermented cereal beverages. *Journal of Functional Foods, 15*: 106-115.

Skendi A, Biliaderis CG, Lazaridou A, Izydorczyk MS (2003). Structure and rheological properties of water soluble β-glucans from oat cultivars of Avena sativa and Avena bysantina. *Journal of Cereal Science, 38*(1): 15-31.

Skujins S (1998). Handbook for ICP - AES (Vartian-Vista). A Short Guide To Vista Series ICP - AES Operation. Variant Int. AG, Zug, version 1.0, Switzerland.

Topal S, Yazıcıoğlu T (1986). Boza mikroflorası üzerine bir araştırma. *Diyabet Yıllığı 1985 XIX. Diyabet Günleri Gençlik Ve Beslenme Kongresi Temel Matbaası, İstanbul*.

Üstün ŞN, Evren M (1998). Değişik hammaddelerden boza üretimi ve üretilen bozaların bileşimi. *Ondokuz Mayıs Üniversitesi Ziraat Fakültesi Dergisi, 13*(3): 95-105.

Wood BJB, Hodge MM (1985). Yeast-lactic acid bacteria interaction and their contribution of fermented foodstuffs *Microbiology of Fermented Foods.1* (pp. 263-293).

Zhu F (2017). Structures, properties, modifications, and uses of oat starch. *Food Chemistry, 229*: 329-340.

Selcuk Journal of Agriculture and Food Sciences

SJAFS

http://sjafs.selcuk.edu.tr/sjafs/index

(2020) 34 (2), 132-136

e-ISSN: 2458-8377

Research Article

DOI:10.15316/SJAFS.2020.206

The Estimation of Trypsin Inhibitor Activities in Soybean Meals Produced in Turkey*

Renan ŞEKER[1,*], Şakir Doğan TUNCER[2]

[1]Necmettin Erbakan University, Faculty of Education, Department of Science Education, Konya, Turkey
[2]Ankara University, Faculty of Veterinary Medicine Department of Animal nutrition. Retired lecturer, Ankara, Turkey

ARTICLE INFO

Article history:
Received date: 07.02.2020
Accepted date14.05.2020

Edited by:
İbrahim AYTEKİN; *Selçuk University, Turkey*

Reviewed by:
Yusuf CUFADAR; *Selçuk University, Turkey*
Halit İMİK; *Atatürk University, Turkey*

Keywords:
Soybean Meal,
Trypsin Inhibitor Activity
Urease Activity

ABSTRACT

This study was conducted in order to determine the nutrient contents and some anti-nutritional factors of of soybean and soybean meal produced in the Adana province of Turkey and imported from various countries. Totally 8 samples, consisting of 5 soybean meals, 1 full-fat soybean meal, 2 raw soybeans, were used in this study as research materials. The trypsin inhibitor activities (TIA) and urease activities (UA) were determined as anti-nutritional factors. Additionally, the degrees of changes in these factors were analyzed in the soybeans which were exposed to heat, humidity and pressure. Besides, the cresol red (CR) test was used to estimate the toasting degree of the samples and to compare with other analysis results. The average TIA, UA and CR values in soybean meal of the soya produced in Turkey used in the experiment, were found to be 2.59 mg/g, 0.05 mg N/g/minute and 4.39 mg/g, respectively. TIA, UA and CR values are determined for the imported soybean meal are 1.62 mg/g, 0.082 N/g/minute and 4.45 mg/g respectively. These values are in the same order for for full-fat soybean, 9.60 mg/g, 0.071 mg N/g/minute and 4.43 mg/g. TIA values of raw soybean (1) which were autoclaved for 35 minutes at 100°C, 110°C, 120°C and 130°C were found as 23.20, 10.27, 2.91, 0.53 and 0.20 mg/g respectively. UA values for same raw soybeans were detected as 8.40, 2.36, 0.23, 0.00 and 0.00 mg N/g/min and CR values were found as 2.42, 2.42, 4.22, 4.46 and 4.95 mg/g respectively. TIA values were 30.00, 27.12, 26.74, 26.29 and 22.74 mg/g in the raw Soybean sample that was subjected to dry heat (heated at 100°C, 110°C, 120°C, 130°C). In the raw soybean sample, where dry heat was applied, the UA values were started with no heat applied, respectively, 8.87, 8.87, 8.80, 8.75 and 4.62 mg N/g/min; CR values were found as 3.03, 3.07, 3.07, 3.22 and 3.28 mg/g in the same order.

1. Introduction

Animal products such as meat, milk and eggs are among the most essential foodstuffs for the world's rapidly growing population to ensure adequate and balanced nutrition. Optimizing animal production is directly related to sufficient and balanced nutrition of livestock. Maintaining the balance of amino acids in poultry and monogastric animals, which have to take exogenous amino acids from the feed, is very important in terms of nutrition. In our country, inadequate production of animal-origin feeds, which are rich in exogenous amino acids, causes problems in balancing rations of animals. Thus, the soybean, which contains a high level of protein and appropriate amino acid balance and the soybean meal, which is produced using soybean, became an alternative to animal-origin feed ingredients (Liu, 2004).

Although it is possible to eliminate the anti-nutrients during processing in the soybean plant, which plays an important role in human and animal nutrition, some of these substances may be passed on to the product in plants which are not properly processed. Analysis such as trypsin inhibitor activity (TIA), urease activity (UA) and dye binding test are the main methods used to determine the quality of these compounds in soybean (Hamerstrand, Black and Glover, 1981; Garlich, 1987; Holmes 1987).

When this study was conducted, soybean production in the world was calculated as 100 million tons and more than half of this amount was produced in USA (Aygar, 1987). Soybean production was reported to be 314 million tons in 2016 (Taşcı and Uçum, 2018). While USA ranked first in the world soybean production amount, it had 106.8 million tons of production in 2015/16 season. Brazil ranked second with 96.5 million

* Corresponding author email: *rseker@erbakan.edu.tr*
*This study was produced from Master's thesis of Renan ŞEKER

tons, Argentina ranked third with 56.8 million tons, China ranked fourth with 11.8 million tons and India ranked fifth with 6.9 million tons (Taşcı and Uçum, 2018). While soybean production in Turkey was 120 000 tons (Anonymous, 1988), 165 000 tons of soybean were grown in 381 804 decares in 2016 (Taşcı and Uçum, 2018).

Soybean has the richest protein among oilseed grains. Most of the proteins in the structure are composed of globulins (Morrison, 1961; Koshiyama et al., 1981). Soybean protein is similar to casein and is therefore also referred to as vegetable casein. Soybean meal has a very stable amino acid composition for monogastric animals. It is especially rich in lysine, which is insufficient in many feed ingredients. However, when only soybean protein is used as a protein source, methionine deficiency is frequently encountered (Becker and Nehring, 1965).

Like most oilseeds, raw soybean has anti-nutritional substances that negatively affects feed conversion and animal production. Trypsin inhibitors, urease, hemagglutinin and lipoxidase are the leading anti-nutritional factors found in the structure of soybean. Temperature, humidity, pressure and chemicals can eliminate the effects of these substances (Balloun, 1980).

Various tests are used to determine the quality of soybean and soybean meal. Measuring the amount of trypsin inhibitor activity is the most significant one (Hamerstrand et al., 1981). A high amount of TIA indicates insufficient heat and time applied in the process. Since the method is difficult and time consuming, urease activity estimations, which are easier to perform in laboratory, are used for this purpose (Garlich, 1987). In order to determine the height of the applied heat, dye binding test is used (Holmes, 1987).

This study was conducted to determine the nutrient contents and levels of anti-nutritional factors in soybean and soybean meal produced in the Adana province of Turkey and compare those with imported soybean and its meal. Therefore, it is expected that this study may contribute to our country's feed industry and animal husbandry.

2. Materials and Methods

2.1. Material

In this study, total samples consisting of 4 soybean meals produced in factories in Turkey, 1 soybean meal imported from the United States, 2 raw soybeans (raw soybean 1 and 2) and 1 full-fat soybean, were used as materials.

2.2. Processes applied to the material

Samples with high oil content were extracted with ether and the oil content was reduced to below 2%. Soybean meals and full-fat soybean were subjected to direct analysis, while raw soybean samples were subjected to heat treatments.

For this purpose, the first, soybean samples were taken into petri dishes in quantities of 50 grams. These petri dishes were numbered from 1 to 5. No treatment was applied to the sample in first Petri dish. 2nd petri dish was kept at 100°C, third at 110°C, fourth at 120°C and fifth at 130°C in dry and unpressurized environment for 35 minutes.

The second sets of soybean samples were taken into petri dishes as in the first one and treated with water vapor and pressure in the autoclave at the same temperatures and time. For this sample also, no treatment was applied to the sample contained in petri dish 1. The second petri dish was kept at 100°C (0.00 Atmosphere), third at 110°C (0.40 Atmosphere), fourth at 120°C (1.00 Atmosphere) and fifth was kept at 130°C (1.72 Atmosphere) in the autoclave for 35 minutes.

2.3. Chemical Analyses

For determination of nutrients in samples, standard wet chemistry analysis were applied to all samples according to AOAC (1980). For the determination of trypsin inhibitor amounts in the samples, method used by Kakade et al. (1974) was applied. Urease activity, cresol red (CR) test estimations and amounts of raw nutrients were found by methods reported by Naumann and Bassler (1985).

2.4. Statistical Analysis

The statistical analysis performed by SPSS version 22 software (SPSS for Windows, version 22.0; SPSS Inc., 247 Chicago, IL)..

3. Results and Discussion

Summary statistics of nutrient contents of the samples used in the study are presented in Table 1, trypsin inhibitory activity, urease activity and cresol red test results of intact samples are given in Table 2. The results of trypsin inhibitor activity, urease activity and cresol red analysis after dry heat application and autoclave retention are given in Tables 3 and 4, respectively.

Table 1
Nutrient contents of the samples used in the study, %

Sample	N		Dry Matter	Organic Matter	Crude Protein	Crude Fiber	Ether Extracts	Crude Ash	Nitrogen Free Extract
Soybean	2	Mean	94.60	88.40	35.8	4.70	21.50	6.20	26.50
		SD	1.53	2.21	1.06	0.19	0.08	0.68	3.54
		CV	1.62	2.50	2.97	4.07	0.37	10.91	13.36
Soybean Meal	4	Mean	92.9	85.40	43.30	6.90	1.20	7.40	34.40
		SD	0.85	1.49	2.30	0.60	1.00	0.68	1.99
		CV	0.92	1.74	5.32	8.75	81.30	9.14	5.79
Soybean Meal (Imported)	1		92.79	86.53	39.50	7.84	0.42	6.26	38.47
Full-fat Soybean	1		95.18	87.96	37.13	5.49	19.13	7.22	26.21

SD= Standard Deviation; CV= Cofficient of variance

Table 2
Trypsin inhibitor activities, Urease activities and Creosol Red Test results in soybean meals and full-fat soybean

Sample	N		Trypsin inhibitor activities, mg/g	Urease activities, mg N/g/minute	Creosol Red Test, mg/g
Soybean Meal	4	Mean	2.59	0.05	4.39
		SD	1.46	0.02	0.33
		CV	56.37	40.00	7.52
Soybean Meal (Imported)	1		1.62	0.082	4.45
Full-fat Soybean	1		9.60	0.071	4.43

SD= Standard Deviation; CV= Cofficient of variance

Table 3
Trypsin inhibitor activities, Urease activities and Creosol Red Test results of raw soybean sample (Soybean 1) autoclaved at different temperatures for 35 minutes

Sample	Trypsin inhibitor activities, mg/g	Urease activities, mg N/g/minute	Creosol Red Test, mg/g
Soybean 1	23.20	8.40	2.42
100 °C (0.00 Atmosphere)	10.27	2.36	2.42
110 °C (0.40 Atmosphere)	2.91	0.23	4.22
120 °C (1.00 Atmosphere)	0.53	0.00	4.46
130 °C (1.72 Atmosphere)	0.20	0.00	4.95

This study was planned to determine the nutrient contents and feeding quality of soybean meals obtained from different part of Turkey (4 samples) and USA (1 sample**)** in 1988. In this study different temperature, humidity and pressure were applied to the raw soybean samples in laboratory conditions. TIA, UA and CR tests were used as quality control criteria in soybean to determine optimum conditions.

The crude protein and ether extracts values of raw soybeans examined in the study are given in table 1. These values obtained from the analysis are in similar with the values reported in the other sources (Crampton and Harris, 1968; Doğan, and Akyıldız, 1985). When the same table is examined, it is seen that the average of 43.30% crude protein value of the soybean meal produced in Turkey. Although the protein values of soybean meal are acceptable within normal limits according to some sources (Crampton and Harris, 1968; Morrison, 1961), it is given as 43.80-50-40% in some sources (Becker and Nehring, 1965; Doğan, and Akyıldız, 1985) remained lower than the values. On the other hand, the meal imported to Turkey 39.50% of the protein present was too small proportion of the meal produced. Except for the values of these samples, all other nutrient amounts of all studied samples were found similar to the values reported in the literature (Becker and Nehring, 1965, Morrison, 1961).

Table 4
Trypsin inhibitor activities, Urease activities and Creosol Red Test results of raw soybean sample (Soybean 2) dry heated at different temperatures for 35 minutes

Sample	Trypsin inhibitor activities, mg/g	Urease activities, mg N/g/minute	Creosol Red Test, mg/g
Soybean 2	30.00	8.87	3.03
100 °C	27.12	8.87	3.07
110 °C	26.74	8.80	3.07
120 °C	26.29	8.75	3.22
130 °C	22.74	4.62	3.28

The average TIA, UA and CR values of the soybean meal produced in Turkey used in the experiment, were found to be 2.59 mg/g, 0.05 mg N/g/minute and 4.39 mg/g, respectively. TIA, UA and CR values are determined as for imported soybean meal are respectively 1.62 mg/g, 0.082 N/g/minute and 4.45 mg/g. These values are in the same order for for full-fat soybean, 9.60 mg/g, 0.071 mg N/g/minute and 4.43 mg/g (Table 2).

The TIA values of the soybean meal used in this study were found to be lower than the results of the TIA values of the samples used in another study conducted in our country (Yücelt, 1985). Similar results can be said in terms of UA values. Veltman et al. (1986) in 4 different soybean meal they added to the chick rations TIA values respectively; 6.5, 5.2, 4.4 and 2.9 mg/g: found the UA values in the same order as 0.19, 0.11, 0.06 and 0.03 mg N/g min.

TIA values in the products examined in the study are between 1-6 mg/g (Anonymous, 1978) reported as the availability limit for soybean products, except for 9.60 mg / g for full-fat soya. In addition, the results obtained were found below the maximum amounts given by Holmes (1987) and indicated as one tenth of the protein value. Accordingly, it can be said that enough heat is applied during the production of soybean meal, other than full-fat soya.

Urease activity in soybean and soybean meal is expressed in very different units in the literature. For this reason, it is not possible to compare the results of urease activity obtained with different methods in researches. As in this study, the normal use limit is reported between 0.05-0.50 mg N/g min in the references that evaluate the activity determination as mg N/g min (Schiller, 1964; Holmes, 1987) In the research the average value of soybean meal produced in Turkey is 0.05 mg N/g which is present as minutei is acceptable as an indication may be subjected to excessive heating process of some of these samples (Table 2).

The most important method used to check whether high temperature is applied in the production of soybean meal is the cresol red (CR) test. Compared to the results reported by Holmes (1987), according to the CR test results obtained (Table 2), it can be said that excess heat is applied to the soybean meals used in this study. However, according to another source (Schiller 1964) stating that the CR value can reach the upper limit of 6.5 mg / g, the CR test values obtained in this research can be accepted within normal limits. However, it is understood that the limit suggested by Schiller 1964 (Table 3) (Schiller 1964) was found to be experimentally considered in raw soybean with 130 ° C heat and 1.72 Atmosphere pressure (Table 3) (Schiller 1964).

Significant reductions in TIA and UA values of raw soybean were observed due to the increase in temperature and pressure in the autoclave (Table 3). In raw soybean, the TIA value of 23.20 mg/g decreased to 0.20 mg/g and UA value of 8.40 mg N/g min decreased to 0.00 mg N/g min when 130 °C heat and 1.72 Atmosphere pressure applied. In dry heat application (Table 4), the TIA value decreased from 30.00 mg/g to 22.74 mg/g with increasing temperature. In dry heat application, decrease in UA test results was not considerable. When raw soybean samples were heated at 100° C, 110° C, 120° C and 130° C under certain pressure in autoclave, the trypsin inhibitor was inactivated at rates of 55.73%, 87.46%, 97.72% and 99.14% respectively. In dry heat application at the same temperature, inactivation levels were realized at the rates of 9.60%, 10.87%, 12.37% and 24.20%. This is consistent with the results of the study, in which Scott, Sandholm, and Hockstets (Scott at al., 1976) indicated that trypsin inhibitors could not be sufficiently inactivated by dry heat application unless very high temperatures reached.

A decrease in TIA and UA values and an increase in CR test values were observed with increasing temperature (Table 3 and 4).

4. Conclusions

Finally, TIA and UA values in 5 soybean meals examined in this study were not too high to cause problems and this may be related to the high temperature applied during production. It can be said that as a result of high temperature application, the amount of usable protein and amino acids in the soybean meal will be significantly reduced and thus growth efficiency can be negatively affected in animals fed with such soybean meals.

5. References

Anonymous (1978). The estimation of trypsin inhibitor activity (TIA), In soy meals and soy products. *Method no R/1/4b. Unilever Research Laboratory.* Colworth

Anonymous (1988). *Tarım Orman Köy İşleri Bakanlığı, Koruma Kontrol Genel Müdürlüğü kayıtları.* Ankara.

AOAC (1980). Official Methods of Analysis. (13th Eds. Dr George Latimer, Jr. Ed.) *Association of Official Analytical Collaboration International.*

Aygar Ö, (1987). Soya Küspesi Açığı ithal edilerek kapatılacak. *Çiftlik Dergisi.* 46, 12:30-31

Balloun SL, (1980). ''Soybean meal poultry Nutrition '' The ovid Bell Press, Inc.; Fulton, Missouri.

Becker M, Und Nehring K (1965). Handbuch der Futttermittel''Zweiter Band. Verlag Paul Parey, Hamburg.

Crampton EW, Harris LE (1968). Applied Animal Nutrition. 2nd edition. W.H. Freeman and company San Francisco.

Doğan K, Akyıldız R. (1985). Soya:Üretimi, kalite kontrolü ve değerlendirilmesi. *Yem Sanayicileri Birliği yayınları*, Ankara.

Garlich JD (1987).Urease-trypsin inhibitor relationship in commercially produced soybean meals. *Poultry Sci*.66:suppl. 1.103.abs.

Hamerstrand GE, Black LT, Glover JD (1981). Trypsin inhibitor in soy products: Modification of the standard analytical procedure. *Cereal Chem.* 58(1): 42-45.

Holmes B (1987). Quality control of raw material and finished product in: '' full fat Soya *A Regional Conference* 14-15, April, 1987,Milan Italy.

Kakade ML, Rackis JJ, Mc Ghee JE, Puski G (1974). Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. *Cereal Chem.* 46:518-526

Koshiyama l, Kikuchi M, Harada K, Fukushima D (1981). 2S Globulins of soybeans seeds. 1.İsolation and characterization of proteın components. *J. Agric. Food Chem.* 28: 336-340.

Koshiyama l, Kikuchi M, Fukushima D (1981). 2S Globulins of soybeans seeds:2. Physicocchemical and biological properties of protease inhibitors in 2s globulins. *J. Agric Food Chem.* 29: 340-343.

Morrison FB (1961). ''Feeds and Feeding Abridged''. Claremont Ontario Canada the Morrison Publishing Company.

Liu K (2004). Soybeans as a Powerhouse of Nutrients and Phytochemicals and Edible Soybean Products in the Current Market. In Soybean as Functional Foods and Ingredients, K. Liu, (ed). pp.1-51, AOCS Press, Champaign, IL, USA

Naumann C, und Bassler R (1983). ''Die chemische Untersuchung von Futtermitteln. Methodenbuch. Bd. 3 mit Ergänzungslieferungen" 1983, 1988, 1993, 1997 2004 und 2006 VDLUFA. Darmstadt, Germany.

Schiller K (1964). Die wirkung von Dampferhitzen(Toasten) auf die biologische Eiweißwertigkeit von Sojaektraktionsschrot. *Zeitschrift für Tierphysiologie Tierernährung und Futtermittelkunde*. *1*9: 322-330..

Scott ML, Sandholm M, Hockstetler HW (1976). Effects of antitrypsin and hemagglutinins in soybeans and other feedstuffs upon feed digestions in chickens. *Proceeding Cornel Nutrition Conference.* p 22-25.

Taşcı R, Uçum İ (2018). Tarımsal Ekonomi ve Politika Geliştirme Enstitüsü (TEPGE) Soya 2017/2018 Durum Tahmin, Yayın no 304. Isbn: 978-605-2207-15-4

Veltman JR, Blaine JR, Hansen J, Tdtanksley JR, Sandra S, Linton DK (1986). Comparison of nutritive value of different commercial soybean meals. Utilization by chicks in practical type rations. *Poultry sci*. 64:2314-2320

Yücelt Ö (1985). Çukurova bölgesinde üretilen soya fasulyesi tane ve küspelerindeki üreaz aktivitesi ve tripsin inhibitör faktör düzeylerinin saptanması üzerine bir araştırma. Yüksek lisans tezi. Ç.Ü. Fen bilimleri Enstitüsü.

Selcuk Journal of Agriculture and Food Sciences

http://sjafs.selcuk.edu.tr/sjafs/index

Research Article

SJAFS
(2020) 34 (2), 137-140
e-ISSN: 2458-8377
DOI:10.15316/SJAFS.2020.207

Investigation of Insulin-Like Growth Factor Binding Protein 3 (IGFBP-3) Polymorphism in Anatolian Black and Holstein Friesian Cattle Breeds*

Marwan FADHIL[1,*], İbrahim AYTEKIN[1], Uğur ZÜLKADIR[1]

[1]Selçuk University, Faculty of Agriculture, Department of Animal Science, Konya, Turkey

ARTICLE INFO

Article history:
Received date: 28.03.2020
Accepted date: 16.05.2020

Edited by:
Duran YAVUZ; *Selçuk University, Turkey*

Reviewed by:
Yasemin GEDİK; *Ankara University, Turkey*
Taki KARSLI; *Akdeniz University, Turkey*

Keywords:
Anatolian Black
Holstein Friesian
IGFBP-3
PCR-RFLP
BsuRI
Polymorphism

ABSTRACT

Insulin-like growth factor binding protein-3 (IGFBP-3) gene is a structural gene which is associated with development and growth in livestock. The present study aimed to investigate the polymorphisms of IGFBP-3 gene in Holstein Friesian (HF) and Anatolian Black (AB) cattle breeds. *BsuRI* (GG↓CC) restriction enzyme was used to detect of IGFBP-3 gene polymorphism. Although Anatolian Black breed was monomorphic (AA genotype), three genotypes (AA, AB and BB) were detected in Holstein Friesian breed by digestion of PCR products with *BsuRI*. The A and B allele frequencies were 0.57 and 0.43, respectively, in Holstein Friesian breed, while A allele frequency was 1.00 in Anatolian Black breed. AA, AB and BB genotype frequencies were 0.32, 0.50 and 0.18, respectively in the Holstein Friesian breed. All three possible genotypes weare detected in Holstein Friesian breed. In the analysis made taking into account Hardy-Weingberg equilibrium, significant deviation was not observed in terms of genotype distributions (P>0.05). In other words, the Holstein Friesian cattle population was found in the Hardy-Weinberg equilibrium.

1. Introduction

Improvement of livestock has focused on the selective breeding of individuals with superior phenotypes. With the development of increasingly advanced statistical methods that maximize selection for genetic gain, this simple approach has been extremely successful in increasing the quantity of agricultural output (Williams, 2005). Milk production is a quantitative trait which is affected by many environmental factors and controlled by many genes (Zhang et al., 2006). Several candidate genes have been identified that affect the productivity of cattle (Fadhil and Zülkadir, 2017; Aytekin and Boztepe, 2013). One of these genes is IGFBP-3. IGFBP-3 is a family of proteins that are a fundamental part of the Insulin-like Growth Factors (IGFs) system (Sudhakar, 2009) and plays a key role in regulating the biological activities of IGFs (Zhang et al., 2006). The IGFBP-3 gene is mapped on chromosome 4 in bovine genome (Priyadi, 2017). The mRNA of IGFBP-3 gene is length 8.407 bp containing 4 non-coding introns and 5 coding exons (Othman, 2014). The polymorphim of IGFBP-3 gene was identified for the first time by Maciulla (1997). Zhang et al. (2006), reported that IGFBP-3 gene affects milk yield at 305 days and protein percentage in Chinese Holstein cattle breed. In addition, it has been reported that IGFBP-3 gene affects serum IgG levels (Choudhary et al. 2006; Choudhary et al. 2007). According to previous studies the IGFBP-3 gene can be used as candidate gene for milk and growth traits. Today, restriction enzyme polymorphisms are commonly used for different candidate genes in many livestock species such as cattle (Saleh et al. 2019; Karslı 2019), goat (Demir et al. 2020), sheep (Ali et al. 2009; Qureshi et al. 2014) and chicken (Karslı et al. 2017).

The aim of this study was to determine the Insulin-Like Growth Factor Binding Protein-3 (IGFBP-3) gene polymorphism by using *BsuRI* (*Hae*III) restriction enzyme in both Anatolian Black and Holstein Friesian cattle breeds.

2. Materials and Methods

* Corresponding author email: *mervan.bayraktar@gmail.com*

*Short communication

In this study, a total of 50 (AB) and 50 (HF) cattle breeds were used for IGFBP-3 gene. Disodium EDTA containing tubes were used to prevent coagulation of blood during collection of samples. Then, blood samples storage was carried out at - 20 ^{o}C until DNA extraction procedures. Blood samples were taken from the *Tail Vein* of animals. Genomic DNA was extracted from whole blood using the Quick Gene DNA whole blood kit S (DB-S) (KURABO, Japan). 651 bg length of IGFBP-3 gene region was amplified with forward (5′-CCAAGCGTGAGACAGAATAC-3′) and reverse (5′-AGGAGGGATAGGAGCAAGTT-3′) primers reported by Maciulla et al. (1997). The PCR was done in a reaction volume of 10 µL according with some modifications. The reaction consists of 5µL of 2X Dream *Taq* Green PCR Master Mix (Thermo Scientific, USA), 0.30µL primer each primer forward and reverse (10 pmol) (Macrogen, Turkey) and 3.4µL ddH2O which finally added to 1 µL genomic DNA. The cycling protocol followed with initial denaturation at 94℃ for 5 min followed by 35 cycles of denaturation at 94℃ for 1 min, annealing at 60℃ for 1 min, extension at 72℃ for 1 min with a final extension at 72℃ for 5 min. The PCR product of each sample (5 µL) and 100 bp DNA ladder (Vivantis, Malaysia) were loaded in 2% (w/v) agarose gels in 0.5X Tris-Borate-EDTA (TBE) buffer staining using ethidium bromide. The electrophoresis was carried out for 45 min at 100 V. The electrophoresis gel was examined on an UV transilluminator and bands were visualized and photographed. The PCR products of IGFBP-3 gene were cleaved by fast digest; amplified fragments were digested with *BsuRI* (Thermo Scientific, #FD0154) at 37°C. The reaction volume was 15 µL consisted of 5 µL PCR product, 8.5 µL ddH2O, 1 µL 10X buffer and 0.5 µL restriction enzyme. The polymorphism of the cleaved fragments recognition was carried out by %2 agarose gel electrophoresis then the digested PCR products was obviously envisioned under UV light and scored in a gel documentation system.

3. Results and Discussion

A total of three genotypes including AA (199 and 164 bp), BB (215 and 164 bp) and AB (215, 199 and 164) (Figure 1 and 2) were detected by digestion of 651 bp of IGFBP-3 gene region with *BsuRI* restriction enzyme. Additionally, 164 and 154 bp fragments were observed on agarose gel as a thick band for all genotypes. The allele and genotype frequencies of each breed are given in Table 1. The results showed differences between the two breeds where the Anatolian black cattle showed one genotype while the Holstein Friesian showed three genotypes. This means that the AB cattle breeds maintains its genetic structure compared to Holstein Friesian breeds. The reason may be that AB is not subject to migration, gene flow, admixture, mutation or selection. The genotype frequencies of IGFBP-3 gene in Holstein Friesian are agreement to Hardy-Weinberg equilibrium (P>0.05). Sun et al. (2002) investigated the relationship between IGFBP-3 gene polymorphism and the beef performance in Qinchuan cattle. The frequency of AA, AB and BB genotypes were found to be 0.70, 0.28 and 0.02 and the allele frequencies were 0.84 and 0.16 for A and B respectively. The result showed that eye muscle area of AA genotype was significantly higher than BB genotype (P<0.05) and beef fat content of AB and BB genotypes were significantly higher than AA. Choudhary et al., (2007) studied polymorphism of IGFBP-3 gene and its association with birth weight and body weight in Hariana, Holstein Friesian and their crossbreds. The frequency of AA, AB and BB genotypes was 0.65, 0.32 and 0.03 in crossbreds and 0.29, 0.65 and 0.06 in Holstein Friesian respectively. The allelic frequency of the A and B allele was 0.81 and 0.19 in crossbreds and 0.62 and 0.38 in Holstein Friesian respectively.

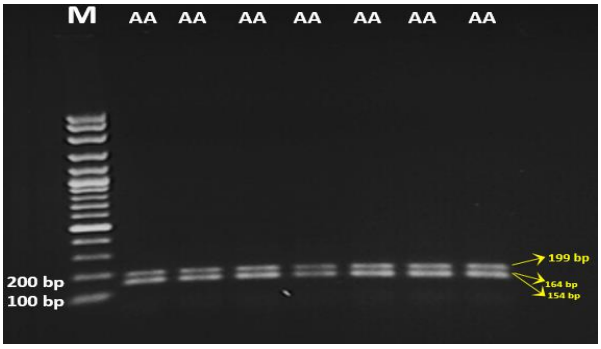

Figure 1
Agarose gel electrophoresis of digested products of IGFBP-3 gene with *BsuRI* restriction enzyme in Anatolian Black cattle; M: 100 bp Plus DNA Ladder (*Vivantis Technologies*), AA: 199 and 164 bp

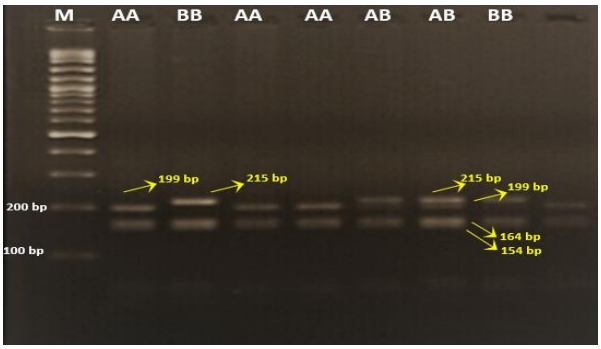

Figure 2
Agarose gel electrophoresis of digested products of IGFBP-3 gene with *BsuRI* restriction enzyme in Holstein Friesian cattle; M: 100 bp Plus DNA Ladder (Vivantis Technologies), AA: 199 and 164; BB: 215 and 164 bp; AB: 215, 199 and 164 bp

Table 1
Allele and genotype frequencies at IGFBP-3 gene in two cattle breeds

Breed	N	Genotype frequencies			Allele frequencies		$\chi 2$ and P
		AA	AB	BB	A	B	
AB	50	1.00	0.00	0.00	1.00	0.00	-
HF	50	0.32	0.48	0.20	0.56	0.44	0.03 (P>0.05)

AB: Anatolian Black; HF: Holstein Friesian; P>0.05: in Hardy-Weinberg equilibrium

Association analysis results showed that a significant effect (P<0.05) of genotypes on birth weight and body weight (weight at 12, 18 and 24 months of age) of the animals. Animals with AB genotype showed higher birth weight and body weight than the animals with AA genotype. Zhang et al., (2006) investigated association between IGFBP-3 gene polymorphisms and milk traits in Chinese Holstein. It was reported A and B allele frequencies for IGFBP-3 gene were 0.574 and 0.426, respectively in Chinese Holstein population. The genotypes of animals at IGFBP-3 locus significantly affected 305-day standard milk yield, protein percentage and somatic cell score. The B allele increased the milk yield, while the AB genotype had a higher protein percentage than AA and BB. Othman et al. (2015) determined the genetic polymorphism of IGFBP-3 gene in Egyptian cattle breeds. The restriction patterns of IGFBP-3/*Hae*III showed that forty-six examined animals were genotyped as AA, CC and AC with frequencies of 0.21, 0.21 and 0.56 respectively.

The previous studies mentioned above imply that IGFBP-3 polymorphism may be used for growth, development, body weight, milk yield, reproduction, immunity, metabolism, and energy balance in cattle. Hence, the present study provides baseline data for future genetic assessments of these populations. The results of the present study revealed that IGFBP-3 polymorphism may be used to improve meat properties and growth characteristics in Holstein Friesian in the future, while it cannot be used for Anatolian Black due to deficiency of diversity. This study is also important in determining the status of these two breeds raised in Turkey and to shed light on those who will work on this area in the future.

4. Acknowledgements

We thank BAP Coordinatorship of Selçuk University for supporting this study with no. 17401120 project. Authors have an equal contribution rate in the research. There is no conflict of interest.

5. References

Ali BA, El-Hanafy, AA, Salem, HH (2009). Genetic biodiversity studies on IGFBP-3 gene in Egyptian sheep breeds. *Biotechnology in Animal Husbandry* 25: 101-109.

Aytekin I, Boztepe S (2013). Associations of Pit-1 Gene Polymorphism with Milk Yield and Composition Traits in Brown Swiss Cattle. *J Anim Plant Sci* 23: 1281-1289.

Choudhary V, Kumar P, Saxena VK, Bhattacharya TK, Bhushan B, Sharma A, Ahmed KA (2006). Effect of Leptin and IGFBP-3 Gene Polymorphisms on Serum IgG Level of Cattle Calves. *Asian-Australasian Journal of Animal Sciences* 19: 1095-1099.

Choudhary V, Kumar P, Bhattacharya TK, Bhushan B, Sharma A, Shukla A (2007). DNA Polymorphism of Insulin-Like Growth Factor-Binding Protein-3 Gene and Its Association with Birth Weight and Body Weight in Cattle. *Journal of animal breeding and genetics* 124: 29-34.

Demir E, Karslı BA, Karslı T, Balcioğlu MS (2020). Determination of SacII and MboII polymorphisms in the Nerve Growth Factor (NGF) gene in four native Turkish goat populations. *Mediterranean Agricultural Sciences* 33: 145-148.

Fadhil M, Zülkadir U (2017). Molecular Characterization of MSTN Gene in Holstein Friesians and Brown Swiss Cattle Breeds. *Selçuk Tarım ve Gıda Bilimleri Dergisi* 31: 151-153.

Karslı T, Balcioğlu MS, Demir E, Hüseyin Göktuğ F, Aslan M, Aktan S, Kamanlı S, Karabağ K, Şahin E (2017). Ankara Tavukçuluk Araştırma Enstitüsü'nde Yetiştirilen Yumurtacı Saf Tavuk Hatlarında Yumurta Verimi ile İlişkili IGF-I ve NPY Aday Genlerindeki Polimorfizmlerin Belirlenmesi. *Türk - Tarım Gıda Bilim ve Teknoloji Dergisi* 5: 1051-1056.

Karslı T (2019). Türkiye'de Yetiştirilen Bazı Sığır Irklarında Asetil Koenzim A Dehidrogenaz Geni (g.2885C>A) Polimorfizminin ARMS-PCR Yöntemiyle Belirlenmesi. *Lalahan Hayvancılık Araştırma Enstitüsü Dergisi* 59: 25-29.

Maciulla JH, Zhang HM, DeNise SK (1997). A Novel Polymorphism in the Bovine Insulin-Like Growth Factor Binding Protein-3 (IGFBP3) Gene. *Animal genetics* 28: 375-375.

Othman EO, Sally SA, Sekena HA (2014). Single Nucleotide Polymorphism in Egyptian Cattle Insulin-Like Growth Factor Binding Protein-3 Gene. *Journal of Genetic Engineering and Biotechnology* 12: 143-147.

Priyadi DA, Panjono P, Bintara S, Hartatik T (2017). Genotype of Brahman and Brahman Cross Cattle based on SNP in Insulin-Like Growth Factor Binding Protein-3 (IGFBP-3) Gene Sequences. *Biodiversitas Journal of Biological Diversity* 18: 795-800.

Qureshi MI, Sabir J. SM, Mutawakil M. HZ, El Hanafy AA, Ashmaoui HE, Ramadan H, Ahmed MM

(2014). Review of modern strategies to enhance livestock genetic performance: From molecular markers to next-generation sequencing technologies in goats. *Journal of Food, Agriculture & Environment*, 12: 752-761.

Sudhakar A (2009). Molecular Polymorphism Analysis of Bmpr1b, Igfbp-3 and Pou1f1 Genes in Nilagiri and Mecheri Sheep (Doctoral dissertation, Tamil Nadu Veterinary and Animal Sciences University).

Saleh AA, Rashad AM, Hassanine NN, Sharaby MA, Zhao Y (2019). Comparative analysis of IGFBP-3 gene sequence in Egyptian sheep, cattle, and buffalo. *BMC research notes* 12: 623.

Sun WB Chen H, Lei XQ, Lei CZ, Zhang YH, Li RB, Zan LS, Hu SR (2003). Polymorphisms of insulin-like growth factor binding protein 3 gene and its associations with several carcass traits in Qinchuan cattle. *Hereditas* 25: 511-516.

Williams JL (2005). The Use of Marker-Assisted Selection in Animal Breeding and Biotechnology. *Revue Scientifique et Technique-Office International des Epizooties* 24: 379.

Zhang RF, Chen H, Lei CZ, Fang XT, Zhang YD, Hu SR, Su LH (2006). Association between PCR-RFLP polymorphisms of five gene loci and milk traits in Chinese Holstein. *Asian-Australasian Journal of Animal Sciences* 20: 166-171.

Selcuk Journal of Agriculture and Food Sciences

http://sjafs.selcuk.edu.tr/sjafs/index

Research Article

SJAFS
(2020) 34 (2), 141-147
e-ISSN: 2458-8377
DOI:10.15316/SJAFS.2020.208

Evaluation of Losses in Cherry Production: A Case Study of Izmir*

Meltem KANTAROĞLU[1], Nevin DEMİRBAŞ[2,*]

[1] Ege University, Institute of Science, Department of Agricultural Economics, İzmir, Turkey
[2] Ege University, Faculty of Agriculture, Department of Agricultural Economics, İzmir, Turkey

ARTICLE INFO

Article history:
Received date: 27.04.2020
Accepted date: 20.05.2020

Edited by:
Zuhal KARAKAYACI; *Selçuk University, Turkey*

Reviewed by:
Ahmet KUBAŞ; *Tekirdağ Namık Kemal University, Turkey*
Özge Can NİYAZ; *Çanakkale Onsekiz Mart University, Turkey*

Keywords:
Cherry
Fruit / Product Losses
Kemalpaşa
İzmir

ABSTRACT

The purpose of this research is to evaluate the causes of losses in cherry orchards. The research was carried out in Kemalpaşa district of İzmir province, which was an important cherry production center. An approach has been followed to evaluate the losses in cherry production both technically and economically. For this purpose, face-to-face surveys were conducted with 102 cherry producers and the data of the research were obtained from these surveys. The 5-point Likert scale was used in determining the participation of the producers in the judgments about losses in cherry production. The reliability of the data obtained with the Likert scale was tested with the Cronbach Alpha coefficient. Kruskal-Wallis test was used to determine whether there is a difference between the groups for continuous variables that do not have normal distribution. For discrete variables, whether there is a statistical difference between the characteristics of producers and enterprises and losses was determined by Chi-Square Analysis (Independence Test).
According to the research results, producers' views on losses in cherry production focus on seasonal factors and incorrect cultural practices at different stages of production. On the other hand, the opinions received from the producers for the solution of the current problems were mostly focused on training studies and labor costs. Adequate and inefficient producer organization in the region also adversely affects the technical and economic aspects of production. It is anticipated that the technical and economic information flow required by the producers will increase with the activation of the organization and thus will create positive results on the losses.

1. Introduction

Quantity and/or quality losses in the supply chain of food produced for human nutrition are defined as food loss (FAO 2013). Food loss can occur from the farm to fork along the supply chain. The main stages in which food losses occur; pre-harvest and post-harvest applications in fruit orchards and storage, processing, distribution and final consumption stages (Gustavsson et al. 2011; Demirbaş et al. 2017; Demirbaş 2018). Losses occur at every stage of the supply chain for different reasons. In developed countries, food losses are mostly seen at the retail and consumption stages (Permanandh 2011; Prusky 2011; EB 2014). Over 95% of food losses in developing countries are unintentionally lost in the early stages of the food supply chain (FAO 2018). The food losses in Turkey usually occur in agricultural production stage (Tatlıdil et al. 2013).

One of the most important subsector of the agricultural sector is the fresh fruit and vegetable sector. Produced fresh fruits and vegetables are spoiled and discarded for various reasons until they reach the consumer and this situation changes according to the types and varieties. In general, it is stated that 4-12% of the losses in fresh fruits and vegetables are in production, 2-8% of the products are transferred to the market and wholesaler, 1-5% are in the consumption phase (Tatlıdil et al. 2013; Ünlü 2015). These losses occurring at different stages along the supply chain can increase up to 50-60% depending on the product.

Losses of fruit in Turkey, is said to be up 12.7% of the total fruit production (Ozturk et al. 2012). In developed countries, there are studies stating that these losses are around 5% (Kader 2005). The main causes of losses in fruit production; misapplications during production, deficiencies and mistakes in the fight against diseases and pests, lack of proper techniques during harvesting and ignorance of necessary practices can be listed (Food Drink Europe 2013; Keding et al. 2013; Dijksma 2015; FAO 2018; Tarabay et al. 2018; TR Ministry of Foreign Affairs 2019). For example, inade-

* Corresponding author email: *nevin.demirbas@ege.edu.tr*
*This article was derived from the first author's Master Thesis

quate water and food management can lead to decreased production quality and high losses. Adverse weather conditions such as heavy rain can also result in high levels of illness (Thompson 2007). In addition, fruits can remain in the tree because they do not meet the quality standards (shape, size, weight) set by processors, retailers or the target market (Stuart 2009).

Cherry is a type of stone fruit that has a considerable sales potential and superior aroma in the world due to its ability to grow in various ecologies. Cherry is more susceptible to physiological and pathological disorders during storage and shelf life, and its storage life is shorter than soft seed fruit species (Çetin 2010; Hasdemir 2011). Physiological and pathological disorders cause post-harvest quality losses. Cracking and pitting, wrinkling and blackening of stalk color are the most important physiological disorders in cherry. Physiological and pathological disorders cause post-harvest quality losses. Cracking and pitting, wrinkling and blackening of stalk color are the most important physiological disorders in cherry (Mitcham et al. 2006). Stalk darkening is a big negativity for the producer in terms of marketing (Schick et al. 2000).

One of the main problems of cherry growing is that regular yields cannot be obtained from the trees every year. Fruit set and flowering are the most important problems. Fertilization is another problem. Another factor affecting the low yield is climate conditions (Lang 2019). The warm winter months cause the flowering time to be delayed, the flowering period to be prolonged and the flowering to be irregular in cherry trees that cannot realize winter rest (Engin and Akçal 2013). In addition, the pollination process may fail due to bad weather or cause color fading due to nutritional deficiencies (Creamer and Johnson 2018). Another important factor in terms of losses in cherry is marketing strategies. For cherry, like other fruit types, harvest time is extremely important in terms of storage and marketing (Crisosto et al. 2003).

The aim of this research is to evaluate the losses in cherry production with the information obtained from the producers. Therefore, this research was held in Kemalpaşa district which is one of Turkey's most important production centers.

2. Materials and Methods

2.1. Methods of sampling and data collection

All of the cherry producers included in the Farmer Registration System (FRS) of Kemalpaşa district of İzmir constituted the main population of the research. In the preliminary study, it was determined that the number of cherry producers registered in FRS was 2190 as of 2019 (Ministry of Agriculture and Forestry, 2019). Proportional sampling formula was used to determine the number of producers to be surveyed (Güneş and Arıkan 1988; Newbold 1995).

$$n = \frac{Np(1-p)}{(N-1)\sigma_{px}^{2} + p(1-p)}$$

In the formula;

n = Sample size

N = Total number of producers (2190)

p = Proportion of producers who have lost production

σpx2 = Variance of the ratio

In order to reach the maximum sample size, the proportion of producers who lost production phase was taken as p: 0.50 and (1-p): 0.50. With this approach, sample size was determined as 102 with 95% significance level and 9.5% margin of error.

The research area consists of the Merkez, Ören, Bağyurdu, Yiğitler and Armutlu villages of Kemalpaşa district where cherry production is intense. Information was also obtained from Kemalpaşa Agriculture and Forestry District Directorate and Chamber of Agriculture for the selection of the villages. The number of producers interviewed in each determined village was found by considering the contribution of the villages to the total number of producers. The number of producers in the research area is 50.41% of the total number of cherry producers in Kemalpaşa. The number of questionnaires per village was determined by converting them into integers (Table 1).

Table 1
Number of producers surveyed by villages (2019)

Villages	Number of Producers	Proportional Contribution of Villages (%)	Number of producers surveyed
Bağyurdu	294	27	28
Ören	265	24	25
Armutlu	251	23	23
Merkez	170	15	15
Yiğitler	124	11	11
Total	1.104	100	102

2.2. Methods of data analysis

In the research, the 5-point Likert attitude scale was used to compile the opinions of the producers about cherry production and the losses that occurred (Tavşancıl 2014). The scale is based on the principle of determining the positive, negative or neutral participation status of the participants in each of the expressions in the item set created to measure a certain structure (Bora and Altunışık 2016). Weighted average method was used to determine the order of importance of the statements directed to the producers (Kalaycı 2008). The Cronbach Alpha coefficient was calculated to test the reliability of the data obtained with the Likert scale. Reliability Analysis, it is an indicator of how reliable any measurement tool measures the feature (Tavşancıl 2014). The coefficient (Cronbach) that takes a value between 0 and 1 is called the Alpha coefficient (Cronbach 1951; Kalaycı 2008). Cronbach Alfa is expressed as follows:

0 <R2 <0.40 unreliable,

0.40 <R2 <0.60 low reliability,

0.60 <R2 <0.80 very reliable,

0.80 <R2 <1.00 high reliability.

In this study, Cronbach Alpha coefficient was calculated as (0.875). The result is an indication that the scale is highly reliable.

Since the variables did not show normal distribution, the Kruskal-Wallis test was used to determine whether there are differences between groups for continuous variables. Whether there is a relationship between the groups of variables for discrete variables was demonstrated with the Chi-square (Independence) test (Newbold 1995; Kalaycı 2008).

3. Results and Discussion

3.1 Demographic characteristics of producers

The average age of the producers is 49.45 years, and the training period is 8.45 years. There is no statistically difference between the group averages for both variables. The average experience of the producers in the agricultural sector is 20.63 years, and the duration of experience in cherry production is 18.92 years. There is statistically difference between the group means ($p < 0.05$). When the distribution of the general characteristics of the producers by the enterprise groups, it was found that the experience in the agricultural sector and the experience in cherry production were highest in the fourth group (Table 2).

Table 2
General characteristics of cherry producers in terms of enterprise size (year)

Enterprise Size (da)	Number of Enterprise	Variables Age	Education	Agricultural Experience*	Experience in cherry production*
Group 1: ≤10	28	48.85	8.85	16.89	16.10
Group 2: 11≤29	44	48.50	8.50	20.93	18.97
Group 3: 30≤49	17	51.44	7.38	22.38	20.88
Group 4: ≥50	13	51.33	8.91	25.66	22.33
Overall Average		49.45	8.45	20.63	18.92
Total	102				
P Value		-	-	0.041	0.049

*According to Kruskal Wallis test, the difference between the groups is significant for $p < 0.05$.

3.2 Participation of producers to agricultural organizations

Increasing the organizational awareness and level of the producers, determining the appropriate agricultural policies, making the production planned, supporting the enterprises financially and technically and increasing the bargaining power of the producers are extremely important (Karlık 2010). Although the level of organization of the producers within the scope of the research is low, it is determined that they are generally registered to more than one agricultural organization. The agricultural institutions that the producers are registered with are the Chamber of Agriculture (36.51%), the Agricultural Sales Cooperative (23.81%), the Agricultural Credit Cooperative (20.11%), the Irrigation Cooperative (15.87%) and the Agricultural Development Cooperative (3.70%) (Table 3).

Table 3
Agricultural organizations in which producers are registered

Agricultural organizations	Number	%
Chamber of Agriculture	69	36.51
Agricultural Sales Cooperative	45	23.81
Agricultural Credit Cooperative	38	20.11
Irrigation Cooperative	30	15.87
Agricultural Development Cooperative	7	3.70
Total	189*	100.00

*Since it is a member of more than one organization, the total is different.

3.3. Loss rates in the enterprises

In the questionnaires conducted with the producers, it was first asked whether there was any loss in the production of cherries. It was determined that more than 3/4 of the producers (77.50%) experienced different rates of loss in production. The proportion of those who have almost no losses in cherry production is around 23%. Then, the producers were asked at which stage of agricultural production and at what rate they lost. The answers given are grouped according to their loss rates. It was determined that 86.49% of the producers in the first group (≤ 5%) experienced cherry losses at the most production stage. In general, it was determined that 63.25% of the producers experienced the loss of cherries at the highest production stage (Table 4)

Table 4
Loss levels in the enterprises by production stages

Lost Rate Groups (%)	Preharvest		Harvest		Transport and Storage (on farm)		Processing and Packaging (on farm)	
	Number	%	Number	%	Number	%	Number	%
Group 1: ≤5	64	86.49	28	84.85	2	40.00	3	60.00
Group 2: 6-15	4	5.40	2	6.06	2	40.00	1	20.00
Group 3:16-21	5	6.75	3	9.09	1	20.00	1	20.00
General*	73	63.25	33	28.21	5	4.27	5	4.27

*Multiple answers were received.

3.4 Production technique preferences of the producers

Fruit production in Turkey, it can be done with different production techniques such as conventional agriculture, ecological agriculture and Good Agricultural Practices (GAP). The application of certified production techniques such as ecological agriculture and GAP is important for possible product losses. As a matter of fact, according to the Chi_Square analysis conducted for this purpose, a statistically significant difference was found between the groups regarding loss rates and making certified production (Table 5). At all levels of loss, it is observed that those who make conventional production experience more losses than those who prefer the production technique that requires certification. Certified production in only 22.55% of the examined enterprises is an important finding in terms of losses.

Table 5
Comparison of certified production preference and the loss rates in the enterprises surveyed

Certified Production	Lost Rate Groups %			Chi-Square	
	0 ≤ 5	6-15	16-20	Value	p*
Yes	17	2	3	3.151	0.037
No	52	3	2		

*Significant for p<0.05

3.5 Comparison of the production amounts and the loss rates

The average age of cherry orchards is 19.81 years. As a result of the Chi-Square analysis conducted to test the assumption that the amount of cherry production may affect losses, a statistically significant difference was found between the cherry production amounts and the loss rate groups (Table 6). The most frequent loss rate is 0≤5%.

Table 6
Comparison of the cherry production amounts and the loss rates

Cherry production amounts (ton)	Lost Rate Groups (%)			Chi-Square	
Groups	≤ 5	6- 15	16 - 20	Value	p*
Group 1: 20 ≥	24	1	1		
Group 2: 21-29	39	3	2	3.58	0.048
Group 3: 30 ≤	6	1	2		

*Significant for p<0.05

3.6 Causes of the product losses

Cherry losses mostly occur in the period preharvest in the studied enterprises. Producers have stated seasonal factors (43.10%) as the most important reason for losses in cherry production. This is followed by diseases and pests (14.70%), worker errors (9.80%), inability to determine appropriate rootstocks (4.90%), loss of spilled fruit (2.90%) and lack of cold storage (2.00%). The rate of those who do not know the cause of the loss is at an important level of 22.50% (Table 7).

Table 7
Producers' opinions on the causes of loss

Reasons	Number	%
Seasonal Factors	44	43.10
Do Not Know the Cause of the Loss	23	22.50
Diseases and Pests	15	14.70
Worker Errors	10	9.80
Inability to Determine Appropriate Rootstocks	5	4.90
Loss of Spilled Fruit	3	2.90
Lack of Cold Storage	2	2.00
Total	102	100.00

3.7 Technical and economic risk sources in cherry production

The technical and economic risk sources faced by the producers in cherry production have been evaluated in detail as they directly affect the product losses. According to this, the most important technical risk sources were low yield (4.82) due to diseases and pests and frost (4.82)(Table8).

Table 8
Technical risk sources in cherry production

Technical risks	1	2	3	4	5	Likert Scale Mean*	Standard Deviation
Low Yield Due to Diseases and Pests	-	-	-	18	84	4.82	0.383
Frost	-	-	-	18	84	4.82	0.383
Selection of Spraying Time	1	1	-	37	63	4.57	0.652
Pruning Time	2	2	1	47	50	4.38	0.784
Soil Selection	2	4	-	47	49	4.34	0.838
Land Location	1	6	3	41	51	4.32	0.869
Insufficiency of Irrigation Water	1	14	1	34	52	4.20	1.062
Rainfall is More Than Needed	-	2	1	23	75	4.17	4.819

Table 8 (Continuation)
Technical risk sources in cherry production

Rootstock Selection	4	15	5	53	25	3.78	1.095
Less Precipitation	3	27	6	23	43	3.75	1.325
Variety Selection	5	28	6	40	23	3.47	1.248
Product Damage Due to Flood	26	25	3	10	38	3.09	1.695

*1 = Strongly Disagree, 2 = Disagree, 3 = Undecided, 4 = Agree, 5 = Strongly Agree

These results show that producers face problems in taking necessary technical measures against climatic conditions. Some of these problems arise from insufficient knowledge and experience, while others are due to economic factors. Indeed, the most important of the economic risk sources (Table 9) faced by the producers in production is the high and constantly increasing input costs (4.97). This is followed by the high irrigation water cost (4.92). In particular, problems in obtaining experienced and skilled workers for harvest constitute an important risk source for losses.

Table 9
Economic risk sources in cherry production

Economic risks	1	2	3	4	5	Likert Scale Mean *	Standard Deviation
High and Constantly Increasing Input Costs	-	-	-	3	99	4.97	0.169
High Irrigation Water Cost	-	-	-	8	94	4.92	0.270
Fluctuations in Product Prices	-	-	-	20	82	4.80	0.398
Fluctuations in Export	1	-	1	37	63	4.58	0.620
High Loan Interest Rates	3	13	2	19	65	4.27	1.170
Problems in Obtaining Experienced and Skilled Workers	14	21	-	18	49	3.66	1.563
Insufficient Family Workforce	16	28	1	14	43	3.39	1.611
Difficulties in Finding Markets	34	17	1	17	33	2.98	1.729

*1 = Strongly Disagree, 2 = Disagree, 3 = Undecided, 4 = Agree, 5 = Strongly Agree

4. Results and Discussion

It has been determined that the producers experienced the most product loss in the pre-harvest period. Most of the factors affecting cherry losses are related to the growing period and cultural measures. Adverse weather conditions such as heavy rain and frost, as a result of which high levels of disease and pest (insect) invasions are shown as the most important causes of loss.

The reasons for the loss in the harvest period may be due to the wrong harvesting techniques due to the workers, the lack of cold storage for summer fruits such as cherries in the region and the fact that the harvest time could not be determined correctly. The establishment of a net system in the gardens may be one of the most important solution suggestions for producers who experience quality and product losses due to seasonal factors (Doğan 2016; Creamer and Johnson 2018; Özdemir Çifçi and Demirbaş 2020). It is envisaged that the methods of pest control applied at the right time and effectively can prevent product losses due to diseases and pests. In order to prevent all these factors mentioned above and the loss of products that may occur as a result of these, producers should be encouraged to switch to certified production methods such as GAP. It is stated that the application of techniques such as GAP, Integrated Struggle with Pests and Integrated Product Growing in production will have a preventive and reducing effect on losses (Demirbaş 2019). Again, according to the results of the analysis, it has been determined that certified producers such as GAP, Organic agriculture experience less losses compared to conventional producers. Mandatory processes such as traceability and supervision can be directly effective in reducing the causes of loss. Training processes, which are an integral part of GAPs, also have a positive effect in terms of reducing losses (SKD 2018).

According to research findings, losses due to diseases and pests and seasonal factors are higher than losses due to lack of cold storage. The same results have been achieved in another study involving the same area of research (Bayraktar 2015). Producers can find solutions to these problems by getting help from Provincial and District Agricultural and Forestry Directorates regarding the technical problems in cherry production. The District Agriculture and Forestry Directorate can contribute to the awareness of the producers by organizing seminars or training meetings on cherry production and providing practical training in the gardens (Başkaya 2011).

It has been determined that the producer organization in the research region is not sufficient. Increasing agricultural production and obtaining quality products depend on the efficient organization of producers. It is stated by the producers that the agricultural chamber does not play an active role in the region. Another important problem is that the Cherry Producers Union, which was operating in the past in the region, does not exist today. With the re-establishment of Cherry Producers Union, technical and financial support will be provided to producers on issues such as production techniques, harvesting, storage and packaging.

5. References

Başkaya Z (2011). Türkiye'de kiraz tarımının coğrafi esasları. *Doğu Coğrafya Dergisi* 16(26):26-45.

Bayraktar ÖV (2015). İzmir Kemalpaşa yöresinde GlobalGAP uygulayan ve uygulamayan kiraz iş-

letmelerinin teknik ve ekonomik yönünün sürdürülebilir tarım açısından değerlendirilmesi. Doktora Tezi, Ege Üniversitesi Fen Bilimler Enstitüsü (Basılmamış), İzmir.

Bora SB, Altunışık R (2016). Pazarlama araştırmalarında Likert tipi ölçeklerin özelliklerinin cevaplama tarzları üzerindeki etkilerinin incelenmesi, *Bartın Üniversitesi İ.İ.B.F. Dergisi* 7(14): 577- 598.

Creamer N, Johnson LK (2018) Introducing Farm-Level Loss into the Food Waste Discussion. Huffington Post. 04.

Crisosto CH, Crisosto GM, Metheney P (2003). Consumer acceptance of "Brooks" and "Bing" cherries is mainly dependent on fruit SSC and visual skin color. *Postharvest Biology and Technology 28 (2003): 159- 167.*

Cronbach LJ (1951). Coefficent alpha and the internal structure of tests, *Psichometrika Journal,*16 (3): 297-334.

Çetin T (2010). Uluborlu ilçesinde kiraz tarımı, *Atatürk Üniversitesi Doğu Coğrafya Dergisi* 15(23):185-220.

Demirbaş N (2018). Dünya'da ve Türkiye'de gıda israfını önleme çalışmalarının değerlendirilmesi. *IBANESS Kongreler Serisi*, 21-22 Nisan, Plovdiv-Bulgaristan, s.521-526.

Demirbaş N (2019). İyi tarım uygulamaları ile meyve bahçelerinde ortaya çıkan üretim, hasat ve hasat sonrası kayıpları azaltılabilir mi? *IBANESS Kongreler Serisi*, Plovdiv- Bulgaristan, 20-21 Nisan, s. 289-296.

Demirbaş N, Niyaz ÖC, Apaydın YM (2017). An evaluation on problems within food supply chain in Turkey in terms of food losses and waste. *International Balkan and Near Eastern Social Sciences Conference Series*, March 4-5, Edirne-Turkey, pp.830-835.

Dijksma S (2015). No More Food to Waste, Global Action to Stop Food Losses and Food Waste, Netherlands.

Doğan B (2016). Kırsal kalkınma yatırımlarının desteklenmesi programı kapsamında kiraz işletmelerinin yatırımlardan yararlanma düzeyi ve işletmelerin yatırım eğilimlerinin belirlenmesi: Kemalpaşa ilçesi örneği. Yüksek Lisans Tezi, Ege Üniversitesi Fen Bilimleri Enstitüsü (Basılmamış), İzmir.

EB (2014). Dünya Bankası: Gıda fiyatlarında düşüş yaşanmakla beraber gıda kayıp ve israfı gıda arz güvenliğini tehdit ediyor. http://blog.ibp.gov.tr/ Erişim tarihi: 15 Şubat 2019.

Engin H, Akçal A (2013). Kiraz Yetiştiriciliği. Çanakkale 18 Mart Üniversitesi Ziraat Fakültesi Yayınları, 1.Baskı, Çanakkale, 133s.

FAO (2013). Report of the Expert Consultation Meeting on Food Losses and Waste Reduction in the Near East Region: Towards a Regional Comprehensive Strategy. Egypt, 33p.

FAO (2018). Save Food: Global: Global Initiative on Food Loss and Waste Reduction.http: www.fao.org/savefood/tr/. Erişim tarihi: 04 Ocak 2020.

Food Drink Europe (2013). Every Crumbs Counts Joint Food Wastage Declaration,http://everycrumbcounts.eu/uploads/static_pages_documents/Joint_Declaration/www.fao.org/savefood/tr/. Erişim tarihi: 04 Ocak 2020.

Gustavsson J, Cedeberg C, Sonesson U, Otterdijk RV, Meybeck A (2011). Global Food Losses and Food Waste: Extend, Causes and Prevention, FAO, Rome.

Güneş T, Arıkan R (1988). Tarım Ekonomisi İstatistiği, Ankara Üniversitesi Ziraat Fakültesi Yayınları. Yayın No: 1049, Ankara Üniversitesi Basımevi, Ankara.

Hasdemir M (2011). Kiraz yetiştiriciliğinde iyi tarım uygulamalarının benimsenmesini etkileyen faktörlerin analizi. Doktora Tezi, Ankara Üniversitesi Fen Bilimleri Enstitüsü (Basılmamış), Ankara.

Kader AA (2005). Increasing Food Availability by Reducing Postharvest Losses of Fresh Produce. http://ucce.ucdavis.edu/files/datastore/234-528.pdf (Erişim tarihi: 12 Aralık 2018).

Kalaycı Ö (2008). SPSS Uygulamalı Çok Değişkenli İstatistik Teknikleri, ISBN: 9799759091148, Ankara.

Karlık Aİ (2010). Türkiye'de ve Avrupa Birliğinde tarımsal örgütlenme modelleri. Yüksek Lisans Tezi, İnönü Üniversitesi Sosyal Bilimler Enstitüsü (Basılmamış), Malatya.

Keding GB, Schneider K, Jordan I (2013). Production and processing of foods as core aspects of nutrition-sensitive agriculture and sustainable diets. *Food Security* 5 (6): 825-846.

Lang GA (2019). Achieving sustainable cultivation of temperate zone tree fruits and berries Volume 2: Case studies, Burleigh Dodds Science Publishing, Cambridge, UK, (ISBN: 978 1 78676 212 2; www.bdspublishing.com).

Ministry of Agriculture and Forestry (2019). Çiftçi Kayıt Sistemi Verileri. Kemalpaşa İlçe Tarım ve Orman Müdürlüğü, Kemalpaşa-İzmir.

Mitcham EJ, Crisosto CH, Kader AA (2006). Recommendations for Maintaining Postharvest Quality-Sweet Cherry. http://postharvest,ucdavis.edu.Produce/ProduceFacts/Fruits/cherry.shtml (Erişim tarihi: 01 Şubat 2019).

Newbold P (1995). Statistics for Business and Economics. Prentice Hall International Editions.

Özdemir Çiftçi R, Demirbaş N (2020). Meyve ve sebze üretiminde ortaya çıkan kayıplar üzerinde etkili olan faktörler: İzmir İli örneği. *Mediterranean Agricultural Sciences 33(1): 85-91.*

Öztürk FP, Karamürsel D, Sarısı HC, Emre M, Kaçal E, Eren İ, Çalhan Ö (2012). 0900 kiraz çeşidinde

hasat ve hasat öncesi kayıpların belirlenmesi. *V. Bahçe Ürünlerinde Muhafaza ve Pazarlama Sempozyumu*, 18-21 Eylül, İzmir, s.171-179.

Permanandh J (2011). Factors affecting food security and contribution of modern Technologies in food sustainability. *Journal of the Science of Food and Agriculture* 91(15):2707-2714.

Prusky D (2011). Reduction of the incidence of postharvest quality losses, and future prospects. *Food Security* 3(4):463-474.

Schick JL, Toivonen P.A (2000). Optimizing Cherry Stem Quality. http://postharvest.tfrec.wsu.edu/pages/PC2000Q (Erişim tarihi: 1 Şubat 2019).

SKD (2018). Sürdürülebilir Tarım İlkeleri İyi Uygulamalar Rehberi. http://www.skdturkiye.org/files/yayin/surdurulebilir-tarim-ilkeleri-iyi-uygulamalar-rehberi_4.pdf (Erişim tarihi: 11 Aralık 2019).

Stuart T (2009). Waste: Uncovering the Global Food Scandal. https://www.telegraph.co.uk/culture/books/bookreviews/5842301/Waste-Uncovering-the-Global-Food-Scandal-by-Tristram-Stuart-review.html (Erişim tarihi : 7 Şubat 2019).

Tarabay PA, Chahine-Tsouvalakisb H, Tawkc ST, Nemera N, Habiba W (2018). Reduction of food losses in Lebanese apple through good harvesting and postharvest practices. *Annals of Agricultural Sciences* 63 (2018) 207–213.

Tatlıdil FF, Dellal İ, Bayramoğlu Z (2013). Food Losses and Waste in Turkey. FAO, 67p.

Tavşancıl E (2014). Tutumların Ölçülmesi ve SPSS ile Veri Analizi, Nobel Yayınları, Ankara, ISBN: 978-605-133-740-1.

Thompson AK (2007). Preharvest Factors on Postharvest Life. https://pub.epsilon.slu.se/9029/1/Ali_L_120906.pdf (Erişim tarihi: 09 Şubat 2019).

TR Ministry of Foreign Affairs (2019). Bazı Meyveler İçin Hasat Sonrası Kayıpların Ekonomik Analiz Raporu. http://www.postharvestproject.com/uploads/outputs/d3270dfd-9d15-4b65-a2b9-9d13567be7e9.PDF (Erişim tarihi: 12 Aralık 2019).

Ünlü M (2015). Yaş Meyve ve Sebzelerde Derim (Hasat) Sonrası Oluşan Kayıplar ve Çözüm Önerileri. Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü, Alata Bahçe Kültürleri Araştırma Enstitüsü, Mersin.

Selcuk Journal of Agriculture and Food Sciences SJAFS

http://sjafs.selcuk.edu.tr/sjafs/index

(2020) 34 (2), 148-153

e-ISSN: 2458-8377

DOI:10.15316/SJAFS.2020.209

Research Article

Influence of Modified Atmosphere Packaging on the Postharvest Quality and Chilling Injury of Tomato Harvested at Different Maturity Stages

Ferhan KÜÇÜKBASMACI SABIR[1], Emine Gül SELÇUK[2], Sevil UNAL[1]

[1]Selçuk University, Faculty of Agriculture, Department of Horticulture, Konya, Turkey
[2]Selçuk University, Graduate School of Natural and Applied Science, Konya, Turkey

ARTICLE INFO

Article history:
Received date: 19.05.2020
Accepted date: 08.06.2020

Edited by:
Zuhal KARAKAYACI; *Selçuk University, Turkey*

Reviewed by:
İhsan CANAN; *Bolu Abant İzzet Baysal University, Turkey*
Şebnem KUŞVURAN; *Çankırı Karatekin University, Turkey*

Keywords:
Lycopersicon esculentum Mill.,
Chilling injury
MAP
Maturity stages

ABSTRACT

The present study was performed to determine effects of modified atmosphere packaging (MAP) on postharvest quality and chilling injury alleviation of tomatoes during low temperature storage. Tomatoes were harvested at two different stages (breaker and pink stage) and packed with MAP (Xtend® bags MAP). Air stored fruits were considered as control. All samples were stored at 5°C with 90% RH for 21 days. Weight loss, firmness, surface color, lycopene, ascorbic acid, total phenol, total antioxidant activity and chilling injury were investigated with intervals of 7 days. At the end of the storage, MAP of either breaker or pink fruits reduced the weight loss, maintained firmness and exhibited less biochemical changes than the control fruit. Moreover, tomatoes stored in MAP have less chilling injury than control at breaker maturity stage. The onset of chilling injury was also delayed by packaging compared to nonpackaged fruits. The general qualities of MAP fruits were better than those of air stored fruits. Overall findings indicate that MAP can be an effective method for enhancing the phytochemical content, delaying the senescence and chilling injury of tomatoes at breaker or pink maturity stages during low temperature storage.

1. Introduction

Consumption of fruits and vegetables is increasing, as consumers are becoming more aware of numerous health benefits associated with fresh agricultural commodities. Tomatoes are an important vegetable crops and are among major contributor of carotenoids (especially lycopene), phenolics, vitamin C and small amounts of vitamin E in daily diets. Results from the epidemiological studies have shown that tomatoes and tomato products may have protective effect against various forms of cancer and cardiovascular diseases (Dumas et al. 2003; Toor and Savage 2005; Javanmardi and Kubota 2006). However, relatively short shelf life of tomatoes limits the long distance commercial transport and availability of this produce around the year. As stated by Benhabiles et al. (2013), postharvest losses of tomatoes may drastically reach up to 50% of total production in countries where harvest amount peaks in short period (Kibar and Sabir 2018).

Low temperature storage has been the main strategy applied in postharvest technology to prolong the shelf life of fruits and vegetables and maintain their quality. For commodities such as tomatoes, however, low temperatures induce chilling injury (El Ghaouth et al. 1992). Optimum storage temperature depends on the maturity of the tomato fruit at harvest. Immature and mature-green tomatoes are more sensitive to chilling temperatures than pink or red tomatoes are. If held for longer than 2 weeks below 10°C or for longer than 6–8 days at 5°C, they may develop chilling injury (CI). As a result of CI, fruits develop symptoms such as a rubbery texture, watery flesh, irregular ripening and pitting or browning. In the cases where its impact is very severe, it brings significant deterioration of the produce and therefore has a great negative effect on its final market value and leads to substantial economic losses (Stevens et al. 2008; Aghdam et al. 2016).

Modified atmosphere packaging (MAP) is a technique used for prolonging the cold storage period of fruit and vegetables. This technique can be described as an alteration in the composition of gases in and around fresh produce by respiration and transpiration in package. Composition of the gas inside the package is modified by respiration of fruits, decreasing O_2 level while CO_2 increases during storage (Thompson 2003; Sandhya 2010). Storage of tomatoes in MAP reduced weight loss and decay, maintained firmness and delayed ethylene production, color change and ripening

* Corresponding author email: *fkbasmaci@selcuk.edu.tr*

in cold storage (Akbudak et al. 2007; Tano et al. 2007; Sabir and Agar 2010). The development of a modified atmosphere and high relative humidity inside the package retards senescence and prevents chilling injury (Wang and Qi 1997; Singh and Rao 2005).Wang and Qi (1997) found that cucumber sealed in low density polyethylene (LDPE) have less CI symptoms than unsealed fruits and LDPE packaging delayed onset of chilling injury. MAP has been studied for efficacy in inhibiting CI and extending shelf life of perishable produces such as papaya (Singh and Rao 2005) and apricot (Ezzat 2018).

The objective of this work was to evaluate the effects of MAP on postharvest quality and alleviation of CI of tomatoes harvested at breaker and pink of maturity stages during low temperature storage (5°C).

2. Materials and Methods

Tomatoes (*Lycopersicon esculentum* Mill. cv. Hakan F_1) were harvested from greenhouse of Selcuk University in Konya-Turkey at breaker and pink ripening stages using the United States Department of Agriculture tomato ripeness color classification chart (USDA 1991). Fruits of tomatoes were transferred to laboratory of Department of Horticulture, Selcuk University. Afterwards, fruits were selected for unity and freedom from defects and blemishes, tomatoes were randomly divided into two equal lots at both maturity stages. First lot was evaluated as a control group unwrapped and stored in plastic box in air. The second lot was placed in modified atmosphere packaging (Xtend®) and then sealed (MAP).

All the samples were stored at 5°C and 85-90% RH for 21 days. At harvest and following 7, 14 and 21 days of cold storage, fruit were analyzed for weight loss, fruit skin color (hue angle), firmness, lycopene, ascorbic acid (AA), total phenols (TP), total antioxidant activity (TAA) and chilling injury (CI).

Tomatoes were weighted before storage and during storage at 7, 14 and 21 days. Results were expressed as percentage of weight loss relative to the initial weight.

Fruit skin color was determined on 8 individual fruits per treatment using a colorimeter (Minolta CR-400), with the Hunter scale (L, a*, b*). Two measurements were performed on fruit equatorial axes and results were calculated as hue angle using equations described by McGuire (1992).

Fruit firmness was measured using a digital penetrometer (fruit pressure tester, model 53205; TR, Forlì, Italy). After removing the epidermis at two equatorial sites, an 8 mm probe was used to measure the fruits firmness and results were expressed in Newton (N).

Ascorbic acid was determined as previously described by Pearson et al. (1970). Tomatoes were ground with a warring blender and 5 g sample was mixed with 0.4% oxalic acid and then filtered via filter paper. One milliliter filtrate and 9 mL 2,6-dichlorophenolindophenol sodium salt solution ($C_{12}H_6C_{l2}NO_2$-Na) was mixed and then read transmittance values at 520 nm in a spectrophotometer. Blank were prepared in the same way but using 1 ml filtrate and 9 ml distilled water. Results were expressed as mg 100 g^{-1}.

Lycopene content of tomatoes was performed as previously described by Sharma and Maguer (1996); Rao et al. (1998) with slight modifications. For lycopene analysis, pericarp tissue of tomatoes was blended with a warring blender for 1 min. One gram of homogeneous tissue and 50 mL hexane:ethanol:acetone (2:1:1, v/v) were shaken for 30 min. After shaking, 10 mL of distilled water were added and shaken for 5 min again. The solution was then placed in a separator funnel and, after phase separation, the upper phase was collected. The extract was filtered via Whatman No. 42 filter paper and lycopene concentration was determined by measuring the absorbance of the solution at 502 nm using a UV-visible spectrophotometer. Results were expressed as mg kg^{-1} fresh fruit weight.

Fruit extracts for antioxidant and phenol analyses were prepared using method described by Thaipong et al. (2006) with certain modifications. Five grams tomato tissue was homogenized in methanol using Ultra-Turrax homogenizer (IKA, T18 digital, Staufen, Germany) for 1 min. The homogenates were kept at 4°C for 14–16 h and then centrifuged at 8000 x *g* for 15 min at 5°C. The supernatants were recovered and stored at −20°C in dark color bottles until analysis.

Total phenols (TP) were determined according to the method of Singleton et al. (1999) with slight modifications. The 0.1 ml extract, 6.0 ml distilled water and 0.5 ml Folin-Ciocalteu's reagent were mixed and then were vortexed. The mixture were incubate 3 min and then 20% sodium carbonate solution supplemented and volume was made up 10 ml distilled water. The solution was incubated at 25°C for 2 h and the absorbance was measured at 760 nm. The content of total phenols was calculate basis of the calibration curve of gallic acid and was expressed as mg 100 g^{-1} FW.

Total antioxidant activity (TAA) was determined by the ferric reducing ability antioxidant power (FRAP) according to the procedure described by Benzie and Strain (1996). For this, 150 µL of extract and 2.85 mL of the FRAP reagent was incubated at 30°C for 30 min. After incubation, reaction mixture was measured at 593 nm on a UV-vis spectrophotometer. Standard curve was prepared using different concentrations of 1 mM trolox and expressed as µmol kg^{-1}.

Chilling injury (CI) index of fruits was evaluated at 20°C for 3 d after 7, 14 or 21 days in cold storage according to Aghdam et al. (2014). The fruits were returned to ambient temperature (20°C) for development of CI symptoms. The severity of the symptoms was assessed visually in a 4-stage scale: 0=no pitting; 1=pitting covering <25% of the fruit surface; 2= pitting covering <50%, but >25% of surface; 3= pitting covering <75%, but >50% of surface and 4= pitting covering >75% of surface. The average extent of cold damage

was expressed as a CI index, which was calculated using the following formula:

CI index (%)= Σ [(CI level) x (number of fruit at the CI level)]/ [(4 x total number of fruit)] x100

The experiment was a completely randomized design with three replications and each replication contained 8 fruits. For each maturity stage, data from analyzed parameters were subjected to analysis of variance separately. Sources of variation were treatment, storage time and their interaction. Means were compared by Student's t-test at $P \leq 0.05$, using JMP statistical software version 5.1 (SAS Institute Inc., Cary, NC, USA).

3. Results and Discussion

Weight loss increased during cold storage for both ripening stages, while the effect of modified atmosphere packaging on weight loss was found statistically significant (Table 1). At the end of the storage, weight loss of breaker tomatoes stored in MAP was 2.49%, while the value for the control fruit was 5.32%. Similarly, weight loss was 4.52% (control) and 1.92% (MAP) in pink tomatoes. MAP has been commonly used to extend the postharvest quality and life of the horticultural commodities. During the storage, the increase in CO_2 concentration inside MAP restricts the respiration of the produces and, by this way, the shelf life of the stored product prolongs. In the present study, effects of MAP on the postharvest quality and chilling injury of tomato harvested at different maturity stages have been investigated. The weight loss, known to be the major determinant of storage life and quality of fresh commodities, was significantly reduced by MAP for both maturity stages used in the study. In tomatoes, the acceptable range of weight loss during the storage ranges from 6 to 7% as indicated by Nunes (2008). Control tomatoes of both maturity stages were approaching to these values. However, weight loss values in the produces stored in MAP were far below the threshold values. The values recorded in the study were quite similar to those obtained in various vegetables such as cucumber, tomatoes, broccoli (Tano et al. 2007; Sabır and Ağar 2008; Jia et al. 2009; Sabir and Agar 2010).

Table 1
Changes in weight loss, firmness, color and chilling injury (CI) index of the tomatoes in response to MAP[x].

Treatment	Day	Breaker stage				Pink stage			
		Weight loss	Firmness	Color	CI index	Weight loss	Firmness	Color	CI index
Control	0	0.00 f	27.8 a	102.7 a	0.00 d	0.00 f	23.8 a	78.1	0.00
	7	1.71 d	24.8 c	88.4 c	0.49 cd	1.20 d	20.2 b	66.4	0.20
	14	3.68 b	21.8 d	72.8 e	1.16 b	2.83 b	18.3 c	59.6	0.28
	21	5.32 a	18.6 e	61.6 g	2.26 a	4.53 a	13.4 d	56.7	0.68
MAP	0	0.00 f	27.8 a	102.7 a	0.00 d	0.00 f	23.8 a	78.1	0.00
	7	0.71 e	26.6 ab	91.9 b	0.00 d	0.57 e	22.3 a	67.1	0.00
	14	1.42 d	25.4 bc	77.9 d	0.16 d	1.29 d	19.5 bc	60.3	0.10
	21	2.49 c	22.5 d	64.5 f	0.79 bc	1.92 c	18.2 c	56.4	0.31
$LSD_{0.05}$		0.58	1.49	1.50	0.51	0.39	1.55	N.S.	N.S.

[x] Means followed by different letters within a column are significantly different at $p \leq 0.05$ according to Student's t-test. N.S.: Nonsignificant

Changes in fruit skin hue angle values during storage were indicated in Table 1. At harvest, hue angle of fruit skin was 102.7° and 78.1° in breaker and pink fruits, respectively. During storage, the hue angle significantly decreased and this decline accelerated rapidly after 7 d in pink fruits which could be related to the ripening process of tomatoes. At the end of the storage, hue angle values ranged from 61.6° (control) to 64.5° (MAP) in breaker fruits while they were 56.7° (control) and 56.4° (MAP) in pink fruits. Color differences between the treatments were insignificant in pink stage.

Firmness of all fruits reduced during the storage period but breaker fruits demonstrated higher firmness when compared with the pink fruits (Table 1). The lowest firmness values were always determined in control while MAPs preserved the fruit firmness during storage. Initial firmness values of breaker and pink fruits were 27.8 N and 23.8 N, respectively. At breaker stage, firmness in control (18.6 N) and MAP (22.5 N) tomatoes were 33.22 and 19.31% lower than those of initial values, respectively. At pink stages, reduction of firmness values was more rapid compared with breaker. At the end of the storage, fruit firmness were 13.4 N (control) and 18.2 N (MAP) in pink fruits. In this ripening stage, fruit firmness was reduced by 43.65% in control and by 23.34% in MAP compared to initial value. Softening in cold storaged tomatoes was more progressive in accordance with the prolonged maturity. Firmness is one of the prime considerations determining the postharvest quality and shelf life of tomatoes (Nunes 2008; Kibar and Sabir 2018). Softening of the fruit texture can be induced through the partial degradation of cells by polygalactronase (PG) and the ripening process increases the activity of PG (Lee 2003). In this study, softening was prevented by MAPs as reported by Nakhasi et al. (1991).

Changes in ascorbic acid (AA) of tomatoes during the storage were presented in Table 2. AA contents of tomatoes increased during storage in both stages but the differences between the treatments were statistically insignificant. At the beginning of the storage, AA was 24.3 mg $100g^{-1}$ in breaker stages. At 21 days, AA content of breaker tomatoes in MAP was measured 34.8 mg $100g^{-1}$ while the content for the control fruit

was 38.7 mg $100g^{-1}$. AA content at harvest was 26.5 mg $100g^{-1}$ at pink tomatoes and this value increased along with the prolonged storage. At the end of the storage, AA content of pink tomatoes stored in MAPs and control were 38.6 mg $100g^{-1}$ and 40.7 mg $100g^{-1}$.

Tomato color is greatly correlated with lycopene content, and as the fruit develops from the mature green stage to the red stage, lycopene concentration increases significantly (Nunes 2008). Lycopene value of all fruits reduced during the storage period and pink fruits demonstrated higher value when compared with the breaker fruits. MAP prevented the lycopene accumulation during the storage period in both ripening stages (Table 2). Lycopene synthesis of MAPs breaker fruits began at 14 days, while accumulation of lycopene was initiated at 7 days for control. At pink stage, initial lycopene value was 10.2 mg kg^{-1} and underwent a remarkable increase with prolonged storage. At the end of the storage, lycopene contents of tomatoes were 39.9 mg kg^{-1} (control) and 29.8 mg kg^{-1} (MAP) in breaker fruits and were 53.1 mg kg^{-1} (control) and 41.9 mg kg^{-1} (MAP) in pink fruits. In marketing of the majority of horticultural produces, skin color is an effective factor on the preference of the consumer. The color in tomatoes is greatly correlated with lycopene content (Sabir and Agar 2011). As tomatoes ripen, the color changes from green in immature fruit to deep dark red in fully mature fruit, lycopene concentration increases significantly (Hobson and Grierson 1993). Decrease in hue angle and the increase in lycopene synthesis were markedly restricted by MAP during the prolonged maturity stage. This was most probably due to lower respiration level of the tomatoes in MAP in comparison to the control produces. Such findings were also recorded by Sabir and Agar (2010) who investigated a restriction effect of MAP on the lycopene synthesis during storage in tomatoes.

Changes in the TP of tomatoes during the storage are illustrated in Table 2. TP values of stored fruits linearly increased during the storage, whereas MAPs significantly inhibited rising in TP. Initial TP values of breaker and pink fruits were 48.2 and 68.7 mg $100g^{-1}$, respectively. At the end of the study, TP was maximum in control fruits for both stages (220.4 and 121.7 mg $100g^{-1}$ for breaker and pink tomatoes, respectively). At 21 d, TP values of breaker and pink fruits stored in MAPs were 151.0 and 101.5 mg $100g^{-1}$, respectively.

Initial TAA for the breaker and pink stage of tomatoes were 1.80 μmol kg^{-1} and 2.82 μmol kg^{-1}, respectively (Table 2). During prolonged storage times, TAA increased either in control or MAP. At 21 d, TAA of tomatoes were 4.18 μmol kg^{-1} (control) and 3.96 μmol kg^{-1} (MAP) in breaker fruits and were 4.64 μmol kg^{-1} (control) and 4.22 μmol kg^{-1} (MAP) in pink fruits.

CI index of stored tomatoes linearly increased during storage, whereas MAP significantly inhibited the increase in CI index especially breaker stages. Chilling injury symptoms started to in control fruits by 7th day of storage in both maturity stage (Table 1). The initial symptoms include small pitting and water soaked areas and which increased in size with the prolonged storage. The onset of CI symptoms in MAPs tomatoes were delayed. At the end of the storage, CI index of tomatoes were 2.26 (control) and 0.79 (MAP) in breaker fruits and were 0.68 (control) and 0.31 (MAP) in pink fruits. Chilling injury is one of the physiological disorders causing significant loses during the storage of horticultural produces. Storing the produces at their cold endurance levels would prevent the cold storage chilling injury. Studies revealed that MAP can decrease the chilling injury by maintaining the moisture content inside the package around the produces (Wang and Qi 1997; Gonzales-Aguilar et al. 2003). The results of the current investigation demonstrated that chilling injury in MAPs was considerably lower than those of control. In certain studies conducted on perishable produces, such as papaya and cucumber, the chilling injury levels were significantly prevented by MAP (Wang and Qi 1997; Gonzales-Aguilar et al. 2003).

4. Conclusions

Tomatoes are one of the most commonly consumed vegetables across the world. However, a greater part of the produced tomatoes are lost after harvest due to their perishable structure and improper postharvest handling processes. Low temperature storage is a prime strategy to maintain the postharvest life of tomatoes. However, certain factors one of which is chilling injury, remarkable restrict the storage potential of the cold sensitive produces like tomatoes. Nonetheless, MAP was proven to protect the commodities against the environmental constraints while it also restricts the respiration in varying levels. In the present study, overall investigations revealed that MAP at cold storage (5 °C) of the tomatoes harvested at two different maturity stages (breaker and pink maturity) was obviously effective on maintenance of postharvest quality, preventing the chilling injury and extending the storage duration of the tomatoes.

Table 2
Changes in lycopene, ascorbic acid (AA), total phenol (TP) and total antioxidant activity (TAA) of the tomatoes in response to MAP[x].

Treatment	Day	Breaker stage				Pink stage			
		Lycopene	AA	TP	TAA	Lycopene	AA	TP	TAA
Control	0	0.0 f	24.3	48.2 d	1.80 d	10.2 g	26.5	68.7 d	2.82 e
	7	12.3 e	34.0	96.9 c	3.91 b	21.6 e	34.7	95.3 c	4.85 a
	14	33.9 b	38.8	163.4 b	4.14 ab	36.7 c	34.6	109.3 b	3.31 d
	21	39.8 a	38.7	220.4 a	4.18 a	53.1 a	40.4	121.6 a	4.64 a
MAP	0	0.0 f	24.3	48.2 d	1.80 d	10.2 g	26.5	68.7 d	2.83 e
	7	0.4 f	29.4	53.7 d	3.01 c	17.1 f	32.9	70.4 d	3.72 c
	14	22.5 d	32.5	148.2 b	3.93 b	28.7 d	37.3	107.2 b	4.02 b
	21	29.8 c	34.8	151.0 b	3.96 ab	41.9 b	38.6	101.5 bc	4.22 b
$LSD_{0.05}$		3.86	N.S.	16.56	0.23	4.38	N.S.	11.03	0.29

[x] Means followed by different letters within a column are significantly different at $p \leq 0.05$ according to Student's t-test. N.S.: Nonsignificant

5. References

Aghdam MS, Asghari M, Babalar M, Sarcheshmeh MAA (2016). Impact of salicylic acid on postharvest physiology of fruits and vegetables. In: Eco-Friendly *Technology for Postharvest Produce Quality* pp. 243-268.

Aghdam MS, Asghari M, Khorsandi O, Mohayeji M (2014). Alleviation of postharvest chilling injury of tomato fruit by salicylic acid treatment. *Journal of Food Science and Technology* 51(10): 2815-2820.

Akbudak B, Akbudak N, Seniz V, Eris A (2007). Sequential treatments of hot water and modified atmosphere packaging in cherry tomatoes. *Journal of Food Quality* 30: 896-910.

Benhabiles MS, Tazdait D, Abdi N, Lounici H, Drouiche N, Goosen MFA, Mameri N (2013). Assessment of coating tomato fruit with shrimp shell chitosan and N,O-carboxymethyl chitosan on postharvest preservation. *Food Measure* 7: 66–74.

Benzie IFF, Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem*. 239: 70-76.

Dumas Y, Dadomo M, Di Lucca G, Grolier P (2003). Effects of Environmental Factors and Agricultural Techniques on Antioxidant Content of Tomatoes. *Journal of the Science of Food and Agriculture* 83:369–382.

El Ghaouth A, Ponnampalam R, Castaigne F, Arul J (1992). Chitosan coating to extend the storage life of tomatoes. *HortScience* 27 (9): 1016-1018.

Ezzat A (2018). Effect of modified atmosphere package on apricot fruit storability. *International Journal of Horticultural Science* 24 (3-4): 30-32.

Gonzalez-Aguilar GA, Buta JG, Wang CY (2003). Methyl jasmonate and modified atmosphere packaging (MAP) reduce decay and maintain postharvest quality of papaya 'Sunrise'. *Postharvest Biology and Technology* 28(3): 361-370.

Hobson G, Grierson D (1993). Tomato, in Biochemistry of fruit ripening, ed.by Seymour G, Taylor J and Tucker G). Chapman & Hall, London pp. 405–442.

Javanmardi J, Kubota C (2006). Variation of Lycopene, Antioxidant Activity, Total Soluble Solids and Weight Loss of Tomato during Postharvest Storage. *Postharvest Biology and Technology* 41:151-155.

Jia CG, Xu CJ, Wei J, Yuan J, Yuan GF, Wang BL, Wang QM (2009). Effect of modified atmosphere packaging on visual quality and glucosinolates of broccoli florets. *Food Chemistry* 114:28-37.

Kibar HF, Sabir FK (2018). Chitosan coating for extending postharvest quality of tomatoes (*Lycopersicon esculentum* Mill.) maintained at different storage temperatures. AIMS *Agriculture and Food* 3 (2): 97–108.

Lee YS (2003). Development of 1- methylcyclopropene package delivery system to control tomato ripening, Michigan State University, PhD thesis. 199 p., USA.

McGuire RG (1992). Reporting of objective color measurements. *HortScience* 27 (12): 1254-1255.

Nakhasi S, Schlimme D, Solomos T (1991). Storage potential of tomatoes harvested at the breaker stage using modified atmosphere packing. *Journal of Food Science* 56 (1): 55-59.

Nunes MCN (2008). Tomato. In: MCN Nunes (ed.), Color atlas of postharvest quality of fruits and vegetables. Blackwell Publishing, Iowa, USA pp. 239-252.

Pearson D, Surry J, Chuchill A (1970). The chemical analysis of food national college of food technology, University of Reading, Weybridge Surry T and A Churchill.

Rao AV, Waseem Z, Agarwal S (1998). Lycopene content of tomatoes and tomato products and their contribution to dietary lycopene. *Food Research International* 31: 737–741.

Sabir FK, Ağar İT (2008). Modifiye atmosfer muhafazanın Çengelköy hıyar çeşidinde meyve kalitesine etkileri. *Alatarım* 7 (1): 29-35.

Sabir FK, Agar İT (2010). Effects of modified atmosphere packaging on postharvest quality and storage of mature green and pink tomatoes. *Acta Horticulturae* 876: 201-208.

Sabir FK, Agar İT (2011). Influence of different concentrations of 1-methylcyclopropene on the quality of tomato harvested at different maturity stages. *Journal of the Science of Food and Agriculture* 91 (15): 2835-2843.

Sandhya (2010). Modified atmosphere packaging of fresh produce: Current status and future needs. LWT-*Food Science and Technology* 43 (3): 381-392.

Sharma SK, Maguer ML (1996). Kinetics of lycopene degradation in tomato pulp solids under different processing and storage conditions. *Food Research International* 29 (3-4): 309–315.

Singh SP, Rao DS (2005). Effect of modified atmosphere packaging (MAP) on the alleviation of chilling injury and dietary antioxidants levels in 'Solo' papaya during low temperature storage. *European Journal of Horticultural Science* 70 (5): 246-252.

Singleton VL, Orthofer R, Lamuela-Ravento RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299: 152–315.

Stevens R, Page D, Gouble B, Garchery C, Zamir D, Causse M (2008). Tomato fruit ascorbic acid content is linked with monodehydro ascorbate reductase activity and tolerance to chilling stress. *Plant, Cell & Environment* 31 (8): 1086-1096.

Tano K, Oule MK, Doyon G, Lencki RW, Arul J (2007). Comparative evaluation of the effect of storage temperature fluctuation on modified atmosphere packages of selected fruit and vegetables. *Postharvest Biology and Technology* 46:212-221.

Thaipong K, Boonprakob U, Crosby K, Cisneros ZL, Byrne DH (2006). Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis* 19: 669-675.

Thompson AK (2003). Fruit and vegetables harvesting, handling and storage. Blackwell Publishing Oxford, UK.

Toor RK, Savage GP (2006). Changes in major antioxidant components of tomatoes during postharvest storage. *Food Chemistry* 99: 724–727.

USDA, 1991. United States Standards for Grades of Fresh Tomatoes, United States Department of Agriculture, Agricultural Marketing Service, s.13.

Wang CY, Qi L (1997). Modified atmosphere packaging alleviates chilling injury in cucumbers. *Postharvest Biology and Technology* 10 (3): 195-20.

Selcuk Journal of Agriculture and Food Sciences

SJAFS

http://sjafs.selcuk.edu.tr/sjafs/index

(2020) 34 (2), 154-161

e-ISSN: 2458-8377

Research Article

DOI:10.15316/SJAFS.2020.210

Determination of Some Agronomic Traits and Their Correlation with Yield Components in Cowpea

Mustafa CERITOGLU[1,*], Murat ERMAN[1]

[1]Siirt University, Faculty of Agriculture, Department of Field Crops, Siirt, Turkey

ARTICLE INFO

Article history:
Received date: 29.05.2020
Accepted date: 26.06.2020

Edited by:
Ali KAHRAMAN; *Selçuk University, Turkey*

Reviewed by:
Mustafa ÖNDER; *SelçukUniversity, Turkey*
Ömer SÖZEN; *Kırşehir Ahi Evran University, Turkey*

Keywords:
Adaptation
Agronomic traits
Chlorophyll (SPAD-502)
Correlation
Heat stress
Vigna unguiculata

ABSTRACT

Cowpea is one of the vital grain legumes used for human and animal nutrition. Due to its rich protein content, cowpea supplies the protein requirement, especially in the African continent. Although cowpea is morphologically similar to common bean, it is a more tolerant species to heat and drought conditions. So, cowpea production has various advantages in semi-arid regions. The aim of this study was to determine some agronomic traits of used cowpea genotypes and evaluate their correlations with yield components. Plant height (PH), stem diameter (SD), leaf surface temperature (LST), total chlorophyll content (TCC), number of pods per plant (NP), number of seeds per plant (NS) and seed yield (SY) changed between 54.6-91.3 cm, 3.1-7.6 mm, 27.9-31.7 °C, 39-56.1%, 25.7-49.1, 307.5-684 and 646-2381 kg ha^{-}, respectively. It is noteworthy that Karagöz produced the maximum SY compared to the other varieties. Besides, it was determined that SY has positive significant correlation with NS (r=0.98**), NP (r=0.96**), TCC (r=0.93**), SD (r=0.91**) and PH (r=0.86**).

1. Introduction

Food legumes play a significant and diverse role in the farming systems and the diets of poor people around the World (Stoilova, Pereira, Sousa and Carnide, 2005). Cowpea, *Vigna unguiculata*, is considered an essential grain legume adapted to sub-Saharan Africa (SSA) where it supplies to the nutrition, health, and income of rural and suburban inhabitants (Boukar et al., 2015).

Cowpea is the most produced grain legume on the World after common bean and chickpea. Additionally, due to its high nutritional value, cowpea is one of the most important legumes for indigenous Africa (Agbicodo, Fatokun, Muranaka, Visser et al., 2009). Cowpea seed contains 24.8% protein, 1.9% lipid, 6.3% fiber, 63.6% carbohydrate, ash, riboflavin, carotene and vitamin B1 (Stancheva et al., 2016). Generally, the production and consumption of cowpea is high in the world, Although, it is lower than other grain legumes in the Turkish market. The cowpea production area was nearly 12.5 million hectares in the World while it was produced in 13.5 thousand hectares in Turkey in 2018 (Food and Agriculture Organization of the United Nations [FAO], 2018; Türkiye İstatistik Kurumu [TUIK], 2018). The main reasons for the low production of cowpea in Turkey are low demand, lack of export opportunities and low grain yield per unit area and farmers turning to more profitable crops (Sert and Ceyhan, 2012).

Some problems occur in common bean cultivation in regions such as Southeastern Anatolia, where temperatures are high and precipitation is very low during the summer season (Sozen and Karadavut, 2017). High temperature has negative effects on plant growth and grain yield in common bean (Kazai, Noulas, Khah and Vlachostergios, 2019). However, cowpea can be easily grown in drought and subtropic regions. Incorporating to hot and dry conditions and minimal soil selectivity are the main reasons for the spread of cowpea cultivation worldwide (Kahraman, 2017; Simion, 2018). Cowpea generally favors hot climate and shows optimum growth in regions in which average temperatures are nearly 25 °C in summer (Boukar et al., 2015).

* Corresponding author email: *ceritoglu@siirt.edu.tr*

Cowpea is an important rotation crop. It forms a symbiosis with appropriate *Rhizobium* bacteria similar to other legumes. Despite the important role acted by biological Nitrogen fixation, little is known of the symbiosis between cowpea varieties and native or recommended *Rhizobium* spp. (Freitas, Silva and Sampaio, 2012). So, it provides more productive soil for the next seasons' plant (Sanchez-Navarro, Zornoza, Faz and Fernandez, 2019). The values are vary depending on the genotypic variation and ecological conditions (Makoi, Chimphango and Dakora, 2009). Moreover, due to their taproot system, cowpea allows aeration in the soil for a more productive and fertile rhizosphere in rotation systems. Simunji, Munyinda, Lungu and Mweetwa (2019) suggested that cowpea provides to improve yield on the second crop in rotation.

This is of concern due to the ecological constraints of the Southeast Anatolian region, the number of crop species adapted to local harsh climate is limited. Region's agriculture and farmers need novel products that have high adaptation potential and added-value. Cover crops in the region are exposed to strong heat stress and almost no rain in summer seasons (Table 2). Many crops can not cope with these ecological conditions. In all but few genera are negatively affected due to high temperature and drought conditions. Therefore, researching new plants that can be grown in the region is a need. The aim of this study was to understand different agronomic variations of various cowpea genotypes and their correlation with yield and yield components in semi-arid and high temperature conditions.

2. Materials and Methods

2.1. Study Site and Location

The study was conducted in Siirt University in 2019. The city where the study was laid out is located on 41^o 57' east longitude and 37^o 55' north latitude, Southeastern Anatolia Region of Turkey. The altitude of the city is 880 m.

2.2. Plant Materials and Experimental Design

In the study, 3 cultivars (Karagöz-86, Karnıkara and Akkız) supplied from a commercial company and 3 local populations (L_1, L_2 and L_3) collected from the Tokat, Samsun and Manisa regions were adopted. The cultivars used in the study are the most growth cowpea varieties in Turkey. The local populations are grown in different regions of Turkey and exhibit hopeful performance.

The study was laid out in a randomized complete block design with 4 replications. The plots were constituted as 4 rows and 9.6 m^2 (0.6 x 4) x 4. Row spacing and Intra-row distances were determined as 60 cm and 10 cm, respectively (Augustine, 2018).

2.3. Soil Analysis and Climatic Traits

According to Table 1, the soil of the trial area was composed of deep and medium-deep soil which is low in organic matter and phosphorus content, enough in potassium. Also, it was a little saline and limy. The texture of soil was clay loam, pH was alkaline near neutral (FMANR, 1990). Based on reference soil analysis results, 4 kg/da Diammonium phosphate was applied with sowing under the seed drill (Daramy, Sardoie-Addo and Dumbuya, 2016). Irrigation was done with a drip system. Weed control was realized with mechanical methods, not any chemical.

Table 1
Properties of soil in the study area

Deepth (cm)	0-20
Structure (Sand: Silt: Clay) (%)	39:6.3:54.6
pH	7.5
EC (dS/cm)	6.64
Lime (%)	9.3
Organic matter (%)	1.4
Phosphorus (kg/da)	1.91
Potassium (kg/da)	149

The region has characteristic temperature and humidity of the terrestrial climate. Temperature and humidity values of vegetation period were similar to the long years' average ranges. However, the rainfall was erratic and excessive compared with the long years average. Some climate data were given in Table 2.

Table 2
Some climate data belonging on area

Months	Rainfall (mm)		Average Temperature (°C)		Relative humidity (%)	
	2019	Long Years Average	2019	Long Years Average	2019	Long Years Average
March	182.0	92.3	8.3	10.1	63.5	59.2
April	175.6	91.7	11.9	15.3	66.8	53.8
May	64.4	69.5	21.9	20.0	41.8	49.6
June	1.2	10.8	29.1	27.0	26.5	28.7
July	2.0	2.6	31.8	31.7	19.9	20.4
August	1.4	1.9	32.0	31.6	19.3	19.6
Total	427	269				
Mean			22.5	22.6	39.6	38.6

2.4. Measurement of Traits

All traits investigated on 10 plants collected from per plot. Plant height (PH), number of pods per plant (NP), number of seeds per plant (NS) and seed yield (SY) were measured according to Erman and Çığ (2009). The Stem diameter (SD) was measured at 1 cm above the soil surface with an electronic digital caliper (Mitutoyo 500-182-30 digital caliper, Co. Ltd., Japan) (Verbree, Singh and Payne, 2015). Leaf surface temperature (LST) was measured with an infrared thermometer (SATO SK-8700, Co. Ltd., South Korea) with a 45^{o} angle and 10 cm distance to the leaf surface on a clear day between 12.00-14.00 during the flowering time (Yu, Wang, Xin and Zheng., 2016). Total chlorophyll content (TCC) of the leaf was measured with portable chlorophyll meter (SPAD-502, Minolta Camera Co. Ltd., Japan) on the upper fully expanded leaf at the beginning of flowering (Dong et al., 2019).

2.5. Statistical Analysis

The Shapiro-Wilk test was applied to evaluate the normality of data (Korkmaz et al., 2014). Data were calculated by analysis of variance in the R v.3.5.2 according to the randomized complete block design. The results were grouped according to the TUKEY test (Mangiafico, 2016). According to the results of multiple comparisons, significant differences ($P<0.01$) were determined between genotypes for all traits except leaf surface temperature. The correlation analysis of all the characters was calculated as per the procedure stated by Al-jubouri, Millar and Robinson (1958).

3. Results and Discussion

3.1.Agronomic traits

According to the results, cv. Karagöz had superior traits compared to others and it exhibited more tolerance in heat condition. PH, SD, LST, TCC changed between 54.6-91.3 cm, 3.1-7.6 mm, 27.9-31.7 °C, 39-56.1%, respectively (Table 4).

3.1.1. Plant height

The highest PH was determined in Karagöz (92.3 cm) while the shortest one (54.6 cm) was observed in L_2 (Table 3). However, the difference between L_2 and L_3 landraces was not significant. Different researchers reported that PH in cowpea changes between 27.9-108.5 cm (El-Naim, Jabereldar, Ahmed, Ismaeil et al., 2012; Bisikwa et al., 2014). Also, Massey, Singh, Nautiyal and Bhatt (2020) stated that PH is genotype-dependent and changes week by week during the growth period. Therefore, the adaptation potential of genotype to the environment has a vital role in PH.

3.1.2. Stem diameter

The thickest SD was in Karagöz while the thinnest one was in L_3 landrace (Table 4). According to these results, SD had a significant variation among genotypes. Verbree et al. (2015) stated that stem diameter, which is an important and easy phenotypic trait, may also be an indicator of response to drought stress in cowpea. Also, thicker stem in plants provides resistance them to lodging which is a vital reason for death in seedlings. Previous reports showed similarities with the result in this study. Ravelombola et al. (2018) stated that stem diameter value in cowpea affected by drought changed between 2.45-3.69 mm.

3.1.3. Leaf surface temperature

Differences among genotypes in terms of the LST was not significant (Table 3). Various researchers stated that changes in temperature affect plant growth and yield parameters (Olatunji et al., 2016; Kirigia, Winkelmann, Kasili and Mibus, 2018). Also, Hall (2004) stated that long term high temperature leads to unfavorable effects on seed yield in cowpea. Besides, Hesketh (1967) stated that the photosynthesis rate and amount of gas input and output through stomata decrease at high temperatures. However, more studies must be conducted with larger sets of genotypes to understand the tolerance and susceptibility levels in cowpea.

3.1.4. Total chlorophyll content

In terms of TCC, statistically significant ($P<0.01$) differences were determined among genotypes (Table 3). While the highest TCC was obtained from Karagöz, the lowest values obtained from L_3 landrace (Table 4). Chlorophyll as one indicator of heat-stricken plants is synthesized with ecological and genetic factors and its amount shows diversity for each species (Hendriyani and Setiari, 2009). So, the measurement of chlorophyll content is an indicator of photosynthesis intolerant plants. Higher chlorophyll content in Karagöz shows that its adaptability to heat conditions is superior compared with the other genotypes. Different studies supported the results (Karuwal, Suharsona, Tjahjoleksona and Hanif, 2017). Also, Barro et al. (2018) stated that TCC varied from 42.20 to 62.00% among the cowpea genotypes with a general mean of 51.38%.

Table 3
Some agronomic traits of genotypes

Genotypes	Plant height (cm)	Stem diameter (mm)	Leaf surface temperature (°C)	Total Chlorophyll Content (%)
L1	62.7cd	4.3c	31.7	49.1bc
L2	54.6d	3.4d	29.1	39.0d
L3	57.1d	3.1d	31.6	36.3d
Karnıkara	68.8c	5.1c	27.9	46.4c
Akkız	78.2b	6.1b	30.6	51.9ab
Karagöz-86	91.3a	7.6a	30.8	56.1a
Mean	68.8	4.9	30.3	46.5
TUKEY	28.8**	2.9**	17.0	14.6**

(**: *P<0.01*)

3.2. Yield components

The analysis of variances for yield parameters was given in Table 4. The Results pointed out significant differences among the genotypes on NP, NS and SY.

According to the results, NP, NS and SY changed between 25.7-41, 307.5-684 and 646-2381 kg ha^{-}, respectively (Table 5). The differences among genotypes are thought to be caused by adaptability to heat stress.

Table 4
Analysis of variance on three selected yield parameters

Source of variation	Number of pods per plant			Number of seed per plant		Seed Yield (kg/ha)	
	DF	MS	F prob.	MS	F prob.	MS	F prob.
Genotypes	5	237.5	**	45.8	**	13178868	**

3.2.1. Number of pod

The results showed that genotypes have different pod yield capacity (Table 5). Karagöz had the highest NP (49) while the L_3 had the least (25.7). Oladejo, Akinwale and Obisesan (2011) reported NP between 34.78-67.25. The NP is one of the most substantial yield components and it is affected by environmental stress factors such as heat or drought that causes the death of pollen grains and denaturation of physiological tissues (Al-Assafi and Abed, 2014; Abed, 2017). It is thought to be caused by genetic differences among genotypes concerning growth potential, nutrient uptake efficiency and yield capacity. Moreover, the adaptability of genotypes also affects yield parameters.

3.2.2. Number of seed

The NS changed based on genotypes. The highest NS was found in Karagöz and the lowest one was in L_3 (Table 5). Oladejo et al. (2011) pointed out that seed yield changes depending on traits of cultivars and environmental factors. As it is seen in growth and yield parameters, some cowpea genotypes, especially Karagöz and Akkız, showed hopeful performance for the region. It can show the reason for this cowpea grows best in the regions where average temperatures vary between 15-25 °C and night temperatures should not be less than 15 °C in growth period (Boukar et al., 2015). So, it is thought that the region is suitable for cowpea cultivation and choosing genotype has a vital role in high yield. Also, it is known that ecological conditions and cultivars have a significant effect on yield parameters. Some researchers stated that yield components change depending on genotypes and their adaptability to the local conditions (Basaran, Ayan, Acar, Mut et al., 2011; Agele, Oyewusi, Fayeun and Famuwagun, 2017). Aliyu, Lawal, Wahab and Ibrahim (2019) stated that NS varied from 22 to 360.

3.2.3. Seed yield

A highly significant variation was observed among the test genotypes under investigations (Table 4). The highest seed yield was obtained from Karagöz (2381 kg ha^{-}) while the lowest one (646 kg ha^{-}) was determined in L3 (Table 5). It is noteworthy that Karagöz variety produced the maximum SY compared to the other varieties. This is so because according to Ogbonnaya et al. (2003), cowpea is recognized to have extreme stomatal control leading to rapid closure of stomata under stress conditions. Also, Reza (2011) stated that seed yield is a polygenic trait. Horn, Shimelis, Sarsu, Mwadzingeni et al. (2018) revealed that genetic diversity affects grain yield both alone and depending on environmental factors. So, it can be understood that while genetic traits of material effect on adaptability to regions, growth parameters, physiological properties, yield components, and it also affect the seed yield, directly or indirectly. Kyei-Boahen, Savala, Chikoye and Abaidoo (2017) denoted that grain yield changed between 1097-1674 kg/ha in cowpea depending on various chemical and biological fertilization applications.

Table 5
Yield and some yield components of genotypes

Genotypes	Number of pods per plant	Number of seed per plant	Seed Yield (kg/ha)
L1	38.6bc	478.9bc	1599c
L2	34.1c	422.8c	1334d
L3	25.7d	307.5d	646e
Karnıkara	36.6bc	478.7bc	1514c
Akkız	41.0b	559.7b	1850b
Karagöz-86	49.0a	684.0a	2381a
Mean	37.5	488.6	1554
TUKEY	14.4**	268.1**	437.3**

3.3. Correlations among agronomic traits in cowpea

The summary of the correlation coefficients between SY and other traits average is presented in Table 6. The results presented positive and significant correlation was observed among the major of the agronomic traits evaluated. It shows that there were significant positive correlations between SY and NS (r=0.98**), NP (r=0.96**), TCC (r=0.93**), SD (r=0.91**) and PH (r=0.86).

It is seed as Table 6, the relationship between NS and SY showed the highest positive correlation (r=0.98**). Besides, NP has the highest correlation after NS. The results are the indicator that NP and NS have a vital role in total grain yield. Various researchers found a high correlation among NP, NS and SY (Alidu, Atokple and Akromah, 2013; Shanko, Andargic and Zelleke, 2014; Patel, Kumar and Meena, 2018).

Table 6
The results of correlation analysis between seed yield and other parameters

	PH	SD	LST	TCC	NP	NS
SD	0.95**					
LST	0.04	-0.05				
CC	0.89**	0.90**	0.03			
NP	0.86**	0.88**	-0.06	0.92**		
NS	0.90**	0.93**	-0.07	0.93**	0.96**	
SY	0.86**	0.91**	-0.08	0.93**	0.96**	0.98**

(PH: Plant height, SD: Stem diameter, LST: Leaf surface temperature, TCC: Total chlorophyll content, NP: Number of pods per plant, NS: Number of seeds per plant, **: p<0.01)

Otherwise, TCC had a significant correlation with NP (r=0.92**), NS (r=0.93**) and SY (r=0.93**). The well-known role of photosynthesis products on plant growth also affects metabolic activities (Duca, 2015). Several researchers stated that there is a direct positive correlation between TCC and yield parameters (Esaghira et al., 2016; Musa, Bashir and Tadda, 2017; Sozen and Karadavaut, 2018). Besides, SD had a significant correlation with NP (r=0.88**), NS (r=0.93**) and SY (r=0.91**). It can be commented that the plants which have thicker stem diameter are more tolerant of heat conditions exhibited superior performance in terms of yield parameters (El-Naim et al., 2012). Also, a significant correlation was determined between PH and NP (r=0.86**), NS (r=0.9**), SY (r=0.86**). Walle, Mekbib, Amsalu and Gedil (2018) demonstrated that genetic correlations are more effective than phenotypic correlations in cowpea and it was revealed that PH has a favorable relationship with yield components.

4. Conclusion

From the results obtained in the study, Karagöz exhibited superior properties compared to others in terms of morphological growth, total chlorophyll content of leaf, seed yield and some yield components. The results of the study indicated that genetic traits have a significant effect on yield parameters and some other traits. The cowpea genotypes have different adaptation capacity due to their genetic traits and show various responses to conditions under investigation. Additionally, it was concluded that cowpea cultivation has some advantages in semi-arid regions. The number of seeds per plant and the number of pods per plant have the highest correlation values with seed yield. It must be laid out further studies on the genotypic variation and local adaptation potentials of cowpea cultivars.

5. Acknowledgments

This research was conducted in the study areas of Siirt University. The authors thank Dr. Ayman El SABAGH and Dr. Harun BEKTAS for their contributions.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

6. References

Abed RD (2017). Effects of genotypes and potassium rates on some of cowpea traits heritability. *Asian Journal of Crop Science* 9(1): 11-19. DOI:10.3923/ajcs.2017.11.19

Agbicodo EM, Fatokun CA, Muranaka S, Visser RG F, Van Der Linden CG (2009). Breeding drought tolerant cowpea: Constraints, accomplishments, and future prospects. *Euphytica* 167: 353-370. https://doi.org/10.1007/s10681-009-9893-8

Agele SO, Oyewusi IK, Fayeun L, Famuwagun IB (2018). Genotype by environment ınteractions and effects on growth and yield of cowpea varieties in the rainforest and derived savanna agroecologies of Nigeria. *International Journal of Plant Breeding and Crop Science* 5(2): 370-382.

Al-Assafi RDA, Abed ZA (2014). Selection effectiveness for pods number in early generations of seed yield of cowpea cultivar imported. *Iraqi Journal of Agricultural Science* 45(8): 909-914.

Al-jubouri HA, Millar PA, Robinson HF (1958). Genotypic and environmental variance and covariance in upland cotton crosses of interspecific origin. *Agronomy Journal* 50: 633-637.

Alidu MS, Atokple IDK, Akromah R (2013). Genetic analysis of vegetative-stage drought tolerance in cowpea. *Greener Journal of Agricultural Science* 3: 481-496. DOI:10.15580/GJAS.2013.6.030613516

Aliyu OM, Lawal OO, Wahab AA, Ibrahim UY (2019). Evaluation of advanced breeding lines of cowpea (*Vigna unguiculata* L. Walp) for high seed yield under farmers' field conditions. *Plant Breeding and Biotechnology* 7(1): 12-23. https://doi.org/10.9787/PBB.2019.7.1.12

Augustine AJ (2018). Effect of Inter-row and Intra-row spacing on phenological characters of cowpea (*Vigna unguiculata* L. Walp) varieties in semi-arid region of North-Western Nigeria. *Journal of Experimental Agriculture International* 27(5): 1-9. DOI:10.9734/JEAI/2018/13986

Barro A, Batieno TBJ, Tignegre JBS, Neya JB, Pale K, Kabore A, Ouedraogo MH, Sawadogo M (2018). Evaluation of agronomic performances of five cowpea lines in the experimental research station of Saria, Burkina Faso. *World Journal of Agricultural Research* 6(3): 82-86. DOI:10.12691/wjar-6-3-2

Basaran U, Ayan I, Acar Z, Mut H, Asci OO (2011). Seed yield and agronomic parameters of cowpea (*Vigna unguiculata* L.) genotypes grown in the Black Sea Region of Turkey. *African Journal of Biotechnology*, 10(62): 13461-13464. DOI:10.5897/AJB11.2489

Bisikwa J, Kawooya R, Ssebuliba JM, Ddungu SP, Biruma M, Okello DK (2014). Effect of plant density on the performance of local and elite cowpea [*Vigna unguiculata* L. (Walp)] varieties in Eastern Uganda. *African Journal of Applied Agricultural Sciences and Technologies* 1(1): 28-41. http://www.naroinfohub.org/RepDetails?pn=1007

Boukar O, Fatokun CA, Roberts PA, Abberton M, Huynh BL, Close TJ, Kyei-Boahen S, Higgins TJV, Ehlers D (2015). Cowpea. In: De Ron, A. M. (ed) Grain Legumes, vol 10, *Springer*, New York. DOI:10.1007/978-1-4939-2797-5

Daramy MA, Sardoie-Addo J, Dumbuya G (2016). The effects of nitrogen and phosphorus fertilizer applicatıon on crude protein, nutrient concentration and nodulation of cowpea in Ghana. *Journal of Agricultural and Biological Science* 11(12): 470-477.

Dong L, Ravelombola W, Weng Y, Qin J, Bhattarai G, Zia B, Zhou W, Wang Y, Mou B, Shi A (2019). Seedling salt tolerance for above ground-related traitsin cowpea (*Vigna unguiculata* (L.) Walp). *Euphytica* 215(53): 1-22.

Duca M (2015). Plant Physiology. In: Greenbaum EE (ed) Photosynthesis, *Springer*, New York, pp. 72-80.

Esaghira C, Yamauchi T, Miyamoto Y, Yuasa T, Ishibashi Y, Iwaya-Inoue M (2016). Physiological response of cowpea (*Vigna unguiculata* (L.) Walp) to drought stress during the pod-filling state. *Cryobiology and Cryotechnology* 1: 69-75.

El-Naim AM, Jabereldar AA, Ahmed SE, Ismaeil FM, Ibrahim EA (2012). Determination of suitable variety and plants per stand of cowpea (*Vigna unguiculata* (L) walp.) in the Sandy Soil, Sudan. *Advances in Life Science* 2(1): 1-5. DOI:10.5923/j.als.20120201.01

Erman M, Çığ F (2009). Farklı bitki sıklığı uygulamalarının börülce (*Vigna unguiculata* L.) Walp.)'de verim ve verim ögelerine etkisi. *Türkiye VIII. Tarla Bitkileri Kongresi*, 19-22 Ekim, Hatay, s. 669-672.

FAO (2018). Cowpea production on the World, Available at: http://www.fao.org/stat

Federal Ministry of Agriculture and Natural Resources (FMANR) (1990). Literature on soil fertility ınvestigation in Nigeria. *Federal Ministry of Agriculture and Natural Resources*, Lagos, s. 40.

Freitas ADS, Silva AF, Sampaio EVSB (2012). Yield and biological nitrogen fixation of cowpea varieties in the semi-arid region of Brazil. *Biomass and Bioenergy*, 45: 109-114. DOI: 10.1016/j.biombioe.2012.05.017

Hall AE (2004). Breeding for adaptation to drought and heat in cowpea. *European Journal of Agronomy* 21(4): 447-454.

Hendriyani IS, Setiari N (2009). Chlorophyll content and growth of yard long (*Vigna sinensis)* at different levels of water supply. *Journal of Sains Matematika* 17(3): 145-150.

Hesketh JD (1967). Effects of light and temperature during plant growth on subsequent leaf CO_2 assimilation rates under standard conditions. *Australian Journal of Biological Science* 21: 235-241.

Horn L, Shimelis H, Sarsu F, Mwadzingeni L, Laing MD (2018). Genotype-by-environment interaction for grain yield among novel cowpea (*Vigna unguiculata* L.) selections derived by gamma irradiation. *The Crop Journal* 6(3): 306-313. https://doi.org/10.1016/j.cj.2017.10.002

Kahraman A (2017). Effect of humic acid doses on yield and quality parameters of cowpea [*Vigna unguiculata* (L.) Walp] cultivars. *Legume Research* 40 (1): 155-159, DOI: 10.18805/lr.v0iOF.3763.

Karuwal RL, Suharsona S, Tjahjoleksona A, Hanif N (2017). Physiological response of some local cowpea from Southwest Maluku (Indonesia) varieties to drought stress. *Biodiversitas* 18(4): 1294-1299. DOI:10.13057/biodiv/d180402

Kazai P, Noulas C, Khah E, Vlachostergios D (2019). Yield and seed quality parameters of common bean cultivars grown under water and heat stress field conditions. *Agriculture and Food* 4(2): 285-302. DOI:10.3934/agrfood.2019.2.285

Kirigia D, Winkelmann T, Kasili R, Mibus H (2018). Development stage, storage temperature and storage duration ınfluence phytonutrient content in cowpea (*Vigna unguiculata* L. Walp.). *Heliyon* 4(6): 1-24. DOI:10.1016/j.heliyon.2018.e00656

Korkmaz S, Goksuluk D, Zararsiz G (2014). MVN: An R Package for Assessing Multivariate Normality. *The R Journal* 6(2): 151-162.

Kyei-Boahen S, Savala CEN, Chikoye D, Abaidoo R (2017). Growth and yield responses of cowpea to inoculation and phosphorus fertilization in different environments. *Frontiers in Plant Science* 8: 646. DOI:10.3389/fpls.2017.00646

Mangiafico SS (2016). Summary and analysis of extension program evaluation in R (version 1.18.1). *Rutgers Cooperative Extension*, New Brunswick.

Makoi JHJR, Chimphango BM, Dakora FD (2009). Effect of legume plant density and mixed culture on symbiotic N_2 fixation in five cowpea (*Vigna unguiculata* L. Walp.) genotypes in South Africa. *Symbiosis* 48(1-3): 57-67. https://doi.org/10.1007/BF03179985

Massey P, Singh MK, Nautiyal MK, Bhatt L (2020). Evaluation of cowpea (*Vigna unguiculata* L. Walp) genotypes for yield and associated traits. *International Journal of Chemical Studies* 8(1): 1709-1711.

Musa M, Bashir KA, Tadda SA (2017). Response of cowpea (*Vigna unguiculata* L. Walp) varieties to phosphorus levels in Sudan Savanna of Nigeria. *International Multidisciplinary Research Journal* 7: 23-29. DOI:10.25081/imrj.2017.v7.3380

Ogbonnaya CI, Sarr B, Brou C, Diouf O, Diop NN, Roy-Macauley H (2003). Selection of cowpea genotypes in hydroponics, pots, and field for drought tolerance. *Crop Science* 43(3): 1114–1120.

Oladejo AS, Akinwale RO, Obisesan IO (2011). Interrelationships between grain yield and other physiological traits of cowpea cultivars. *African Crop Science Journal* 19(3): 189-200.

Olatunji OA, Okunlola GO, Komolafe ET, Afolabi AM, Tariq A, Odeleye AA (2016). Yield and growth characteristics of cowpea (*Vigna unguiculata*) as affected by prior heat stress and nutrient addition. *African Journal of Agricultural Research* 11(43): 4269-4276. DOI:10.5897/AJAR2016.11651

Patel PS, Kumar P, Meena R (2018). Correlation and Path analysis in Cowpea [(*Vigna unguiculata* (L.) Walp.)]. *International Journal of Pure and Applied Bioscience* 6(5): 142-146. DOI:http://dx.doi.org/10.18782/2320-6941

Ravelombola W, Shi A, Qin J, Weng Y, Bhattarai G, Zia B, Zhou W, Mou B (2018). Investigation on various aboveground traits to identify drought tolerance in cowpea seedlings. *Hortscience* 53(12): 1757-1765. https://doi.org/10.21273/HORTSCI13278-18

Reza GA (2011). Assessment of relationship between seed and oil yield with agronomic traits in spring safflower cultivars under drought stress condition. *Journal of Research in Agricultural Science* 7(2): 109-113.

Sert H, Ceyhan E (2012). Hatay ili ekolojik şartlarında börülce (*Vigna sinensis* (l.) savi) çeşitlerinin tane verimi ve bazı tarımsal özellikleri üzerine farklı bitki sıklıklarının etkileri. *Selçuk Tarım ve Gıda Bilimleri Dergisi*, 26(1): 34-43.

Sanchez-Navarro V, Zornoza R., Faz A, Fernandez JA (2019). Does the use of cowpea in rotation with a vegetable crop improve soil quality and crop yield and quality? A field study in SE Spain. *European Journal of Agronomy* 107: 10-17. https://doi.org/10.1016/j.eja.2019.03.007

Shanko D, Andargic M, Zelleke H (2014). Interrelationship and path coefficient analysis of some growth and yield characteristics in cowpea (*Vigna unguiculata* L. Walp) genotypes. *Journal of Plant Science* 2(2): 97-101. DOI:10.11648/j.jps.20140202.13

Simion T (2018). Adaptability performances of cowpea [*Vigna unguiculata* (L.) Walp] genoty-

pes in Ethiopia. *Food Science and Quality Management* 72: 43-47.

Simunji S, Munyinda KL, Lungu OI, Mweetwa AM, Phiri E (2019). Evaluation of cowpea (*Vigna unguiculata* L.walp) genotypes for biological nitrogen fixation in maize-cowpea crop rotation. *Sustainable Agriculture Research* 8(1): 82-93. DOI:10.5539/sar.v8n1p82

Sozen O**,** Karadavut U (2017)**.** Determination of direct and indirect relations between some yield characters of red lentil cultivars. *Pakistan Journal of Botany*, 49(6): 2339-2346.

Sozen O, Karadavut U (2018)**.** Correlation and path analysis for yield performance and yield components of chickpea (*Cicer arietinum* L.) genotypes cultivated in Central Anatolia. *Pakistan Journal of Botany*, 50(2): 625-633.

Stancheva I, Nedyalkova K, Geneva M, Donkova R, Hristozkova M, Perfanova I, Sichanova M, Petkova G, Djonova E, Valchovski H (2016). Nutritional value of cowpea (*Vigna Unguiculata* L. Walp) grain grown under different soil moisture as affected to the dual inoculation with nitrogen fixing bacteria and arbuscular mycorrhizal fungi. *Bulgarian Journal of Soil Science* 1(2): 112-121.

Stoilova T, Pereira G, Sousa MMT, Carnide V (2005). Diversity in common bean landraces (*Phaseolus vulgaris* L.) from Bulgaria and Portugal. *Journal of Central European Agriculture* 6(4): 443-448.

TUIK (2018). Harvested area for cowpea in Turkey. Available at: http://www.tuik.gov.tr/UstMenu.do?metod=temelist

Verbree DA, Singh, BB, Payne WA (2015). Genetics and heritability of shoot drought tolerance in cowpea seedlings. *Crop Science* 55(1): 146-153. DOI:10.2135/cropsci2014.02.0137.

Walle T, Mekbib F, Amsalu B, Gedil M (2018). Correlation and path coefficient analyses of cowpea (*Vigna unguiculata* L.) landraces in Ethiopia. *American Journal of Plant Science* 9: 2794-2812. DOI:10.4236/ajps.2018.913202

Yu L, Wang W, Xin Z, Zheng W (2016). A review on leaf temperature sensor: measurement methods and application. International conference on computer and computing technologies in agriculture, 12-15 August, *Springer*, China, pp. 1-15. DOI:1010.1007/978-3-319-48357-3_21

Selcuk Journal of Agriculture and Food Sciences

http://sjafs.selcuk.edu.tr/sjafs/index

Research Article

SJAFS
(2020) 34 (2), 162-168
e-ISSN: 2458-8377
DOI:10.15316/SJAFS.2020.211

The Effects of Malch Applications on the Seedling Quality of 110R and Fercal Grape Rootstocks*

Zeki KARA[1,*], Mohammed Salah Mohammednoorı FAKHAR[2]

[1]Selçuk University, Graduate School of Natural and Applied Sciences, Department of Horticulture, Konya, Turkey
[2]Selçuk University, Faculty of Agriculture, Department of Horticulture, Konya, Turkey

ARTICLE INFO

Article history:
Received date: 19.06.2020
Accepted date: 30.06.2020

Edited by:
Ali SABIR; *Selçuk University, Turkey*

Reviewed by:
Hatice BİLİR EKBİÇ; *Ordu University, Turkey*
Aydın UZUN; *Erciyes University, Turkey*

Keywords:
Grapevine rootstock,
Propagation,
Biodegradable mulch,
Organic mulch,
Plastic mulch

ABSTRACT

Viticulture, in Turkey as well as all over the world is one of the most important agricultural activities as socio-economic issues. Significant quantities of high-quality grapevine seedlings are needed to maintain and improve the current state of viticulture at national and global levels. Soil cultivation, irrigation and plant protection activities in the sapling production process not only increase the production cost, but also affect the yield and quality of the seedlings. In this study, mulch applications (Black plastic BP, Black plastic jute PJ, Biodegradable plastic BD, Wheat stalk OM and Control) of 110R and Fercal grape rootstocks obtained from the Sub-Union of Sapling Producers, from standard graftable quality virus free cuttings, in open area conditions was done. Effects of applications on seedling yield and quality (soil temperature (°C), leaf temperature (°C), stomatal conductivity (mmol m^{-2} s^{-1}), leaf chlorophyll content (spad value), leaf area (cm2), leaf number (pieces), leaf weight (g), shoot length (cm), shoot diameter (mm), pruning residue weight (g), shoot development level (0-4 scale), root numbers (pieces) and diameter (mm), root fresh and dry weight (g), root growth level (0-4 scale) and seedling efficiency (%) were examined. While mulch applications in general provided improvement in all parameters examined, the efficiency varied according to grapevine rootstocks and examined properties. In terms of seedling efficiency, BP application in Fercal rootstock and BD application in 110R rootstock were most effective. According to the data obtained from this study, the positive effects of BP and BB, PJ and OM mulch applications were determined in the production of grape rootstock seedlings in open areas and especially in areas where the relative humidity was very low.

1. Introduction

Viticulture, in Turkey as well as all over the world is one of the most important agricultural activities as socio-economic issues. Turkey is 416 907 ha of vineyards and grape production by 4.2 million tons, the world's 5th largest grape producer countries (Faostat 2020). Since the phyloxerae (*Daktulospharia vitifoliae* Fitch) moved from America to Europe at the end of the 19th century, seedling production, seedling yield and quality of grapevine rootstocks is one of the primary issues in the viticulture industry.

Traditional agricultural practices can affect the efficiency and durability of soil and environmental ecosystems, leading to soil degradation. Some common practices in traditional viticulture, such as the continuous use of herbicides, may lead to increased soil quality and overall sustainability losses of the grape production system (Ingels 1992). In order to use water resources more efficiently, water saving methods have become mandatory (Kanber et al. 1991).

Mulching is a protective layer consisting of organic or inorganic materials applied on the soil to create a suitable environment for the soil surface, plant growth, development and efficient production around the plant (Bakshi et al. 2015). Viticulture experts want to protect a healthy and productive soil. Depending on the situation, mulch may be an appropriate option (Ross 2010).

Mulches affects soil temperature, moisture level, nutrition level, microorganism activity (Ross 2010; Mundy and Agnew 2002), suppresses weeds, and provides advantages by increasing seedling yield and quality in nurseries (Nauleau 1997; Wheeler et al. 2005; Nazrala 2008; Bakshi et al. 2015; Watson 2006; Chan et al. 2010; Arslan and Uygur 2014; Cowan 2013; Król-Dyrek and Siwek 2015; Zengin 2019; Dağ 2017;

* Corresponding author email: *zkara@selcuk.edu.tr*

* This manuscript has been produced from the master thesis Mohammed Salah Mohammednoorı Fakhar supported by Selcuk University Scientific Research Projects Board (SU BAP 20201020).

Küçükyumuk 2009; Zenginoğlu 2015; Zhang et al. 2014; Ferrara et al. 2012; Hostetler et al. 2007).

In this study, the effects of OM, BD, BP and PJ mulch applications used to improve seedling yield and quality in sapling production parcels created by direct planting of Fercal and 110R grape rootstocks for the production of rooted grapevine seedlings in open area conditions were examined.

2. Material ve Metot

OM (Wheat stalk): The wheat stalk in the bale form was laid in rows in the sapling production plot just before planting 80 mm wide and 10 cm thick vine rootstock cuttings.

BP and PJ as inorganic malches were produced by Filesan İskenderun TR company for use as agricultural mulch, and they were laid at the 80 cm width of the sapling production parcel rows, 80 cm wide, just before planting of the rootstocks.

Biodegradable mulch (BD): The product, which was produced and introduced to the market for agricultural use through the Ankara Agricultural Market TR company, was laid just before planting 80 cm wide vine rootstock cuttings in the rows of seedlings production plots.

Control: It was a sapling production plot created directly under the influence of environmental extracts without applying any mulch.

Fercal [(*Vitis vinifera* L. × *Vitis berlanderi* L.) × 333 EM] and *110R* **(**Berlandieri Resseguier No: 2 × Rupestris Martin) were used as roorstock cuttings. Both of them are supplied as standard rootstock cuttings (TS 3981, TS 3912) from the Sapling Produrers Sub-Association. Trial 3 repetitive random blocks were established according to the trial pattern and the number of cuttings in the parcel was 30.

Following the soil preparation, which was initiated in autumn and repeated in early spring, cutting were planted for the trial in early April as 15 cm x 80 cm between cuttings, routine cultural practices was carried out in the summer period, records were kept and post-harvest measurements were carried out by harvesting rooted saplings in autumn (in November).

The trial plots soil temperatures (°C), leaf temperature (° C, by SC-I Leaf Porometer), stomatal conductivity (mmol m^{-2} s^{-1}, by SC-I Leaf Porometer), leaf chlorophyll content [(spad value by Minolta Spad Meter 520,(Kara et al. 2017)], leaf area (cm^2), leaf number (pieces), leaf weight (g), shoot length (cm), shoot diameter (mm), pruning residue weight (g), shoot development level (0-4 scale), number of roots (pieces) and root diameter (mm), root fresh and dry weight (g), root growth level (0-4 scale) and seedling efficiency (first and second grade %) were examined.

Statistical analyses

A complete randomized block design with three replicates and 30 cuttings sample in each replicate for mulch applications, for both grape rootstcks were established. The numerical data obtained were compared with Student's t-test at 0.05 significance level using SPSS 17.0 and JMP 7 statistical programs.

3. Results and Discussion

Stomatal conductivity (mmol m^{-2} s^{-1})

Mulch applications on stomatal conductivity were significant ($p < 0.05$) (Figure 1). Generally, the values obtained from 110R were higher than Fercal, but when the measurement times and applications were evaluated together, there was no stable situation in both rootstocks. On 30 July, the highest stomatal conductivity was recorded as 261.86 mmol m^{-2} s^{-1} in Fercal control and 296.33 mmol m^{-2} s^{-1} in BD application in 110 R. On August 20, the highest values [(Fercal 316.80 mmol m^{-2} s^{-1}, 110R 331.73 mmol m^{-2} s^{-1})] were detected in BP application, on August 10, the maximum values were detected in Fercal control as 313.30 mmol m^{-2} s^{-1} and in 110R PJ application as 347.27 mmol m^{-2} s^{-1}. On August 20, the highest values were detected in [(Fercal 316.80 mmol m^{-2} s^{-1}, 110R 331.73 mmol m^{-2} s^{-1})] BP aplication. On September 10, the highest values were 31.30 mmol m^{-2} s^{-1} in Fercal control and 347.27 mmol m^{-2} s^{-1} in 110R PJ aplication. On September 1, the maximum values were determined in BD application in Fercal (265.53 mmol m^{-2} s^{-1}) and PJ applications in 110R (368.63 mmol m^{-2} s^{-1}). In a similar study, Zengin (2019), 99 R, 44-53 M, Rupestris du Lot and 41B grape rootstock seedlings did not show a stable situation in the effect of mulch applications on stomatal conductivity.

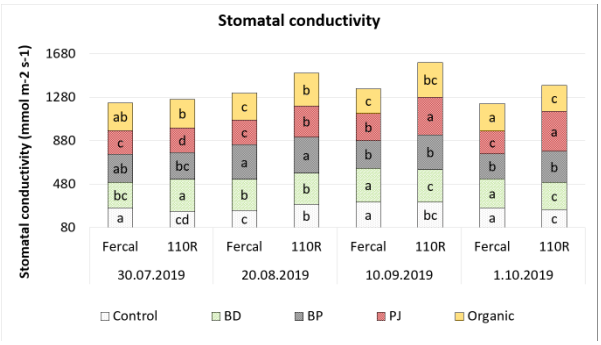

Figure 1
Effects of mulch applications on stomatal conductivity (mmol m^{-2} s^{-1}).

Leaf temperature (°C)

The effects of mulch applications on leaf temperature (Figure 2) were also important in 4 measurement periods ($p < 0.05$). While the BP application in the Fercal rootstock gave the highest leaf temperature as a value of 31.02 °C on July 30, the PJ application gave the highest value in 110R rootstock as 32.06 °C. On 20 August, OM application on both rootstocks [(Fercal 29.40 °C and 110R 29.60 °C)] gave the highest leaf temperature values. On September 10, BP applied samples were on both rootstocks [(Fercal 31.57 °C and 110R 31.33 °C)] had the highest temperature value. In the determinations dated 1 October, the application of OM in both rootstocks [(Fercal 30.23 °C and 110R 30.23 °C)] gave the highest leaf temperature. According to the data obtained, it increased the leaf temperature of BP, OM and PJ applications, respectively. In a previous study, Doğan (2020) reported that the effects of different mulch applications were significant in the

grape variety of Trakya İlkeren, and were listed as Control (33.46 °C), Straw (32.80 °C), BP (32.79 °C) and Pumice (32.61 °C) mulches.

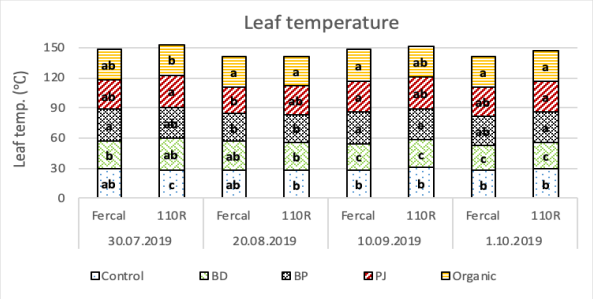

Figure 2
Effects of mulch applications on leaf temperature (°C)

Leaf chlorophyll content (spad value, mg kg^{-1})

The effects of mulch applications on leaf chlorophyll content (Figure 3) were also significant in 4 measurement periods ($p < 0.05$), BP and PJ increased leaf chlorophyll content more than the other applications. In the measurements performed on 30 July and 20 August, PJ application gave the highest Spad values in the Fercal rootstock as 23.42 mg kg^{-1} ve 26.48 mg kg^{-1} and in 110R rootstock BP application has the highest value as 7.29 mg kg^{-1} and 27.50 mg kg^{-1} respectively. On September 10, BP mulch had the highest leaf chlorophyll value in both rootstocks [(Fercal 26.91 mg kg-1 and 110R 25.27 mg kg^{-1})]. On October 1, PJ application in Fercal rootstock gave the highest SPAD value as 27.92 mg kg^{-1}, while OM gave the highest leaf chlorophyll value as 26.43 mg kg^{-1} in 110R.

In a similar study, Doğan (2020), reported that in the combination of Trakya İlkeren / 1103P, in 50% irrigation and BP mulch has the highest leaf chlorophyll content as 38.8 mg kg^{-1} compared to other mulch applications. Zengin (2019), reported that synthetic mulch applications significantly increased leaf chlorophyll content in their study with different rootstocks of 99R, 44-53M, Rupestris du Lot and 41B. Curtis (2013), reported that leaf chlorophyll content was greater than control as 39.9 mg mg kg^{-1} in those who applied OM in the *Vitis vinifera* vineyard, and increased vine growth and productivity.

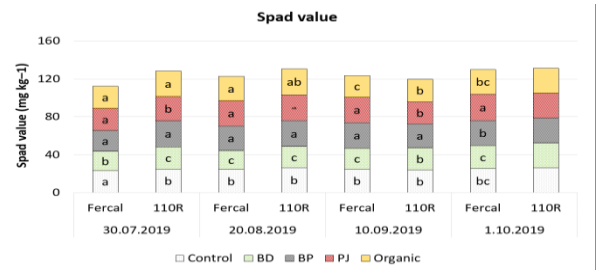

Figure 3
Effects of mulch applications on leaf clorophil content

Soil temperature (°C)

The effects of mulch applications on soil temperature (Figure 4) were significant ($p < 0.05$). According to the applications in both rootstocks, a stable condition was not observed in soil temperature values. The soil was the hottest in both rootstocks (Fercal 23.17 °C and 110R 23.00 °C) on which BP was applied at July 30. On August 20, PJ application in Fercal rootstock gave the highest soil temperature as 23.17 °C, and 110R rootstock as 22.80 °C. On September 10, in 110R rootstock, the highest soil temperature was determined in OM application as 21.83 °C, while the effects of applications on soil temperature in Fercal rootstock were statistically insignificant. In the last determinations made on 1 October, Fercal control gave the highest soil temperature value, while the effects of the applications on soil temperature were insignificant in 110R.

In one of the previous studies, Dağ (2017), achieved the highest soil temperature in BP application the Michele Palieri / 41B combination as 25.3 °C and indicated that this value was 3.7 °C higher than the control. Abramova (1984), reported that the application of synthetic mulch in the production of Areni and Burmunk grape vine saplings increased the soil temperature by 1.5-3 °C. Küçükyumuk (2009), stated that in the production of Alphonse Lavallée grapevine grafted onto 140 Ru, 5BB, 41B rootstocks, soil temperatures are listed as BP, Rose pulp, Control and Grass residue mulch applications from high to low. Zenginoğlu (2015), stated that in the combination of Sultani Çekirdeksiz / 1613, in the production of vine saplings, different mulch applications were compared and the soil temperature was higher in BP and upper surface gray bottom surface black mulch applications, and mulch applications had an effect on increasing soil temperature.

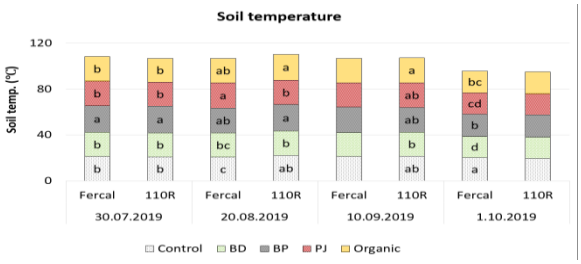

Figure 4
Effects of mulch applications on soil temperature (°C),

Leaf area (cm2)

The effects of mulch applications on leaf area (Figure 5) were significant ($p < 0.05$) and differed by rootstocks. In Fercal grape vine rootstock, all applications were above the control and the highest value as 143.50 cm2 was in the BP application, while in 110R rootstock the highest value as 66.75 cm2 was recorded in OM application. In a similar study previously conducted, Zengin (2019) reported that in the 41B rootstock, BP and OM applications increased leaf temperature up to 51% and 48%, respectively.

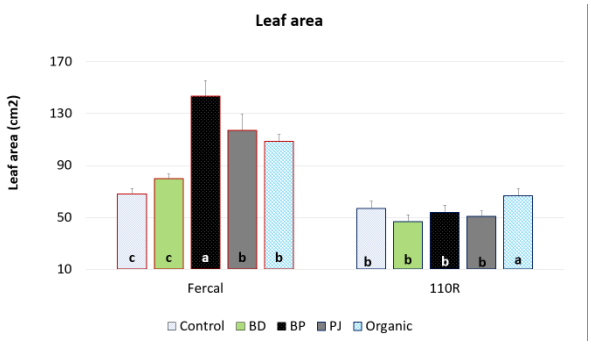

Figure 5
Effects of mulch applications on leaf area (mg kg^{-1}).

Number of leaves and roots (pieces)

The effects of mulch applications on the number of leaves (Figure 6) were significant ($p < 0.05$) and differed by rootstocks. OM application gave the highest number of leaves in both rootstocks (Fercal as 30.13 pieces and 110R as 29.05 pieces. All mulch applications in Fercal rootstock, BP and OM applications in 110R rootstock increased the number of leaves. While the highest number of roots was determined as 28.83 in BP application in Fercal, it was determined as 15.28 in OM application in 110R rootstock. In similar studies, In a similar study, Zengin (2019) reported that the application of OM on 99 R, 44-53 M, Rupestris du Lot and 41B grape rootstocks increased the number of leaves up to 30%. Dağ (2017), Zenginoğlu (2015), Küçükyumuk (2009) obtained the highest number of roots from BP application.

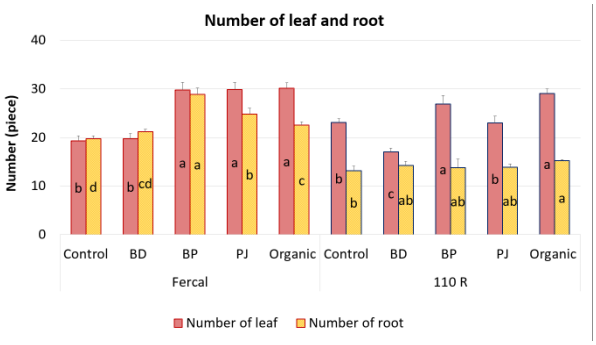

Figure 6
Effects of mulch applications on number of leaf and root (pieces).

Leaf weight (g)

The effects of mulch applications on leaf weights (Figure 7) were significant ($p < 0.05$). In Fercal, BP (3.30 g fresh 1.14 g dry) mulch gave the highest fresh leaf weight, whereas fresh and dry leaf weights were above the control in all applications. While the highest fresh leaf weight value was determined in OM (1.50 g) application in 110R, the difference between dried samples was insignificant ($p < 0.05$). In similar studies by Zengin (2019), and Ross (2010), mulch applications have been reported to increase fresh leaf weight. Zengin (2019), reported that the application of leaf dry weight BP (0.93 g) increased in the Rupestris du Lot rootstock.

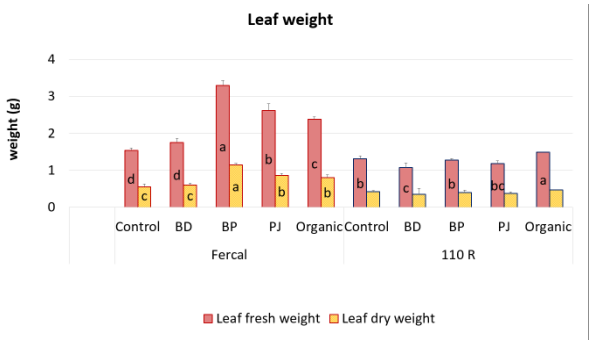

Figure 7
Effects of mulch applications on leaf weight (g).

Shoot length (cm)

The effects of mulch applications on total and lignified shoot length (Figure 8) were significant ($p < 0.05$) and the values were more than 110R in Fercal. In Fercal rootstock, the highest total as 130.73 cm and woody as 121.87 shoot length was recorded in BP application, followed by PJ and OM applications. In this rootstock, the shoot length values obtained from the BD application remained under control. In 110R, OM application came to the fore with the total as 94.76 cm and woody as 86.31 cm shoot length value. In previous studies where BP application increased the length of shoots, it was reported by Zengin (2019), Dağ (2017), Zenginoğlu (2015), Küçükyumuk (2009), Van der Westhuizen (1980).

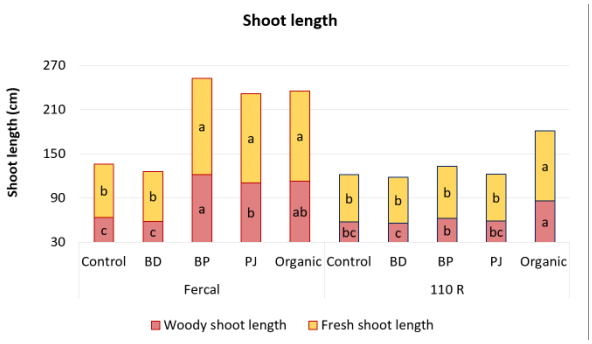

Figure 8
Effects of mulch applications on shoot length (cm)

Shoot and root diameter (mm)

The effects of mulch applications on shoot and root diameter (Figure 9) were significant ($p < 0.05$), differed by rootstocks (Figure 2), and in the same trend with shoot length, and the values were more than 110R in Fercal. The highest shoot length in BP application as 5.97 mm in Fercal and OM application as 4.89 mm in 110R gave the highest values. OM application [(Fercal 2.65 mm and 110R 2.80 mm)] gave the highest root diameter in both rootstocks, followed by PJ and BP applications with close values. In similar studies, where in BP application increased the diameter of the shoot, it was reported by Zengin (2019), Dağ (2017), Küçükyumuk (2009).

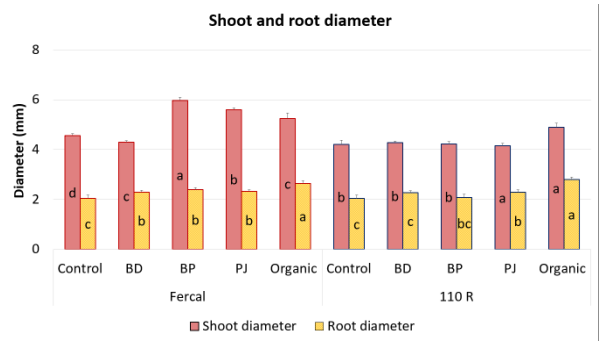

Figure 9
Effects of mulch applications on shoot and root diameter (mm).

Pruning residue weight (g)

The effects of mulch applications on pruning residue weight were significant ($p < 0.05$) and different from rootstocks (Figure 10) and the values were more than 110R in Fercal. The OM application gave the highest pruning residue weight as 31.88 g in Fercal rootstock and 17.54 g in 110R rootstock. Similar effects of BP application were reported by Dağ (2017) and Küçükyumuk (2009).

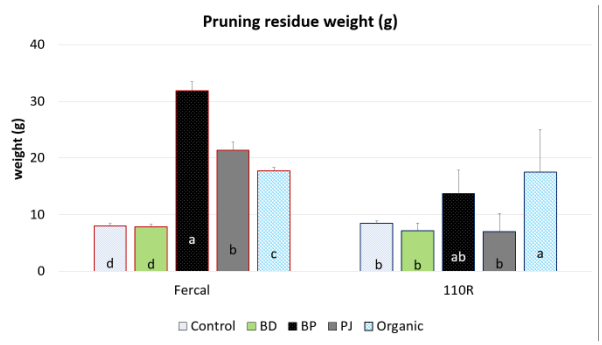

Figure 10
Effects of mulch applications on Pruning residue weight (g).

Shoot and root development level (0-4 scale)

BP, PJ, and OM applications significantly increased the shoot growth ($p < 0.05$) (Figure 11) in Fercal and the only OM in 110R, and all mulche inreased root developemnt in both rootstocks. In Fercal, BP (3.09) and PJ (3.03) gave higher values than the control and BD applications. The highest shoot development in 110R was obtained by the OM (2.49) application. Root growth level in Fercal gave the highest root scale in PJ (3.83) application, while BD (3.06) application in 110R.

In previous studies, Zenginoğlu (2015) reported that Sultani Çekirdeksiz / 1613 saplings were the highest shoot development level in BP (2.9) application. Küçükyumuk (2009) reported that BP application significantly increased the level of shoot growth compared to other mulch applications in the production of Alphonse Lavallée grapevine grafted onto 140 Ru, 5BB, 41B. Dağ (2017), Michele Palieri / 41B had the highest shoot growth level in sapling production as 2.10 scale from BP application. In similar studies conducted by Dağ (2017), Zenginoğlu (2015), Küçükyumuk (2009) it was reported that BP applications significantly increased the level of root development.

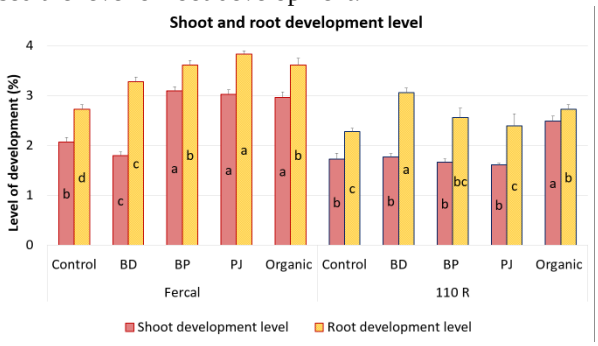

Figure 11
Effects of mulch applications on shoot and root development level (0-4 scale).

Root fresh and dry weight (g)

All of the mulch applications significantly increased the root fresh and dry weight ($p < 0.05$) (Figure 12) and the values were more than 110R in Fercal. In Fercal, BP (25.78 g) and OM (24.90 g) applications significantly increased the root fresh and dry (12.00 g and 11.97 g) weight. The highest root in Fercal rootstock was obtained from BP application with its fresh weight. The highest root fresh (7.05 g) and dry weight (3.54 g) were determined in OM application at 110R rootstock. In similar studies, Dağ (2017), Küçükyumuk (2009) reported that the application of BP increases the root age weight of 41B grapevine seedling production. Van der Westhuizen (1980) reported that vines with plastic mulch have higher root weight due to higher soil moisture. Van Huyssteen and Weber (1980) obtained higher hanging root density from OM than other mulch applications.

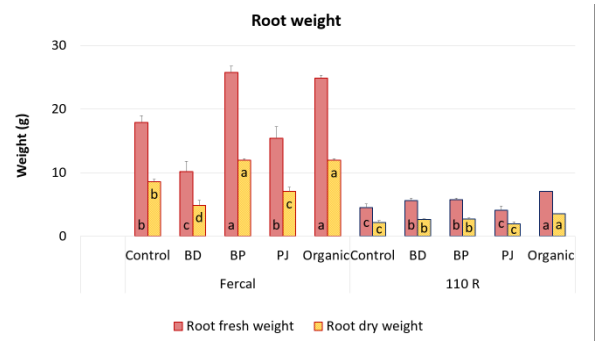

Figure 12

Effects of mulch applications on root weight (g).

Sapling efficiency (%)

BP and OM applications increased the 1st and 2nd grade seedlings efficiency ($p < 0.05$) (Figure 13) and the values were more than 110R in Fercal. The highest 1st (28.33%) and 2nd grade (44.17%) seedlings yield in Fercal were obtained by BP application, and in 110R, it was obtained by BD and OM application (49.17%, 20.00%, respectively). In similar studies, Dağ (2017), Küçükyumuk (2009), Zenginoğlu (2015) BP application significantly increased the yield of 1st and 2nd grade seedlings in vine sapling production, Zengi-

noğlu (2015), black mogul (17.5) mulch application in the production of open-rooted vine seedlings. He reported that he gave a high 2nd grade sapling yield value. Akman (2009), reported that in the production of seedlings in the combination of Tekirdağ Çekirdeksizi / 5BB, the second-grade seedlings yield was 14.84 in control, while the efficiency in mulch applications remained lower with 12.25.

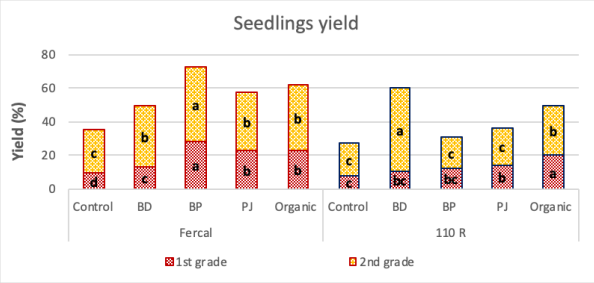

Figure 13
Effects of mulch applications on sapling yield (%).

4. Conclusion

Mulch practices had significant effects on all the characters examined and the overall efficacy rank varied according to the character and rootstocks. Vegetative development was more than 110R in Fercal. Stomatal conductance values were higher in 110R than Fercal, but when measurement times and applications were evaluated together, there was no stable situation in both rootstocks. While the leaf temperature increasing effect, order was determined as SP, OM and PJ, and the efficiency of increasing leaf chlorophyll content was listed as SP, PJ and OM. No stable variance was observed in soil temperature values, the activity from high to low was listed as BP and PJ and OM, BD.

In the Fercal grapevine rootstock, the leaf area was above the control in all applications and the efficacy order was determined as BP, PJ, OM, BD and Control, while the single value above the control in 110R was determined in the OM application.

While OM, BP and PJ applications increased the number of leaves in both rootstocks, others were in the same group with control.

The order of activity in increasing leaf weight was BP, PJ, OM, BD in Fercal. In the 110R rootstock, the effect of BD and PJ remained limited, while OM and BP increased leaf weights.

The effects of mulch applications on total and woody shoot length were listed as in Fercal, BP, OM and PJ, while BD and BD were in the same group with control. In the 110R rootstock, OM application increased the total and lignified shoot length, while the others were in the same group with control.

While the pruning residue weight increased in Fercal BP and PJ, OM applications significantly, BD was in the same group with the control. In the 110R rootstock, OM and BP applications increased the pruning residue weight, while PJ, BD and control were in the same group.

All the applications of mulch increased the level of shoot and root development. While the activity was ranked as BP, PJ and OM in Fercal, BD remained under control. In the rootstock 110R, only OM significantly increased the level of growth, while other applications were in the same group with control. While all applications increased in root development levels, the ranking was BD, OM, BP, PJ and Control.

BP, PJ, OM and BD applications in Fercalde, and OM and PJ applications in 110R increased the diameter of shoots. In Fercal root diameter was affected by the applications in the same way as shoots and the activity was listed as BP, PJ, OM and BD. In 110R, OM, PJ and BP applications increased the root diameter.

When the root fresh and dry weight were evaluated together, in Fercal BP and OM, and in 110R, OM, BP and BD came to the fore.

The efficiency of the applications for total seedling efficiency and 1st and 2nd grade seedlings efficiency were listed in Fercal in the same way (BP, OM, PJ and BD) and BB, OM, PJ and BP in 110 R.

Since, significant differences were found at the level of the examined characteristics in both rootstocks, it may be recommended to select mulches according to the effect expected from mulch applications in the future studies.

Acknowledgements

The authors are grateful to Selcuk University Research Funds (Project No: 20201020 as the master thesis of M.S.M. Fakhar) for supporting this study.

Conflict of interest

Z. Kara, M.S.M. Fakhar, declare that they have no competing interests.

5. References

Abramova L (1984) The use of black polyethylene in raising grape vine transplants. Horticultural Abstracts 54 (7):4374

Akman B (2009) Tekirdağ şartlarında Tekirdağ çekirdeksizi üzüm çeşidi fidanlık parsellerinde damla sulama yöntemiyle farklı sulama uygulamalarının fidan randımanı ve kalitesi üzerine etkileri. Yüksek Lisans Tezi, Namık Kemal Üniversitesi Fen Bilimleri Enstitüsü 50s

Arslan ZF, Uygur FN (2014) Sebze üretiminde sorun olan yabancı otlara karşı bazı yeni ve etkili yöntemler: malç tekstili, fırçalama aleti ve keser çapa. Bitki Koruma Bülteni 54 (3):219-232

Bakshi P, Wali VK, Iqbal M, Jasrotia A, Kour K, Ahmed R, Bakshi M (2015) Sustainable fruit production by soil moisture conservation with different mulches: A review. AJAR 10 (52):4718-4729

Chan K, Fahey D, Newell M, Barchia I (2010) Using composted mulch in vineyards-effects on grape yield and quality. IJFS 10 (4):441-453

Cowan JS (2013) The use of biodegradable mulch for tomato and broccoli productıon: crop yield and quality, mulch deterioration, and growers'perceptions. Ph.Doctor, Washıngton State Unıversıty, 198p

Curtis MA (2013) Influence of cover crop residue management on soil moisture, vine growth, and productivity in a pre-production vineyard in the Willamette Valley. Masters Thesis, Oregon State University, 95p

Dağ S (2017) Michele palieri \ 41B aşılı asma fidanı üretiminde farklı alıştırma süreleri ve siyah plastik malç uygulamasının fidan randımanı ve kalitesi üzerine etkileri. Yüksek Lisans Tezi, Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü 53s

Doğan G (2020) Trakya ilkeren üzüm çeşidinde sulama ve malç Uygulamalarının verim, kalite ve Ekofizyolojik özelliklere etkisi. Yüksek Lisans Tezi, Çukurova Üniversitesi Fen Bilimleri Enstitüsü 77s

Faostat (2020) Access date. http://wwwfaoorg/faostat/en/#data/QC: 30.06.2020

Ferrara G, Fracchiolla M, Al Chami Z, Camposeo S, Lasorella C, Pacifico A, Aly A, Montemurro P (2012) Effects of mulching materials on soil and performance of cv. Nero di Troia grapevines in the Puglia region, southeastern Italy. AJEV 63 (2):269-276

Hostetler GL, Merwin IA, Brown MG, Padilla-Zakour O (2007) Influence of geotextile mulches on canopy microclimate, yield, and fruit composition of Cabernet franc. AJEV 58 (4):431-442

Ingels C (1992) Sustainable agriculture and grape production. AJEV 43 (3):296-298

Kanber R, Tekinel O, Baytorun N, Kumova Y, Alagöz T (1991) Harran Ovası Koşullarında pamuk sulama aralığı ve su tüketiminin belirlenmesinde açık su yüzeyi buharlaşmasından yararlanma olanakları. . TC Başbakanlık GAP Kalkınma İdaresi Başkanlığı GAP Yayın (44):15-25

Kara Z, Sabır A, Yazar K, Akçay A (2017) Klinoptilolitik mikronize zeolit uygulamalarının asma anacı fidanlarının vegetatif gelişme ve kalitesine etkileri. Selçuk Tarım Bilimleri Dergisi 3 (2):253-260

Król-Dyrek K, Siwek P (2015) The influence of biodegradable mulches on the yielding of autumn raspberry (Rubus idaeus L.). Folia Horticulturae 27 (1):15-20

Küçükyumuk C (2009) Aşılı asma fidanı üretiminde farklı sulama aralıkları ve malç uygulamalarının fidan randımanı ve kalitesi üzerine etkileri. Doktora Tezi, Süleyman Demirel Üniversitesi, Fen Bilimleri Enstitüsü 188s

Mundy D, Agnew R (2002) Effects of mulching with vineyard and winery waste on soil fungi and botrytis bunch rot in Marlborough vineyards. N Z Plant Prot 55:135-138

Nauleau F (1997) Nouvelles techniques d'entretien des sols viticoles. Conséquences oenologiques Synthése de 5:188-190

Nazrala JJB (2008) Influencia del manejo del suelo y las coberturas vegetales en el microclima de la canopia de la vid, la composición de la uva y el vino. Rev Fac Cienc Agrar 40 (1):85-104

Ross OC (2010) Reflective mulch effects on the grapevine environment, Pinot noir vine performance, and juice and wine characteristics. Master of Applied Science, Lincoln University, 89p

Van der Westhuizen J (1980) The effect of black plastic mulch on growth, production and root development of chenin blanc vines under dryland conditions. SAJEV 1 (1):1-6

Van Huyssteen L, Weber H (1980) The effect of selected minimum and conventional tillage practices in vineyard cultivation on vine performance. SAJEV 1 (2):77-83

Watson (2006) Mulch softens the big dry. Australian Viticulture 10, 74 - 75

Wheeler SJ, Black A, Pickering G (2005) Vineyard floor management improves wine quality in highly vigorous Vitis vinifera'Cabernet Sauvignon'in New Zealand. New Zeal J Crop Hort:317-328

Zengin H (2019) Farklı malç uygulamalarının bazı asma anaçlarının gelişimi üzerine etkileri. Yüksek Lisans Tezi, Selçuk Üniversitesi, Fen Bilimleri Enüstitüsü 43s

Zenginoğlu M (2015) Açık köklü asma fidanı üretiminde farklı malç materyalleri ve gölgeleme oranlarının fidan randıman ve kalitesine etkileri. Yüksek Lisans Tezi, Gaziosmanpaşa Üniversitesi, Fen Bilimleri Enstitüsü 55s

Zhang Q, Wang S, Li L, Inoue M, Xiang J, Qiu G, Jin W (2014) Effects of mulching and sub-surface irrigation on vine growth, berry sugar content and water use of grapevines. AWM 143:1-8

Selcuk Journal of Agriculture and Food Sciences

http://sjafs.selcuk.edu.tr/sjafs/index

Review Article

SJAFS
(2020) 34 (2), 169-177
e-ISSN: 2458-8377
DOI:10.15316/SJAFS.2020.212

Doubled Haploid Production in Cereals Using Microspore Culture

Neslihan Doruk[1,*], Nejdet KANDEMİR[2], Sabri GÖKMEN[1]

[1]Selçuk University, Faculty of Agriculture, Department of Field Crops, Konya, Turkey
[2] Tokat Gaziosmanpaşa University, Faculty of Agriculture, Department of Field Crops, Tokat, Turkey

ARTICLE INFO

Article history:
Received date: 04.05.2020
Accepted date: 25.05.2020

Edited by:
Ali KAHRAMAN; Selçuk University, Turkey

Reviewed by:
Ertan SAit KURTAR; Selçuk University, Turkey
Selçuk KODAZ; Atatürk University, Turkey
Mustafa YILDIZ; Ankara University, Turkey

Keywords:
Albino plant
Co-cultivation
Embryo induction
Green plant
Microspore isolation
Pretreatment

ABSTRACT

Doubled haploids are extremely useful in plant breeding since they provide rapid homozygosity. However, the success rate of doubled haploid production in cereals is still not high enough, and there is a special problem involving the formation of high percentage of albino plants. Nevertheless, the success rate in microspore culture in cereals is higher than classical anther cultures, and the method has the advantage of spontaneous chromosome doubling. On the other hand, this method has some critical stages such as pretreatments and microspore isolation, and these stages need to be optimized for the successful use of the technique in plant breeding. For this aim, there have been studies in recent years about combining the pretreatment practices, supplementing growth media with a variety of ingredients, improving the various co-culturing practices and decreasing the albino plant percentage. This technique has been commonly used in the world especially in barley and wheat breeding. Improving the success rate of the technique will be useful for its integration into modern breeding techniques such as apomictic crops and transgenics.

1. Introduction

Haploids are plants that have half the number of chromosomes that a species normally has. These plants are mostly sterile since they can't form gametes. In order to avoid the sterility, doubled haploid plants are produced from haploids through doubling their chromosome numbers using various methods. These plants attaining homozygosity in just one generation have potential uses in plant breeding. However, their low production efficiency is a major obstacle for their common use in plant breeding (Devaux and Kasha 2009).

Haploid plants can naturally occur with apomixis mechanisms such as parthenogenesis and semigamy (Bashaw 1980). Artificially, they can be produced using relatively traditional techniques such as anther culture (Germana 2011), ovule or ovary cultures (Chen et al. 2011), chromosome elimination (Houben et al. 2011) and irradiated pollen (Sestili and Ficcadenti 1996) and using advanced methods such as microspore culture (Ferrie and Caswell 2011) and centromere engineering (Tek et al. 2015). The method of choice largely depends, among other factors, on the degree of success rates in the relevant crop species.

One of the methods that have been heavily researched in recent years for the production of doubled haploid plants in cereals is the microspore culture (Esteves and Belzile 2019; Wang et al. 2019; Zur et al. 2019). Being a more sophisticated method, microspore culture has a higher success rate since the impact of the pretreatment methods on the microspores used to divert them from the gametophytic to sporophytic pathway is direct without the protective tissues of the anther. Compared to anther culture, microspore culture was reported to increase plant regeneration by 5-200 times (Hoekstra et al. 1992; Davies and Morton 1998). The method also has additional advantages such as producing only haploid plants and having the ability of spontaneous chromosome doubling (Li and Devaux 2001). However, this method has many critical procedures compared to anther culture, and optimizing them is a necessity for successful use of the method in plant breeding.

* Corresponding author email: *neslihan.doruk@selcuk.edu.tr*

Microspore culture, one of the methods used to achieve rapid homozygosity in plant breeding, has strong potential to increase the effectiveness of plant breeding (Kasha et al. 2003; Li and Devaux 2001). The successful use of this method could significantly accelerate the breeding of pureline and hybrid varieties. However, such an effect can only be achieved by elaboration of many stages involved with this method. The aim of the present review is to introduce various critical stages of the microspore culture method and to discuss the ways to improve them with the current research.

2. Use of haploids or doubled haploids

Doubled haploid plants are 100% homozygous for all loci, and are very useful for plant breeding purposes. They could be used for rapid production of true-breeding lines from the segregating progeny of crosses, inbred lines in hybrid variety programs and doubled haploid mapping populations for quantitative trait mapping purposes (Rajcan et al. 2011). Homozygosity, traditionally achieved in 6-8 generations, is achieved in only one generation with doubled haploidy method (Jahne and Lörz 1994). Doubled haploids have been increasingly used in breeding of cereal species. For example, it has been estimated that about half of the registered barley varieties in Europe has been produced using doubled haploidy (Forster et al. 2007).

The haploidy or doubled haploidy method is used to produce interspecific hybrids for plant breeding. Cultivated potatoes with tetraploid genome composition produce sterile triploids when crossed to diploid wild species for an aim of transferring useful genes from them. However, when the chromosome number of potatoes is reduced to diploid level using haploidy method, resulting diploid plants could be easily crossed with diploid wild relatives and produce fertile seeds (Jansky 2006). The chromosome number of these diploid interspecific hybrids could be doubled to restore the original tetraploid genome of the cultivated potato. Thus, valuable genes could be introgressed to other potato varieties in future crosses with potato cultivated varieties.

Doubled haploid mapping populations from biparental crosses are commonly used for mapping the quantitative traits. Since they have all loci in homozygous conditions, their genetic analyses are more robust. Besides, since they could be multiplied indefinitely, doubled haploid lines allow producing seeds enough to plant rows or even plots to observe quantitative traits that cannot be observed in single plants such as lodging or grain yield. Thus, it could even be possible to conduct multilocational trials for more complex traits (Humphreys and Knox 2015).

Mutation or transgenic studies carried on haploid materials have the advantage of producing homozygous material. Thus, no extra generation is needed to convert heterozygous (mutants) or hemizygous (transgenics) plants into true breeding homozygous lines to observe the effects of the genetic changes created (Shariatpanahi and Ahmadi 2016).

3. Microspore development in plants

Microspore mother cells are formed in pollen sacs of the anthers. These cells with diploid (2n) chromosome sets undergo meiosis and give rise to four daughter cells with haploid (n) chromosomes called microspore (uninucleate stage). Then, each microspore nucleus (n) undergoes mitosis and forms pollen grain with two nuclei. For a great majority of the plants, the best microspore developmental stage for production of embryos (i.e. androgenesis) was reported to be mid-late uninucleate stage (Zheng 2003). Determination of the developmental stage of the microspores and using the ones in correct stage is crucial for the success of the microspore cultures. Nuclei in the microspores are observed to determine their developmental stages.

Appearance of plants at the best stage for microspore culture could change by the genotype. In barley, this is a stage at a point from the extension of the last internode between the flag leaf and penultimate leaf to the awn appearance, and is mostly when this internode is 2 cm. However, this appearance could vary by the growing conditions of donor plants such as fields, greenhouses or growth chambers. Therefore, it is a necessity to observe the best stage of microspores before conducting microspore culture for a set of plants. After such a determination, the plants can be judged based on their appearance only. For such an observation, anthers from the middle of the spikes of a series of plants with consecutive developmental stages (Figure 1) are extracted and squashed in an eppendorf tube containing 2% acetocarmine solution using a pipet tip. After a two-hour incubation period in acetocarmine, microspores are observed under a microscope. In uninucleate stage, only the nucleus is stained, and the remaining parts of the microspore are relatively clear. On binucleate stage, the second nucleus is generally diffused and rarely observed as a compact structure. However, this stage is easily distinguished by the staining of all microspore due to the starch accumulated (Figures 2 and 3).

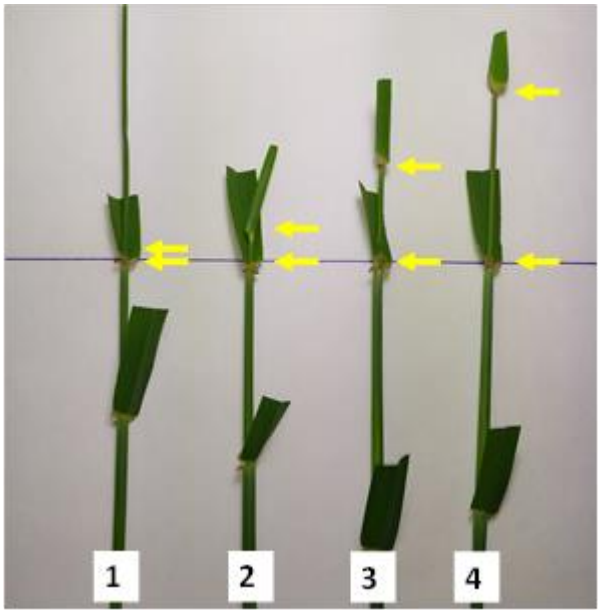

Figure 1
Determining the appropriate stage of spike development for microspore culture. After examination of acetocarmine-stained microspores, the best stage in which most of the microspores were at mid-late uninucleate stage was found to be 3.

4. Microspore culture procedure

Microspore culture is the method of isolating and culturing immature microspores on special media under aseptic conditions to produce plants with haploid chromosome numbers (Chen et al. 2011). It is a more elaborate procedure compared to anther culture and involves many critical stages. Among them are isolation and purification of microspores and determination of their number and viability. The success rate in microspore culture is dictated by factors such as genotype, growing conditions of donor plants, microspore isolation methods, pretreatments, culture media and growing conditions in tissue culture.

4.1. Genotype

As in all tissue culture procedures, genotype is a major determinant of the success of microspore culture. First of all, the responses of plant species to microspore culture are different, which could be extremely low in some species (Soriano et al. 2013). In general, androgenic response is not high in cereal crop species. Although the efficiency of doubled haploidy could be sufficient for plant breeding purposes in barley and wheat, success rate is not satisfactory with oat and rice (Ferrie and Caswell 2011). The androgenic response could also vary at genotype level within the species. Two-row barley genotypes were reported to have better response to microspore culture compared to six-row cultivars. Two-row winter barley cultivar Igri is used as standard in microspore culture studies of barley (Davies 2003). In wheat, on the other hand, cvs. Chris, Pavon 79 and Bob White are well known for their superior androgenic response (Kasha et al. 2003).

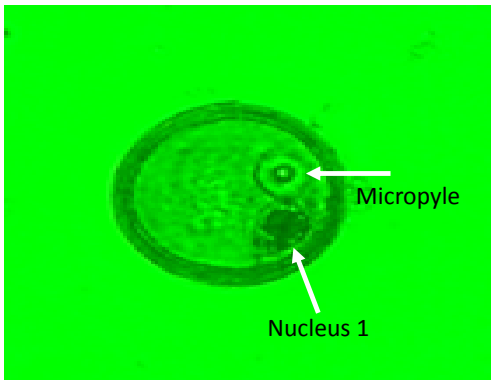

Figure 2
Late uninucleate stage when the single nucleus is close to microphyll opening and the rest of the microspore is not well stained. This stage is appropriate for microspore culture

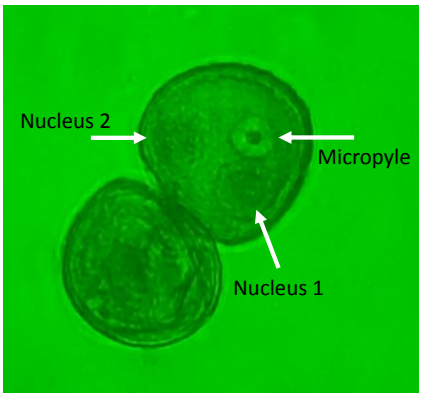

Figure 3
Binucleate stage where microspore is thoroughly stained due to starch accumulation. This stage is late for microspore culture.

4.2. Growing conditions of donor plants

Stimulating the microspores to induce embryos in vitro is highly dependent on the growing conditions of the plants from which the microspores are obtained. Donor plants need to be strong, healthy and devoid of all kinds of stress conditions. Environmental factors such as water status, diseases, temperature, moisture, aeration, photoperiod, light quality and intensity and plant nutrition during the period in which plants are grown can have an impact on the success of microspores obtained from those plants.

Donor plants for microspore culture can be grown in fields, greenhouses and growth chambers. Field conditions provide strong plants with high androgenic response compared to greenhouse grown plants (Ouyang et al. 1993), but they have a higher risk of contamination in tissue culture. Growth chambers are places with strictly controlled environmental conditions. However, due to their limited space, they may not be suitable for large scale studies. Climate-

controlled greenhouse conditions are generally places preferred for growing the donor plants.

Water stress during the growing period of donor plants could lead to smaller plants and microspores. Success rate for haploid plant production is low with this kind of small microspores (Ferrie and Caswell 2011). Similarly, plants should not be overwatered during their growth period.

Necessary precautions should be taken to prevent plants from getting sick and to protect them from pests. When diseases or pest infestations are encountered, the problem should be remedied as early as possible using appropriate chemicals.

Temperature preferences of cereal species during the growth periods vary considerably. When the donor plants of winter barley genotypes are grown at 15/12 °C day/night temperatures, their microspores give the best androgenic response, while 18/15 °C temperatures are better for spring barley cultivars (Kasha et al. 2003). For growing donor wheat plants, 18/15 °C day/night temperatures are optimal (Hu et al. 1995), whereas 24/20 °C are best for maize (Gaillard et al. 1991). A photoperiod regime of 16 hours light and 8 hours dark is appropriate for most species. The relative humidity in plant growing area should be about 70-80% (Jacquard et al. 2003). Optimal light density to grow donor plants for cereal microspore culture was reported to be about 18,000-20,000 lux (Foroughi-Wehr et al. 1976).

Under greenhouse conditions, plants should be grown in pots no smaller than 3-4 liters. A growing mix of soil, manure, perlite and peat is suitable. Some sand could be added to the mixture if the soil texture is heavy. The pots should be fertilized with nitrogen during or before planting the seeds. However, in order to prevent plants to suffer from nutrient deficiency, they should be fertilized weekly or fortnightly using fertilizers which have micronutrients or nutrient solutions such as Hoagland's.

4.3. Pretreatments

Microspores need to be exposed to some pretreatments in order to switch them from the pathway they are programmed for, which is the development of functional pollen, to another pathway of producing embryos. A number of different pretreatments could be employed to do this. Among them are cold, heat shock, starvation and high osmotic pressure treatments, various chemical pretreatments and their combinations (Oleszczuk et al. 2006). These pretreatments could be applied to tillers, spikes, anthers or microspores.

***Cold pretreatment*:** In this pretreatment method, tillers carrying the spikes are kept at 4 °C for 28 days (Esteves and Belzile 2014a). After the pretreatment period, spikes are taken from the tillers and surface sterilized (one minute at 70% alcohol, five minutes at 1% sodium hypochlorite solution containing a few drops of Tween-20, followed by four rinses with sterile distilled water). Then, microspores are isolated. Cold pretreatment was reported to produce 35.3 green plants per spike used in barley, but a high percentage of albino plants were observed (Esteves and Belzile 2014a). Cold pretreatment was found to increase androgenic response in other cereals such as maize, wheat and rice, and was commonly used in microspore cultures of these plants (Oleszczuk et al. 2006).

***High osmotic pressure*:** Spikes are stored in a chemical solution such as mannitol which exerts high osmotic pressure. For this aim, the spikes at the appropriate microspore developmental stage are surface sterilized, and immersed in a 0.3 M sterile mannitol solution. Asif et al. (2014) reported that the production of embryo-like structures and green plants were significantly higher in 9.1 g/l mannitol pretreatment compared to the control. Besides, green/albino plant ratio was also higher with this pretreatment. Mannitol pretreatment was also found useful in microspore cultures of rice (Raina and Ifran 1998), oats (Ferrie et al. 2014), rye (Zieliński et al. 2020) and triticale (Gland-Zwerger et al. 1994). It is common to combine mannitol pretreatment with heat shock or cold pretreatment methods.

***Heat shock pretreatment*:** Spikes exposed to heat shock of about 26-33 °C have better androgenic response. Heat shock pretreatment is usually applied after a cold pretreatment or in combination with other pretreatment methods such as high osmotic pressure, starvation or use of various chemicals (Oleszczuk et al. 2006). Liu et al. (2002) reported that success rates of chemical pretreatment methods were better when they were combined with heat shock pretreatment of 33 °C. In barley, green plant production rate of 35.3 per spike increased to 65.4 when the mannitol pretreatment was combined with a 48-hour heat shock treatment at 26 °C, and it was mentioned that this increase was due to decreased albino plant percentage (Esteves and Belzile 2014a).

***Starvation*:** Using media lacking nitrogen or sugars increases the androgenic response of the microspores. This treatment is also commonly used together with cold or high osmotic pressure pretreatment methods. This method was reported to be effective in recalcitrant genotypes (Oleszczuk et al. 2006).

Chemical pretreatments: It was reported that treating isolated microspores with some chemicals for short periods (38-52 hours) at temperatures around 25 °C increases the androgenic response of microspores. Colchicine is one of these chemicals successfully used to increase haploid plant production, which also doubles the chromosomes of the plants produced (Zhao et al. 1996). Zheng et al. (2001), on the other hand, used 10 different chemicals, including 2HNA, and reported that nine of them increased the microspore viability 1.5-2-fold, and produced almost twice as many green plants. Pechan and Keller (1989), on the other hand, stored rapeseed inflorescences in 15% ethanol for 2-4 days as a pretreatment, and observed that embryo production in microspore culture increased almost twice.

4.4. Microspore isolation methods

Microspore isolation is extracting the microspores from the anthers at the correct stage and culturing on growth media. Various methods could be employed to isolate the microspores. Some of them use blenders to homogenize spikes, while some macerate the anthers with a pestle, and some collect the microspores spontaneously shed into a storage solution. In blender method, the spikes are placed in blender's chamber along with about 20-25 ml cold mannitol solution (0.3 M), and blender is run at a low speed for 15-25 seconds. Blender chamber is washed with mannitol and the remaining microspores are collected. In maceration method, anthers are squashed using a glass or Teflon rod after pretreatment (Olsen 1991). In shed-microspore method, anthers are stored in a solution in petri dishes generally in combination with a heat shock pretreatment (25-32 °C), and they are spontaneously shed into the solution after storing for two-three days (Ziauddin et al. 1990). Vortex, sonication or magnetic stirrer could be used in all these methods for a better isolation of all microspores from the anthers.

The resulting slurry after using above-mentioned methods to release the microspores into a solution contains both microspores and spike pieces, and is passed through 100 µm nylon sieves. Microspores passing through the sieve and separated from plant debris is collected using centrifugation performed at about 100 RCF (relative centrifugal force) for four or five minutes. Microspore pellet is cleaned through resuspensions in 0.3 M mannitol for three or four times. Then, microspores are suspended in 21-23% maltose solution, and empty, dead or broken microspores are removed through a density gradient centrifugation at about 100 RCF for three to five minutes. Finally, microspores are suspended in 200 µl liquid induction medium. Microspore concentration is determined using a hemocytometer or by counting all microspores in a small volume (e.g. 20 µl), and the density is adjusted to 10^6 microspore/ml using liquid induction medium.

Quality and viability of the microspores need to be checked after the isolation. They could be small, empty or damaged during the isolation process. Sustaining the microspore viability is one of the critical factors affecting the success of the culture. There are some methods to check the quality of microspores. The most common ones are Tetrazolium test (TTC) and acetocarmine staining. Besides, iodine-potassium iodide and aniline blue methods could also be used.

In acetocarmine staining, a small amount of microspore preparation is added to a 2% acetocarmine solution and 30 minutes later microspores are observed under microscope (Malayeri et al. 2012). Living microspores are stained in red while dead or empty ones are not (Figure 4).

For tetrazolium test, 1% triphenyl tetrazolium chloride (TTC) dissolved in 5% sucrose is used. TTC solution is dropped on a microscope slide and a cover glass is put on it. Microspores are observed under a light microscope. Living microspores are stained in red while others remain colorless (Vizintin and Bohanec 2004).

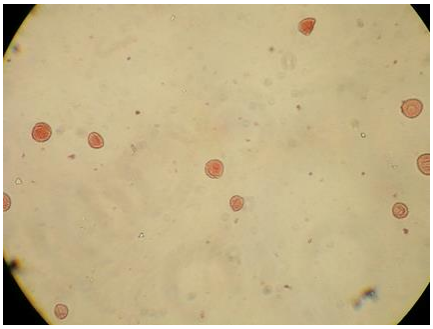

Figure 4
Acetocarmine-stained living microspores

4.5. Culturing the isolated microspores

Isolated microspore culture is basically a cell culture method, and its management in tissue culture is more challenging than other commonly used tissue culture explants. Microspores cannot survive on solid media, whereas they are submerged and cannot get enough oxygen in liquid media. In case of culturing in liquid medium, the cultures need to be aerated through continuous shaking, which necessitates an intensive use of shaker equipment, which may not be available in all laboratories. Instead, microspores are commonly cultured as small droplets placed directly on the bottom of petri dishes or on a solid media in a two-layer system (Jahne et al. 1994). A small amount of liquid medium (about 1 ml) containing microspores could also be spread on a 3 ml solid medium in a 5 cm petri dish. The density of the microspores is important for the success of the culture. About 100.000 microspores are cultured on a 5 ml petri dish (Esteves and Belzile 2014a).

4.6. Microspore growth media

Two types of growth media are used in microspore cultures: first, induction medium in which microspores are induced to produce somatic embryos, and then regeneration medium which is used to produce green plants from somatic embryos.

Induction medium

The induction medium is basically a modified Murashige Skoog basal medium containing low amounts of nitrogen (165 mg ammonium nitrate rather than 1,650 mg in regular MS medium) and 60 g maltose. The induction medium could include 32 g/l mannitol. In a two-layer induction medium system, the solid medium in the bottom is solidified using an agar, while the liquid medium containing the microspores does not include any solidifier. A cytokinin (e.g. 1 mg/l BAP or TDZ) and a small amount of auxin (e.g. 0.3 mg/l dicamba) are added to the induction medium (Esteves and Belzile 2014a). Besides, in order to help the viability of microspores in the culture, induction media are supplemented with 50 mg/l gum arabic and arabinogalactan proteins (Letarte et al. 2006). Microspores cul-

tured on induction medium are incubated under dark conditions at 28 °C for 20-30 days (Asif et al. 2014). Developing embryo-like structures (ELSs) are kept under light conditions (16 hours light in 24 hours, with a light intensity of 80 µmol m^{-2} s^{-1}) for two or three days. ELSs which show green segments are transferred to regeneration medium.

Regeneration medium

Regeneration media used in microspore culture are basically modified MS media, and have the same salts, vitamins and organic compounds as the induction media. They may have gum arabic and arabinogalactan proteins, but they do not have mannitol. They include 30 g maltose instead of 60 g/l, and have different hormone content. Regeneration media generally contain low levels of cytokinin (e.g. 0.2 mg/l BAP or 0.1 mg/l metatopoline) and auxin (e.g. 0.1 mg/l IAA) (Esteves and Belzile 2014b). ELSs developing in regeneration media is kept in growth chambers under light conditions. Two weeks after transferring to regeneration media, the number of green sectors in each petri dish is counted, and they are transferred to germination media, which are basically the same as the regeneration media except that they do not include any hormones. Two weeks later, green and albino plants are counted. This method could produce more than 100 green plants per spike in barley (Esteves and Belzile 2014b) and about 90 in wheat (Wang et al. 2019).

4.7. Co-cultivation

Although its exact mechanism is not known, co-cultivation of cereal microspores along with various flower parts were reported to increase the androgenic response of microspores (Oleszczuk et al. 2006). It was hypothesized that this effect could be due to hormone-like substances released from the ovaries (Köhler and Wenzel 1985) or due to small molecules released from immature pistils (Lipman et al. 2015). The whole floret, ovary or pistil could be used for co-cultivation. There is even a report that mentions the failure of wheat microspores to undergo cell division to produce ELSs without ovary co-cultivation (Patel et al. 2004). Co-cultivation with ovary was reported to cause 2.1- and 2.4-fold increases in ELS and green plant productions, respectively, in a recalcitrant barley cultivar (Li and Devaux 2001). In addition, co-cultivation of barley microspores with pistils cut and placed in microspore medium was reported to cause a five-fold increase in the number of embryogenic calli (Lipman et al. 2015). However, pistils needed to be changed in every four days. Microspores themselves in culture are known to exert a "self-feeder effect" and increase the androgenic response. Lipman et al. (2015) reported that when the microspore density was less than 1000 per milliliter medium, no embryogenic callus was produced.

4.8. Albinism problem in cereal microspore cultures

One of the major challenges with cereal microspore cultures is the formation of albino plants. Previously, albinism was thought to be due to the breakdown of plastid DNA (Caredda et al. 2000). However, now it has been understood that inactivation of the plastid ribosome is the culprit (Torp and Andersen 2009).

There are several factors which play roles in albinism in cereal cultures. One of them is the genotype. Durum wheats are known to have a higher degree of albinism (Zheng 2003). Differences were also reported among bread wheat cultivars (Broughton 2008). It was found that culturing microspores on solid media increases the albino plant percentage (Zheng 2003). The position of the spike in the plant is another factor affecting the albinism, and it was reported that the highest green plant percentage was obtained from the second tiller, which was the third spike on the plant after the main shoot and the first tiller (Jacquard et al. 2006). Embryo inductions under light conditions were reported to result in the formation of more than 90% albino plants (Ziegler et al. 1990). Microspore isolation method was mentioned to be one of the factors affecting albino plant percentage, and isolation using a glass rod and homogenizer caused significant decreases in albino plant production compared to microspore isolation carried out using blenders (Islam et al. 2013). Finally, pretreatment methods affect albinism considerably, and albino plants are more common in cold pretreatment (Oleszczuk et al. 2006).

In eliminating the albinism problem, modifying the pretreatment method seems to be the most feasible one. Use of a 48-hour heat shock at 26 °C instead of cold pretreatment reduced the albino plant production prominently (Esteves and Belzile 2014a). Makowska et al. (2017) reported that increasing the copper content of growing medium by 100 folds resulted in a higher number of total plant regeneration and lower percentage of albino plants. Wojnarowiez et al. (2004) found that use of osmotic stress agents decreased albino plant percentage, but there were differences among different osmotic stress agents such as sorbitol, mannitol, sucrose and PEG. Growth regulator composition of the medium could also be modified to alleviate the albinism problem. BAA + IAA combination was reported to be associated with production of fewer albino plants compared to Kinetin + 2,4-D combination in bread wheat (Broughton 2008).

4.9. Chromosome doubling

Haploid plants are sterile. Their chromosome numbers need to be restored to the regular chromosome number of the species to be used in plant breeding. In microspore culture, chromosome doubling mostly occurs spontaneously. It was reported that 75-85% of the plants produced in wheat or barley microspore cultures were spontaneously doubled. Cold pretreatments commonly used in cereal microspores were mentioned as the origin of spontaneous chromosome doubling (Oleszczuk et al. 2006). Spontaneous chromosome doubling is relatively less common in triticale (10-30%) and maize (4.5-6.5%) microspore cultures.

When the microspore culture-derived plants are haploids, their chromosomes can be doubled through

applying colchicine to their growing points. Colchicine prevents the formation of microtubules and chromosomes are not pulled to opposite poles. Thus, ploidy level increases. Ploidy levels of the plants could be determined by physical appearance of the plants, number of stomata underneath the leaves, cytogenetic observations, hemocytometer counting or DNA markers. A commonly used method is cytogenetic observation with a chromosome dye such as acetocarmine carried out on cells of root tips whose cell division is stopped at metaphase. Another robust method is counting the stomata since haploid plants have a higher number of smaller stomata compared to diploid plants.

4.10. Acclimatization

Plants produced in microspore culture are transferred to soil when they have enough roots to support them. However, they will die immediately when directly transferred to soil because they do not have cuticular wax on their leaves which protects them against the water loss and their capillary roots are not well developed yet. They need to be acclimatized first. For this aim, plantlets from the microspore culture are transplanted to a few cm thick water-saturated peat in a pot. Water is sprayed on them, and the top of the pot is sealed using a stretch film tightened with rubber bands. Three to four holes are made on the stretch film on the following day, and these holes are expanded during the next two or three days. Finally, acclimatized seedlings are transplanted to the pots where they will be grown to maturity.

In conclusion, microspore culture has a higher success rate for haploid plant production compared to anther culture. In anther culture, microspores could be protected by the surrounding anther tissues against the external shock of the pretreatments used to divert them into embryo formation. In microspore culture, however, microspores are directly exposed to such effects. Microspore culture also has the advantage of spontaneous chromosome doubling. The success rate for haploid plant production is still not satisfactory in some cereal species especially due to albino plant formation, and this problem should be remedied for an efficient use of the method in plant breeding programs. It is especially important to improve pretreatment methods and growth media compositions to sustain the viability of microspores in culture. Microspore cultures with better success rates would contribute to the modern plant breeding technologies such as apomixis and transgenics.

5. References

Asif M, Eudes M, Randhawa H, Amundsen E and Spaner D (2014). Induction medium osmolality improves microspore embryogenesis in wheat and triticale. *In Vitro Cellular and Developmental Biology - Plant* 50:121–126.

Bashaw EC (1980). Apomixis and its application in crop improvement. In: Fehr WR and Hadley HH (ed) Hybridization of Crop Plants, American Society of Agronomy Crop Science Society of America Soil Science Society of America, Madison pp. 45–63.

Broughton S (2008). Ovary co-culture improves embryo and green plant production in anther culture of Australian spring wheat (*Triticum aestivum* L.). *Plant Cell Tissue and Organ Culture* 95:185–195.

Caredda S, Doncoeur C, Devaux P, Sangwan RS, Clement C (2000). Plastid differentiation during androgenesis in albino and non-albino producing cultivars of barley (*Hordeum vulgare* L.). *Sexual Plant Reproduction* 13 (2): 95-104.

Chen J, Cui L, Malik AA, Mbira K (2011). In vitro haploid and dihaploid production via unfertilized ovule culture. *Plant Cell Tissue and Organ Culture* 104: 311–319.

Davies PA (2003). Barley isolated microspore culture (IMC) method. In: Maluszynski M, Kasha KJ, Forster BP, Szarejko I (eds.) Doubled haploid production in crop plants: A manual, Kluwer, Dordrecht, pp 49–52.

Davies P and Morton S (1998). A comparison of barley isolated microspore and anther culture and the influence of cell culture density. *Plant Cell Reports* 17: 206–210.

Devaux P and Kasha KJ (2009). Overview of barley doubled haploid production. In: Touraev A, Forster BP and Jain SM (eds.) Advances in Haploid Production in Higher Plants, Heidelberg, Springer: 47–63.

Esteves P and Belzile FJ (2019). Isolated microspore culture in barley. In: Harwood W. (ed.) Barley: Methods in Molecular Biology, vol 1900. Humana Press, New York, NY.

Esteves P and Belzile F (2014a). Improving the efficiency of isolated microspore culture in six-row spring barley: I-optimization of key physical factors. *Plant Cell Reports* 33 (6): 993–1001.

Esteves P, Clermont I, Marchand S, Belzile F (2014b). Improving the efficiency of isolated microspore culture in six-row spring barley: II-exploring novel growth regulators to maximize embryogenesis and reduce albinism. *Plant Cell Reports* 33 (6): 871–879.

Ferrie AMR and Caswell KL (2011). Isolated microspore culture techniques and recent progress for haploid and doubled haploid plant production. *Plant Cell Tissue and Organ Culture* 104: 301–309.

Ferrie A M R, Irmen KI, Beattie AD, Rossnagel BG (2014). Isolated microspore culture of oat (*Avena sativa* L.) for the production of doubled haploids: effect of pre-culture and post-culture conditions. *Plant Cell Tissue and Organ Culture* 116: 89–96.

Forster BP, Heberle-Bors E, Kasha KJ, Touraev A (2007). The resurgence of haploids in higher plants. *Trends in Plant Science* 12: 368–375.

Foroughi-Wehr, B, Mix G, Gaul H and Wilson H M (1976). Plant production from cultured anthers of *Hordeum vulgare*. *L. Z. Pflanzenzucht* 77: 198-204.

Gaillard A, Vergne, P, Beckert M (1991). Optimization of maize microspore isolation and culture conditions for reliable plant regeneration. *Plant Cell Reports* 10: 55 58.

Germana M A (2011). Anther culture for haploid and doubled haploid production. *Trends Plant Science* 104: 283–300.

Gland-Zwerger A, Javornik B, Bohanec B, Kreft I (1994). Production of doubled haploid lines through anther culture in barley and triticale. In: Javornik B and Bohanec B (eds.) Proceed. International Colloquium. Impact of Plant Biotechnology on Agriculture. December 5-7, Rogla, Slovenia: 9-14.

Hoekstra S, van Zijderveld M H, Louwerse J D, Heidekamp F, van der Mark F (1992). Anther and microspore culture of *Hordeum vulgare* L. cv Igri. *Plant Science* 86: 89-96.

Houben A, Sanei M, Pickering R (2011). Barley doubled-haploid production by uniparental chromosome elimination. *Plant Cell Tissue and Organ Culture* 104: 321–327.

Hu TC, Ziauddin A, Simion E, Kasha KJ (1995). Isolated microspore culture of wheat (*Triticum aestivum* L.) in a defined media. I. Effects of pretreatment, isolation methods, and hormones. *In Vitro Cell and Developmental Biology* 31: 79–83

Humphreys DG and Knox RE (2015). Doubled haploid breeding in cereals. In: Al-Khayri J, Jain S and Johnson D. (eds.) Advances in Plant Breeding Strategies: Breeding, Biotechnology and Molecular Tools, Springer, Cham.

Islam SSM, Ara I, Tuteja N, Subramaniam, S (2013). Efficient microspore isolation methods for high yield embryoids and regeneration in rice (*Oryza sativa* L.). *International Journal of Biological Science and Engineering* Vol: 7 No: 12.

Jacquard C, Wojnarowiez G, Clement C (2003). Anther culture in barley. In: *Doubled haploid production in crop plants*, Springer, Berlin, Heidelberg, Tokyo, pp. 21-28.

Jacquard C, Asakaviciute R, Hamalian AM, Sangwan R S, Devaux P, Clement C (2006). Barley anther culture: effects of annual cycle and spike position on microspore embryogenesis and albinism. *Plant Cell Reports* 25: 375–381.

Jahne A, Becker D, Brettschneider R, Lörz H. (1994). Regeneration of transgenic, microspore-derived, fertile barley. *Theoretical and Applied Genetics* 89 (4): 525-33.

Jansky S (2006). Overcoming hybridization barriers in potato. *Plant Breeding* 125 (1): 1-12.

Kasha KJ, Simion E, Miner M, Letarte J, Hu TC (2003). Haploid wheat isolated microspore culture protocol. In: Maluszynski M, Kasha KJ, Forster BP and Szarejko I (eds.) Doubled haploid production in crop plants: A manual, Kluwer, Dordrecht, pp 77–81.

Köhler F and Wenzel G (1985). Regeneration of isolated barley microspores in conditioned media and trials to characterise the responsible factor. *Journal of Plant Physiology* 121: 181–191.

Letarte J, Simion E, Miner M, Kasha J (2006). Arabinogalactans and arabinogalactan-proteins induce embryogenesis in wheat (*Triticum aestivum* L.) microspore culture. *Plant Cell Reports* 24: 691.

Li H and Devaux P (2001). Enhancement of microspore culture efficiency of recalcitrant barley genotypes. *Plant Cell Reports* 20: 475–481.

Lippmann R, Friedel S, Mock HP, Kumlehn J (2015). The low molecular weight fraction of compounds released from immature wheat pistils supports barley pollen embryogenesis. *Frontiers in Plant Science* 6: 498.

Liu W, Zheng MY, Polle EA, Konzak CF (2002). Highly efficient doubled-haploid production in wheat *Triticum aestivum* L. via induced microspore embryogenesis. *Crop Science* 42: 686–692.

Makowska K, Oleszczuk S, Zimny J (2017). The effect of copper on plant regeneration in barley microspore culture. *Czech Journal of Genetics and Plant Breeding* 53 (1): 17–22.

Malayeri B E, Noori M, Jafari M (2012). Using the pollen viability and morphology for fluoride pollution biomonitoring. *Biological Trace Element Research* 147: 315–319.

Oleszczuk S, Sowa S, Zimny J (2006). Androgenic response to preculture stress in microspore cultures of barley. *Protoplasma* 228: 95–100.

Olsen FL (1991). Isolation and cultivation of embryogenic microspores from barley (*Hordeum vulgare* L.). *Hereditas* 115: 255-266.

Ouyang J W, Zhou SM, Jia SE (1993). The response of anther culture to culture temperature in *Triticum aestivum*. *Theoretical and Applied Genetics* 66: 101–109.

Patel M, Darvey NL, Marshall DR, Berry JO (2004). Optimization of culture conditions for improved plant regeneration efficiency from wheat microspore culture. *Euphytica* 140: 197–204.

Pechan PM and Keller WA (1989). Induction of microspore embryogenesis in *Brassica napus* L. by gamma irradiation and ethanol stress. *In Vitro Cellular and Developmental Biology - Animal* 25: 1073–1074.

Raina SK and Irfan ST (1998). High-frequency embryogenesis and plantlet regeneration from isolated microspores of indica rice. *Plant Cell Reports* 17: 957–962.

Rajcan I, Boersma JG, Shaw EJ (2011) Plant genetic techniques: Plant Breeder's Toolbox. *Comprehen-*

sive Biotechnology (Second Edition), Volume 4. Elsevier.

Sestili S and Ficcadenti N (1996). Irradiated pollen for haploid production. In: Jain, SM, Sopory SK and Veilleux RE (eds.) In vitro haploid production in higher plants. Springer, Netherlands, pp. 263–274.

Shariatpanahi ME and Ahmadi B (2016). Isolated microspore culture and its applications in plant breeding and genetics. In: Anis M and Ahmad N (eds.) Plant Tissue Culture: Propagation, Conservation and Crop Improvement. Springer, Singapore.

Soriano M, Li H, Boutilier K (2013). Microspore embryogenesis: establishment of embryo identity and pattern in culture. *Plant Reproduction* 26: 181–196.

Tek A, Stupar R, Nagaki K (2015). Modification of centromere structure: a promising approach for haploid line production in plant breeding. *Turkish Journal of Agriculture and Forestry* 39 (4): 557-562.

Torp AM and Andersen SB (2009). Albinism in microspore culture. In: Touraev A et al. (eds.) Advances in haploid production in higher plants, Springer, New York.

Vizintin L and Bohanec B (2004). In vitro manipulation of cucumber (*Cucumis sativus* L.) pollen and microspores: Isolation procedures, viability tests, germination, maturation. *Acta Biologica Cracoviensia Series Botanica* 46: 177–183.

Wang HM, Enns JL, Nelson KL (2019). Improving the efficiency of wheat microspore culture methodology: evaluation of pretreatments, gradients, and epigenetic chemicals. *Plant Cell Tissue and Organ Culture* 139: 589–599.

Wojnarowiez G, Caredda S, Devaux P, Sangwan R, Clement C (2004). Barley anther culture: assessment of carbohydrate effects on embryo yield, green plant production and differential plastid development in relation with albinism. *Journal of Plant Physiology* 161: 747–755.

Zhao JP, Simmonds DH, Newcomb W (1996). Induction of embryogenesis with colchicine instead of heat in microspores of *Brassica napus* L. cv. Topas. *Planta* 198: 433-439.

Zheng MY, Liu W, Weng Y, Polle E, Konzak CF (2001). Culture of freshly isolated wheat (*Triticum aestivum* L.) microspores treated with inducer chemicals. *Plant Cell Reports* 20: 685–690

Zheng M Y (2003). Microspore culture in wheat (*Triticum aestivum*) – doubled haploid production via induced embryogenesis. *Plant Cell Tissue and Organ Culture* 73: 213–230.

Ziauddin A, Simion E, Kasha KJ (1990). Improved plant regeneration from shed microspore culture in barley (*Hordeum vulgare* L.) cv. Igri. *Plant Cell Reports* 9: 69-72

Ziegler G, Dressler K, Hess D (1990). Investigations on the anther culturability of four German spring wheat cultivars and the influence of light on regeneration of green vs. albino plants. *Plant Breeding* 105: 40-46.

Zieliński K, Krzewska M, Żur I, Juzoń K, Kopeć P, Nowicka A, Moravčiková J, Skrzypek E, Dubas E (2020). The effect of glutathione and mannitol on androgenesis in anther and isolated microspore cultures of rye (*Secale cereale* L.). *Plant Cell, Tissue and Organ Culture* 140: 577–592.

Zur I, Dubas E, Krzewska M, Zieliński K, Fodor J, Janowiak F (2019). Glutathione provides antioxidative defence and promotes microspore-derived embryo development in isolated microspore cultures of triticale (× *Triticosecale* Wittm.). *Plant Cell Reports* 38: 195–209.