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RESEARCH PAPER

HORTICULTURAL STUDIES

Determination of Oil Content and Fatty Acid Composition of Twenty-Six Pecan Cultivars Grown in Türkiye

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Abstract

The aim of this study was to investigate the total oil content and fatty acid composition of 26 pecan cultivars oils. Significant differences were observed between the total oil contents and fatty acid composition of pecan cultivars ($P<0.05$). The oil contents of the cultivars changed between 69.35 (Comanche) and 77.08% (Curtis). The fatty acid composition of the pecan oils ranged from 56.17 to 71.55% monounsaturated fatty acids, from 20.23 to 34.78% polyunsaturated fatty acids, and 7.34 to 9.49% saturated fatty acids. The major fatty acid was oleic acid (55.91-71.27%), followed by linoleic (19.38-33.45%), palmitic (5.05-6.68%), stearic (1.97-3.42%), linolenic (0.79-1.55%), 11-Eicosenoic (0.22-0.30%) and arachidic acids (0.10-0.33%), respectively. The highest oleic acid content was found in the Choctaw cultivar. Tejas and Western cultivars showed the highest levels of linoleic acid. As a result, the data of this study may contribute to future breeding programs and the food industry regarding the selection of pecans with improved health and nutritional quality. It is suggested that pecan oil should be consumed due to its high-unsaturated fatty acid content for health benefits.

1. Introduction

Pecan [*Carya illinoinensis* (Wangenh) C. Koch] belongs to the Juglandaceae family and is native to North America. It has been cultivated in many countries including USA, Mexico, Australia, South Africa, Israel, Argentina, and Brazil (da Parto et al., 2013). The South of Turkey has very favorable climatic characteristics for the pecan cultivation. Firstly, the pecan was brought to Türkiye in 1953 as a seed from the USA. Then, adaptation studies on pecans were started in Antalya with 14 cultivars brought from Israel through FAO in 1969 (Alkan et al., 2014). Recently, pecan cultivation areas have been increased especially in the Aegean, Mediterranean, and Southeast Anatolia Regions (Apak and Akşit, 2016). Pecans contain major nutrients, such as carbohydrates, proteins, dietary fiber, and fat. In addition, they are a rich source of phenolics, phytosterols, tocopherols, and minerals (Atanasov et al., 2018; Ribeiro et al., 2020). Pecans are known to have a higher oil content than many other nuts. The amount of pecan oil varies between 65 and 75% (Wakeling et al., 2001). Pecan oil is added to salad sauces and meals due to its distinctive flavor, and is also used as an ingredient in lotions, soaps, perfumes, and massage oils in the cosmetic industry (Salvador et al., 2016). Pecan oil is one of the healthy oils because of its high content of unsaturated fatty acids (UFAs) and low amounts of saturated fatty acids (SFAs) (Rivera-Rangel et al., 2018). The fatty acid composition of pecan oil generally consists of oleic, linoleic and linolenic, palmitic, and stearic acids (Jia et al., 2019). Consumption of oils rich in UFAs is associated with positive health effects. Recent studies have shown

that the consumption of monounsaturated fatty acids (MUFAs) can lower LDL (low-density lipoprotein) cholesterol, defend against coronary heart disease, modulate blood pressure, and may have notable effects on coagulation factors, inflammation, and endothelial activation (Rajaram et al., 2001; Alonso et al., 2006). Similarly, the intake of polyunsaturated fatty acids (PUFAs) in the daily diet has beneficial effects on reducing the risk of cardiovascular and inflammatory diseases (Finley and Shahidi, 2001). Besides, PUFAs have antithrombotic and antiatherosclerotic properties and may inhibit the development of some diseases such as arterial hypertension and insulin resistance (Simopoulos, 1991; Hermansen, 2000; Manco et al., 2004). Moreover, Garman et al. (2009) reported that PUFAs exhibit protective effects against diabetic kidney diseases. The amount and composition of pecan oil can vary widely depending on the cultivar, as well as environmental and agricultural practies such as growing region and conditions, harvest year, maturity state, and the age of trees (Toro-Vazquez, 1999; Alvarez-Parrilla et al., 2018). Although there are many studies on pecan cultivars in the world, few comprehensive studies are available about the fatty acid composition of pecan cultivars in Türkiye. This study aimed to determination of the total oil content and the fatty acid composition of the oils obtaining from twenty-six pecan cultivars grown in Antalya province.

2. Material and Methods

In this study, 26 pecan cultivars (Big Z, Bradley, Burkett, Cape Fear, Cheroke, Cheyenne, Choctaw, Comanche, Curtis, Desirable, Green River, Harris Super, Hasting, Mahan, Mohawk, Mahan*Stuart, Moneymaker, Royal, Schley, Shannee, Shoshoni, Stuart, Tejas, Western, Wichita, Woodard) grown in the Kayaburnu genetic resources parcel of the Batı Akdeniz Agricultural Research Institute in Antalya were used as materials. All cultivars were collected from mature trees (30 years old) in the 2018 and 2019 harvesting seasons (20 November-1 December). Twenty fruits per cultivar were used for each experiment.

2.1. Oil extraction

The fruits were shelled, and the edible parts were dried in a hot air oven at 70°C until reaching final dry matter content about 96% w.b. (weight basis). Dried samples were ground with a laboratory type miller (Retsch Grindomix GM200, Retsch GmbH & Haan, Germany) before oil extraction. Then, the oils were extracted with petroleum ether in a soxhlet fat extractor (E-500, Buchi, Flawil, Switzerland) for 3 hours.

The total oil content of pecan cultivars was expressed as a percentage of dry matter according to AOAC (2005).

2.2. Analysis of fatty acid composition

Firstly, fatty acid methyl esters (FAMEs) were prepared from pecan oils for the fatty acid composition analysis (Da Porto et al., 2012). Briefly, the oil samples (0.1 g) were mixed in a screw cap tube with 200 mL of methanolic potassium hydroxide (2N). Then, 2 mL of n-hexane was added. After separation of phase, the supernatant containing methyl esters was transferred into a vial for analysis.

Fatty acid methyl esters of samples were analyzed by gas chromatography (GC) system (Agilent 7890A) equipped with flame-ionization detector (FID) and mass spectrometry (Agilent 5975C). An HP Innowax capillary column (60.0 m*0.25 mm; film thickness: 0.25 µm) was used for the separation. Helium was used as a carrier gas at a flow rate of 0.8 mL min^{-1} and an average velocity was set to 19.314 cm sec^{-1}. The temperatures of the injector and detector were 250°C and 260°C, respectively. The column temperature program employed in the analysis was as follow; started from 150°C and raised to 200°C with an increment of 10°C per minute, hold at 200°C for 5 minute, then increased to 250°C with 5°C per minute increments and hold 250°C at 15 minute. The total analysis time was 35 minutes. The split ratio was 1:40 and the injection volume was 1 µL. Mass spectra were obtained by scanning in the mass range 35-450 AMU (Atomic Mass Unit) and the ionization mode used was electronic impact at 70 eV. The percentages of the fatty acid components were calculated from the integration of the peaks in FID chromatograms. The identification of the peaks was performed using WILEY7N, NIST05 and OIL ADAMS libraries of the MS (Mass Spectrometric) detector (Gölükcü et al., 2016).

2.3. Statistical analysis

The oil extractions were carried out in duplicate and the fatty acid analyses were performed in two replicates for each year. Results were expressed as mean values ± standard errors of two years. Data were subjected to analysis of variance using SAS software (Version 6.12, SAS Institute, Cary, NC, USA). The differences were determined by Duncan's Multiple Range Tests at $P<0.05$ confidential interval.

3. Results and Discussion

3.1. Oil contents of pecan cultivars

Total oil contents of pecan samples ranged from 69.35 to 77.08% (Figure 1). The highest oil content was found in Curtis (77.08%), followed by Stuart (75.67%), Green River (75.27%), Mohawk (75.23%) and Woodard (73.97%). The lowest oil content was obtained from the cultivar Comanche (69.73%).

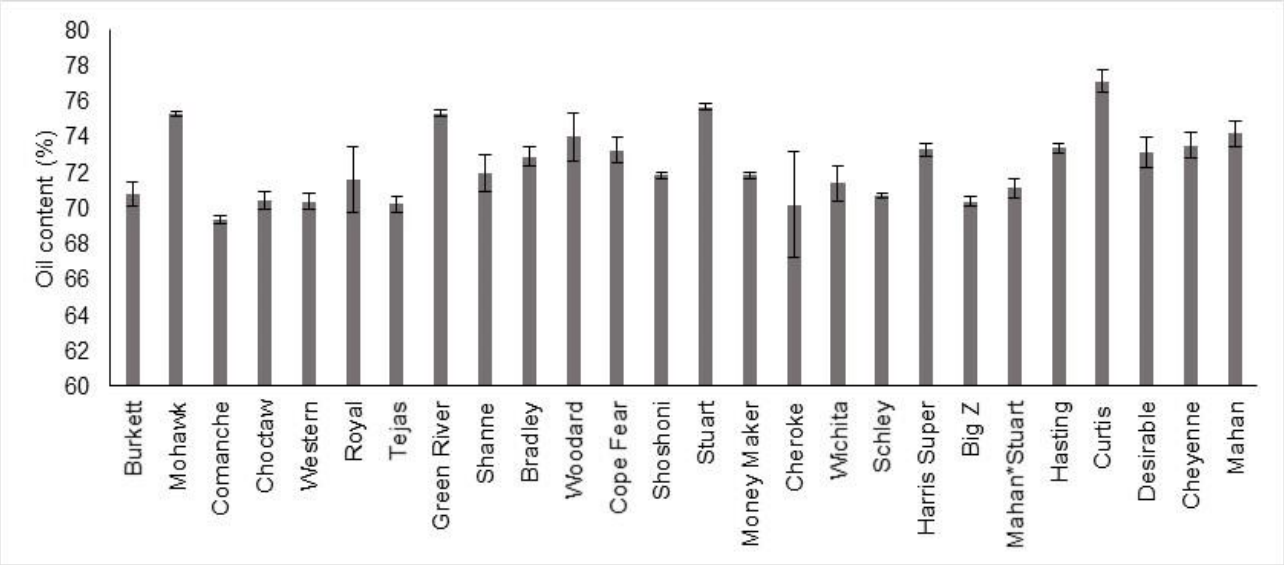

Figure 1. Total oil contents of 26 pecan cultivars.

Previously, Jia et al. (2019) reported the oil contents of fourteen pecan cultivars grown in China ranged from 70.1 to 79.7%. Oil content of desirable cultivar was reported as 66.18 % by Venkatachalam and Sathe (2006). Toro-Vazquez et al. (1999) reported total lipid contents in pecan kernels obtained from central Mexico was found between 70 and 79% on dry basis. In another study, Ribeiro et al. (2020) determined that the total lipid contents of eleven pecan cultivars varied between 52.69-69.76% grown in Brazil.

Our results are compatible with previous reports mentioned above. In addition, the pecan cultivars analyzed in the current study had a higher oil content than the other nuts (pistachios, hazelnuts, almonds, macadamias, pinenuts, walnuts, cashew nuts, Brazil nuts) reported in the literature (Yıldız et al., 1998; Amaral et al., 2006; Sathe et al., 2008; Venkatachalam and Sathe, 2006; Doğan and Akgül, 2005; Bakkalbaşı et al., 2010). The oil content and fatty acid composition of pecans can vary depending on cultivar, harvest year, growing location and condition, ripening, extraction method, soil type and climate (Wakeling et al., 2001; Villarreal-Lozoya et al., 2007; Bouali et al., 2013; Dominguez-Avila et al., 2013; Rivera-Rangel et al., 2018; Ribeiro et al., 2020).

3.2. Fatty acid composition

The UFAs and SFAs of oil from twenty-six cultivars are presented in Table 1 and Table 2, respectively. The results revealed that there are statistically significant differences in fatty acid composition among the pecan cultivars ($P<0.05$). Seven fatty acids were determined in the oils of pecans. The oils of all pecan cultivars was mainly composed of UFAs which account for 90.51-92.66% of total fatty acids (Table 1). The highest UFAs were determined in Cape Fear (92.30%), Harris Super (92.33%), Woodard (92.66%) and Shoshoni (92.57%) cultivars. Monounsaturated fatty acids were dominant over PUFAs in all pecan samples (Figure 2). The major MUFA in the cultivars was oleic acid which ranged between 55.91% (Tejas) and 71.27% (Choctaw). Oleic acid was also the predominant fatty acid detected in the analyzed pecan oils. The percentages of 11-Eicosenoic acid, another MUFA, were between 0.22% (Wichita) and 0.30% (Western and Burkett).

Polyunsaturated fatty acids contents of the cultivars changed between 20.23% and 34.78% (Figure 2). Polyunsaturated fatty acids detected in the analyzed oils of pecan cultivars were linoleic and linolenic acids. Linoleic acid was the most prevalent PUFA in the all pecan oils. Linoleic acid, also known as omega 6, is an essential fatty acid that cannot be synthesized by the animal and human organism. Therefore, it must be taken by the diet (Atanasov et al., 2018). The highest linoleic acid was found in Western (33.45%), the lowest was in Choctaw (19.38%). Linolenic acid content varied from 0.79% (Mahan*Stuart) to 1.55% (Wichita) (Table 1).

The percentages of SFAs (7.34-9.49%) were lower than the mono and polyunsaturated fatty acids (Table 2). Palmitic acid was determined to be the predominant SFA and ranged between 5.05 and 6.68%. Tejas had the highest palmitic acid content, while Bradley had the lowest palmitic acid content. In addition, stearic acid was the second SFA and changed between 1.97 and 3.42%. The contents of arachidic acid in the pecan oils varied between 0.10 and 0.33%, and that of the Curtis cultivar was significantly higher than the others ($P<0.05$).

Venkatachalam et al. (2007) found that the dominant fatty acid in 24 different pecan cultivars grown in the USA was oleic acid (52.52-74.09%), followed by linoleic (17.69-37.52%), palmitic (4.16-7.36%), stearic (1.00-3.15%), linolenic (0.65-1.64%) and arachidic acid (0.06-0.13%). Villarreal-Lozoya et al. (2007) reported that seven pecan

Table 1. Unsaturated fatty acid components (%) of 26 pecan cultivars*.

Cultivars	Linoleic acid (C:18:2)	Oleic acid (C:18:1)	Linolenic acid (C:18:3)	11- Eicosenoic acid (C:20:1)	ΣUFAs
Cheroke	30.75±4.20 ac	59.01±4.78 ej	1.35±0.12 ae	0.28±0.00 af	91.39±0.62 ad
Comanche	26.98±1.57 bg	63.26±1.66 ch	1.10±0.04 ci	0.26±0.002 cf	91.60±0.07 ae
Cape Fear	20.46±1.56 hi	70.72±1.74 ab	0.84±0.05 gi	0.28±0.004 af	92.30±0.15 a
Cheyenne	28.85±0.97 af	61.47±1.00 dj	1.01±0.004 di	0.29±0.01 ad	91.62±0.04 ae
Green River	25.32±0.27 ci	64.77±0.39 ag	1.14±0.02 bi	0.28±0.01 ae	91.51±0.11 ae
Harris Super	22.49±0.92 fi	68.73±0.95 ac	0.83±0.03 hi	0.28±0.01 af	92.33±0.08 a
Hasting	31.23±0.27 ac	58.83±0.36 fj	1.15±0.05 bh	0.25±0.008 eg	91.46±0.03 ae
Mahan*Stuart	19.90±0.54 i	69.58±0.18 ac	0.79±0.05 i	0.24±0.01 fg	90.51±0.42 f
Desirable	25.71±1.99 ch	65.04±2.14 ah	1.05±0.07 ci	0.27±0.007 af	92.07±0.35 ac
Mohawk	26.45±3.01 ch	64.15±3.46 ah	0.85±0.09 gi	0.28±0.009 ae	91.73±0.35 ad
Mahan	26.63±1.22 ci	63.05±1.35 ci	1.19±0.008 bg	0.24±0.008 fg	91.11±0.14 bf
Money Maker	25.32±0.23 ci	64.92±0.24 ag	1.06±0.01 ci	0.27±0.002 af	91.57±0.02 ae
Royal	30.09±1.39 ae	59.90±1.50 dj	1.38±0.07 ac	0.30±0.007 ab	91.67±0.07 ae
Shanne	24.14±0.93 di	66.32±0.68 ae	1.19±0.02 bg	0.29±0.009 ac	91.94±0.47 ac
Schley	23.90±1.40 ei	66.91±1.57 ad	0.91±0.09 fi	0.27±0.006 af	91.99±0.08 ac
Shoshoni	27.26±2.75 ag	63.92±3.03 bh	1.12±0.10 ci	0.27±0.01 af	92.57±0.20 a
Stuart	30.47±1.47 ad	59.27±1.98 gj	1.36±0.01 ad	0.27±0.01 af	91.37 ±0.65 ae
Tejas	33.29±2.55 ab	55.91±2.80 j	1.49±0.11 ab	0.26±0.01 df	90.95±0.16 cf
Western	33.45±1.89 a	56.18±1.83 ji	1.32±0.06 ae	0.30±0.008 a	91.25±0.13 bf
Wichita	31.47±0.94 ac	57.50±0.73 hj	1.55±0.22 a	0.22±0.002 g	90.74±0.19 ef
Woodard	29.86±0.84 ae	61.19±0.95 dj	1.32±0.07 ae	0.29±0.006 ad	92.66±0.05 a
Burkett	26.79±3.31 ch	63.63±3.59 bh	1.23±0.09 ae	0.30±0.008 a	91.95±0.21 ac
Bradley	23.88±3.69 ei	67.08±4.08 ad	1.00±0.08 ei	0.27±0.01 bf	92.23±0.33 ab
Big Z	31.74±0.69 ac	58.84±0.96 fj	1.16±0.13 bh	0.28±0.007 af	92.02±0.14 ac
Choctaw	19.38±2.06 i	71.27±2.20 a	0.85±0.06 gi	0.28±0.01 ae	91.78±0.15 ad
Curtis	22.07±0.72 gi	67.49±1.65 ae	1.33±0.38 ae	0.26±0.01 df	91.15±1.00 bf
Retention time (min)	18.923	18.156	19.973	21.591	

*Different letters within the same column show significant differences between pecan cultivars (*P<0.05*).

cultivars contained 53-75% oleic, 15-36% linoleic, 5-6% palmitic, 2-3% stearic and 1% linolenic acids. Ribeiro et al. (2020) determined 65.53–72.99% oleic acid, 16.27-22.50% linoleic acid, 5.64-6.53% palmitic acid and 2.46-4.67% stearic acid in oils from eleven pecan cultivars grown in Brazil. They also reported that the pecan cultivars were rich in UFAs. In the study done by Yılmaz et al. (2021) oleic acid and linoleic acids were determined as the major fatty acids in five pecan cultivars. The present study fatty acid results are generally consistent with these literature findings. On the other hand, Rivera-Rangel et al. (2018) reported that the predominant fatty acid in pecan varieties cultured in Chihuahua, Mexico was linoleic acid followed by oleic acid, which is different from our results. In another report, oleic acid contents (47.43-57.18%) found in pecan oils by Dominguez-Avila et al. (2013) were lower than our values. Yılmaz et al. (2021) determined linoleic acids for the Western cultivar as 29.81% which is lower than our result. Wakeling et al. (2001) determined average linolenic acid content to be %1.74 in Western Schley and Wichita pecans. This content was found to be higher compared to the values of our study. These differences can be explained by factors such as genotype, ripening, harvest year, climate, growing region, horticultural practices, oil extraction method, and soil type (Rudolph et al., 1992; Wakeling et al., 2001; Venkatachalam and Sathe, 2006; Dominguez-Avila et al., 2013; Bouali et al., 2013).

Regarding the fatty acid groups, it was revealed that the oils obtained from the pecan cultivars were rich in MUFA and PUFA and contained low levels of SFA (Figure 2). The significant variations were detected among the pecan cultivars in terms of fatty acid groups (*P<0.05*). Tejas and Western cultivars had the highest percentage of PUFAs, known as essential fatty acids, due to their high linoleic content. The highest MUFA was determined in the Choctaw cultivar with the highest oleic acid content. Also, the linoleic acid content of this cultivar had the lowest value. Several studies on pecan oil have reported an inverse relationship between oleic acid and linoleic acid contents (Domínguez-Avila et al., 2013; Ribeiro et al., 2020; Rivera-Rangel et al., 2018). This may be due to the fact that oleic acid is the biosynthetic precursor of linoleic acid (Toro-Vazquez, 1999; Domínguez-Avila et al., 2013). It is a desirable feature that oleic acid is higher than linoleic acid in edible oils since the oils rich in oleic acid have high oxidative stability (Alvarez-Parrilla et al., 2018). In this respect, pecan oil may be used in cooking applications. Many studies on fatty acids

Table 2. Saturated fatty acid components (%) of 26 pecan cultivars*.

Cultivars	Palmitic acid (C:16)	Stearic acid (C:18:0)	Arachidic acid (C:20:0)	ΣSFAs
Cheroke	6.44±0.09 ac	2.07±0.01 ef	0.10±0.002 b	8.61±0.11 cg
Comanche	5.81±0.12 ci	2.46±0.09 ce	0.13±0.006 b	8.40±0.03 di
Cape Fear	5.31±0.24 gk	2.27±0.09 cf	0.12±0.006 b	7.70±0.15 km
Cheyenne	6.18±0.03 af	2.10±0.06 ef	0.10±0.004 b	8.38±0.04 di
Green River	5.72±0.06 ej	2.65±0.13 c	0.12±0.002 b	8.49±0.06 di
Harris Super	5.08±0.11 jk	2.45±0.03 ce	0.14±0.008 b	7.67±0.08 km
Hasting	6.45±0.05 ac	1.97±0.002 f	0.12±0.00 b	8.54±0.05 ch
Mahan*Stuart	5.92±0.005 bg	3.42±0.40 a	0.15±0.01 b	9.49±0.42 a
Desirable	5.78±0.15 ci	2.03±0.03 ef	0.12±0.005 b	7.93±0.12 il
Mohawk	5.81±0.37 ci	2.34±0.01 cf	0.12±0.002 b	8.27±0.34 ej
Mahan	6.23±0.05 ae	2.55±0.10 cd	0.11±0.005 b	8.89±0.12 bd
Money Maker	5.76±0.02 di	2.55±0.02 cd	0.12±0.002 b	8.43±0.04 di
Royal	6.27±0.23 ae	1.96±0.12 f	0.10±0.004 b	8.33±0.11 dj
Shanne	5.89±0.15 bg	2.07±0.08 ef	0.10±0.004 b	8.06±0.06 fk
Schley	5.83±0.06 ch	2.07±0.009 ef	0.11±0.004 b	8.01±0.05 hk
Shoshoni	5.15±0.26 ik	2.17±0.08 df	0.11±0.004 b	7.43±0.17 lm
Stuart	6.43±0.19 ad	2.09±0.08 ef	0.11±0.002 b	8.63±0.10 cf
Tejas	6.68±0.16 a	2.26±0.05 cf	0.11±0.00 b	9,05±0,16 ac
Western	6.26±0.10 ae	2.37±0.07 cf	0.12±0.002 b	8.75±0,03 be
Wichita	6.54±0.07 ab	2.61±0.14 c	0.11±0.004 b	9.26±0.22 ab
Woodard	5.18±0.05 hk	2.04±0.02 ef	0.12±0.004 b	7.34±0.04 m
Burkett	5.35±0.56 gk	2.57±0.32 cd	0.13±0.01 b	8.05±0.23 gk
Bradley	5.05±0.35 k	2.59±0.03 cd	0.13±0.004 b	7.77±0.35 jm
Big Z	5.87±0.08 cg	2.00±0.01 f	0.11±0.002 b	7.98±0.10 hl
Choctaw	5.54±0.24 fk	2.56±0.13 cd	0.12±0.006 b	8.22±0.14 ek
Curtis	5.49±0.08 gk	3.03±0.07 b	0.33±0.21 a	8.85±0.08 bd
Retention time (min)	13.792	17.591	21.129	

*Different letters within the same column show significant differences between pecan cultivars (P<0.05).

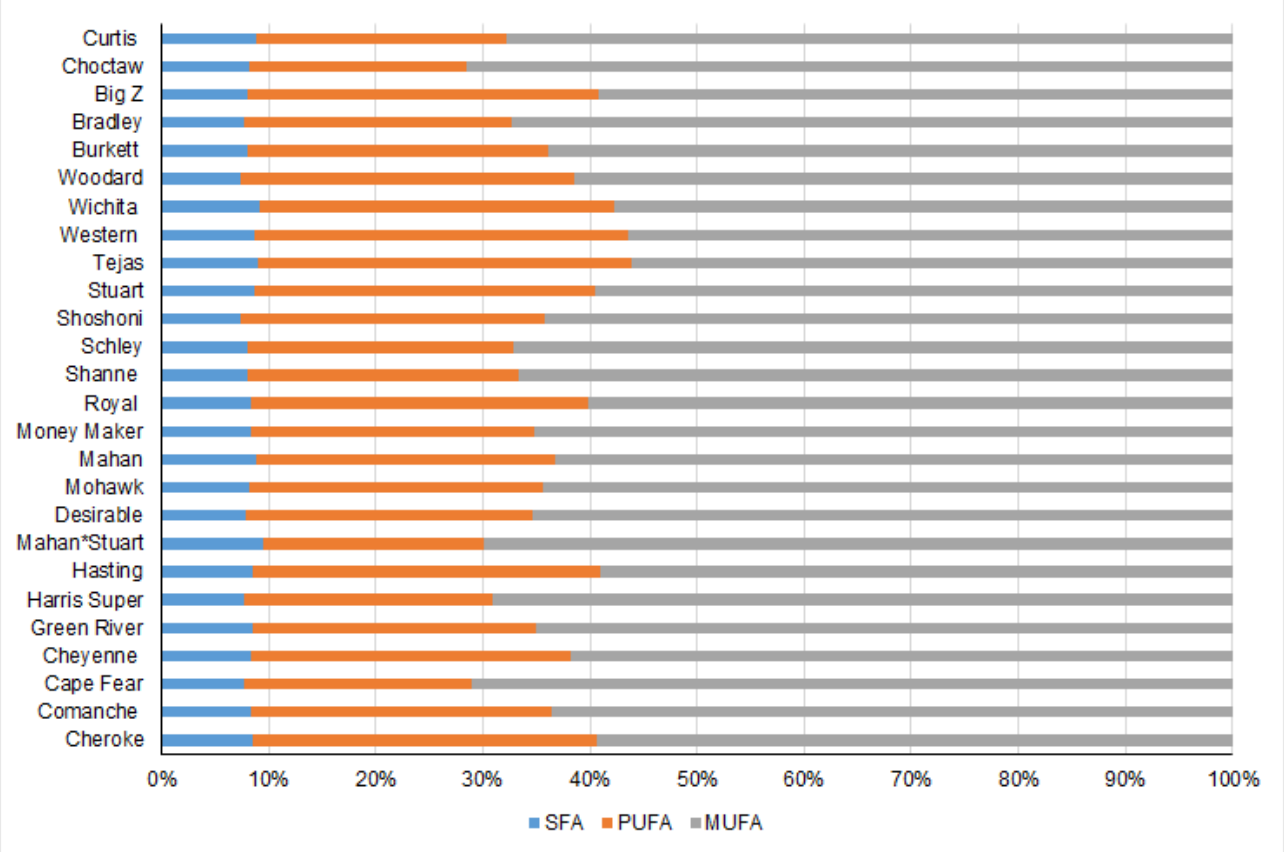

Figure 2. Fatty acid groups of 26 pecan cultivars (SFA: Saturated fatty acids, PUFA: Polyunsaturated fatty acids, and MUFA: Monounsaturated fatty acids).

suggested that diets containing MUFAs and PUFAs have a positive impact on the health of human (Simopoulos 1991; Alonso et al., 2006; Garman et al., 2009). However, high SFA consumption can cause chronic diseases such as obesity, diabetes and cancer (Ribeiro et al., 2020).

4. Conclusion

In this study, the oil contents and fatty acid compositions of 26 pecan cultivars grown in Türkiye were investigated. The results showed that there were significant differences between cultivars in terms of both oil contents and individual fatty acids. The oil contents and fatty acid compositions of pecan oils were generally similar to those previously reported in the literature. Curtis, Stuart, Green River, Mohawk, and Woodard cultivars had high oil content.

Seven fatty acids were detected in the oil samples including oleic, linoleic, palmitic, stearic, linolenic, 11-Eicosenoic and arachidic acids. More than 90% of the oils from the pecan cultivars consisted of UFAs, which protect against coronary heart disease and reduce LDL (low-density lipoprotein) cholesterol. Among the pecan cultivars, Choctaw had the highest MUFAs and oleic acid contents. High levels of PUFAs and linoleic acid were determined in Tejas and Western cultivars. As a result, the data of this study may contribute to future breeding programs and the food industry regarding the selection of pecans with improved health and nutritional quality.

It is suggested that pecan oil should be consumed due to its high unsaturated fatty acid content for health benefits. There is a need for studies investigating the usability of pecan in the food, cosmetic and pharmaceutical industries to improve the production and consumption of pecan in Türkiye.

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References

Alkan, G., Algül, B.E., & Dalkılıç, Z. (2014). Determination of germination speed of pecan seeds. *Journal of Adnan Menderes University Agricultural Faculty*, 11(2):1-6.

Alonso, A., Ruiz-Gutierrez, V., & Martínez-González, M. Á. (2006). Monounsaturated fatty acids, olive oil and blood pressure: epidemiological, clinical and experimental evidence. *Public Health Nutrition*, 9(2):251-257.

Alvarez-Parrilla, E., Urrea-López, R., & de la Rosa, L.A. (2018). Bioactive components and health effects of pecan nuts and their byproducts: A review. *Journal of Food Bioactives*, 1: 56-92.

Amaral, J.S., Casal, S., Citová, I., Santos, A., Seabra, R.M., & Oliveira, B.P. (2006). Characterization of several hazelnut (*Corylus avellana* L.) cultivars based in chemical, fatty acid and sterol composition. *European Food Research and Technology*, 222(3):274-280.

AOAC. (2005). Association Official Analytical Chemist, Official Methods of Analysis. 18th edition, Horwitz W., Latimer GW. (ed.), AOAC International, Gaithersburg, Maryland.

Apak, F.K., & Akşit, T. (2016). Natural enemies and population dynamics of the blackmargined aphid (Monellia caryella (Fitch) Aphididae, Hemiptera) on pecan trees in Aydın, Turkey. *Journal of the Entomological Research Society*, 18(3):49-60.

Atanasov, A.G., Sabharanjak, S.M., Zengin, G., Mollica, A., Szostak, A., Simirgiotis, M., Huminiecki L., Horbanczuk, O.K., Nabavi, S.M., & Mocan, A. (2018). Pecan nuts: A review of reported bioactivities and health effects. *Trends in Food Science & Technology*, 71:246-257.

Bakkalbaşı, E., Yılmaz, Ö.M., & Artık, N. (2010). Physical properties and chemical composition of some walnut cultivars grown in Turkey. *Akademik Gıda*, 8(1):6-54 (in Turkish).

Bouali, I., Trabelsi, H., Abdallah, I. B., Albouchi, A., Martine, L., Grégoire, S., Bouzaien, G., Gandour, M., Boukhchina, S., & Berdeaux, O. (2013). Changes in fatty acid, tocopherol and xanthophyll contents during the development of tunisian-grown pecan nuts. *Journal of the American Oil Chemists' Society*, 90(12):1869-1876.

Da Porto C., Decorti D., Tubaro F. (2012). Fatty acid composition and oxidation stability of hemp (*Cannabis sativa* L.) seed oil extracted by supercritical carbon dioxide. *Industrial Crops and Products*, 36:401-404.

De la Rosa, L.A. do Prado, A.C.P., Manion, B.A., Seetharaman, K., Deschamps, F.C., Arellano, D.B., & Block, J.M. (2013). Relationship between antioxidant properties and chemical composition of the oil and the shell of pecan nuts [*Carya illinoinensis* (Wangenh) C. Koch]. *Industrial Crops and Products*, 45:64-73.

Dogan, M., & Akgul, A. (2005). Fatty acid composition of some walnut (*Juglans regia* L.) cultivars from east Anatolia. *Grasas y aceites*, 56(4):328-331.

Domínguez-Avila, J.A., Alvarez-Parrilla, E., González-Aguilar, G.A., Villa-Rodríguez, J., Olivas-Orozco, G. I., Molina-Corral, J., Gómez-García, M.C., & de la Rosa, L.A. (2013). Influence of growing location on the phytochemical content of pecan (*Carya illinoinensis*) oil. *Journal of Food Research*, 2(5):143.

Finley, J.W., Shahidi, F. (2001). The chemistry, processing, and health benefits of highly unsaturated fatty acids: an overview. pp. 2–11. In: Omega-3 fatty acids, chemistry, nutrition and health effects, Shahidi, F., Finely, J.W. ed. American Chemical Society, Washington, DC: ACS.

Garman, J.H., Mulroney, S., Manigrasso, M., Flynn, E., & Maric, C. (2009). Omega-3 fatty acid rich diet prevents diabetic renal disease. *American Journal of Physiology-Renal Physiology*, 296(2):F306-F316.

Gölükcü, M., Toker, R., Tokgöz, H., & Cinar, O. (2016). The effect of harvesting time on seed oil content and fatty acid composition of some lemon and mandarin cultivars grown in Turkey. *Journal of Agricultural Sciences,* 22(4):566-575.

Hermansen, K. (2000). Diet, blood pressure and hypertension. *British Journal of Nutrition*, 83(S1):113-119.

Jia, X., Luo, H., Xu, M., Wang, G., Xuan, J., & Guo, Z. (2019). Investigation of nut qualities of pecan cultivars grown in China. *Journal of Plant Sciences*, 7(5):117-124.

Manco, M., Calvani, M., & Mingrone, G. (2004). Effects of dietary fatty acids on insulin sensitivity and secretion. *Diabetes, Obesity and Metabolism*, 6(6):402-413.

Rajaram, S., Burke, K., Connell, B., Myint, T., & Sabate, J. (2001). A monounsaturated fatty acid–rich pecan-enriched diet favorably alters the serum lipid profile of healthy men and women. *The Journal of Nutrition*, 131(9):2275-2279.

Ribeiro, S.R., Klein, B., Ribeiro, Q.M., Dos Santos, I.D., Genro, A.L.G., de Freitas Ferreira, D., Haman, J.J., Barin, J.S., Cichosk, A.J., Fronza, D., Both, V., & Wagner, R. (2020). Chemical composition and oxidative stability of eleven pecan cultivars produced in southern Brazil. *Food Research International*, 136:109596.

Rivera-Rangel, L.R., Aguilera-Campos, K.I., García-Triana, A., Ayala-Soto, J.G., Chavez-Flores, D., & Hernández-Ochoa, L. (2018). Comparison of oil content and fatty acids profile of Western Schley, Wichita, and native pecan nuts cultured in Chihuahua, Mexico. *Journal of Lipids*, 1-6.

Rudolph, C.J., Odell, G.V., Hinrichs, H.A., Hopfer, D.A., & Kays, S.J. (1992). Genetic, environmental, and maturity effects on pecan kernel lipid, fatty acid, tocopherol, and protein composition. *Journal of Food Quality*, 15(4):263-278.

Salvador, A.A., Podestá, R., Block, J.M., & Ferreira, S.R.S. (2016). Increasing the value of pecan nut [*Carya illinoinensis* (Wangenh) C. Koch] cake by means of oil extraction and antioxidant activity evaluation. *The Journal of Supercritical Fluids*, 116:215-222.

Sathe, S.K., Seeram, N.P., Kshirsagar, H.H., Heber, D., & Lapsley, K.A. (2008). Fatty acid composition of California grown almonds. *Journal of Food Science*, 73(9):C607-C614.

Simopoulos, A.P., Kifer, R.R., Martin, R.E., & Barlaw, S.M. (1991). Health effects of ω-3 polyunsaturated fatty acids. *World Review of Nutrition and Dietetics*, 66:39-43.

Toro-Vazquez, J.F., Charó-Alonso, M.A., & Pérez-Briceno, F. (1999). Fatty acid composition and its relationship with physicochemical properties of pecan (*Carya illinoensis*) oil. *Journal of the American Oil Chemists Society*, 76(8):57-965.

Venkatachalam, M., & Sathe, S.K. (2006). Chemical composition of selected edible nut seeds. *Journal of Agricultural and Food Chemistry*, 54(13):705-4714.

Venkatachalam, M., Kshirsagar, H.H., Seeram, N.P., Heber, D., Thompson, T.E., Roux, K.H., & Sathe, S. K. (2007). Biochemical composition and immunological comparison of select pecan [*Carya illinoinensis* (Wangenh.) K. Koch] cultivars. *Journal of Agricultural and Food Chemistry*, 55(24):9899-9907.

Villarreal-Lozoya, J.E., Lombardini, L., & Cisneros-Zevallos, L. (2007). Phytochemical constituents and antioxidant capacity of different pecan [*Carya illinoinensis* (Wangenh.) K. Koch] cultivars. *Food Chemistry*, 102(4): 241-1249.

Wakeling, L.T., Mason, R.L., D'Arc, B.R., & Caffin, N.A. (2001). Composition of pecan cultivars Wichita and western Schley [*Carya illinoinensis* (Wangenh.) K. Koch] grown in Australia. *Journal of Agricultural and Food Chemistry*, 49(3):1277-1281.

Yıldız, M., Gürcan, Ş.T., & Özdemir, M. (1998). Oil composition of pistachio nuts (*Pistacia vera* L.) from Turkey. *Lipid/Fett*, 100(3):84-86.

Yilmaz, R., Yildirim, A., Çelik, C., & Karakurt, Y. (2021). Determination of Nut Characteristics and Biochemical Components of Some Pecan Nut Cultivars. *Yuzuncu Yil University Journal of Agricultural Sciences*, 31(4):906-914.

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RESEARCH PAPER

Effect of Different Concentrations of IBA and Time of Taking Cutting on Propagation of Black and White Myrtle (*Myrtus communis* L.) Cuttings

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Abstract

This study aimed to evaluate the effect of different time of taking cutting and IBA concentrations on the rooting of *Myrtus communis* L. White and black myrtle types were used as plant materials. The cuttings were obtained from Batı Akdeniz Agricultural Research Institute (BATEM) collection garden. The effects of 0 (control), 500, 1000, 2000, 4000 and 8000 mg L^{-1} doses of IBA applications and time of taking cutting (February, March, April and May) on the rooting were investigated. The highest rooting percentage (76.67%) in black myrtle was obtained from cuttings taken in April and applied 1000 mg L^{-1} IBA, while the lowest rooting (10.00%) was obtained from cuttings applied 500 mg L^{-1} taken in May. White myrtle was rooted at a lower rate than black myrtle. It was determined that the highest rooting percentage (43.33%), shooting percentage (43.33%), rooted-shooted rates (43.33%) and average root number (1.63 pcs) in the white myrtle were 500 mg L^{-1} IBA dose in April. In addition, the study showed that the best rooting of white myrtle was in the cuttings taken in April and applied 500 mg L^{-1} IBA. Based on results, it can be concluded that time of taking cuttings in propagation and different concentration of IBA applications had a different impact on the success of black and white myrtle rooting.

1. Introduction

The myrtle (*Myrtus communis* L.) is one of the important medicinal plants used in traditional medicine in many parts of the World. *Myrtus communis* L. belongs to the family of Myrtaceae. The myrtle plant also known as true myrtle, is a perennial, evergreen shrub or small tree, typical of the Mediterranean region. In Türkiye, it spreads from the lowest altitude up to 600 meters in the Mediterranean, Aegean and Marmara Regions. Myrtle is also known by names such as erduran, erdüren, sazak, zazak, murt, mort. Naturally grown myrtle plant is resistant to both diseases and pests as well as drought and soil stress factors. This feature ensures that myrtle is grown organically (Uzun et al., 2014). The leaves and berries are a source of essential oils that have medicinal, insecticidal and sensory value (Khosh-Khui et al., 1984; Mulas et al., 1996; Milhau et al., 1997). Myrtle fruits are white and black coloured. White myrtles, which are called hambeles, are mostly consumed mainly as fresh fruit in Türkiye. Wild myrtles found in nature are grafted with hambeles or grown as border trees on the edges of the land. The black myrtle fruits are mainly consumed as fresh fruit (edible), dried fruit and fruit tea, and are also used in marmalade and jam production (Baytop, 2007; Alım and Uzun, 2017). The myrtle leaf and fruit are widely used as traditional folk medicine to treat many disorders and diseases. Its fruits, leaves, and seeds have a significant amount of phenolic substances, anthocyanins and antioxidant compounds (Alım et al., 2019).

Generally, myrtles grown wild in nature are grafted with white myrtle. In addition, this type of myrtle is grown as a border tree on the edges of the land. In our country, it is produced in the form of a closed garden in a very limited area. The demand for the myrtle fruits has increased in recent years because of their antioxidant and phenolic compound content. It was determined that especially black myrtle contains more phenolic substances than white fruit. Myrtle fruits naturally grown in Turkey were collected from nature by local people and it was sold at the district bazaars and herbalists in the coastal parts of the Mediterranean region (Şan et al., 2015a; Alım and Uzun, 2017; Alım, 2020).

Myrtle is generally propagated by seeds or woody stem cuttings (Ruffoni et al., 2010). Propagation with cuttings is widely used due to its advantages such as being simple and easy to apply, obtaining many seedlings from a unit area and obtaining high-quality seedlings with the same genetic structure as the rootstock plant. Differences in root ability of cuttings are due to anatomical, physiological, biochemical and environmental factors (Babaie et al., 2014). Cutting is at the moment the easiest and cheapest technique for mass propagation and production of plants more uniform and genetically similar to the genitors (Hartman et al., 2011). As in most fruit trees that are propagated by woody cuttings, physiological stage of the mother plant and the type of growth regulators are very important factors for the success of rooting cutting. In addition, the time of cuttings taken is very important in rooting yield in woody plants, but the optimum time for rooting must be established individually for each species (Howard, 1996; Chojnowska, 2004; Elgimabi, 2008).

For general use, for cuttings propagation of the majority of plant species, treatment with auxins is recommended (Davis and Haissig, 1990). Auxin has an effect on speed and increases the percentage of rooting of the stem cuttings. Plants produce natural auxin in young shoots and leaves, but synthetic auxin should be used for successful rooting to cuttings (Stefanic et al., 2007; Kasim and Rayya, 2009). IBA is a synthetic growth regulator, which belongs to the class of auxins (Hartmann and Kester, 2002). Treatment with rooting stimulators and especially IBA is generally effective in increasing the rooting rate of cuttings and making the root system stronger (Kaşka and Yılmaz, 1990).

The myrtle plant provides raw material for the pharmaceutical, cosmetic, ornamental and food industries (Medda and Mulas, 2021). Myrtle fruits and leaves, which are used as raw materials, are collected from wild plants and natural areas. This situation puts pressure on myrtle grown in the natural area. The increase in demand for myrtle in recent years creates uncontrolled pressure on wild plants. Therefore there is a great need for this plant to be produced as a standard and serial. The rooting ability of myrtle varies considerably, and some growers state that the plant is difficult to root (Mulas et al., 1996; Klein et al., 2000). However, limited studies have been made on the possibilities of producing myrtle plants with cutting, which is easier and more suitable for mass production. So far, studies on the propagation of myrtle species are not enough. The purpose of the present study was to improve the rooting of black and white myrtle cuttings by determination of the best concentration of IBA and time of cutting cultivation under mist propagation.

2. Material and Methods

2.1. Material

The study was carried out in the Batı Akdeniz Agricultural Research Institute Aksu campus rooting greenhouse in 2018. The distance of the research area to the sea is 20 km and the altitude is 46 m. In the province of Antalya, Türkiye where the trial area is located, summers are hot and dry, and winters are warm and rainy. Antalya 4th Regional Directorate of Meteorology has determined the average temperature as 19.65°C, the highest temperature of 32.51°C, the lowest temperature of 9.32°C, the annual average precipitation as 52.67 mm and the average relative humidity as 71.81% in 2018 (Table 1) (Anonymous, 2018).

In this research white (Hambeles) and black (Yakup) myrtle genotype which was not registered taken from the collection garden in the Aksu campus of the Batı Akdeniz Agricultural Research Institute, were used as plant materials (Figure 1). The fruits of hambeles are larger and have fewer seeds compared to the black fruits. Black myrtle fruits contain many (16 pieces fruit^{-1}) and hard seeds. Also, its fruits are more aromatic (Uzun et al., 2016).

2.2. Preparation of cuttings

Cuttings were taken from 5-year-old plants grown in the BATEM collection garden in four periods (February, March, April and May) in 2018. It was prepared from 1-year shoots (woody cuttings), which were about 15 cm long, bearing 3-4 leaves and eyes on the cuttings. Before planting date, all cuttings were soaked in 50% captan solution.

2.3. IBA treatment

In this study, 0 (control), 500, 1000, 2000, 4000 and 8000 mg L^{-1} doses of IBA doses were applied to the cuttings taken at different time of taking cutting. Before the applications, 250 mL 8000 mg L^{-1} IBA stock solution was prepared. For this purpose, 2000 mg L^{-1} of IBA (Sigma-57310) in pure powder form was weighed into a 250 mL measuring cylinder on precision scales and 30 mL of ethyl alcohol (96%) was added to dissolve. After the powder was

Table 1. Some climate data of Antalya 4th Regional Directorate of Meteorology in 2018.

Months	Average temperature (°C)	Highest temperature (°C)	Lowest temperature (°C)	Precipitation (mm)	Average relative humidity (%)
January	10.8	20.9	1.7	93.0	72.2
February	12.8	21.2	3.4	91.0	83.0
March	15.0	25.8	6.8	94.0	78.9
April	18.5	35.2	6.7	2.0	68.7
May	23.2	35.6	11.9	19.0	66.2
June	25.5	38.0	16.3	65.0	72.8
July	28.5	43.3	18.2	18.0	65.8
August	28.0	40.8	17.2	0.0	71.2
September	25.9	40.7	15.2	13.0	65.1
October	20.4	35.5	7.2	24.0	67.3
November	15.7	31.5	7.2	57.0	72.5
December	11.5	21.6	0.0	156.0	78.0
Mean	19.6	32.5	9.3	52.6	71.8

Figure 1. Black and white myrtles.

dissolved, it was completed to 125 ml with 96% ethanol and then to 250 ml with distilled water. For each application 500, 1000, 2000, 4000 and 8000 mg L^{-1} IBA were prepared from the stock solution. In the control application, distilled water was applied. It was applied to IBA cuttings by fast dipping method and 1 cm of the cuttings from the bottom were immersed in IBA solutions for 5 seconds. For each type and time of taking cutting, they were planted 2.5 × 2.0 cm apart in plastic crates (410 × 620 × 255 mm) filled with propagation medium (peat and perlite 1:1 v/v).

The crates were placed on rooting tables irrigated with a fogging system. The fogging system was set to operate for 5 seconds at 10 minute intervals throughout the experiment. In the study, 2 cultivars (white and black myrtles), 4 period (February, March, April, May), 6 doses 0 (control), 500, 1000, 2000, 4000 and 8000 mg L^{-1}, 3 replications and 10 cuttings in each replication, a total of 1440 cuttings were used. The cuttings were cut on the 7th day of February, March, April and May. The rooting percentage (%), the shooting percentage (%), the rooted-shooted rate (%) and the average number of roots (pcs) were determined in the cuttings removed four months after planting. The rooting percentage was found by dividing the number of rooted cuttings to the total number of cuttings. Shooting rate is the number of shooting cuttings by dividing the total number of cuttings. The average root number was found by counting the roots formed in the cuttings.

2.4. Statistical analysis

The research was established according to the Randomized complete block design, with 3 replications. The significance of treatment means was tested by one-way analysis of variance (ANOVA) on the transformed square root of rooting percentages, shooting percentages and rooted-shooted rates. Where significant differences were found ($P<0.05$) treatment means were compared using LSD test (Düzgüneş et. al., 1987).

3. Results and Discussion

Rooting percentage, shooting percentage, rooted-shooted rates and average root number of cuttings were taken in February, March, April and May and IBA was applied at doses (500, 1000, 2000, 4000 and 8000 mg L^{-1}) in black and white myrtles. The results were given in Table 2 and 3. The interaction of time of taking cutting and IBA dosages was determined as important as $P<0.05$ level in terms of rooting rate, shooting rate, rooted-shooted cutting rate and average root number at black and white myrtles.

The highest rooting percentage of black myrtle cuttings in 1000 mg L^{-1} IBA application taken in April were determined as 76.67% (Figure 2). The lowest value was determined in the cuttings taken in May and applied 500 mg L^{-1} (10.00%) IBA application (Figure 2). Rooting percentage decreased in

Table 2. Rooting percentage (%), shooting percentage (%), rooting-shooting rates (%) and average root number (pcs) according to time of taking cutting and IBA doses in black myrtle.

Parameters	IBA doses (mg L^{-1})	Time of taking cutting				
		February	March	April	May	Mean
Rooting percentage (%)	Control	40.00 (5.95) dh*	60.00 (7.68) af	66.67 (8.12) ac	26.67 (5.14) h	48.34
	500	46.67 (6.76) ah	63.33 (7.95) ad	73.33 (8.53) ab	10.00 (2.54) i	48.33
	1000	36.67 (5.95) dh	63.33 (7.79) ae	76.67 (8.75) a	33.33 (5.51) gh	52.50
	2000	40.00 (6.20) ch	73.33 (8.55) ab	36.67 (5.89) eh	36.67 (5.95) dh	46.67
	4000	73.33 (8.54) ab	66.67 (8.12) ac	53.33 (7.27) ag	33.33 (5.70) fh	56.67
	8000	66.67 (8.07) ac	66.67 (8.12) ac	56.67 (7.38) ag	46.67 (6.72) bh	59.17
	Mean	50.56	65.56	60.56	31.11	51.95
Shooting percentage (%)	Control	60.00 (7.67) ac	60.00 (7.69) ac	66.67 (8.12) ab	26.67 (5.14) f	53.34
	500	60.00 (7.69) ac	66.67 (8.15) ab	73.33 (8.53) ab	10.00 (2.54) g	52.50
	1000	60.00 (7.67) ac	66.67 (8.02) ab	66.67 (8.12) ab	33.33 (5.51) ef	56.67
	2000	60.00 (7.59) ad	73.33 (8.55) ab	36.67 (5.89) cf	36.67 (5.95) cf	51.67
	4000	76.67 (8.75) a	70.00 (8.35) ab	56.67 (7.47) bd	33.33 (5.70) df	59.17
	8000	70.00 (8.25) ab	66.67 (8.12) ab	56.67 (7.38) ae	46.67 (6.72) bf	60.00
	Mean	64.45	67.22	59.45	31.11	55.56
Rooted-shooted rates (%)	Control	40.00 (5.95) dg	60.00 (7.68) ae	66.67 (8.12) ab	26.67 (5.14) g	48.34
	500	43.33 (6.51) bg	63.33 (7.95) ac	73.33 (8.53) ab	10.00 (2.54) h	47.50
	1000	33.33 (5.51) fg	63.33 (7.79) ad	66.67 (8.12) ab	33.33 (5.51) fg	49.17
	2000	36.67 (5.95) cg	73.33 (8.55) a	36.67 (5.85) dg	36.67 (5.95) cg	45.84
	4000	73.33 (8.54) ab	66.67 (8.12) ab	53.33 (7.27) af	33.33 (5.70) eg	56.67
	8000	66.67 (8.07) ab	66.67 (8.12) ab	56.67 (7.38) af	46.67 (6.72) ag	59.17
	Mean	48.89	65.56	58.89	31.11	51.11
Average root number (pcs)	Control	1.33 jm	3.80 cg	2.60 fj	0.80 lm	2.13
	500	1.53 ıım	4.53 ad	2.83 ei	0.20 m	2.27
	1000	2.07 hl	4.67 ad	3.30 dh	1.60 im	2.91
	2000	2.27 hl	5.60 ab	1.73 ıl	1.37 im	2.74
	4000	4.20 be	5.17 ac	2.27 hl	1.03 km	3.17
	8000	4.03 cf	5.70 a	2.47 gk	1.50 ıım	3.43
	Mean	2.57	4.91	2.53	1.08	2.77

* IBA dosage × time of taking cutting interaction is indicated with small characters (a) (*P<0.05*). While grouping the percentage values, the square root values written in parentheses were taken as basis.

Table 3. Rooting percentage (%), shooting percentage (%), rooted-shooted rate (%) and average root number (pcs) according to time of taking cutting and IBA doses in white myrtle.

Parameters	IBA doses (mg L^{-1})	Time of taking cutting				
		February	March	April	May	Mean
Rooting percentage (%)	Control	3.33 (1.05) gh*	0.00 (0) h	23.33 (4.80) ad	10.00 (3.59) bg	9.17
	500	3.33 (1.05) gh	16.67 (4.02) af	43.33 (6.57) a	10.00 (2.54) ch	18.33
	1000	6.67 (2.10) dh	8.33 (1.66) eh	10.00 (2.54) ch	3.33 (1.05) gh	7.08
	2000	3.33 (1.05) gh	16.67 (4.02) af	33.33 (5.51) ab	1.82 (0.00) eh	13.33
	4000	6.67 (1.49) fh	12.50 (3.53) bg	20.00 (4.37) ae	13.33 (3.59) bg	13.13
	8000	3.33 (1.05) gh	8.33 (2.35) dh	10.00 (2.54) ch	26.67 (5.14) ac	12.08
	Mean	4.44	10.42	23.33	10.56	12.19
Shooting percentage (%)	Control	13.33 (3.60) ad	0.00 (0.00) e	16.67 (4.04) ad	10.00 (3.60) ad	10.00
	500	3.33 (1.05) de	20.83 (4.40) ac	43.33 (6.57) a	10.00 (2.54) be	19.37
	1000	10.00 (2.54) be	12.50 (2.85) be	13.33 (2.88) be	3.33 (1.05) de	9.79
	2000	3.33 (1.05) de	16.67 (3.22) bd	33.33 (5.52) ab	0.00 (0.00) e	13.33
	4000	10.00 (1.83) ce	12.50 (3.54) ad	20.00 (4.37) ac	13.33 (3.60) ad	13.96
	8000	3.33 (1.05) de	8.33 (2.36) be	10.00 (2.54) be	26.67 (5.14) ab	12.08
	Mean	7.22	11.81	22.78	10.56	13.09
Rooted-shooted rates (%)	Control	3.33 (1.05) ef	0.00 (0.00) f	16.67 (4.04) ae	10.00 (3.60) ae	7.50
	500	3.33 (1.05) ef	16.67 (4.02) ae	43.33 (6.57) a	10.00 (2.54) bf	18.33
	1000	6.67 (2.10) cf	8.33 (1.67) df	10.00 (2.54) bf	3.33 (1.05) ef	7.08
	2000	3.33 (1.05) ef	12.50 (2.85) bf	33.33 (5.52) ab	0.00 (0.00) f	12.29
	4000	10.00 (1.83) df	12.50 (3.54) ae	20.00 (4.37) ad	13.33 (3.60) ae	13.96
	8000	3.33 (1.05) ef	8.33 (2.36) cf	10.00 (2.54) cf	26.67 (5.14) ac	12.08
	Mean	5.00	9.72	22.22	10.56	11.86
Average root number (pcs)	Control	0.03 ef	0.00 f	0.57 cf	0.67 bf	0.32
	500	0.17 df	0.83 bd	1.63 a	0.20 df	0.72
	1000	0.30 df	0.25 df	0.30 df	0.17 df	0.24
	2000	0.07 ef	0.37 df	1.33 ab	0.30 df	0.43
	4000	0.27 df	0.41 df	0.70 be	0.63 cf	0.48
	8000	0.10 ef	0.42 df	0.60 cf	1.17 ac	0.55
	Mean	0.16	0.31	0.86	0.51	0.46

* IBA dosage × time of taking cutting interaction is indicated with small characters (a) (*P<0.05*). While grouping the percentage values, the square root values written in parentheses were taken as basis.

Figure 2. Black myrtle cuttings taken in April and applied 1000 mg L^{-1} IBA.

a

b

Figure 3. Black myrtle cuttings taken in February and applied 4000 mg L^{-1} IBA (a) and white myrtle cuttings taken in April and applied 500 mg L^{-1} IBA (b).

cuttings taken in later periods. The lowest value in both of them was in the cuttings taken in May (31.11%). When evaluated in terms of creep rate, the highest was in the 4000 mg L^{-1} (76.67%) application taken in February, the lowest was in the cuttings taken in May and 500 mg L^{-1} (10.00%) IBA application (Figure 3a). The highest shooting percentage was in February (64.45%), March (67.22%) and April (59.45%) in black myrtle. The lowest shooting rate was taken in May (31.11%). The rate of rooted-shooded rate cutting was highest in 2000 mg L^{-1} (73.33%), 4000 mg L^{-1} (66.67%) in March. The lowest rooted-shooted rate was determined in the cuttings applied (10.00%) 500 mg L^{-1} IBA taken in May. Rooted-shooted rates were found to be significant in the time of taking cuttings. The rate of rooted-shooted cutting decreased as the time of taking cutting progressed, and it was obtained at least in May (31.11%). The highest rooted-shooted cutting rate was in the cuttings taken in March (65.56%) in black myrtle. We have also measured root number of the cutting during in rooting experiment. The data showed significant differences in root number of the cutting which ranged from 0.2 to 5.7 pcs (Table 2). The average root number of black myrtle increased in parallel with the increase in IBA dose in cuttings taken in February and March. In our study, the highest average number of roots was obtained from the cuttings taken in March and applied 8000 mg L^{-1} (5.70 pcs) IBA. The lowest average root number was 500 mg L^{-1} in May (0.20 pcs). As the time of taking cutting progressed, the average root number of the cuttings decreased. It was determined that the highest average root number was in March (4.91 pcs) and the lowest was in the cuttings taken in May (1.08 pcs).

In terms of rooting rate, white myrtle was found to be lower than black myrtle. It was determined that the highest rooting rate (43.33%), shooting rate (43.33%), rooting-shooting rate (43.33%) and average root number (1.63 pcs) in the white myrtle

were 500 mg L^{-1} IBA dose in April (Figure 3b). In this myrtle type, there were no rooting or shooting in the control cuttings taken in March and the 2000 mg L^{-1} IBA application taken in May. The lowest average root shooted rate results were in control (0.00%) in February, 2000 mg L^{-1} IBA (0.00%) in May.

Application of IBA concentration and date of cutting collection can affect and promote root formation of hardwood cuttings in some genotypes by influencing the endogenous auxin and carbohydrate contents of the tissues. Furthermore, a correlation among polarity, root differentiation, and auxin movement can be made (Hartman, 1985; Hartmann and Kester, 2002). It was reported that the sampling time had a clear effect on the rooting percentage of the myrtle cuttings and the weld of the cuttings was negligible. In the study conducted by the researchers, while approximately 70% of the cuttings taken during the winter months (December-February) were rooted, 20% of the cuttings taken in May-August showed rooting (Klein et. al., 2000). Additionally, Abd El Hameed (2018), in his study on *Myrtus communis*, recommended the application of 4000 mg L^{-1} IBA dose to terminal cuttings taken in March and September. The lowest values were obtained from base cuttings without IBA applied in June and December. Our results confirmed the findings that time of taking cutting and IBA applications were important factors affecting plant propagation. Additionally, Pignatti and Crobeddu (2005) reported that applied 0.80% NAA in powder formulation to *Myrtus communis* cuttings. Holcomb and Michalas (1992) reported that 58.30-88.90% of the cuttings of *Myrtus communis* taken from mature plants and 86.10-91.70% of the cuttings taken from young plants were rooted. Researchers stated that *Myrtus communis* is suitable for mass production with young material. In addition, peat:perlite mixture was found to be superior to peat alone in promoting myrtle rooting. It is known that both genotype and growth regulators significantly affect rooting in myrtle. Şan et al. (2015b) reported that in myrtus in vitro study, higher rooting was obtained than our results. In the study, the explants were rooted in ½MS containing indole-3-butyric acid (IBA), naphthalene acetic acid (NAA) and activated charcoal (AC). IBA applications induced more rooting than NAA. The medium supplemented with 1.0 mg L^{-1} IBA + 2.0 g L^{-1} AC resulted in the highest rooting ratio (80%). Addition of AC into the medium resulted in slight increase in the rooting ratio, significant increase in shoot length, and reduced darkening in the rooting area. Acclimatization was successful for 86% of the rooted plants. Hatzilazarou et al. (2001) investigated that rooting capability of two M. communis clones, with large and small leaves. Shoots transferred to WPM medium supplemented with various concentrations (0, 0.5, 1.0 or 2.0 µM) of IBA, IAA or NAA in rooting assays. According to this study, the best rooting was achieved with the application of 0.5 µM IBA (96% rooting) and 1.0 µM IAA (100% rooting) for large leaves and small leaves, respectively.

Similar results to our results were found in studies conducted on different species. Palanisamy and Pramod (1997) stated that in *Pongamia pinnata*, rooting was best in March cuttings and IBA induced 100% rooting. Tewfik (2002) showed that 6000 mg L^{-1} IBA concentration has the highest rooting percentage in Nemaguard peach. It has been reported that the cutting time and IBA doses are important in the success of rooting. Meanwhile, Thirunavoukkarasu and Brahmam (1999) reported that auxin treatments stimulated sprouting of *Enterolobium cyclocarpum* cutting in but only cuttings prepared in January, March responded for rooting. On the other hand, Ercisli et al. (2002), in their study on kiwi fruits, the highest rooting percentage, maximum root length and number were obtained with 6000 mg L^{-1} IBA application. In addition, cuttings taken in February were rooted better than in January. Houle and Babeux (1998), stated that IBA significantly increased percentage of rooting and root length of *Pinus ocarpa* and increased enhanced the number of roots /cuttings and average roots length. A similar effect has also been observed by Ingle (2008) in Stevia. According to the results, on rooting of Stevia that increasing the concentration of IBA from 50 to 500 mg L^{-1}, caused an increase in rooting percentage, root number, root length and fresh weight of root. In addition, De Souza and De Lima (2005) obtained the best rooting percentages for *Prunus* cuttings at 2000 mg L^{-1} IBA concentration. Siddiqui and Hussain (2007) examined the effect of IBA applications (between 0-5000 mg L^{-1}) on the rooting of *Ficus Hawaii*. The researchers showed that the maximum root length and number, maximum shoot and leaf number per cutting were obtained at 4000 mg L^{-1} IBA concentration.

4.Conclusion

When both myrtle types were evaluated together, the rooting rate, shooting rate, rooted-shooted cutting ratio and average root number changed between time of taking cutting and IBA applications. Rooting ability of black myrtle was higher than white myrtle. Rooting properties of black myrtle decreased as time of taking cutting was delayed. The best time of taking cutting for white myrtle was April. As a result, IBA applications of 4000 and 8000 mg L^{-1} in February, 2000, 4000 and 8000 mg L^{-1} in March, 500, 1000 ve control in April can be recommended for black myrtle propagation by cuttings. In addition, propagation was suitable with black (control) cuttings taken in April and not applied IBA. In the production of myrtle with white, 500 mg L^{-1} IBA can be applied to the cuttings taken in April. In further studies, it is aimed to investigate

different applications to increase the propagation rate of myrtle**.**

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References

Abd El Hameed, N.S. (2018). Effect of indole butyric acid (IBA), cutting type and planting date on cuttings rooting of *Myrtus communis*. *Middle East Journal of Agriculture Research*, 7(3):1135-1145.

Alım, E., & Uzun, H.İ. (2017). Effects of GA3 applications on seedlesness and fruit quality in black myrtle (*Myrtus communis* L.). *Horticultural Studies*, 34(2):113-121.

Alım, E., Uzun, H.İ., & Cinar, O. (2019). Effects of GA_3 applications on biochemical properties in black myrtle (*Myrtus communis* L.) fruits. *Acta Horticulturae*, 1254:295-302.

Alım, E. (2020). The effects of the gibberellic acid applications to the seedless and fruit size of black myrtle (*Myrtus communis* L.). PhD Thesis, Akdeniz University, Antalya, Türkiye.

Anonymous, (2018). Meteoroloji 4. Bölge Müdürlüğü, Antalya.

Babaie, H., Zarei, H., Nikde, K., & Najar Firoozjai, M. (2014). Effect of different concentrations of ıba and time of taking cutting on rooting, growth and survival of *Ficus binnendijkii* 'Amstel Queen' cuttings. *Notulae Scientia Biologicae* 6(2):163-166.

Baytop, T. (2007). Türkçe Bitki Adları Sözlüğü. Türk Dil Kurumu Yayınları, 515 s, Ankara (in Turkish).

Chojnowska, E. (2004). The effect of cutting dates, temperature and different rooting stimulators on the rooting of cuttings of aubrieta cultoru. *Ejpau*, 7(2):04.

Davis, T.D., & Haissig B.E. (1990). Chemical control of adventitious root formation in cuttings. *Plant Growth Regulation Society America Quarterly*, 18:1-17.

De Souza, F.X., & De, Lima, R.N. (2005). Rooting of cuttings of different hog plum stock plants treated with IBA. *Revista Ciência Agronômica*, 36:189-194.

Düzgüneş, O., Kesici, T., Kavuncu, O., & Gürbüz, F. (1987). Araştırma ve Deneme Metotları (İstatistik Metotları II). Ankara Üniversitesi Ziraat Fakültesi Yayınları, 295 s, Ankara (in Turkish).

Elgimabi, M.E.N.E. (2008). Effect of season of cutting and humidity on propagation of (*Ixora coccinea*). *Advances in Biological Research*, 2(5-6):108-110.

Ercisli, S., Anapali, O., Esitken, A., & Sahin, U. (2002). The effects of IBA, rooting media and cutting collection time on rooting of kiwifruit. *Gartenbauwissenschaft*, 67(1):34-38.

Hartman, W. (1985). Investigations on propagating ability of self-rooted, Buhler prune using soft-wood cultivars. *Deutsche Baumschule*, 37(10):418-420.

Hartmann, H.T., & Kester, D.E. (2002). Plant Propagation: Principles and practices. 7th Ed. *Prentice Hall*, Upper Saddle River, NJ, 980 p.

Hartman, H.T., Kester, D.E., Davies, J.F.T., & Geneve, R.L. (2011). Plant Propagation: principles and practices, 8th Ed. Boston: *Prentice-Hall,* 915 p.

Hatzilazarou, S., Grammatikos, H., Economou, A.S., Rifaki, N., & Ralli, P. (2001). Rooting in vitro and acclimatization of *Myrtus communis* microcuttings. *Acta Horticulturae*, 616:259-264.

Holcomb, E.J., & Michalas, P.J. (1992). Myrtle as a flowering potted plant. Bull. *Pennsylvania Flower Growers*, 413:5-7.

Houle, G., & Babeux, P. (1998). The effects of collection date IBA, plant gender, nutrient availability, and rooting volume on adventitious root and lateral shoot formation by *Salix planifolia* stem cuttings from the ungava Bay area (Quebec, Canada). *Canadian Journal of Botany* 76(10):1687-1692.

Howard, B.H. (1996). Relations between shoot growth and rooting of cuttings in three contrasting species of ornamental shrub. *Journal of Horticultural Science*, 71:591-605.

Ingle, M.R. (2008). Effect of growth regulators and environments on rooting of Stevia cuttings (*Stevia rebaudiana* Bertoni). MSc Thesis, University of Agricultural Sciences, India.

Kasim, N.E., & Rayya, A. (2009). Effect of different collection times and some treatments on rooting and chemical in terminal constituents of bitter almond hardwood cutting. *Journal of Agricultural and Biological Science*, 5(2):16-122.

Kaşka, N., & Yılmaz, M., (1990). (Hartmann H.T. ve D.E. Kester' den çeviri) Bahçe Bitkileri Yetiştirme Tekniği, Ç.Ü. Ziraat Fakültesi Yayınları: 79, Ders Kitabı: 52, Adana, 247 s. (in Turkish).

Khosh-Khui, M., Shekafandeh, A., & Azarakhsh, H. (1984). Micropropagation of myrtle (*Myrtus communis* L.). *Scientia Horticulturae*, 22:139-146.

Klein, J.D., Cohenb, S., & Hebbe, Y. (2000). Seasonal variation in rooting ability of myrtle (*Myrtus communis* L.) cuttings. *Scientia Horticulturae*, 83(1):71-76.

Medda, S., & Mulas, M. (2021). Fruit quality characters of Myrtle (*Myrtus communis* L.) selections: Review of a domestication process. Sustainability, 13: 8785.

Milhau, G., Valentin, A., Benoit, F., Mallie, M., Bastide, J.M., Pelissier, Y., & Bessiere, J.M. (1997). In vitro antimalarial activity of eight essential oils. *Journal of Essential Oil Research*, 9:329-333.

Mulas, M., Cani, M.R., & Pank, F. (1996). Variability of rooting ability of softwood cuttings in myrtle germplasm. *Proceedings of The International Symposium on Breeding Research on Medicinal and Aromatic Plants*, 2(1):191-194.

Palanisamy, K., & Pramod, K. (1997). Seasonal variation of adventitious rooting in branch cuttings of *Pongamia pinnata* Pierre. *Indian Forester*, 123(3):236-239.

Pignatti, G., & Crobeddu, S. (2005). Effects of rejuvenation on cutting propagation of Mediterranean shrub species. *Forest* 2(3):290-295.

Ruffoni, B., Mascarello, C., & Savona, M. (2010). In vitro propagation of ornamental myrtus (*Myrtus communis*). In: Jain S.M., Ochatt S.J. (eds) protocols for in vitro propagation of ornamental plants. *Springer Protocols*, Humana Press, pp. 257-270.

Siddiqui, M.I., & Hussain, S.A. (2007). Effect of indole butyric acid and types of cutting on root initiation of *Ficus hawaii*. *Sarhad Journal of Agriculture*, 23(4):919-925.

Stefanic, M., Stamper, F., Veberic, R., & Oster, G. (2007). The level of IAA, IAAsp and some phenolics in cherry rootstock, Gisela5, leafy cutting pretreated with IAA and IBA. *Scientia Horticulture*, 112:399-405.

Şan, B., Karakurt, Y., & Dönmez, F. (2015a). Effects of Thidiazuron and Activated Charcoal on in vitro Shoot Proliferation and Rooting of Myrtle (*Myrtus communis* L.). Journal of Agricultural Sciences, 21:177-183.

Şan, B., Yıldırım, A.N., Polat, M., & Yıldırım, F. (2015b). Chemical composition of myrtle (*Myrtus communis* L.) genotypes having bluish–black and yellowish–white fruits. *Erwerbs–Obstbau*, 57:203-210.

Şimşek, Ö., Biçen, B., Dönmez, D., & Aka Kaçar, Y. (2017). Effects of different media on micropropagation and rooting of myrtle (*Myrtus communis* L.) in *ın vitro* conditions. *International Journal of Environmental & Agriculture Research (IJOEAR),* 3(10):54-59.

Tewfik, A.A. (2002). Effect of IBA, planting media and type of cutting on rooting of nemaguard peach rootstock under Egyptian conditions. *Acta Horticulturae,* 592(24):169-175.

Thirunavoukkarasu, M., & Brahmam, M. (1999). Vegetative propagation of *Enterolobium cyclocarpum* (Jacq.) Griseb by coppice shoot cuttings. *Journal of Sustainable Forestry,* 8(2):27-34.

Uzun, H.İ., Aksoy, U., & Gözlekçi, Ş. (2014). Endüstriyel Amaçlı Organik Siyah Mersin Yetiştiriciliğinin Geliştirilmesi. TAGEM-10/ARGE-02, Proje Sonuç Raporu (in Turkish)

Uzun, H.İ., Aksoy, U., Gözlekçi, Ş., Bayır Yeğin, A., & Selçuk, N. (2016). Studies on the yield and quality characteristics of Myrtle (*Myrtus communis* L.) grown in two different ecologies. *Horticultural Studies*, 33(2):159-174.

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RESEARCH PAPER

HORTICULTURAL STUDIES

Determination of Self-Compatibility of the 'Arsel' Olive Cultivar Obtained by Hybridization Breeding

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Abstract

This study was aimed to determine the fertilization biology of the 'Arsel' olive cultivar obtained by hybridization breeding. For this reason, treatments of open pollination, cross pollination, and self-pollination were performed for 2 years and self-compatibility and appropriate pollinators of this new cultivar were investigated. Among the varieties included as pollinators (‘Memecik’, ‘Gemlik’, ‘Uslu’, ‘Ayvalık’ and ‘Eğriburun Nizip’), the highest fruit set was achieved with ‘Memecik’ and ‘Gemlik’ varietes in both years (2.64%, 1.37% and 1.75%, 1.39%). For this reason, ‘Memecik’ and ‘Gemlik’ cultivars were the best pollinator for ‘Arsel’ olive was thought. On the other hand, considering the statistical analyzes and productivity index values, ‘Arsel’ variety was found to be self-incompatible. However, the data also indicated that cross pollination was effective in increasing fruit set. Therefore, it is thought that the use of pollinator cultivar in orchard establishment would be beneficial in terms of yield. Pollen viability and germination tests were performed by using 2,3,5 Triphenyl Tetrazolium Chloride (TTC) and agar in petri (15% sucrose + 1% agar + 100 ppm H_3BO_3) methods in the study. The highest pollen viability rate was observed in ‘Memecik’ cultivar in 2020 and there isn’t statistical differences between olive cultivars in 2018. The highest pollen germination rate was observed in ‘Arsel’ cultivar in 2018. Accordingly, differences were determined between pollen viability and germination rates of examined olive cultivars in terms of years.

1. Introduction

Olive (*Olea europea* L.), which is produced all over the Mediterranean Basin and used as oil and table olives, is one of the most important products of this geography. It is widely cultivated in Türkiye, especially in the Aegean, Marmara and Mediterranean Regions. According to data of the Turkish Istatistical Institute (TUİK), there are almost 192,3 million olive trees with about 1.74 million tons of production in Türkiye. Türkiye ranks fourths in the world in terms of production amount (TUİK, 2021). It will be possible to have a say in the world market with the development of new varieties and the spread of production.

Determination of suitable pollinator variety and pollen quality are of great importance in terms of fruit breeding, adaptation and breeding studies. High efficiency ranks first among production targets. For this, first of all, successful pollination and fertilization are required in fruit species. Because low fruit set in olive species is a common problem. Significant production losses are observed due to the absence or insufficient use of the pollinator variety with the main variety. Similarly, self-compatibility of 13 olive species was investigated at

the Olive Land Gene Bank of the 'Olive Research Institute' in Izmir for 2 years. As a result, it was determined that 8 varieties were self-incompatibility, 2 varieties were partially fertile, and 3 varieties were fertile. In addition, it was determined that two hybrid candidates were self-incompatible. As a result, researchers stated that self- incompatibility is common in olives (Gül, 2020). Thus, Mete et al. (2016) reported that although the 'Hayat' olive variety obtained from hybridization studies is self-fertile, it would be appropriate to have a pollinator variety in the orchard in order to increase the fertilization efficiency and fruit set.

There are many studies on the determination of viability and germination of pollen in different olive cultivars. Pollen viability and germination tests are important in fertilization biology studies. It is stated that pollen performances vary according to genotypes and pollen tube development is very slow and does not reach the embryo sac in self-pollination (Porlings and Voyiatsiz, 1976; Palasciano et al., 2008; Selak et al., 2013; Mete et al., 2015).

It is necessary to include 10% pollinator varieties in the orchard, where olive pollen is carried by the wind for very long distances and for an effective pollination. It is reported that this ratio may vary depending on the topography of the region and the wind and air temperature during the flowering period (Lavee and Datt, 1978). In this context, it was determined that the stigma remained receptive for 12 days in the 'Manzanilla' olive variety and the fertilization ratio was 26% in the first 3 days and 55% at the end of the 8th day (Cuevas et al., 2009). In the current study, it was aimed to determine the appropriate pollinator to be uses as a pollinator, self-compatibility and pollen quality of the 'Arsel' olive variety obtained by hybridization breeding by the Olive Research Institute in İzmir.

2. Materials and Methods

The experiment of this study was conducted at the observation plot of registration of Olive Research Institute in İzmir/Kemalpaşa, Türkiye between 2018 and 2020. The study interrupted in 2019, due to strong alternate-bearing tendency of ‘Arsel’ cultivar. Cultivars like ‘Memecik’, ‘Gemlik’, ‘Uslu’, ‘Ayvalık’ and ‘Eğriburun Nizip’ cultivars were used as pollinator for ‘Arsel’ cultivar. Moreover, open pollination and self pollination applications were conducted. ‘Arsel’ olive cultivar was developed by hybridization studies and registered on February 2019. This cultivar which showed good performance particularly got with 24.69% oil and 88.24% pulp ratio. Flower clusters were isolated with oily paper bags at the balloon stage of buds to obtain pollens. After the anthesis stage bags were removed and pollens were sieved and protected in the refrigerator in glass bottles. For open-pollination treatments, flowers in clusters, as for the self-pollination, flower buds on clusters take place on one-year-old shoots were counted at balloon stage buds before the flower were counted, isolated and labeled. At the end of the receptivity of pistil, bags were removed and the isolation was completed. The number of flowers in three combinations as open pollination, self pollination and cross pollination was predicted as at least 500 (Figure 1).

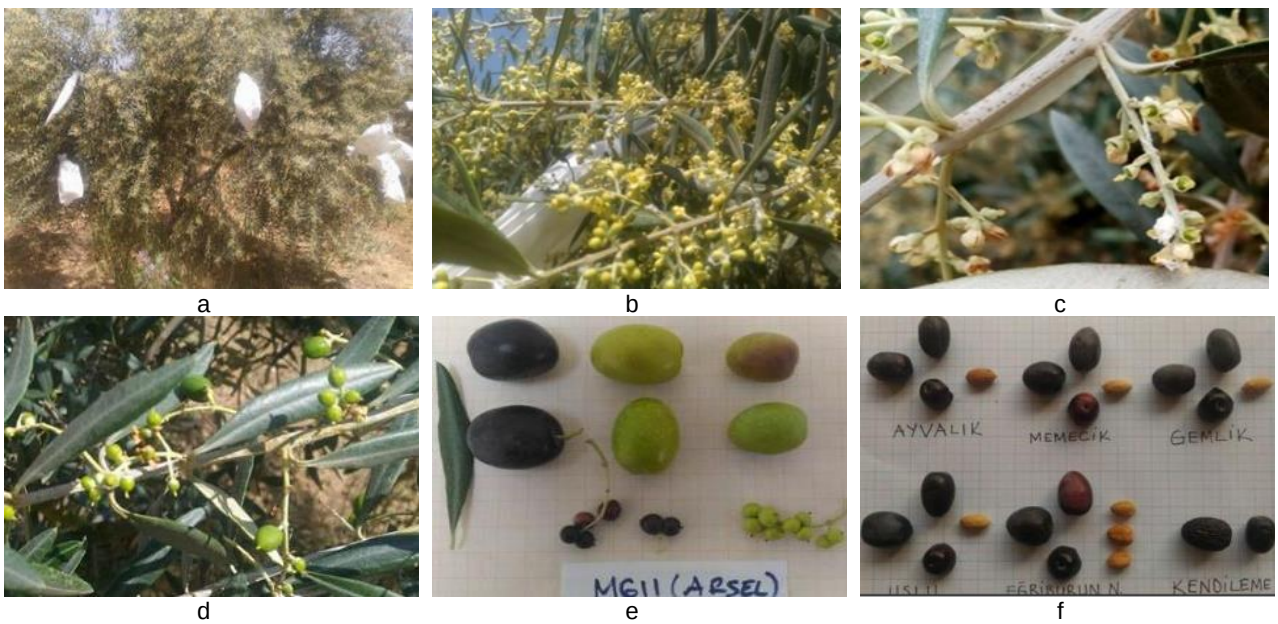

Figure 1. Arsel cultivar (a), beginning of blooming (b), full bloom (c), fruits and unfertilized parthenocarpic fruits 10 days after full bloom (d), fertilized and unfertilized parthenocarpic fruits (e), changes in fruit and seed with the effect of pollinator varieties 3 months after fruit set (f).

Self-fertility of examined cultivars and the degree of fertility of pollinator cultivars were calculated according to productivity index (R) and the obtained figures were evaluated by considering the Table 1 (Moutier, 2002).

$$R = \frac{SP}{OP}$$

where, R is productivity index, SP is percent fruit set obtained from self-pollination or pollinator, and OP is percent fruit set obtained from open-pollination cultivar.

2,3,5 Triphenyl Tetrazolium Chloride (TTC) solution was used to determine the pollen viability rates (Norton, 1966; Heslop-Harrison and Heslop-Harrison, 1970). In this test, which is based on the dyeing of pollen, the red pollen was considered as alive. Unstained pollen was classified as inanimate. In the pollen germination test, in the agar-petri method; 15% sucrose + 1% agar + 100 ppm H_3BO_3 medium was used (Mete, 2009).

The experiment was conducted on a randomize block design with five replications. Variance analysis was applied to the obtained data and Student's T-Test was used to compare the averages.

3. Results and Discussion

The percentages of pollen viability and germination belonging to 'Arsel' olive cultivar, this was used as the main cultivar and the other; pollinator cultivars were given in Table 2.

With the exception of pollen viability rates in 2018, the treatments showed statistically significant differences at *(P<0,001)* level. The highest rate of pollen viability in 2020 was determined in 'Memecik' (79%) and 'Arsel' (74%) cultivars. These cultivars were followed by 'Uslu' (65%), 'Eğriburun Nizip' (62%), 'Ayvalık' (58%) and 'Gemlik' (49%) cultivars respectively. In terms of pollen germination rates, the highest figure was determined in 'Arsel' cultivar, while the lowest one was determined in 'Eğriburun Nizip' in the first year of the study. As for the second experiment year, two statistical groups occurred. Accordingly, 'Gemlik', 'Eğriburun Nizip', 'Ayvalık', 'Uslu' and 'Memecik' took place in the first group and 'Arsel' was in the last group.

Pollen viability rates of olive cultivar could be variable, and the possible reasons of this situation might be related to climatic factors, cultural practices, tendency to alternate-bearing together with genetic factors as formerly exposed in different studies (Ferri et al., 2008; Palasciano et al., 2008; Mete., 2009; Gierdani et al., 2012; Mete et al., 2012; Manzzeo et al., 2014; Abacı and Asma, 2015; Karabıyık and Eti, 2015).

Pollen viability values were higher than germination values in this study which were with the former results of Rovira and Tous (2002). One of possible reasons of this situations, despite the acceptance of the viable pollens have a good germination capacity, as a result of the insufficient in-vitro conditions for pollen germination, lower germination rates could be generally be obtained as formerly suggested by Yıldız and Kaplankıran (2014). Similar result was emphasized in various stone fruit species (Eroğlu and Mısırlı, 2016) Moreover, in 'Arsel' cultivar which was determined in self-incompatible category in accordance with pollination biology in this study, obtained pollen germination rate as 19% in 2020 might be one of the possible causes of self-incompatibility was thought. Accordingly, the low pollen germination rate can be resulted with the decreases in germination and fruit set consecutively.

The fruit set and productivity index (R) values related with the combinations of controlled pollinations that conducted in 'Arsel' cultivar, which was used as the main cultivar have seen in Table 3. Statistically significant *(P<0,001)* differences were predicted between treatments in both years (2018-

Table 1. Classification of self-fertility and activity level of pollinators.

(R)	0.00	0.15	0.15	0.30	0.30	1.00
	Self-incompatibility		Partially self-compatibility		Self-compatibility	
(R)	0.00	0.33	0.33	0.66	0.66	1.00
	Bad pollinator		Passable pollinator		Good pollinator	

Table 2. Pollen viability and germination ratio (%).

Cultivars	Pollen viability ratio		Pollen germination ratio	
	2018	2020	2018	2020
Arsel	89.82	74.00 ab	64.28 a	19.00 b
Memecik	93.05	79.00 a	36.47 c	26.00 a
Uslu	78.37	65.00 bc	35.44 c	29.00 a
Eğriburun Nizip	87.60	62.00 c	24.12 d	37.00 a
Ayvalık	84.01	58.00 c	34.89 c	35.00 a
Gemlik	81.93	49.00 d	45.69 b	37.00 a
CV%	1	9	2	6

Means were grouped according to Student's t test (P<0,001).

2020) and data were in parallel with normal separation. Fruit set rate of 1-2% in olive was accepted as sufficient by different researchers. The combination in which the 'Memecik' cultivar was used as pollinator for 'Arsel' cultivar, fruit set rates were 2.64% and 1.37% respectively. In terms of Number of Fruits Per Inflorescence (NFPI) in 2018, 'Memecik' cultivar was ranking first and it was followed by 'Gemlik', open pollination, 'Ayvalık', 'Uslu', 'Eğriburun Nizip' and self-pollination respectively. According to Productivity Index (R) values of 2018, while 'Memecik', 'Gemlik', 'Ayvalık' and 'Uslu' cultivars were in good pollinator class, 'Eğriburun Nizip' took place in acceptable pollinator class. Moreover, 'Arsel' cultivar was classified as self-incompatible. In terms of NFPI in 2020, 'Memecik', 'Gemlik' and open pollination took place in the same statistical group in both years and gave the highest fruit set values as well *(P<0,001).* According to Productivity Index (R) values, while 'Memecik' and 'Gemlik' cultivars in good pollinator class, 'Ayvalık' took place in acceptable pollinator class. On the contrary, 'Uslu' cultivar was in bad pollinator class as it was calculated. 'Arsel' cultivar was also self-incompatible like the first year.

In Kemalpasa location, blooming period was observed between April 27 and May 15 in 2018. As the start of blooming in April 27 and May 3-4 when the full blooming was observed in 'Arsel' cultivar, daily maximum temperatures ranged between 28-33°C and these values did not cause any problem related with pollination biology was determined (Figure 2).

Moreover, after quite low precipitations measured as 16 and 5 mm, on May 6 and 11, resulted with proper conditions for pollination biology was thought. In fact, results were quite satisfactory clue to fruit setting values and 2,64% fruit setting was determined in combination, in which 'Memecik' cultivar was used as pollinator and more than 1% fruit settings were also obtained with the other combinations (Table 3).

In 2020, daily maximum temperatures were higher than 35˚C at the start of blooming (May 14) and during full blooming (May 17-18) have seen in Figure 3.

However, these high temperatures, which may adversely affect the fruit set, dropped after the precipitation occurred on May 21. After that, lower daily maximum temperatures were measured to the

Table 3. Fruit set rates depending on pollination combinations in 'Arsel' female parent.

Application	Fruit set ratio (%)		NFPI		Productivity index (R)	
	2018	2020	2018	2020	2018	2020
Memecik	2.64 a	1.37 a	0.43 a	0.17 a	1.00	1.00
Gemlik	1.75 ab	1.39 a	0.28 b	0.17 a	1.00	1.00
Uslu	1.17 bc	0.42 b	0.17 bc	0.04 b	0.73	0.28
Ayvalık	1.11 bc	0.46 b	0.19 bc	0.05 b	0.82	0.35
Eğriburun Nizip	0.83 c	-	0.10 cd	-	0.43	-
Open pollination	1.47 bc	1.08 a	0.23 bc	0.14 a	1.00	1.00
Self-pollination	0.15 d	0.13 b	0.02 d	0.01 b	0.08	0.07
(CV, %)	19	21	8	5		

NFPI: Number of fruits per inflorescence.The means were classified according to the original values by Student's t test, and the groups were classified according to the transformed values (P<0,001). The data fit the normal distribution.

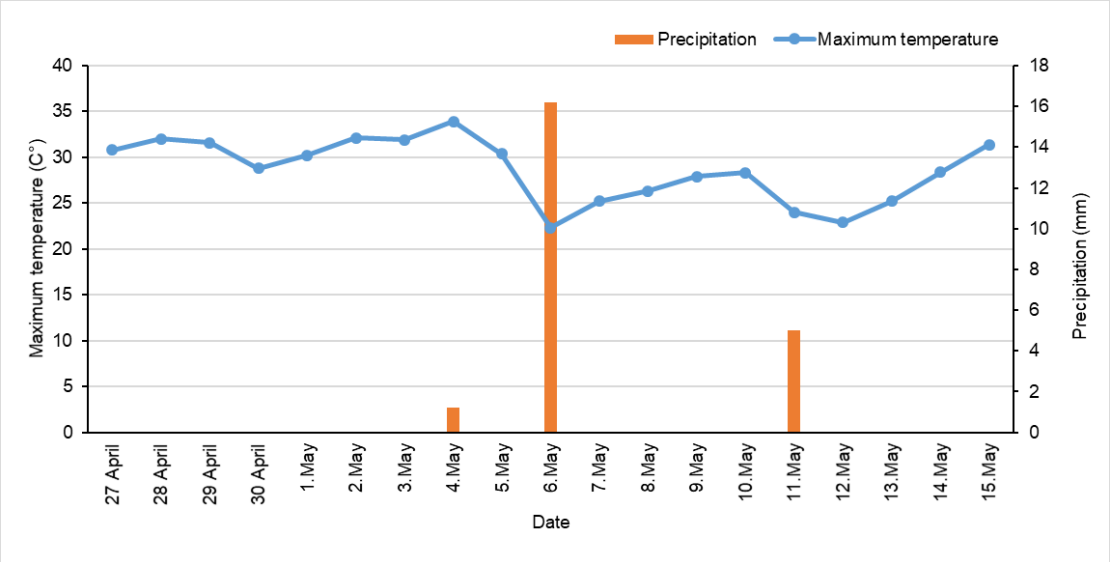

Figure 2. The daily maximum temperature and precipitation in the blooming period of 2018.

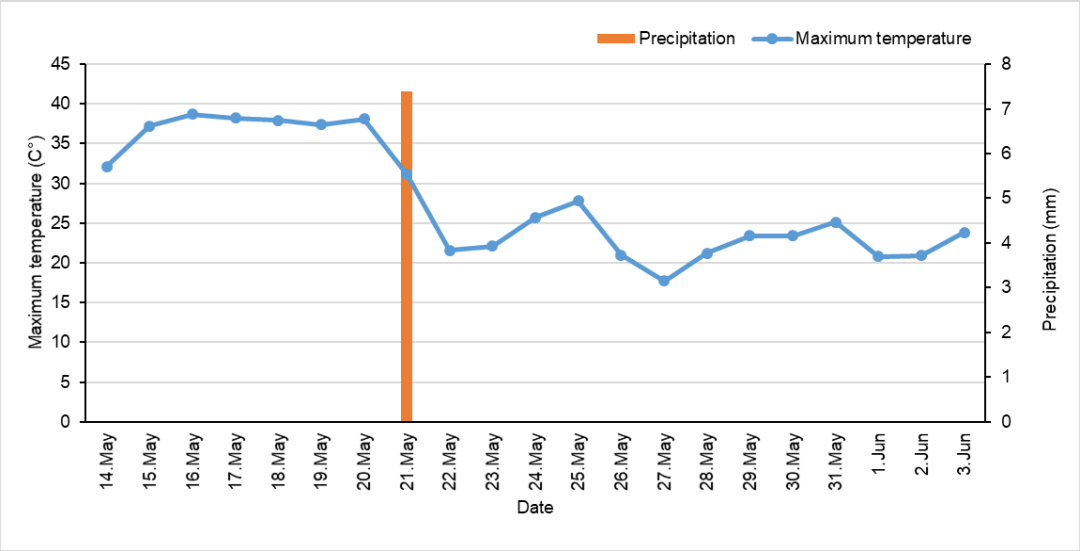

Figure 3. The daily maximum temperature and precipitation in the blooming period of 2020.

end of blooming (June 3), which gave rise to quite proper conditions for pollination biology was observed. Considering the all combinations of the second pollination were done after the precipitation on May 21, in terms of fruit set rates obtained as 1,37% from 'Memecik' and 1,39% from 'Gemlik' combinations were quite good figures was thought. Fruit set at 1-2% have been accepted as sufficient for olive, in general. In both years of the study, in accordance with the pollinator cultivars, quite satisfactory fruit set rates occurred. However, when the meteorological data were considered, fruit set rates obtained in 2018 were higher compared with 2020, due to the more proper daily maximum temperature values. Moreover, a difference of 10-17 days between inflorescence initiation and blooming was determined in relation with the warmer weather conditions during blooming period in 2018.

4. Conclusion

For 'Arsel' olive cultivar, 'Memecik' and 'Gemlik' cultivars were the combinations that provided the highest level of fruit sets related either with fruit number per inflorescence, which is basic for evolution, or values of productivity index (R). For this reason, mentioned cultivars were the proper pollinator for 'Arsel' cultivar olive. Moreover, higher fruit set was obtained compared with open pollination treatment. Plantations of 'Arsel' should include a minimum of 10% of trees of 'Memecik' or 'Gemlik' in order to ensure a good compatible pollination. According to this study, for 'Arsel' cultivar which was obtained with the hybridization of 'Gemlik' and 'Memecik' cultivars, determination of parent cultivars as the most proper pollinator should be evaluated as a quite interesting result for olive breeding programs.

References

Abacı, T.Z., & Asma, M.B. (2015). Pollen vıtalıty, germınatıon condıtıons and pollen tube lenght investigation of hybrid apricot genotypes. *Anadolu Journal of Agricultural Science*, 29(1):12-19.

Cuevas, J., Pınıllos, V., & Polito, S. (2009). Effective pollination period for 'Manzanillo' and 'Picual' olive trees. *Journal of Horticultural Science & Biotechnology,* 84(3):370–374.

Eroğlu, Z., & Mısırlı, A. (2016). The determination of pollen quality in some peach cultivars and types. *Ege University Agriculture Faculty Journal,* 53(1): 83-88.

Ferri, A., Giordani, E., Padula, G., & Bellini, E. (2008). Viability and in vitro germinability of pollen grains of olive cultivars and advanced selections obtained in Italy. *Advances in Horticultural Science,* 22(2):116-122.

Gierdani, E., Ferri, A., Trentacoste, E., & Radice, S. (2012). Viability and ın vıtro germinabilty of pollen grains of olive cultıvars grown ın different environments. *VII International Symposium on Olive Growing Acta Hortıculture*.1057.5.

Gül, H. (2020). Bazı zeytin çeşitlerinin kendine verimlilik durumlarının saptanması. MSc Thesis, Ege University, 48 p (in Turkish).

Heslop-Harrison, J., & Heslop-Harrison, Y. (1970). Evaluation of pollen viability by enzmatically induced flourescense. Intraceluler hydrolysis of fluorescein diacetate. *Stain Technology*, 45(3):115-120.

Karabıyık, Ş., & Eti, S. (2015). Determination of pollen viability, germination levels and amount of pollen production of some loquat cultivars at different flowering periods. *Fruit Science Journal*, 2(1):42-48.

Lavee, S., & Datt, Z. (1978). The necessity of cross pollination for fruit set of Manzanillo olives. *Journal of Horticultural Science,* 53: 61-266.

Manzzeo, A., Palasciano, M., Gallotta, A., Camposeo, S., Pacifico, A., & Ferrara, G. (2014). Amount and quality of pollen grains ın four olive (*Olea europaea* L.) cultivars as affected by 'on' and 'off' years. *Scientia Horticulturae*, 170(7):89-93.

Mete, N. (2009). Bazı Zeytin çeşitlerinin Döllenme Biyolojisi Üzerinde Araştırmalar. MSc Thesis, Ege University, 21 p. (in Turkish).

Mete, N., Mısırlı, A., & Çetin, Ö. (2012). Determining the biology of fertilization and pollinators in some olive cultivars. *Proceedings of the 4th International Conference on "Olive Culture and Biotechnology of Olive Tree Products".* 69-74 pp.

Mete, N., Şahin, M., & Çetin, Ö. (2015). Determining of pollen viability and germination in some olive cultivars. *Olive Science*, 5(1):9-12.

Mete, N., Şahin, M., & Çetin, Ö. (2016). Determination of self-fertility of the 'Hayat' olive cultivar obtained by hybridization breeding. *Journal of Tekirdag Agricultural Faculty,* 2016:13.

Moutier, N. (2002). Self-fertility and inter-compatibilities of sixteen olive varieties. *Acta Horticulturea,* 586.

Norton, J.D. (1966). Testing of plum pollen viability with tetrazolium salts. *Proceedings of the American Society for Horticultural Science,* 89:132-4.

Palasciano, M., Camposeo, S., Ferara, G., & Godini, A, (2008). Pollen production by poplular olive cultivar. *Acta Horticulturea,* 791:489-492.

Porlingis, I.C., & Voyiatzis, D., (1976). Effect of growth substances on fruit-set in a partly self-incompatible olive cultivar. *Journal of the American Society for Horticultural Science,* 101:432-434.

Rovira, M., & Tous, J. (2002). Pollen viability in several 'Arbequina' olive oil clones. *Acta Horticulturea,* 586:197-200.

Selak, G.V., Perica, S., & Poljak, M., (2013). The effect of temperature and genotype on pollen performance in olive (*Olea europaea* L.) *Scientia Horticulturae,* 156(7):38-46.

TUİK, 2021. Türkstat, Crop Production Statistcs. http://tuikapp.tuik.gov.tr/bitkiselapp/bitkisel.zul, Access Date: 08.02.2021.

Yıldız, E., & Kaplankıran, M. (2014). The pollen viability and germination ratio of different persimmon genotypes. *Ege University Agriculture Faculty Journal,* 51(2):117-123.

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RESEARCH PAPER

HORTICULTURAL STUDIES

The Use of Silver Nitrate as an Elicitor to Increase Bioactive Compounds in Artichoke [*Cynara cardunculus* var. *scolymus* (L.) Fiori] Callus Culture

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Abstract

The globe artichoke belongs to the Asteraceae family and has become more and more popular among other vegetables due to its beneficial health-promoting features that are related to bioactive compounds present in leaves. The plant materials have inadequate proportions of valuable bioactive compounds in nature, so researchers are emphasizing on how to enhance their amounts. *In vitro* techniques with integrated novel practices can be employed to enhance phytochemicals from any plant. The current study aimed to determine and assess valuable bioactive components in 3 artichoke cultivars via callus cultures which were subjected to a treatment of 4 different concentrations (2.5, 5.0, 10.0, and 15.0 mg L^{-1}) of silver nitrate. Results indicated that well-balanced levels of plant growth regulators were necessary for stimulating the callus formation of globe artichoke. The findings of the current study also revealed the importance of cultivar differences regarding callus formation. Experimental results revealed that variation in silver nitrate concentrations had a significant effect on biomass, total phenolic content and total antioxidant activities. The results of the current, study may offer a good strategy by promoting bioactive compounds of globe artichoke leaves for utilizing in large-scale industries, pharmacology, and food supplements.

1. Introduction

Several researchers believe that in the upcoming years, the potential adverse consequences of global warming on agricultural ecosystems will become considerably more pronounced than before. If agricultural production becomes less productive, it will be difficult for people to get healthy food. This has led to greater interest in research aimed at improving the existing nutritional value of our goods, increased consumer awareness of the significance of consuming high-quality meals, and a shift toward functional foods. Plants/vegetables have the potential to be viewed as functional foods due to the phytochemicals they have, and among vegetables, in this context, artichoke is known to be rich in valuable bioactive compounds.

The globe artichoke [*Cynara cardunculus* var. *scolymus* (L.) Fiori] plant has been used to meet a variety of human requirements, such as culinary and medicinal reasons since ancient times. Nowadays, it is appreciated as a functional food. The value of the artichoke plant in terms of being a rich source of vitamins, minerals, and bioactive compounds has once more come to light recently due to the growing interest in functional foods coupled with healthy and conscientious nutrition.

To meet the growing demand for functional foods, it is critical to expedite research into whether these valuable bioactive components, which can vary depending on the stage of plant development,

can be produced using *in vitro* techniques and whether there is a chance of increasing the amounts of the produced components. The artichoke plant may be used to make extracts, like many other plants. High-level contents of the desired extracts are is also significant in this regard. Yet, as long as they are not impacted by harsh conditions, the contents of extracts obtained from conventionally grown plants are at a certain steady level. The beneficial bioactive chemicals already present in plants may be amplified utilizing *in vitro* methods and that approach has been tried on different crops. Based on *in vitro* approach, callus culture technique has been also used in previous studies by treating callus with different elicitors to see their effect on the contents of biomass and bioactive components.

Instead of helping individuals grow and develop, bioactive components offer "improvement in quality of life" by lowering the risk of developing certain medical problems (Pandino et al., 2011). They also assist in the process of plant growth and development while protecting them against biotic or abiotic stresses (Abu-Reidah et al., 2013). The quantity of medicinally important herbal bioactive components in plants is low in concentration, and they are synthesized at different growth and developmental phases of the plant, thus production rates often remain low. Nowadays, certain biotechnological techniques are applied to boost these important compounds' production rates. They may be acquired in this situation utilizing the *in vitro* callus cultivation approach in high quantities and under controlled circumstances and regardless of time. It is a known fact that callus culture is used on a large scale in different industries including pharmacy, cosmetics, and agriculture (Ozsan and Onus, 2020).

Elicitors are agents that cause stress and activate secondary pathways, which trigger the creation of bioactive substances. To rapidly produce a large number of phytochemicals, elicitors (biotic and abiotic) can be used in *in vitro* techniques. Regarding the subject, plant callus cultures have great potential to get beneficial phytochemicals rich in antioxidant activity. Besides, the integration of elicitors to callus cultures may enhance the accumulation of valuable phytochemicals in plants (Ali et al., 2018; Al-Khayri and Naik, 2020). It is known that silver nitrate has effects on enzyme activity, gene expression, and the synthesis of secondary metabolites in plants (Alirezaei et al., 2017), and that is why silver nitrate is a preferred abiotic elicitor to increase the synthesis of bioactive compounds in plant tissue culture experiments (Winson et al., 2020). Based on above stated information present study was conducted to provide solutions to issues like whether the culture process and elicitation might be improved the total phenolic contents and total antioxidant activities in artichoke. In this content, the total phenol and total antioxidant activities of the extracts produced as a consequence of the silver nitrate elicitation treatments on callus culture of three different artichoke cultivars (Sakız OP, Bayrampaşa OP, and Olympus F_1 hybrid).

2. Material and Methods

2.1. Plant material

As plant materials two open-pollinated (OP) Sakız, Bayrampaşa, and one F_1 hybrid Olympus cultivar, that were grown in open fields of Faculty of Agriculture at Akdeniz University were employed. Plant collecting and surface sterilization of plant materials was conducted according to Ozsan and Onus (2020). Leaves were separately harvested for each cultivar from the inner part of the plants and subjected to the surface sterilization process. After washing by running the tap water leaves were kept for 15 min in an antibacterial soap solution with 5% then washed again. The following step for surface sterilization step was performed at laminar flow cabinets by utilizing a hypochlorite solution with 20% (active substance 5%) for ten minutes, then three rinsing with autoclaved distilled water.

2.2. The media preparation, callus cultures and physical culture conditions

In the current study, optimizing studies on callus formation were conducted with 22 different media combinations (Table 1). As the basic media Gamborg B5 (Gamborg et al., 1968) was employed with various concentrations (0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 mg L^{-1}) 6-Benzylaminopurine (BAP) and naphthalene acetic acid (NAA). Approximately 0.5-1.0 cm size leaf explants were inoculated to prepared media and maintained the growths at certain physical culture room conditions, 24 ± 2°C, 16h/8h - light/dark, 3000 µ $E.m^{-2}.s^{-1}$ light intensity.

2.3. Evaluation of calli formation

The success of callus formation of media combinations was expressed as 0-40% of cultured leaf explants as insufficient growth (+), 41-75% as moderate growth (++), and 76-100% as fine growth (+++). Healthy growing calli in different media combinations were weighed as 1.0 g per treatment and cultivar under aseptic conditions for further elicitor treatments (Table 2).

2.4. Elicitor treatments

All obtained calli were sub-cultured 5 times. At the end of the 5^{th} sub-culture, silver nitrate at different concentrations (2.5, 5.0, 10.0, and 15.0 mg L^{-1}), which were separately filter-sterilized with a 0.22 µm filter, was added to 10-day-old callus cultures for 7 days.

2.5. Growth kinetics and biomass production

Table 1. Media compositions for callus formation.

No	Media-Gamborg B5 (g L^{-1})	Media compositions			
		BAP (mg L^{-1})	NAA (mg L^{-1})	Sucrose (g L^{-1})	Plant agar (g L^{-1})
1	3.2	-	-	30.0	6.0
2	3.2	0.5	0.5	30.0	6.0
3	3.2	0.5	1.0	30.0	6.0
4	3.2	0.5	2.0	30.0	6.0
5	3.2	0.5	3.0	30.0	6.0
6	3.2	0.5	4.0	30.0	6.0
7	3.2	0.5	5.0	30.0	6.0
8	3.2	1.0	1.0	30.0	6.0
9	3.2	1.0	2.0	30.0	6.0
10	3.2	1.0	3.0	30.0	6.0
11	3.2	1.0	4.0	30.0	6.0
12	3.2	1.0	5.0	30.0	6.0
13	3.2	2.0	2.0	30.0	6.0
14	3.2	2.0	3.0	30.0	6.0
15	3.2	2.0	4.0	30.0	6.0
16	3.2	2.0	5.0	30.0	6.0
17	3.2	3.0	3.0	30.0	6.0
18	3.2	3.0	4.0	30.0	6.0
19	3.2	3.0	5.0	30.0	6.0
20	3.2	4.0	4.0	30.0	6.0
21	3.2	4.0	4.0	30.0	6.0
22	3.2	5.0	5.0	30.0	6.0

Differences in callus biomass in response to varied elicitor treatments were measured as fresh (FW) and dry (DW) weights after 7 days of elicitor treatments. Calli were taken out from petri dishes, pressed softly on filter paper to the excess water, weighed, and expresses as fresh weight. The calli were harvested, weighed, and oven dried at 60°C for 48 h to determine the dry weight (DW).

2.6. Determination of total phenolic content

Total phenolic content was measured using the Folin-Ciocalteu method as defined by Škerget et al. (2005). The sample (1.0 g) is extracted in 100 mL of extraction solution. The sample at the volume of 0.5 mL was added to 2.5 mL Folin-Ciocalteu reagent which was 10 times diluted with water. After adding 2.0 mL sodium carbonate (75.0 g L^{-1}) the mixture was maintained for five minutes at 50°C and then cooled. The absorbance values of samples were measured at 760 nm. The total phenolic content was expressed as mg gallic acid equivalent per 100 g dry weight of callus.

2.7. Determination of total antioxidant activity

The free radical-scavenging activity (2,2-diphenyl-1-picrylhydrazyl - DPPH) was analyzed according to Fernández-León et al. (2013). A 1.0 g dry sample was dissolved in a 20 mL extraction solution (80% methanol) for 1 minute with the help of ultraturrax. For analysis, 50 µL of the diluted extract was transferred to an Eppendorf tube and 950 µL of diluted DPPH was added into it and mixed with vortex for 30 minutes and kept in the dark. The absorbance of the DPPH solution prepared for analysis was determined and spectrophotometrically readings were recorded at a wavelength of 515-517 nm. The total antioxidant activity was expressed as mg trolox equivalent per 100 g dry weight of callus.

2.8. Statistical analysis

The experiments of the current study were performed with a completely randomized factorial design in triplicates. The data obtained were subjected to variance analysis in the JMP package program and the differences between the averages were determined by the least significant difference (LSD) test also the differences were determined statistically significant at $P<0.05$.

3. Results and Discussion

3.1. Evaluation of media combinations, callus formation and biomass accumulation

In vitro calli formation and maintenance as well as bioactive compound accumulation in cultivated explants are known to be influenced by a variety of variables, e.g. genotype, plant explant type, media, plant growth regulators, and physical circumstances of culture (Siatka, 2019). One of the most crucial variables to consider when measuring growth is the cell's biomass. Types, concentrations, and combinations of plant growth regulators that added medium had an important effect on callus formation and medium No.15 and medium No.19 were determined as the most responsive media combinations.

The experimental findings demonstrated that there were variations in cultivars' callus formation in

Table 2. Effects of different media combinations on cultivars.

No	Media composition	Cultivars		
		Sakız OP	Bayrampaşa OP	Olympus F_1
1	Gamborg B5 (G.B5 - control)	-	-	-
2	G.B5 + 0.5 mg L^{-1} BAP + 0.5 mg L^{-1} NAA	+		
3	G.B5 + 0.5 mg L^{-1} BAP + 1.0 mg L^{-1} NAA	+	+	+
4	G.B5 + 0.5 mg L^{-1} BAP + 2.0 mg L^{-1} NAA	+	+	+
5	G.B5 + 0.5 mg L^{-1} BAP + 3.0 mg L^{-1} NAA	+	+	+
6	G.B5 + 0.5 mg L^{-1} BAP + 4.0 mg L^{-1} NAA	+	+	+
7	G.B5 + 0.5 mg L^{-1} BAP + 5.0 mg L^{-1} NAA	+	+	+
8	G.B5 + 1.0 mg L^{-1} BAP + 1.0 mg L^{-1} NAA	+	+	+
9	G.B5 + 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA	+	+	+
10	G.B5 + 1.0 mg L^{-1} BAP + 3.0 mg L^{-1} NAA	+	+	+
11	G.B5 + 1.0 mg L^{-1} BAP + 4.0 mg L^{-1} NAA	+	+	+
12	G.B5 + 1.0 mg L^{-1} BAP + 5.0 mg L^{-1} NAA	+	+	+
13	G.B5 + 2.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA	+	+	+
14	G.B5 + 2.0 mg L^{-1} BAP + 3.0 mg L^{-1} NAA	+	++	+
15	G.B5 + 2.0 mg L^{-1} BAP + 4.0 mg L^{-1} NAA	+++	+++	+
16	G.B5 + 2.0 mg L^{-1} BAP + 5.0 mg L^{-1} NAA	+	+	+
17	G.B5 + 3.0 mg L^{-1} BAP + 3.0 mg L^{-1} NAA	+	+	+
18	G.B5 + 3.0 mg L^{-1} BAP + 4.0 mg L^{-1} NAA	++	+	+
19	G.B5 + 3.0 mg L^{-1} BAP + 5.0 mg L^{-1} NAA	+++	+++	+
20	G.B5 + 4.0 mg L^{-1} BAP + 4.0 mg L^{-1} NAA	+	+	+
21	G.B5 + 4.0 mg L^{-1} BAP + 5.0 mg L^{-1} NAA	+	+	+
22	G.B5 + 5.0 mg L^{-1} BAP + 5.0 mg L^{-1} NAA	++	++	+

Table 3. Calli fresh and dry weights (biomass).

$AgNO_3$ treatments (T)	Fresh weight (g)		Dry weight (g)		Mean differences values – $AgNO_3$ treatments	
	Sakız OP (SOP)	Bayrampaşa OP (BOP)	Sakız OP (SOP)	Bayrampaşa OP (BOP)		
1. Medium No.15 (control) (X)	6.22 ef	0.00 h	0.55 df	0.00 h	3.11 E	0.27 EF
2. X + 2.5 mg L^{-1} $AgNO_3$	6.25 ef	5.43 f	0.57 cf	0.45 fg	5.84 C	0.51 C
3. X + 5.0 mg L^{-1} $AgNO_3$	7.19 be	7.51 bd	0.69 bd	0.71 bc	7.35 B	0.70 B
4. X + 10.0 mg L^{-1} $AgNO_3$	6.81 ce	6.31 df	0.62 be	0.54 ef	6.56 BC	0.58 C
5. X + 15.0 mg L^{-1} $AgNO_3$	10.41 a	8.25 b	0.89 a	0.74 b	9.33 A	0.81 A
6. Medium No.19 (control) (Y)	3.62 g	0.00 h	0.37 g	0.00 h	1.81 F	0.18 F
7. Y + 2.5 mg L^{-1} $AgNO_3$	7.64 bc	0.00 h	0.71 bc	0.00 h	3.82 DE	0.35 DE
8. Y + 5.0 mg L^{-1} $AgNO_3$	8.10 b	0.00 h	0.77 ab	0.00 h	4.05 D	0.38 D
9. Y + 10.0 mg L^{-1} $AgNO_3$	7.09 be	0.00 h	0.65 be	0.00 h	3.54 DE	0.32 DE
10. Y + 15.0 mg L^{-1} $AgNO_3$	6.05 ef	0.00 h	0.55 df	0.00 h	3.02 E	0.27 EF
Mean differences values-cultivars (C)	Fresh weight (SOP:6.94 A, BOP:2.75 B), Dry weight (SOP:0.64 A, BOP:0.24 B)					
LSD values-fresh weight	LSD (C) = 0.386, LSD (T) = 0.864, LSD (C × T)= 1.223					
LSD values-dry weight	LSD (C) = 0.043, LSD (T) = 0.097, LSD (C × T)= 0.137					

Different letters between cultivars and $AgNO_3$ treatments denote significant differences (LSD test, $P<0.05$).

all investigated media compositions. The cultivar with the best response regarding callus formation was "Sakız" OP, which was followed by "Bayrampaşa" OP. "Olympus" F_1 did not form enough amount of callus and that is why excluded from further silver nitrate elicitor treatments.

3.2. Effect of silver nitrate on fresh and dry weights of calli

The supplementation of different concentrations of silver nitrate had a positive impact on calli fresh and dry weights (biomass) (Table 3). Experimental results revealed that calli fresh and dry weights were affected positively by 15.0 mg L^{-1} silver nitrate treatment for successful media combinations of No. 15, No. 19 which was followed by silver nitrate treatment concentration of 5.0 mg L^{-1}.

When the cultivars are evaluated among themselves, it is seen that the Sakız cultivar gives a more positive result than Bayrampaşa to silver nitrate concentrations regarding biomass. In previous studies some researchers indicated that the use of silver nitrate as an elicitor in the right quantities might have beneficial effects (Yan et al., 2006; Deepthi and Satheeshkumar, 2016). According to Yan et al. (2006), the addition of 15 µm Ag+ to *Salvia miltiorrhiza*'s hairy root culture significantly promotes growth and increases root dry weight. In addition, Zaker et al. (2015) reported that 25 and 50 µM Ag+ treatment decreased root growth whereas 5 µM of Ag+ application boosted root growth in adventitious root cultures of *Perovskia abrotanoides*.

3.3. Effect of silver nitrate on total phenolic content and total antioxidant capacity of calli

Table 4, display the total phenolic content and antioxidant capacity of calli samples that had been

Table 4. Total phenolic contents and total antioxidant activity of calli.

$AgNO_3$ treatments (T)	Total phenolic content		Total antioxidant activity		Mean differences values – $AgNO_3$ treatments	
	Sakız OP (SOP)	Bayrampaşa OP (BOP)	Sakız OP (SOP)	Bayrampaşa OP (BOP)	Total phenolic content	Total antioxidant activity
1. Medium No.15 (control) (X)	417.42 b	0.00 i	331.35 a	0.00 o	208.71 D	165.67 E
2. X + 2.5 mg L^{-1} $AgNO_3$	375.34 c	383.48 c	283.59 c	261.11 d	379.41 A	272.35 A
3. X + 5.0 mg L^{-1} $AgNO_3$	302.96 d	440.99 a	197.89 i	304.66 b	371.97 A	251.27 B
4. X + 10.0 mg L^{-1} $AgNO_3$	229.45 f	410.69 b	199.30 h	231.61 f	320.07 B	215.45 D
5. X + 15.0 mg L^{-1} $AgNO_3$	228.89 f	269.85 e	223.18 g	233.01 e	249.37 C	228.09 C
6. Medium No.19 (control) (Y)	179.82 h	0.00 i	192.18 k	0.00 o	89.91 F	96.09 G
7. Y + 2.5 mg L^{-1} $AgNO_3$	197.47 g	0.00 i	185.25 l	0.00 o	98.73 E	92.62 H
8. Y + 5.0 mg L^{-1} $AgNO_3$	193.25 g	0.00 i	172.60 m	0.00 o	96.62 EF	86.30 I
9. Y + 10.0 mg L^{-1} $AgNO_3$	189.05 gh	0.00 i	138.89 n	0.00 o	94.52 EF	69.44 J
10. Y + 15.0 mg L^{-1} $AgNO_3$	196.62 g	0.00 i	196.49 j	0.00 o	98.31 EF	98.24 F
Mean differences values-cultivars (C)	Total phenolic content (SOP= 251.03 A, BOP= 150.50 B) Total antioxidant activity (SOP= 212.07 A, BOP= 103.03 B)					
LSD values-Total phenolic content	LSD (C) = 3.885, LSD (T) = 8.688, LSD (C × T)= 12.287					
LSD values-Total antioxidant activity	LSD (C) = 7.467 x 10^{-7}, LSD (T) = 1.669 x 10^{-6}, LSD (C × T)= 2.361 x 10^{-6}					

Different letters between cultivars and $AgNO_3$ treatments denote significant differences (LSD test, $P<0.05$).
**The total phenolic content (TPC) was expressed as mg gallic acid equivalent per 100 g dry weight of callus (mg GAE/g DW).
*** The total antioxidant activity (TAA) was expressed as mg trolox equivalent per 100 g dry weight of callus (mg trolox/g DW).

stimulated with various concentrations of silver nitrate. Accordingly, the highest total phenolic content among various silver nitrate concentrations was obtained from 2.5 mg L^{-1}, while the media with non-added silver nitrate (control) created undesirable results. It is seen that as the silver nitrate concentration increases, the total phenolic content decreases except for media with the non-application of silver nitrate regardless of cultivars and media. Besides, with regards to the total phenolic content between cultivars, Bayrampaşa was by far the best while Sakız has the highest total antioxidant activity. It was also determined that the calli of the Sakız cultivar obtained from the medium without silver nitrate application had the highest antioxidant capacity.

In some previous studies, exogenous treatment with elicitors has been shown to increase the synthesis of bioactive compounds in plant cells (Gheisary et al., 2018). Several researchers have shown that elicitor treatments are particularly successful in plant cell proliferation and the augmentation of phytochemicals (Namdeo, 2007). The efficiency of elicitor treatments can be increased by taking into account factors including the culture age, the length of exposure to elicitors, and the kind and concentration of the elicitor (Nazir et al., 2019). Açıkgöz (2020) reported that medium with the non-elicitor application (control) and medium treated with silver nitrate at 100 µM concentration has the lowest total phenolic content. In his research, it was determined that the treatment with silver nitrate as an abiotic elicitor increased the total contents of phenolic and antioxidants. According to previous research, employing low quantities of silver nitrate may enhance the overall quantity of phenolics and antioxidants, similar to the present report's findings (Cai et al., 2013; Nadeem et al., 2018; Açıkgöz, 2020). It is known that cells can respond to certain elicitor treatments at low concentrations and in a short time. On the other hand, some of the elicitor treatments can respond at high concentrations and over a prolonged time. Gonçalves et al. (2019) reported that 50 µM silver nitrate treatments enhanced the total phenolic content by 12.5% at the end of six weeks, while Yan et al. (2006) demonstrated that for increasing the total phenolic content, the treatment with 15 µM Ag+, which was at low concentration, was more efficient at the 4th day after application.

Previous researches reported the differences between callus tissues of total phenolic content and total antioxidant capacities to be associated with the phenolics of cell and flavonoid types and concentrations (Sabir et al., 2012; Xu et al., 2016). As a result, discrepancies in antioxidant capabilities can be attributed to variations in phenolic acid and flavonoid concentration that emerge throughout elicitor treatment (Krishnan et al., 2015; Sarkate et al., 2017).

4. Conclusion

The present experiment results reveal the different elicitor concentrations' effects on biomass, total phenolic content, and total antioxidant activity from the callus culture of two globe artichoke cultivars. Using silver nitrate as an elicitor increased the biomass of Sakız and Bayrampaşa artichoke cultivars when applied at the concentration 15.0 mg L^{-1}. For further studies, higher concentrations of silver nitrate should be tested to determine the optimum concentration of silver nitrate for biomass increase. Regarding total phenolic and antioxidant contents, the low concentration of silver nitrate treatment as 2.5 mg L^{-1} had a positive effect on the phenolic content of Sakız and Bayrampaşa cultivars, while silver nitrate treatment had no positive effect on antioxidant

contents. The current findings are expected to help those working on increasing globe artichoke's values as a functional food.

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References

Abu-Reidah, I.M., Arraez-Roman, D., Segura-Carretero, A., & Fernandez-Gutierrez, A. (2013). Extensive characterisation of bioactive phenolic constituents from globe artichoke (*Cynara scolymus* L.) by HPLC-DAD-ESI-QTOF-MS. *Food Chemistry*, 141:2269-2277.

Açıkgöz, M.A. (2020). Establishment of cell suspension cultures of *Ocimum basilicum* L. and enhanced production of pharmaceutical active ingredients. *Industrial Crops & Products*, 148:112278.

Ali, A.M.A., El-Nour, M.E.M., & Yagi, S.M. (2018). Total phenolic and flavonoid contents and antioxidant activity of ginger (*Zingiber ocinale* Rosc.) rhizome, callus and callus treated with some elicitors. *Journal of Genetic Engineering and Biotechnology*, 16:677–682.

Alirezaei, F., Kiarostami, K., & Hussein Zadeh Namin, M. (2017). Evaluation effect of silver nitrate on antioxidant activity and antioxidant compounds in calli frome leaves of *Lavandula angostifolia* Mill. *Applied Biology,* 30(1):137–158.

Al-Khayri, J.M. & Naik, P.M. (2020). Elicitor-Induced Production of Biomass and Pharmaceutical Phenolic Compounds in Cell Suspension Culture of Date Palm (*Phoenix dactylifera* L.). *Molecules,* 25:4669.

Cai, Z., Kastell, A., Speiser, C., & Smetanska, I. (2013). Enhanced resveratrol production in *Vitis vinifera* cell suspension cultures by heavy metals without loss of cell viability. *Applied Biochemistry and Biotechnology,* 171(2):330–340.

Deepthi, S., & Satheeshkumar, K. (2016). Enhanced camptothecin production induced by elicitors in the cell suspension cultures of *Ophiorrhiza mungos* Linn. *Plant Cell Tissue Organ Culture,* 124(3):483–493.

Fernández-León, M.F., Fernández-León, A.M., Lozano, M., Ayuso, M.C., Amodio, M.L., Colelli, G., & González-Gómez, D. (2013). Retention of quality and functional values of broccoli 'Parthenon' stored in modified atmosphere packaging. *Food Control*, 31:302-313.

Gamborg, O.L., Miller, R.A., & Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research,* 50(1):151–158.

Gheisary, B., Hosseini, B., Hassanpour, H., & Rahimi, A. (2018). Effects of Silicon and $AgNO_3$ Elicitors on Biochemical Traits and Antioxidant Enzymes Activity of Henbane (*Hyoscyamus reticulatus* L.) Hairy Roots. *Journal of Medicinal Plants and By-products,* 2:135-144.

Gonçalves, S., Mansinhos, I., Rodríguez-Solana, R., Pérez-Santín, E., Coelho, N., & Romano, A. (2019). Elicitation improves rosmarinic acid content and antioxidant activity in *Thymus lotocephalus* shoot cultures. *Industrial Crops and Products,*137:214–220.

Krishnan, V., Ahmad, S., & Mahmood, M. (2015). Antioxidant potential in different parts and callus of *Gynura procumbens* and different parts of *Gynura bicolor*. *BioMed Research International,* 2015:1-7.

Nadeem, M., Abbasi, B.H., Garros, L., Drouet, S., Zahir, A., Ahmad, W., Giglioli-Guivarc'h, N., & Hano, C. (2018). Yeast-extract improved biosynthesis of lignans and neolignans in cell suspension cultures of *Linum usitatissimum* L. *Plant Cell Tissue Organ Culture,* 135(2):347–355.

Namdeo, A.G. (2007). Plant cell elicitation for production of secondary metabolites: a review. *Pharmacognosy Reviews,*1(1):69–79.

Nazir, M., Tungmunnithum, D., Bose, S., Drouet, S., Garros, L., Giglioli-Guivarc'h, N., Abbasi, B.H., & Hano, C. (2019). Differential production of phenylpropanoid metabolites in callus cultures of *Ocimum basilicum* L. with distinct *in vitro* antioxidant activities and in vivo protective effects against UV stress. *Journal of Agricultural and Food Chemistry,* 67(7):1847–1859.

Ozsan, T., & Onus, A.N. (2020). Callogenesis Optimization of Some Globe Artichoke [*Cynara cardunculus* var. *scolymus* (L.) Fiori] Cultivars Based on *in vivo* and *in vitro* Leaf Explants. *Notulae Botanicae Horti Agrobotanici Cluj Napoca*, 48(4):1873-1884.

Pandino, G., Lombardo, S., Mauromicale, G., & Williamson, G. (2011). Profile of polyphenols and phenolic acids in bracts and receptacles of globe artichoke (*Cynara cardunculus* var. *scolymus*) germplasm. *Journal of Food Composition and Analysis*, 24(2):148–153.

Sabir, S.M., Ahmad, S.D., Hamid, A., Khan, M.Q., Athayde, M.L., Santos, D.B., Boligon, D.B., & Rocha, J.B.T. (2012). Antioxidant and hepatoprotective activity of ethanolic extract of leaves of *Solidago microglossa* containing polyphenolic compounds. *Food Chemistry,* 131(3):741–747.

Sarkate, A., Banerjee, S., Mir, J.I., Roy, P., & Sircar, D. (2017). Antioxidant and cytotoxic activity of bioactive phenolic metabolites isolated from the yeast-extract treated cell culture of apple. *Plant Cell Tissue Organ Culture,* 130(3):641–649.

Siatka, T. (2019). Effects of growth regulators on production of anthocyanins in callus cultures of *Angelica archangelica*. *Natural Product Communications,* 2019:1-4.

Škerget, M., Kotnik, P., Hadolin, M., Hraš, A.R., Simonič, M., Knez, Z. (2005). Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chemistry*, 89:191–198.

Winson, K.W.S., Chew, B.L., Sathasivam, K., & Subramaniam, S. (2020). The establishment of callus and cell suspension cultures of *Hylocereus costaricensis* for the production of betalain pigments with antioxidant potential. *Industrial Crops and Products,* 115:112750.

Xu, Y., Fan, M., Ran, J., Zhang, T., Sun, H., Dong, M., Zhang, Z., & Zheng, H. (2016). Variation in phenolic compounds and antioxidant activity in apple seeds of seven cultivars. *Saudi Journal of Biological Sciences,* 23(3):379–388.

Yan, Q., Shi, M., Ng, J., & Wu, J.Y. (2006). Elicitor-induced rosmarinic acid accumulation and secondary metabolism enzyme activities in *Salvia miltiorrhiza* hairy roots. *Plant Science,* 170(4):853–858.

Zaker, A., Sykora, C., Gössnitzer, F., Abrishamchi, P., Asili, J., Mousavi, S.H., & Wawrosch, C. (2015). Effects of some elicitors on tanshinone production in adventitious root cultures of *Perovskia abrotanoides* Karel. *Industrial Crops and Products,* 67:97–102.

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HORTICULTURAL STUDIES

RESEARCH PAPER

Determination of Shelf-Life of New Satsuma (Rize) Mandarin (*Citrus unshiu* Marc.) Cultivar Candidates Obtained by Clonal Selection

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Abstract

Satsuma (Rize) Mandarin is Türkiye's most widely produced and exported mandarin variety. Due to its early ripeness and seedlessness, Satsuma mandarin (*Citrus unshiu* Marc.) is a popular citrus fruit in domestic and foreign markets. The present study was conducted at Recep Tayyip Erdogan University, Faculty of Agriculture, Department of Horticulture in 2020-2021 to determine the shelf-life of Satsuma mandarin genotypes obtained as a result of clonal selection carried out in Rize province and its districts. The harvested mandarin variety candidates were pomologically analyzed and stored in an environment containing at 15°C ± 1 temperature and 55-60% humidity. The changes in fruit weight (g), fruit juice content (%), rind thickness (mm), total soluble solid (%, TSS), and titratable acid (%, TA) content were examined at one-week intervals during the storage period. It was determined that weight loss (%) and TSS (%) increased steadily, while fruit juice content (%), rind thickness (mm), and titratable acid (%) decreased steadily during storage in all genotypes and control. Also, the differences detected in terms of the properties examined were lower on 7 and 14 days than the initial value, whereas they were higher on 21 days. As a result of the findings, it was determined that the quality losses of mandarin genotypes showed differences during the shelf-life, but they could be stored for 14 days without much loss in quality.

1. Introduction

A total of 161,800,880.88 tons of citrus fruits are produced worldwide on a total area of 10,222,415 ha. Orange is the type with the highest production amount in citrus production. Orange production is followed by mandarin, lemon, and grapefruit. Türkiye ranks 8th in the world with 5,362,615 tons of citrus production in 166,417 ha. Türkiye's citrus production consists of 1,742,000 tons of oranges, 1,819,000 tons of mandarin, 1,550,000 tons of lemon, and 249,000 tons of grapefruit (FAO, 2021). Türkiye Statistical Institute (TSI) has reported that Türkiye's mandarin production were increased in 2020, 2021, and 2022. The most produced mandarin variety in Türkiye is Satsuma mandarin (854,720 tons), with a ratio of 45.90%. Satsuma mandarin is followed by Klemantin (86,121 tons), King (5,793 tons), and other varieties (918,366 tons).

Satsuma mandarin is a low-acid, easy-to-peel, seedless, aromatic, and high-quality variety that ripens earlier than other mandarin varieties. It is suitable for storage and transportation and is popular worldwide (Campbell et al., 2004; Cantuarias-Avilés et al., 2010; Turgutoğlu, 2020). Satsuma mandarin was introduced to Türkiye via Batumi, spread to the Eastern Black Sea region and then spread to areas that can grow in the Aegean and Mediterranean regions.

Cultivation of tropical and semitropical citrus fruits is concentrated in subtropical regions (Davies

and Albrigo, 1994). In Türkiye, ecological conditions facilitate the economic cultivation of citrus fruits in the Mediterranean and Aegean Regions (Kaplankıran et al., 2005). However, Satsuma mandarin, also known as Rize mandarin, the most exported fruit in Türkiye, has found the best ecology in the Eastern Black Sea Region, especially in Rize province.

The Black Sea Agricultural Region has 11.8% of Türkiye's fruit production, and the Eastern Black Sea Region ranks 3rd in citrus production after the Mediterranean and Aegean regions (TSI, 2020). Citrus fruits and especially Satsuma (Rize) mandarin, which are in the subtropical fruit group, have found a place to grow individually in home gardens as a border tree in the region, although there are few closed orchards. However, in recent years, it is known that the fruits of the scattered trees are collected and sold at particular sales points in the districts, mandarin jam is also produced in Rize province, and studies have been initiated to establish a mandarin jam factory with increasing demand (Yazıcı et al., 2020).

Fruits maintain their vitality after harvest; in other words, they continue their physiological respiration (Çavuşoğlu et al., 2019). Many factors, such as respiration rate, appropriate harvest time, presence of pathogens, the resistance of the variety to preservation, orchard maintenance conditions, storage temperatures, oxygen, the composition of the packaging atmosphere, and many other factors, affect the shelf-life of fruits. Also, the shelf-life of fresh fruits is shortened due to ethylene, a vital phytohormone associated with the ripening process (Kafa and Canıhoş, 2010; Nayik and Muzaffar, 2014; Çavuşoğlu et al., 2019; Hu et al., 2019).

It is known that citrus fruits have a longer shelf-life than other tropical fruits, but if post-harvest processing is not carried out correctly and stored under the appropriate conditions, the amount of marketable fruit will decrease (Strano et al., 2017). It has also been reported that Mandarins (*Citrus reticulata* Blanco) are more sensitive to post-harvest storage than other citrus species (Cohen et al., 1990).

Approximately half of the world's citrus production is for the fresh market. Therefore, the post-harvest shelf-life in non-climacteric citrus fruits is vital to reduce post-harvest losses and extend the time to market. Citrus fruits have a relatively long post-harvest life when kept under the suitable conditions, but they are prone to developing numerous post-harvest disorders that reduce rind quality. The citrus fruit rind contains oil glands filled with essential oils that are easily oxidized. Exposure of fruits to stressful conditions can lead to the breakdown of gland cells and the release of essential oil content, which can cause injury and damage. Furthermore, the rotting of citrus fruits after harvest due to fungal infection is quite common (Lafuente and Zacarías, 2006; Petracek et al., 2007; Lado et al., 2019; Zacarías et al., 2020).

It has been reported that the shelf-life of citrus fruits can be increased by reducing mechanical damage during post-harvest packaging and packaging processes and by expanding cold system transportation (Strano et al., 2017).

To increase the storage and shelf-life of mandarins, the use of UV-C radiation (Shen et al., 2013a), pre-harvest gibberellic acid application (Rokaya et al., 2016a), investigation of packaging packages with different properties (Mahajan et al., 2016), laurel and wax applications (Doğan, 2017), 1% sodium metabisulfite (SMBS) application (Özdemir et al., 2020), post-harvest salicylic acid application (Haider et al., 2020), chitosan and wax applications (Zan and Özdemir, 2022), and hot water dipping (Dündar et al., 2020). The quality can be maintained with these applications, even under normal storage conditions, and they are widely used worldwide (Bisen et al., 2012).

It has been known that preservation and packaging facilities are not sufficient in the Eastern Black Sea region (Akbulut et al., 2017). In Rize province, Satsuma mandarin is sold in special sales places, especially in a very long period after harvest, and applications are not made to increase the storage period. Therefore, the fruits are kept on the tree, and since the harvest period coincides with the winter months, snowfall and adverse weather conditions cause product losses. Some producers keep the mandarin fruits in covered open areas and offer them for sale in parts. No studies have been carried out in the region for Rize mandarin's shelf-life and storage. It has been known that it is crucial to know the shelf-life of the varieties sought, especially in domestic and foreign markets, and to develop appropriate storage and transportation methods in retail sales places such as Rize province. Also, it has been reported that appearance, robustness, and shelf-life are essential for wholesalers and retail producers (Brasil and Siddiqu, 2018). Therefore, the first shelf-life study was carried out in the Rize mandarin, and the results were evaluated in the present study.

Satsuma Mandarin entered our country for the first time in Rize through Russia. Considering that different genotypes have been formed by the vegetative propagation of this mandarin variety until today, it has been accepted that Rize province has an important genetic resource. With two projects completed by Yazıcı et al. (2017, 2021), somaclonal differences in mandarins were determined, and important Satsuma mandarin cultivar candidates for our country were determined. Among these candidates, there are cold-resistant, early, and late varieties and a variety of candidates were identified that could be effective in extending the mandarin season not only for the Black Sea region but also in Türkiye. The present study was conducted at Recep Tayyip Erdogan University, Faculty of Agriculture, Department of Horticulture to determine the shelf-life of new mandarin variety candidates determined by clonal selection in Rize province.

2. Material and Methods

2.1. Plant materials

In the study, eight Satsuma mandarin genotypes were selected by clonal selection from Rize region, grafted on trifoliate rootstock (*P. trifoliata* (L.) Raf.) and established in the orchard with 4×4 m spacing were used as the material, and a registered Satsuma mandarin variety grafted on trifoliate rootstock was used as the control. Mandarin cultivar candidates used as material in this study are high yield and quality cultivar candidates. Among these cultivar candidates, Yu 2 and Tek 1 are early season, Pa 2, Tek 4 and Yu 3 are late season, Tek 3, Tek 8 and İslm are medium late season characteristics.

2.2. Methods

The harvested fruit samples of each mandarin genotype were divided into four groups, and the first group was initially subjected to pomological analyses, then stored at 15°C ± 1 temperature and 55-60% humidity. To determine the quality losses in the fruit samples during the shelf period, weight loss (%), TSS (%), titratable acid content (%), fruit juice content (%), rind thickness (mm) changes were determined at one-week intervals on the 2nd group on day 7, 3rd group on day 14 day and 4th group on day 21. The experiment was established according to the factorial experimental design in randomized plots with three replicates. The data obtained from the experiment was analyzed with the JMP statistical package program, and the LSD (*P<0.05*) multiple comparisons test was applied to determine the differences between the means.

Weight loss was calculated in percentile for each treatment in each analysis period by comparing the initial weight with a precision balance of 0.01 g. Rind thickness was determined by cutting the fruits transversely from the equator and measuring the flavedo and albedo thickness with the help of calipers. The fruit juice was weighed individually by squeezing the juice of each fruit. After the pulp weight was determined, the pulp content % was calculated. The TSS content was determined in percentile using a hand refractometer after the juices were filtered through the cheesecloth. Titratable acid (TA) content was calculated in % citric acid by titrating the juice samples with 0.1 N NaOH.

3. Results and Discussions

3.1. Weight loss

The weight losses of fruit samples of different mandarin genotypes after three weeks of storage at 15°C ± 1 temperatures and 55-60% humidity is given in Table 1. According to the statistical analysis results, the differences between shelf periods and genotypes in terms of weight losses were found to be statistically significant (*P< 0.05*). As seen in Table 1, weight losses increased in all mandarin types in parallel with the increase in shelf period. During the 21-days shelf-life, the lowest weight loss was found in Tek 1 (18.78%), İslm (18.84%), and Control (19.75%) fruit groups, followed by Tek 4 (22.06%), Tek 3 (22.08%), Pa 2 (23.20%), and Yu 2 (26.57%). The highest weight loss was found in the Yu 3 (31.55%) and Tek 8 (28.27%) mandarin genotypes. It was also reported by different researchers (Dal and Gözen, 2010; Yadav et al., 2010; Kaur and Kumar, 2014; Rokaya et al., 2016b; Majahan et al., 2016; Zan and Özdemir, 2022) that weight loss in fruits increases in parallel with the shelf period.

Dal and Gözen (2010) reported that the weight loss of three different types of Satsuma mandarins, which were applied to wax and kept at 20 ±1°C and 55-60% humidity for 10 days, was 8.46-8.97-10.09% in the control plants. Kaur and Kumar (2014) determined the shelf-life of Kinnow mandarins and the weight loss as 21.22% on day 15 and 33.33% on day 30. Rokaya et al. (2016a) examined the effects of post-harvest treatments on the shelf-life and quality of mandarins (*Citrus reticulata* Blanco) and found that the weight loss of mandarins stored for four weeks at 14ºC - 18ºC and 45% - 73% humidity was 21% in untreated fruits. Majahan et al. (2016) determined that the rate of weight loss in Kinnow mandarin increased during the shelf period, and the weight loss was 15% on day 21. Pekmezci (1984) determined that Satsuma mandarins can be successfully stored for 2-2.5 months at 3ºC and 85-90% relative humidity. In the study, it was also reported that the thin rind of mandarins accelerated their weight loss.

3.2. Fruit juice content

Changes in the fruit juice value during the shelf-life of Rize mandarin genotypes are given in Table 2. At the beginning of the shelf-life, the average fruit juice content was determined to be 40.95% and 35.30% at the end of the 21-day shelf-life. During the shelf-life period, Yu 2, İslm, Tek 1, Tek 3, Tek 4, Tek 4, Tek 8, and Pa 2 genotypes showed a decrease in the fruit juice values, whereas the Yu 3 and control genotypes showed an increase. Both genotypes and shelf-life changes in the fruit juice values were statistically significant (*P< 0.05*). Similar to the results obtained in the present study, Kaur and Kumar (2014) determined the fruit juice content in Kinnow mandarins to be 45% on day 15th day and 43.95% on 30th day. Rokaya et al. (2016a) reported that the juice content of mandarins for four weeks decreased from 47.26% to 34.65%. During the shelf-life of the genotypes, there was a regular increase in the fruit juice values in İslm, Tek 1, Tek 3, and Tek 8 genotypes, whereas fluctuations were observed in Control, Yu 2, Yu 3, Pa 2, and Tek

Table 1. Weight loss (%) of genotypes during storage.

Genotypes	Shelf life			Average (Genotypes)
	7th day	14th day	21st day	
Control	11.80	16.27	31.17	19.75 d
Yu 2	12.68	32.19	34.84	26.57 b
Yu 3	14.02	35.44	45.02	31.55 a
Pa 2	8.05	27.33	34.23	23.20 bd
İslm	10.91	15.04	30.58	18.84 d
Tek 1	10.20	15.92	30.21	18.78 d
Tek 3	13.99	20.18	32.06	22.08 cd
Tek 4	7.88	23.85	34.46	22.06 cd
Tek 8	14.05	21.86	41.80	25.90 bc
Average (Storage period)	11.50 c	25.14 b	37.01 a	

LSD 5% =3.53 (Storage period), LSD 5% =6.11 (Genotypes), LSD 5% = N.S. (Genotypes×Storage period)

Table 2. Changes in the fruit juice values (%) in genotypes during storage.

Genotypes	Shelf life				Average (Genotypes)
	Inception	7th day	14th day	21st day	
Control	40.23 fj	52.50 a	37.84 gk	37.05 gm	41.65 ab
Yu 2	41.92 eg	33.45 lp	46.61 cd	34.62 kp	38.90 cd
Yu 3	34.80 kp	47.45 c	40.40 eh	39.62fı	40.32 bc
Pa 2	34.89 kp	33.18 mp	31.86 np	34.17 kp	33.27 f
İslm	43.96 df	44.43 ce	36.36 hm	36.32 ım	40.02 bc
Tek 1	41.81 eg	39.75 fı	35.31 jo	34.20 kp	37.52 de
Tek 3	40.50 fı	36.40 hm	36.34 hm	30.68 p	35.73 e
Tek 4	52.31 a	31.60 op	45.62 cd	35.70 ın	43.31 a
Tek 8	38.15 gm	37.28 gl	37.07 gm	35.35 jo	36.72 e
Average (Storage period)	40.95 a	39.56 b	38.60 b	35.30 c	

LSD 5% =1.35 (Storage period), LSD 5% =2.03 (Genotypes), LSD 5% =4.06 (Genotypes×Storage period)

Table 3. Changes in rind thickness (mm) of genotypes during storage.

Genotypes	Shelf life				Average (Genotypes)
	Inception	7th day	14th day	21st day	
Control	2.45	2.85	1.91	1.66	2.21 b
Yu 2	2.89	2.41	2.37	1.98	2.41 a
Yu 3	3.22	2.10	1.87	1.64	2.20 b
Pa 2	2.46	2.18	2.04	1.70	2.10 c
İslm	2.35	1.93	1.93	1.40	1.90 d
Tek 1	2.75	2.17	2.15	1.89	2.24 b
Tek 3	2.90	2.28	2.28	2.06	2.40 a
Tek 4	2.53	2.39	2.07	1.72	2.17 bc
Tek 8	2.54	2.16	2.02	1.75	2.11 c
Average (Storage period)	2.68 a	2.29 b	2.07 c	1.76 d	

LSD 5% =0.13 (Storage period), LSD 5% =0.19 (Genotypes), LSD 5% = N.S. (Genotypes×Storage period)

4 genotypes. It has been reported that fluctuations were observed in the fruit juice content during storage (Rokaya et al., 2016b; Didin et al., 2018; Dündar et al., 2018; Özdemir et al., 2020; Güvenç and Dündar, 2021; Zan and Özdemir, 2022). These fluctuations were associated with the moisture loss of the fruit rind and solubility of compounds other than carbohydrates-sugars (Echeverria and Ismail, 1990; Özdemir et al., 2020; Zan and Özdemir, 2022).

3.3. Rind thickness

Regarding rind thickness, the differences between storage periods and genotypes were found to be statistically significant ($P< 0.05$). The rind thickness of the genotypes decreased throughout the shelf-life analysis. The rind thickness, which was 2.68 mm at the beginning, was determined to be 1.76 mm on day 21. At the end of the shelf period, the rind thickness was 2.41 mm in Yu 2 and 2.21, 2.41, 2.20, 2.10, 1.90, 2.24, 2.40, 2.17, 2.11 in control, Yu 2, Yu 3, Pa 2, İslm, Tek 1, Tek 3, Tek 4 and Tek 8, respectively.

Different researchers have also reported a decrease in fruit rind thickness during storage (Wang et al., 2019; Shahzad et al., 2022). Shahzad et al. (2022) reported that the rind thickness of Tango mandarin was 1.99 mm at the beginning of the storage period and became 1.73 mm at the end

Table 4. Changes in the TSS content (%) of genotypes during storage.

Genotypes	Shelf life				Average (Genotypes)
	Inception	7th day	14th day	21st day	
Control	8.40 e	8.80 c	8.60 d	8.80 c	8.65 b
Yu 2	7.80 h	8.20 f	8.40 e	8.47 de	8.22 c
Yu 3	8.60 d	8.80 c	9.00 b	9.20 a	8.90 a
Pa 2	7.40 j	7.60 ı	7.60 ı	7.20 k	7.45 f
İslm	8.00 g	8.00 g	8.00 g	8.20 f	8.05 d
Tek 1	7.20 k	7.40 j	7.20 k	7.40 j	7.30 g
Tek 3	8.00 g	8.00 g	8.00 g	8.10 g	8.02 d
Tek 4	7.80 h	7.80 ı	7.80 ı	8.00 g	7.85 e
Tek 8	8.20 f	8.00 g	8.31 e	8.40 e	8.22 c
Average (Storage period)	7.93 d	8.06 c	8.10 b	8.20 a	

LSD 5% = 0.05 (Storage period), LSD 5% =0.08 (Genotypes), LSD 5% =0.16 (Genotypes×Storage period)

Table 5. Changes in TA content (%) of genotypes during storage.

Genotypes	Shelf life				Average (Genotypes)
	Inception	7th day	14th day	21st day	
Control	1.00 eg	0.93 ıj	0.92 ıj	0.77 qs	0.91 de
Yu 2	0.96 gı	0.74 s	0.99 eg	0.87 km	0.89 e
Yu 3	1.14 a	1.01 df	1.06 bc	1.06 bc	1.07 a
Pa 2	0.98 fh	0.98 fh	0.96 gı	0.91 jk	0.96 c
İslm	1.04ce	1.12 a	1.05 cd	0.94 hı	1.04 b
Tek 1	0.83 np	0.85 mo	0.83 mp	0.79 pr	0.83 f
Tek 3	0.98 fh	0.84 mp	0.81 oq	0.75 rs	0.84 f
Tek 4	0.90 jl	0.90 jl	1.10 ab	0.80 oq	0.92 d
Tek 8	0.96 gı	0.86 ln	0.98 fh	0.84 mo	0.91 de
Average (Storage period)	0.97 a	0.91 b	0.97 a	0.86 c	

LSD 5% = 0.02 (Storage period), LSD 5% =0.02 (Genotypes), LSD 5% =0.05 (Genotypes×Storage period)

of the storage period. Wang et al. (2019) have reported that the rind thickness of pomelo (*Citrus grandis* Osbeck) varieties decreased during storage on the tree and with the storage period in the closed storage environment.

3.4. Total soluble solid (TSS) content

The differences between mandarin genotypes in terms of TSS, storage periods, genotypes, and genotype × storage period interactions were found to be statistically significant ($P< 0.05$). During the shelf-life of the Satsuma mandarin genotypes, the average TSS content increased from 7.93% at the beginning to 8.20% at the end of day 21. At the end of the shelf-life, the highest TSS content among genotypes was determined in Yu 3 (8.90%), followed by Control (8.65%), Yu 2 (8.22%) and Tek 8 (8.22%). The genotypes with the lowest TSS content were determined in Tek1 (7.30%) and Pa 2 (7.45%) (Table 4). Camilla et al. (2016) stated that water loss during storage increased the sugar content in the fruit. In shelf-life studies, it has been reported by many researchers that the TSS content increases during shelf-life (Kim et al., 1998; Şen and Karaçalı, 2005; Altuntaş et al., 2009; Dal and Gözen, 2010; Yadav et al., 2010; Çalhan et al., 2012; Kaur and Kumar, 2014; Rokaya et al., 2016a; Dündar et al., 2018). Şen and Karaçalı (2005) reported that 'Satsuma' mandarin generally did not change the TSS value and slightly decreased the TA value in the post-harvest period. Zan and Özdemir (2022) have reported that, on day 90 of Owari Satsuma mandarin stored in natural refrigerated storage and cold storage, the TSS value increased to 11.06% in natural refrigerated storage and 10.90% in cold storage. Rokaya et al. (2016a) have reported that the TSS content of the *Citrus reticulata* Blanco mandarin fruits increased during the shelf period, and the TSS value, which was 10.92% at the beginning, increased to 12.88% at the end of storage. In another study conducted to determine the shelf-life of Kinnow mandarins, Kaur and Kumar (2014) reported that the TSS content was 12.20% on day 15 and 12.53% on day 30. Our findings were similar to those of these studies. According to Pantastico, changes in sugars are insignificant and slow in fruits that do not show climacteric properties. Although their amounts increase slightly at the beginning of storage, they decrease in long-term storage (Özdemir and Dündar, 1999). In our study, there was an increase in the amount of TSS before the 7th and 14th storage days and a decrease in some genotypes on the 21st day.

3.5. Titratable acid (TA)

After the analysis of the titratable acid content of the genotypes during the shelf-life, it was observed

that while the initial average was 0.97%, it decreased to 0.86% on day 21. At the end of the 21-day period, it decreased to 0.86%. In terms of titratable acid content, the differences between storage periods, genotypes, and genotype × storage period interactions were found statistically significant (*P< 0.05*) (Table 5).

At the end of the shelf period, the highest titratable acid content was determined in Yu 3 (1.07%), followed by İslm (1.04%), Pa 2 (0.96%), Tek 4 (0.92%), Tek 8 (0.91%) and Control (0.91%), and Yu 2 (0.89%). The genotypes with the lowest acid content were Tek 1 (0.83%) and Tek 3 (0.84%). Also, all genotypes showed a decrease in titratable acid content during the storage period. Many researchers have also reported that the acid content of Satsuma mandarin decreases in post-harvest storage conditions (Şen and Karaçalı, 2005; Hong et al., 2007; Tietel et al., 2010; Santos et al., 2010; Shen et al., 2013b; Özdemir et al., 2020; Zan and Özdemir, 2022).

Dal and Gözen (2010), in their shelf-life study on three different Satsuma mandarin genotypes, determined that TA values were decreased on day 10 of the shelf period and decreased to 1.30%-1.57%-1.67%. Zan and Özdemir (2022) determined that the titratable acid value of Owari Satsuma mandarin in natural refrigerated storage was 1.10% and 0.92% on day 90 in cold storage. Rokaya et al. (2016a) determined that the juice acid ratios of mandarins were 0.86%-0.75%-0.65%-0.53% on weeks 1, 2, 3, and 4 during the shelf-life, respectively. Yadav et al. (2010), in their study on Kinnow mandarins, found that the fruit juice acidity decreased from 0.64% to 0.57% in the control groups during the shelf-life. In their study to determine the shelf-life of the same variety, Kaur and Kumar (2014) determined the acid ratios as 0.58% on day 15 and 0.47% on day 30 of the shelf-life period.

4. Conclusion

The present study was carried out to determine the shelf-life of eight satsuma genotypes obtained by selection. As a result of the study, it was determined that shelf-life varied depending on both the storage period and the genotypes. Losses occurred in the fruit weights of the genotypes during the storage period. The highest weight loss (37.01%) was detected on day 21. Among the genotypes, the highest weight loss was found in the Yu 3 genotype (45.02%). During the shelf period, the fruit juice values of the fruits generally increased, whereas the rind thickness decreased. There was a continuous decrease in the titratable acid values. As a result of the findings, it was determined that the quality losses of mandarin genotypes showed differences during the shelf-life, but they could be stored for 14th days without much loss in quality.

References

Akbulut, M., Yazıcı, K., Karataş, A., Bakoğlu, N., Göksu, B., & Büyükdinç, D.T. (2017). The Storage Status of Horticultural Products in the Eastern Black Sea Region. *Fruit Science*, 1:25-27.

Altuntaş, E., Kaya, C., Yıldız, M., & Tekelioğlu, O. (2009). Determination of physico-mechanical properties of Amasya and Granny Smith apple cultivars during shelf life periods*. International Journal of Agricultural and Natural Sciences*, 2(2):7-13.

Bisen, A., Pandey, S.K., & Patel, N. (2012). Effect of skin coatings on prolonging shelf life of kagzi lime fruits (*Citrus aurantifolia* Swingle). *Journal of Food Science and Technology,* 49:753-759.

Brasil, I.M., & Siddiqu, M. (2018). Postharvest quality of fruits and vegetables: An overview. *Preharvest Modulation of Postharvest Fruit and Vegetable Quality*, 1:1-40.

Camilla de, A.P., Evandro, H.S., Mariangela C-Y., Marcos, A.M., & Fernando, A de A. (2016). Fremont mandarin: Fruit with a long shelf life for the fresh fruit market. *African Journal of Agricultural Research*, 11(45):4600–4609.

Campbell, B.L., Nelson, R.G., Ebel, R.C., Dozier, W.A., Adrian, J.L., & Hockema, B.R. (2004). Fruit quality characteristics that affect consumer preferences for satsuma mandarins. HortScience, 39(7):1664-1669.

Cantuarias-Avilés, T., Mourão Filho, F.D.A.A., Stuchi, E.S., da Silva, S.R., & Espinoza-Núñez, E. (2010). Tree performance and fruit yield and quality of 'Okitsu' Satsuma mandarin grafted on 12 rootstocks. *Scientia Horticulturae*, 123(3):318-322.

Cohen, E., Shalom, Y., & Rosenberger, I. (1990) Postharvest ethanol build up and off-flavor in 'Murcott' tangerine fruits. *Journal of the American Society for Horticultural Science*, 115(5):775-778.

Çalhan, Ö., Eren, İ., Onursal, C.E., & Güneyli, A. (2012). The effects of DCA storage on "Granny Smith" apple fruit. V. Bahçe Ürünlerinde Muhafaza ve Pazarlama Sempozyumu, 18-21 Eylül 2012, İzmir/Türkiye, p:145-152. (in Turkish).

Çavuşoğlu, Ş., Keskin, N., & Türkoğlu, N. (2019). Possibilities of use of edible films and coatings for post-harvest fruit cold storage. II. International Agriculture and Forestry Congress, 8-9 November 2019, İzmir/Türkiye, p:57.

Dal, B., & Gözen, B. (2010). The effects of post-harvest waxing on shelf life of satsuma mandarin types. *Acta Horticulturae*, 877:57–764.

Davies, F.S., & Albrigo, L.G. (1994). Citrus, crop production science in horticulture 2. ISBN 13: 978-0851988672, pp 254.

Didin, Ö., Özdemir, A., Çandir, E., Kaplankiran, M., & Yildiz, E. (2018). Effects of rootstocks on cold storage of 'Robinson' mandarin. *Çukurova Journal Agriculture Food Sciences*, 33(2):1-16.

Doğan, Y.L. (2017). Effects on The Stability, Fruit Quality and Biochemical Content of The Defense and Wax Applications in the Satsuma Mandarin Variety. MSc. Thesis, Yüzüncü Yil University, Van.

Dündar, Ö., Özkaya, O., Demircioğlu, H., Eroğlu, O., Dündar, B., & Kükürt, E. (2018). Effect of preharvest applications on storage and quality properties of Miho Wase mandarin. *Yüzüncü Yil Üniversitesi Journal of Agricultural Sciences*, 28(Special Issue):57-62.

Dündar, Ö., Demircioğlu, H., Belik, M., & Özkaya, O. (2020). Effect of hot water application on storage and quality properties of 'Miho Wase' mandarin. *Acta Horticulturae*, 1275:445-450.

Echeverria, E., & Ismail, M. (1990). Sugars unrelated to Brix changes in stored citrus fruits. *HortScience*, *25*:6.

FAO. (2023). Food and Agricultural Organization. https://www.fao.org/faostat/en/#data/QCL. Access date: 07.02.2023.

Güvenç, B., & Dündar, Ö. (2021). Determination Storage Performance of hybrid fruits obtained by hybridization of Clemantine mandarin and Orlando tangelo. *Alatarım*, 20(1):32-44.

Haider, S.A., Ahmad, S., Khan, A.S., Anjum, M.A., Nasir, M., & Naz, S. (2020). Effects of salicylic acid on postharvest fruit quality of "Kinnow" mandarin under cold storage. *Scientia Horticulturae*, 259:1-11.

Hong, S.I., Lee, H.H., & Kim, D. (2007). Effects of hot water treatment on the storage stability of satsuma mandarin as a postharvest decay control. *Postharvest Biology and Technology*, 43(2):271-279.

Hu, B., Sun, D. W., Pu, H., & Wei, Q. (2019). Recent advances in detecting and regulating ethylene concentrations for shelf-life extension and maturity control of fruit: A review. *Trends in Food Science & Technology*, 91:66-82.

Kafa, G., & Canıhoş, E. (2010). Turunçgil Yetiştiriciliği. Türkiye Cumhuriyeti Tarım ve Köyişleri Bakanlığı Teşkilatlanma ve Destekleme Genel Müdürlüğü Televizyon Yoluyla Yaygın Çiftçi Eğitimi Projesi (Yayçep). Yayın 54. (in Turkish).

Kaplankıran, M., Demirkeser, T.H., & Yıldız, E. (2005). The effects of some citrus rootstocks on fruit yield and quality for Okitsu Satsuma during the juvenility period in Dortyol (Hatay, Turkey) conditions. In: 7th International Society of Citrus Nurserymen Congress. Cairo, Egypt, Sept. 17–21.

Kaur, N., & Kumar, A. (2014). Impact of post harvest treatments on shelf life of Kinnow mandarin. International *Journal of Advanced Research*, 2(5):290-295.

Kim, W.T., Lee, S.Y., Kim, J.Y., Kang, C.H., & Koh, J. S. (1998). Effects on the storage life of satsuma mandarin by the pretreatment at various temperatures. *Applied Biological Chemistry*, 41(3):228-233.

Lado, J., Cronje, P.J.R., Rodrigo, M.J., & Zacarias, L. (2019). Postharvest physiological disordes of citrus fruits. In: de Freitas, S.T., Pareek, S. (Eds.), Postharvest Physiological Disorders of Fruits and Vegetables. CRC Press, Boca Raton, FL, pp. 321–342.

Lafuente, M.T., & Zacarías, L. (2006). Postharvest physiological disorders in citrus fruit. *Stewart Postharvest Rev*. 2:1–9.

Mahajan, B.V.C., Singh, R., & Kumar, M. (2016). Quality assurance and shelf-life extension of Kinnow mandarin fruit under supermarket conditions. International Journal of Fruit Science, 16(1):94-102.

Nayik, G.A., & Muzaffar, K. (2014). Developments in packaging of fresh fruits-shelf life perspective: A review. *American Journal of Food Science and Nutrition Research*, 1(5):34-39.

Özdemir, A.E., & Dündar, Ö. (1999). Derim sonrası sıcak su uygulamalarının bazı portakalların muhafazasına etkileri. Türkiye III. Ulusal Bahçe Bitkileri Kongresi, Ankara, Türkiye, p:126-131. (in Turkish).

Özdemir, A.E., Didin, M., Bayazıt, S., Alakraa, N., Gürses, H.K., & Akı, M.K. (2020). Effects of sodium metabisulphite applications on cold storage of 'Owari Satsuma' mandarin variety. *Bahçe,* 49(1):173–183.

Pekmezci, M. (1984). Satsuma' ve 'Klemantin' mandarinlerinin soğukta muhafazası üzerinde araştırmalar. *TÜBİTAK Yayınları*, 587(118):99-116 (in Turkish).

Petracek, P.D., Kelsey, D.F., & Grierson, W (2007). Physiological peel disorders. pp. 397–442.In: Wardowski, W.F., Miller, W.M., Hall, D.J., Grierson, W. (Eds.), Fresh Citrus Fruits.

Rokaya, P., Baral, D., Gautam, D., Shrestha, A., & Paudyal, K. (2016a). Effect of Postharvest Treatments on Quality and Shelf Life of Mandarin (*Citrus reticulate* Blanco). American Journal of Plant Sciences, 7:1098-1105.

Rokaya, P.R., Baral, D.R., Gautam, D.M., Shrestha, A.K., & Paudyal, K.P. (2016b). Effect of pre-harvest application of gibberellic acid on fruit quality and shelf life of mandarin (*Citrus reticulata* Blanco). *American Journal of Plant Sciences*, 7:1033-1039.

Santos, L.O., Durigan, J.F., Martins, R.N., Durigan, M.F.B., & Stuchi, E.S. (2010). Postharvest storage of 'Ponkan', 'Satsuma Okitusu' and 'Fremont' tangerines and their minimally processed products using refrigeration and controlled atmosphere. *In XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on August 2010*, pp:583-589.

Shahzad, F., Vashisth, T., Ritenour, M.A., & Brecht, J.K. (2022). Quest for desirable quality of Tango Mandarin in the citrus greening era: The promise of integrated approaches. *LWT*, 161:113321.

Shen, Y., Sun, Y., Qiao, L., Chen, J., Liu, D., & Ye, X. (2013a). Effect of UV-C treatments on phenolic compounds and antioxidant capacity of minimally processed Satsuma mandarin during refrigerated storage. *Postharvest Biology and Technology*, 76:50-57.

Shen, Y., Zhong, L., Sun, Y., Chen, J., Liu, D., & Ye, X. (2013b). Influence of hot water dip on fruit quality, phenolic compounds and antioxidant capacity of Satsuma mandarin during storage. *Food Science and Technology International*, 19(6):511-521.

Şen, F., & Karaçalı, İ. (2005). The Effects of UV-C Light and Other Protected Treatments on Quality and Resistance Capacity of Satsuma Mandarins. *Derim*, 22(1):10-19.

Strano, M.C., Altieri, G., Admane, N., Genovese, F., & Di Renzo, G.C. (2017). Advance in Citrus Postharvest Management: Diseases, Cold Storage and Quality Evaluation.p.139-159. In: Harsimran Gill (ed.), Citrus Pathology, IntechOpen, United Kingdom. doi: 10.5772/66518.

Tietel, Z., Weiss, B., Lewinsohn, E., Fallik, E., & Porat, R. (2010). Improving taste and peel color of early-season Satsuma mandarins by combining high-temperature conditioning and degreening treatments. *Postharvest Biology and Technology*, 57(1):1-5.

Turgutoğlu, E. (2020). Mandarin Yetiştiriciliği. T.C. Tarım ve Orman Bakanlığı Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü Batı Akdeniz Tarımsal Araştırma Enstitüsü. (in Turkish).

TSI, (2022). Turkish Statistical Instıtute. Date of access: 07.02.2023.

Wang, Y., He, W., Fu, X., Chen, Q., & Wang, X. (2019). Effect of on-tree storage on fruit quality of three pummelo (*Citrus grandis* Osbeck) cultivars. *In IOP Conference Series: Earth and Environmental Science,* 330:1-6.

Yadav, M., Kumar, N., Singh, D.B. & Singh, G.K. (2010). Effect of postharvest treatments on shelf life and quality of kinnow mandarin. *Indian Journal of Horticulture*, 67:243-248.

Yazıcı, K., Göksu B., Bakoğlu N., & Akbulut M. (2017). Satsuma (Rize) Mandarininin Klon Seleksiyonu ile Islahı. Yükseköğretim Kurumları Destekli Proje, 2013 – 2017. (in Turkish).

Yazıcı, K., Göksu, B., Korkmaz, Ö., Akbulut, M., Bakoğlu, N., Tepe., G., & Ömür, R. (2020). Breeding of Rize (Satsuma) mandarin by clonal selection. *Recep Tayyip Erdogan University Journal of Science and Engineering*, 1(1):1-15.

Yazıcı, K., Göksu B., Akbulut M., & Bakoğlu, N. (2021). Seleksiyonu sonucu elde edilen Rize (Satsuma) mandarini klonlarından yeni çeşit adaylarının seçimi (Seleksiyon II Aşaması). Yükseköğretim Kurumları Destekli Proje, 2018 – 2021. (in Turkish).

Zacarias, L, Cronje, P., & Palou, L. (2020). Postharvest technology of citrus fruits. pp. 421-446. Talon, M., Caruso, M., Gmitter, F.G. (ed), Woodhead Publishing. https://doi.org/10.1016/C2016-0-02375-6.

Zan, R., & Özdemir, A.E. (2022). Effect of Chitosan and Wax Treatments on Storage of 'Owari Satsuma' Mandarin in Common and Cold Store. *Alatarım,* 21(2):67-78.