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HORTICULTURAL STUDIES

RESEARCH PAPER

Effect of Potassium Silicate, Glycine Betaine and Proline on Fruit Quality of Peaches in Newly Reclaimed Land Exposed to Heat Stress

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Abstract

The current study was performed on a 6-year-old "Florida" peach cultivar (*Prunus persica*) during 2022 and 2023 respectively. Twenty-four peach trees uniformly were selected and sprayed two times at the beginning of pit starts hardening and at the end of phase two of fruit growth with the following treatments: the control, glycine betaine at 400 mg l^{-1}, potassium silicate at 200 mg l^{-1}, potassium silicate at 400 mg l^{-1}, potassium silicate at 200 mg l^{-1} + glycine betaine at 400 mg l^{-1}, potassium silicate at 400 mg l^{-1} + glycine betaine at 400 mg l^{-1}, proline at 400 mg l^{-1} and potassium silicate at 200 mg l^{-1} + proline at 400 mg l^{-1}. The results indicated that both treatments of potassium silicate at 200 mg l^{-1} + proline at 400 mg l^{-1} and potassium silicate at 400 mg l^{-1} + glycine betaine at 400 mg l^{-1} resulted in a significant increase in concentrations of anthocyanin and total carotenoid contents in the skin. Moreover, total soluble solid, vitamin C and total soluble solid / acidity were shown with high concentrations as compared with the control. Overall, the use of potassium silicate at 200 mg l^{-1} + proline at 400 mg l^{-1} or potassium silicate at 400 mg l^{-1} + glycine betaine at 400 mg l^{-1} two times is recommended.

1. Introduction

Peach is one of the important fruit crops in Egypt, with an estimated total fruit production quantity of both peaches and nectarines at about 337,910 tons. (FAO, 2020). Climate change is one of the main challenges in agricultural production which has many negative consequences in agricultural fields. Heat stress, an abiotic stress resulting from climate changes, is caused by high temperatures and leads to numerous adverse effects throughout various developmental stages, such as carbon assimilation, respiration, fertilization, cell differentiation, and fruit maturity. Furthermore, fruit crops such as apples (Austin et al., 1999; Sugiura et al., 2013) are negatively impacted by heat stress, particularly in terms of their maturity and fruit quality characteristics. The typical air temperature throughout the growing season for cultivated peach trees in Egypt ranges between 35 and 40ºC, while nighttime temperatures in the field range between 25ºC and 28ºC. It was recently reported that KU-PP2, a peach cultivar in Japan, has minimal chill requirements. For example, when trees were exposed to 30ºC, various adverse effects were observed, including decreases in fruit size, photosynthesis, stomata density, leaf size, thickness, and chlorophyll content. Additionally, high temperatures accelerated the harvesting season (Sikhandakasmita et al., 2021a,b). Meanwhile, growth and development could be slowed down by heat stress, which is reflected in peach fruit size, weight, and sweetness. Peach trees grown in the newly reclaimed areas in Egypt are exposed to many adverse effects due to heat, which disrupts the balance between plants and water. Problems with water uptake, imbalances in transpiration, stomatal malfunctioning, disruption of sap flow, inhibition of chlorophyll biosynthesis, ultimately leading to a decline in photosynthesis and damage to thylakoid membranes, result in increased membrane leakage (Prasad et al., 2008). The objectives of this study were to investigate the effects of some osmoregulators on peach trees and fruits exposed to heat stress under field conditions, and to assess the impact of potassium silicate, glycine betaine, and proline on fruit quality under such stressful conditions.

2. Material and Methods

The current study was carried out on the "Florida" peach cultivar (*Prunus persica*) during two consecutive seasons in 2022 and 2023. The six-year-old trees, planted in a private orchard in Sadat City, Menoufia Governorate, Egypt, were spaced at 4×5 meters and were irrigated with drips. Throughout the season, trees had been subjected to standard agricultural practices. The soil had a sand-like texture, and drip irrigation was used. The block design for the treatments was fully randomized. Three replications were used for each treatment and one peach tree represented one replication. For this study, twenty-four trees were used in each season. Eight treatments, applied by spraying twice at the beginning of pit hardening and then at the end of phase two from the double sigmoid curve of fruit growth, were randomly assigned to twenty-four standard "Florida" peach trees. The treatments included the following: tap water spray (as the control), glycine betaine at 400 mg l^{-1}, potassium silicate at 200 mg l^{-1}, potassium silicate at 400 mg l^{-1} + glycine betaine at 400 mg l^{-1}, proline at 400 mg l^{-1}, and potassium silicate at 200 mg l^{-1} + proline at 400 mg l^{-1}. To lower the surface tension and raise the contact angle of sprayed droplets, the non-ionic surfactant Top film was added to all treatments at 0.05% (V/V). The average air temperature measured during spray times was between 33ºC and 37ºC, while the nighttime temperature in the field is between 15 and 20ºC. These treatments were set up in a Randomized Complete Block Design (RCBD) Analysis of Variance (ANOVA). Statistical analysis was done according to (Gomez and Gomez,1984), using Costat (Version 6.303, cohort, USA, 1998–2004).

2.1. Physical characteristics

Samples of five fruits per tree (replicate) were collected randomly and the fruit weight was measured by using an electronic balance so as to determine peel and seeds weight percentage, fruits were peeled and the weight of total seeds, peel (g) and juice volume (ml) were calculated. Fruits diameter (mm) were measured by vernier caliper. Fruit firmness was measured by a hand Effigy-Penetrometers attached with a plunger 8 mm diameter was utilized to estimate this parameter as kg cm^{-2} (Southwick et al., 1996).

2.2. Chemical characteristics

Vitamin C was estimated by a 2,6-dichlorophenol indophenol method (Mazumadar and Majumder, 2003). A known amount of edible portion of "Florida prince" peach fruits extracted with 3% metaphosphoric acid by thorough crushing. The filtered extract was made up to a known volume with 3% metaphosphoric acid then titrated with the standard indophenol dye solution to a pink endpoint (persisting for fifteen sec). Electrolyte leakage (EL %) was determined from the following equation by a Conductivity meter (CD-4301) according to Fan and Sokorai (2005).

$$EL = \left(\frac{C_{60} - C_1}{CT}\right) \times 100$$

According to the AOAC (1994), total soluble solids (TSS %) is estimated in fruit juice by using a Carl-Zeiss hand refractometer and total titratable acidity (%) is expressed as g malic acid / 100 ml juice. Soluble solids content (SSC) / acid ratio was calculated from the results recorded for fruit juice SSC and TA. The spectrophotometer (APEL, PD-303S Japan) is used to measure the amount of vitamin C present (Desai and Desai, 2019). When bromine water is added, ascorbic acid oxidizes and becomes dehydroascorbic acid. A coupling reaction occurs when 2, 4 dinitrophenyl hydrazine is heated to 37°C for three hours. After treating the solution with 85% H_2SO_4, which produces a colored complex after three hours, the absorbance was measured at 491 nm.

Total carotenoids were extracted using the method given by Ranganna (1995). A known weight of sample, i.e. 5 g fresh peach fruit was weighed and ground finely with mortar and pestle in acetone till the residue became colorless. The acetone extract was collected in a conical flask. Separating funnel was used for the separation of the carotenoid pigments. The carotenoid extract was transferred to a separating funnel and then added petroleum was added with the addition of 10% Na_2SO_4. The funnel was swirled to separate the carotenoid layer. The isolated carotenoids were collected in a volumetric flask. The process was repeated till remained extract showed no color. The absorbance was read at 452 nm spectrophotometrically. Total carotenoids were estimated using the following formula:

$$Tc = \left(\frac{3.87 \times A \times Vm \times Df \times 100}{Sw \times 1000}\right)$$

Where; Tc: total carotenoids (mg 100 g^{-1} fresh weight^{-1}), A: absorbance (452 nm), Vm: volume make up, Df: dilution factor, and Sw: sample weight (g).

The procedure described by Onayemi et al., (2006) was used to determine the anthocyanin content of the extracts. One gram of fruit skin samples were ground up using a 1.5 M HCl (by volume) solution and 20 ml of 85% ethanol. The samples were stored in the deep freeze for the entire night while covered. After the extracts were finished using 50 milliliters of the solvent, a spectrophotometer (Unico 1200-USA) was used to measure the absorbance of the mixture at 530 nm. The outcome is given in mg 100 g^{-1} of fresh fruit. The following (Lees and Francis,1971) equation was used to calculate total anthocyanin.

$$Ta = \left(\frac{A530 \times V}{98.2 \times W}\right)$$

Where Ta: total anthocyanin (mg 100 g^{-1}), A530 is the rate of absorption of the sample at the wavelength of the subtitle A. For example, A530 is the absorption at a wavelength of 530 nm. V= total volume of extract (ml), W= weight of sample (g).

3. Results and Discussion

3.1. Physical characteristics

3.1.1. Fruit weight

The effect of various applications and the control on some physical characteristics of “Florida” peach fruits during the two study seasons is shown in Table 1. The data revealed that many treatments were effective in increasing fruit weight at harvest time especially the combination of potassium silicate at 200 mg l^{-1} plus proline at 400 mg l^{-1} in both seasons which had a superior influence on fruit weight as compared with all treatments in a consistent manner in both seasons and compared to the control. Such positive influence on fruit weight was followed in order by potassium silicate at 400 mg l^{-1} plus glycine betaine at 200 mg l^{-1}. Both combinations had a significant effect on fruit weight when compared with either proline alone or potassium silicate alone at the same concentration in both seasons. Additionally, the application of potassium silicate at either 400 or 200 mg l^{-1} significantly increased fruit weight consistently across both seasons. Similarly, the individual application of either Proline or glycine betaine resulted in a significant increase in fruit weight relative to the control in the two seasons. Thus, it was better to combine potassium silicate with either proline or with glycine betaine to have the most favorable influence on fruit weight at harvest. Thus, the combined influence of the enhancer of photosynthesis addition to the presence of the osmo-regulator such as proline or glycine betaine had the greatest potential to increase fruit weight of “Florida” peach fruits.

3.1.2. Stone weight

Changes in stone weight of “Florida” peach fruits in response to used applications were reported in Table 1. The data confirmed the previous trend that the combination of potassium silicate at 200 mg l^{-1} plus proline at 400 mg l^{-1} resulted in the highest stone weight as compared with the control and with many other treatments such as potassium silicate at 400 mg l^{-1} plus glycine betaine at 400 mg l^{-1} in addition to proline alone or potassium silicate alone at both rates at 200-400 mg l^{-1}. Moreover, the individual application of glycine betaine at 400 mg l^{-1} had a similar influence on stone weight compared to the control (which had the lowest stone weight), especially during the first season. However, the application of proline alone at 400 mg L-1 resulted in higher stone weight compared to that observed with glycine betaine at the same concentration in both seasons. Furthermore, the combined

Table 1. The effect of pre-harvest applications on some physical characteristics of "Florida" peach during the 2022 and 2023 seasons.

Treatments	Fruit weight (g)		Stone weight (g)		Fruit diameter (cm)		Fruit firmness (kg cm^{-2})	
	2022	2023	2022	2023	2022	2023	2022	2023
Control	105.65 g*	87.07 g	4.70 f	4.13 g	4.90 e	4.40 f	6.70 h	5.80 e
Glycine betaine (400 mg l^{-1})	112.01 f	93.42 f	4.94 f	4.61 f	5.57 d	4.70 e	7.00 g	5.80 e
Potassium silicate (200 mg l^{-1})	115.69 e	98.58 e	5.50 e	5.24 e	5.97 c	5.50 c	7.70 e	6.90 c
Potassium silicate (400 mg l^{-1})	121.02 d	102.98 d	6.20 d	5.73 d	5.73 cd	5.17 d	7.40 f	6.50 d
Potassium silicate (200 mg l^{-1}+ glycine betaine (400 mg l^{-1})	125.71 c	109.50 c	6.78 c	6.24 c	6.33 b	5.73 bc	8.17 d	7.30 b
Proline (400 mg l^{-1})	126.41 bc	111.81 bc	7.02 c	6.70 b	6.37 b	5.93 b	8.27 c	7.43 b
Potassium silicate (400 mg l^{-1})+ glycine betaine (400 mg l^{-1}l)	128.80 b	114.17 b	7.41 b	6.89 b	6.57 ab	5.97 b	8.50 b	7.50 ab
Potassium silicate (200 mg l^{-1}) + Proline (400 mg l^{-1})	133.12 a	119.33 a	7.98 a	7.37 a	6.83 a	6.23 a	8.73 a	7.87 a
LSD $_{at\ 5\%}$	2.61	2.36	0.25	0.24	0.29	0.24	0.09	0.37

* Values in each column when accompanied with similar letters, were not significantly different by using the least significant difference at 5 % for comparing the means.

treatment had a greater influence on stone weight than the individual applications, consistently across both seasons.

3.1.3. Fruit diameter

The response of fruit diameter of “Florida” peach fruits to various treatments before harvest was shown in Table 1. The data revealed that again the greatest fruit diameter was obtained with the combination of potassium silicate at a rate of 200 mg l^{-1} plus proline at 400 mg l^{-1} in both seasons. Similarly, the combination of potassium silicate at 400 mg l^{-1} plus glycine betaine at 400 mg l^{-1} had a similar influence to the first combination, especially in the first season followed by the sole treatment of proline at 400 mg l^{-1}. Even the individual treatment of potassium silicate whether at 200 or 400 mg l^{-1} caused a significant increase in fruit diameter as compared with the control in both season. However, the highest magnitude was obtained with the combination of potassium silicate at 200 mg l^{-1} plus proline at 400 mg l^{-1}.

3.1.4. Fruit firmness

The influence of pre-harvest application on the firmness of “Florida” peach fruits at harvest was reported in Table 1. The data revealed that many combination treatments were able to delay the less of flesh firmness especially the combination of potassium silicate at 200 mg l^{-1} plus proline at 400 mg l^{-1} in both seasons which resulted in a great magnitude of increase in the flesh firmness in a sensitive climacteric fruit such as peach fruit. The individual application of potassium silicate or proline at the same similar concentration was also able to delay the loss of firmness and even the osmo-regulators whether proline or potassium silicate alone when compared with the control in both seasons. When we compare the influence of proline with potassium silicate at 400 mg l^{-1} (similar concentration), proline had superiorly in delaying the loss of flesh firmness in a consistent manner in the two seasons.

3.2. Chemical characteristics

3.2.1. Total soluble solids (TSS)

The data regarding the changes in TSS in response to various applications before harvest to “Florida” peaches is shown in Table 2. The data revealed that the highest increase in TSS was found with the combinations of potassium silicate at 400 mg l^{-1} plus glycine betaine at 400 mg l^{-1} in both seasons as compared with the control and with other combinations followed by potassium silicate at 200 mg l^{-1} plus proline at 400 mg l^{-1}. However, individual treatment of proline alone or potassium silicate alone at 400 mg l^{-1} had potential to cause a significant increase in TSS as compared with the control while potassium silicate at 200 mg l^{-1} did not result in a significant change in TSS. Furthermore, glycine betaine at 400 mg l^{-1} resulted in a slight increase in TSS as compared with the control. Thus, there was in general, an additive influence from combining either proline or glycine betaine to potassium silicate either at 200 or at 400 mg l^{-1}. This trend of results is favored since proline or glycine betaine are natural osmo-regulators while potassium silicate is an enhancer of the photosynthates in mature leaves.

3.2.2. Juice acidity

Change in juice acidity of “Florida” peaches in response to various pre-harvest treatments were reported in Table 2. The data provided evidences about the magnitudes of reductions in juice acidity by many treatments since the highest acidity values were found with the control fruits in both seasons. It was clear that the used combination treatments resulted in the highest acidity reduction such as proline plus potassium silicate at 200 mg l^{-1} and the combination of potassium silicate at 400 mg l^{-1} plus glycine betaine at 400 mg l^{-1}. However, lower but significant drop of juice acidity occurred with the application of potassium silicate at 200 mg l^{-1} plus glycine betaine at 400 mg l^{-1} in a consistant manner in both seasons. Meanwhile, all individual treatments were able to reduce the juice acidity relative to the control especially proline that was much more effective on lowering juice acidity as compared with potassium silicate even at the higher rate at 400 mg l^{-1}.

3.2.3. TSS/acidity ratio

The influence of field applications of some osmo-regulators and potassium silicate at two concentrations on the TSS to acidity ratio of “Florida” peach fruits were reported in Table 2. The data indicated that the combination treatments had the greatest TSS to acidity values starting with potassium silicate at 200 mg l^{-1} plus proline at 400 mg l^{-1} followed by potassium silicate at 400 mg l^{-1} plus glycine betaine at 400 mg l^{-1}. Even with the third used treatment potassium silicate at 200 mg l^{-1} plus glycine betaine at 400 mg l^{-1}. All these combinations achieved higher ratios of TSS to acidity than the control and every more than use of the sole treatments at different rates such as the application of potassium silicate at 200 mg l^{-1} as compared with the control in a consistent manner in both seasons. Even proline alone at 400 mg l^{-1} was effective on increasing TSS to acidity in the juice of “Florida” peaches by the harvest time much more than the control in both seasons. The increase observed was even greater than that resulting from the individual application of the other osmoregulator, specifically glycine betaine alone at 400 mg l^{-1}.

Table 2. The effect of pre-harvest applications on some chemical characteristics of "Florida" peach during the 2022 and 2023 seasons.

Treatments	TSS (°Brix)		Acidity (%)		TSS/Acidity		Vitamin C (mg 100 g^{-1})	
	2022	2023	2022	2023	2022	2023	2022	2023
Control	8.11 g*	7.58 g	0.94 a	0.86 a	8.60 g	8.86 g	11.28 g	10.48 g
Glycine betaine (400 mg l^{-1})	8.54 f	7.77 f	0.88 b	0.80 b	9.75 f	9.76 f	11.74 f	10.97 f
Potassium silicate (200 mg l^{-1})	8.28 g	7.64 fg	0.83 c	0.76 b	10.94e	10.57 e	11.51 fg	10.68 fg
Potassium silicate (400 mg l^{-1})	9.37 d	8.06 e	0.75 d	0.69 c	12.45d	12.31 d	12.77 d	12.04 d
Potassium silicate (200 mg l^{-1}+ glycine betaine (400 mg l^{-1})	9.04 e	8.61 c	0.73 d	0.65 d	13.52c	13.33 c	12.39 e	11.54 e
Proline (400 mg l^{-1})	9.81 c	8.44 d	0.67 e	0.61 e	15.41b	14.81 b	13.14 c	12.41 c
Potassium silicate (400 mg l^{-1})+ glycine betaine (400 mg l^{-1}l)	10.82 a	9.21 a	0.62 f	0.54 f	17.37a	17.20 a	14.09 a	13.44 a
Potassium silicate (200 mg l^{-1}) + Proline (400 mg l^{-1})	10.36 b	8.96 b	0.58 g	0.52 f	17.98a	17.15 a	13.70 b	12.99 b
$LSD_{\text{at 5\%}}$	0.22	0.14	0.04	0.04	0.91	0.68	0.36	0.31

* Values in each column when accompanied with similar letters, were not significantly different by using the least significant difference at 5% for comparing the means.

3.2.4. Vitamin C content

The results obtained for vitamin C are given in Table 2. The data indicated that the highest content was obtained with the combination treatments especially with the combination of potassium silicate at 200 mg l^{-1} plus proline at 400 mg l^{-1} followed by potassium silicate at 400 mg l^{-1} plus glycine betaine at 400 mg l^{-1}. Moreover, proline alone was able to cause a significant increase in vitamin C content of fruit juice in the two seasons. Thus, there was an additive effect when proline was added to potassium silicate at 200 mg l^{-1} in terms of the significant increase in vitamin C, even though the sole application of potassium silicate at 200 mg l^{-1} alone did not cause a significant increase in vitamin C content of fruit juice consistently. However, when potassium silicate was applied at 400 mg l^{-1}, there was a consistent increase in vitamin C content compared to the control. Even the osmo-regulator, namely glycine betaine alone was still able to increase vitamin C but in a lower magnitude than that obtained with proline alone in both seasons.

3.2.5. Total carotenoid

The influence of various used treatments on total carotenoid content of “Florida” peaches was shown in Table 3. The data revealed that the highest on total carotenoid at harvest was obtained with the combination of potassium silicate at 200 mg l^{-1} plus proline at 400 mg l^{-1} followed by the combination of potassium silicate at 400 mg l^{-1} plus glycine betaine at 400 mg l^{-1} as compared with control in both seasons. Meanwhile, all individual treatments proved to have an effective, significant influence on enhancing the on total carotenoid of treated peaches when compared with the control in both seasons.

Higher the concentration of applied potassium silicate greater the influence on carotene content. However, the application of glycine betaine at 400 mg l^{-1} in a combination resulted in greater influence on on total carotenoid than its individual treatments in both seasons.

3.2.6. Anthocyanin content of the skins

With regard to the changes in anthocyanin content in the “Florida” skin in response to various applications. The data reported in Table 3 revealed these changes. It was evident again that the greatest content of anthocyanin was found with the combination of potassium silicate at 200 mg l^{-1} plus proline at 400 mg l^{-1} in a consistent manner in both seasons. Moreover, when each compound in such combination was applied individually. It resulted in an increase of anthocyanin content but in a smaller magnitude than the combination. This conclusion is true for second combination of potassium silicate at 400 mg l^{-1} plus glycine betaine at 400 mg l^{-1} and even for the third combination of potassium silicate at 200 mg l^{-1} plus glycine betaine at 400 mg l^{-1}. Thus, it was desired to have the treatments combined as shown in Table 3. Moreover, the sole application of proline at 400 mg l^{-1} was able to cause a significant increase in anthocyanin content of the peach skins consistently in both seasons.

3.2.7. Electrolyte leakage of fruit tissues

The leakage of electrolytes as an indicator to the membrane integrity of fruit tissue at harvest time was also reported in Table 3. Lower the leakage indicates to more tissue integrity. The data revealed that the control fruits had the greatest leakage in both seasons. The least magnitude of such leakage was found with the combination of potassium silicate at 200 mg l^{-1} plus proline at 400 mg l^{-1}. Moreover, all the other applied combinations had less leakage of electrolytes than its individual components. Even in the case of applied proline individually, it was able to reduce electrolyte leakage in both seasons as compared with the control. When proline was included in a combination, it resulted in a better influence on the tissue integrity than that obtained when glycine

Table 3. The effect of pre-harvest applications on some chemical characteristics of "Florida" peach tissue during the 2022 and 2023 seasons.

Treatments	Total carotenoid (mg 100 g^{-1})		Anthocyanin (mg 100 g^{-1})		Electrolyte leakage (%)	
	2022	2023	2022	2023	2022	2023
Control	0.773 h*	0.640 h	4.09 h	3.70 g	18.67 a	19.72 a
Glycine betaine (400 mg l^{-1})	0.881 e	0.654 g	4.87 e	3.82 fg	17.93 b	19.10 b
Potassium silicate (200 mg l^{-1})	0.819 f	0.670 f	4.51 f	3.97 f	16.47 d	18.00 d
Potassium silicate (400 mg l^{-1})	1.010 c	0.745 d	5.49 c	4.73 d	17.28 c	18.63 c
Potassium silicate (200 mg l^{-1}+ glycine betaine (400 mg l^{-1})	0.792 g	0.710 e	4.32 g	4.35 e	15.38 f	16.83 f
Proline (400 mg l^{-1})	0.952 d	0.780 c	5.17 d	5.02 c	16.76 d	18.23 cd
Potassium silicate (400 mg l^{-1})+ glycine betaine (400 mg l^{-1}l)	1.072 b	0.808 b	5.91 b	5.27 b	15.87 e	17.51 e
Potassium silicate (200 mg l^{-1}) + Proline (400 mg l^{-1})	1.143 a	0.840 a	6.28 a	5.61 a	14.87 g	16.30 g
LSD $_{at\ 5\%}$	0.004	0.013	0.19	0.20	0.43	0.42

* Values in each column when accompanied with similar letters, were not significantly different by using the least significant difference at 5% for comparing the means.

betaine was individually applied. Plant defense against salt, heat, and water scarcity is largely dependent on proline (Ashraf and Harris, 2004). Proline foliar spraying alleviated the growth inhibition that heat-stressed crops caused (Makela et al., 1998). According to (Farag and Shehata, 2023), applying proline spray three times may improve the colour of pomegranates, particularly when fruit bagging is present. This finding was farther supported by the results on peach fruits since Proline applications resulted in higher anthocyanin in the fruit as compared with the control. Proline accumulation appears to be related to temperature stress. Either at a low temperature (Tarnizi and Marziah, 1995; Wang and Cui, 1996) or a high temperature (Ashraf et al., 1994). Proline functions as a molecular chaperone and an osmotic adjustment mediator of stress tolerance (Lehmann et al., 2010; Kavi Kishor and Sreenivasulu, 2014). Such improvement in plant tolerance was supported by our findings since Proline plus potassium silicate were able to reduce electrolyte leakage of treated peaches even with the sole application of Proline which reduced electrolyte leakage as compared with the control. Rising temperatures brought on by climate change have become a significant challenge for modern crop production in the last few decades, particularly in southern Mediterranean regions (Wang et al., 2020). As a result, efforts to maximize crop yields in order to guarantee food security for a growing global population continue to be difficult. Heat stress impairs the oxygen evolving complex, from photosystem II, RuBisCo, and energy-producing (ATP) processes, which all have a detrimental physiological impact on photosynthesis (Tan et al., 2020; Parrotta et al., 2020). Meanwhile, according to Sorwong and Sakhonwasee (2015), the reduction in leaf gas exchange traits caused by heat stress was mitigated by the application of exogenous glycine betaine (GB). Plants grow larger amounts of GB when subjected to abiotic stress (Storey et al., 1977). Numerous lines of transgenic plants that accumulate GB show significantly increased resistance to different forms of abiotic stress. As a result, it improves CO_2 assimilation's tolerance to high-temperature stress. Several reviews of the relationship between GB and tolerance to abiotic stress have recently been published Sakamoto and Murata (2002), Chen and Murata (2008) and Takabe et al. (2006). Yang et al. (2007) indicated that the accumulation of GB in vivo seemed to reduce the accumulation of ROS during heat stress by maintaining or increasing the activities of ROS scavenging enzymes (namely, catalase, ascorbate peroxidase, glutathione reductase, dehydroascorbate, and monodehydroascorbate reductase). According to Epstein (1999), silicon is the second most abundant element on Earth. It plays a significant role in the formation of clay minerals in soils. Potassium silicate is a highly available source of silicon and potassium that is primarily used as a silica amendment in agricultural systems, with the added benefit of providing potassium. In a number of horticultural crops, potassium nutrition can improve shelf life, ascorbic acid concentrations, fruit colour, soluble solids, fruit size, tree yields, and shipping quality (Kanai et al., 2007). According to Romero- Aranda et al. (2006), the application of potassium silicate does not release any environmentally persistent or hazardous byproducts because it does not enclose any volatile organic compounds. Numerous studies have demonstrated how potassium silicate helps reduce a variety of stress factors, such as drought, oxidative damage, salinity, and nutrient shortages (Chen et al., 2016; Gomaa et al., 2021). Additionally, silicate may promote plant pigments, cell division, photosynthesis, and the uptake of nutrients and water (Ma, 2004). Such mitigation or alleviation of tissue injury was in agreement with our findings since electrolyte leakage of fruit tissues was significantly reduced by proline alone or proline plus potassium silicate at 200 mg l^{-1} as shown in Table 3. Meanwhile, total carotenoid as a defense mechanism against abiotic stresses was significantly increased in fruit tissue especially with the above treatments. Thus, the increase in the protective pigments, namely carotenes and anthocyanins, were in agreement with the trend of our results that indicated to a further reduction of electrolyte leakage since less the leakage means more tissue integrity.

4. Conclusions

Summing up the results, it can be concluded that the use of potassium silicate at 200 mg l^{-1} + proline at 400 mg l^{-1} treatment or potassium silicate at 400 mg l^{-1} + glycine betaine at 400 mg l^{-1} two times caused an improved quality of peach fruits in reclaimed land.

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HORTICULTURAL STUDIES

RESEARCH PAPER

Assessment Results of Salinity Stressed F_2 Population Originated from Interspecific Hybridization of Eggplant with Wild Relative *Solanum incanum* L.

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Abstract

Salinity, which is one of the major abiotic stresses, prevails in mostly arid and semiarid areas that is nearly 20% of the world's cultivated area. Excessive amounts of salt around the plant root zone are detrimental to vegetative growth and economic yield. Today salinization is still severely expanding and posing a great threat to the development of sustainable agriculture. Although eggplant (*Solanum melongena* L.) is considered moderately sensitive, soil salinity mitigates strictly the growth and yield. Eggplant has significant crop wild relatives (CWRs) which are thought to be more tolerant to abiotic stresses and it is substantial to exploit their potential against salinity in hybrid breeding studies. It has previously been proven that *Solanum incanum* L. has tolerance to salinity stress. This study aimed to improve salinity-tolerant pure eggplant lines. Therefore, the acquired F_2 population from interspecific hybridization between the pure line (BATEM-TDC47) with distinctive features from BATEM eggplant gene pool and *S. incanum* L., were subjected to salinity stress at 150 mM NaCl level with its parents and F_1 plants. On the 12th day after the last salt treatment, the plants were evaluated using a 0-5 visual scale. Among the 256 stressed plants, 50 F_2 individuals were determined to be salt tolerant. Additionally, some of their morphological and physiological features, such as shoot length, stem diameter, number of leaves, anthocyanin presence, prickliness, malondialdehyde (MDA), and proline levels, were studied and compared to the controls of their parent and F_1 plants. Results showed that shoot length and stem diameter decreased dramatically under salt stress. According to the analysis, the average MDA and proline levels of the F_2 population were identified as 10.9 μ mol g^{-1} FW and 8.4 μ mol g^{-1} FW, respectively. The distinguished 50 F_2 plants that showed salinity tolerance were transferred to the greenhouse and self-pollinated to produce the F_3 generation.

1. Introduction

In order to supply the food demand of the world's constantly expanding population, the total yield per unit area should be increased. However, agricultural production is gradually decreasing due to the negative effects of various factors such as climate change (Hemathilake and Gunathilake, 2022). Climate change is a multifaceted system that affects biotic and abiotic components on the world (Chaudhry and Sidhu, 2022). Drought, salinity, and heat are the main abiotic stresses that cause reduced growth and crop yield (Giordano et al., 2021).

Salinization occurs due to the accumulation of salt ions (mostly Na^+ and Cl^-) in soil and it is expressed with electrical conductivity (EC). When soil EC reaches 4 dS m^{-1} (equivalent to 40 mM NaCl), the yield of most crops is significantly reduced (Munns and Tester, 2008). In addition, excessive amounts of Na^+ inhibit water conductivity, soil porosity, and aeration (Singh et al., 2018).

Salinity is one of the alarming abiotic stresses and it threatens the sustainable agriculture. Harmful impacts are expected to spread to nearly 50% of total agricultural land by 2050 (Singh et al., 2018; Kumar et al., 2020). Vegetables are more sensitive to saline conditions when compared to the other crops (Chinnusamy et al., 2005). Due to salinity, reduced nutrient mobilization causes poor plant growth and decreases in yield. The negative effects of salt stress on plants are mitigated by some external applications such as, arbuscular mycorrhizal fungi (Porcel et al., 2012; Hanci et al, 2014; Evelin et al., 2019), plant growth promoting bacteria (Etesami and Noori, 2019) and some seed priming methods (Ibrahim, 2016; Johnson and Puthur, 2021). However, the use of these techniques has not yet become practical. Therefore, the development of salt-tolerant crop varieties remains important and necessary to achieve economic yields in affected areas.

High salt concentration near the plant root zone damages to vegetative growth and economic production in all crops. Plants respond to salt stress in a number of ways in terms of morphological, physiological, and biochemical ways. Mainly, 100-200 mM NaCl concentration in soil can limit growth or lead to plant mortality (Tang et al., 2015; Brenes et al., 2020a). Salt stress escalates the toxicity of some ions such as Na^+ and Cl^-, which causes water stress and prevents nutrient uptake in plants (Mbarki et al., 2018). Increasing salinity causes an increase in osmotic pressure, reducing the water uptake or even preventing it completely (Chen and Jiang, 2010). This leads to a reduction in leaf growth, reducing the amount of photosynthesis during plant development, and ultimately limiting plant growth under salinity.

Reactive oxygen species (ROS) are generated in abundance under abiotic stress conditions (Sharma et al., 2019). Anthocyanins, a type of flavonoid, have antioxidant functions. Anthocyanins usually associated with increased stress tolerance in plants and they are activated in response to abiotic stresses (Li and Ahammed, 2023). Unbalanced ROS activity results in cell damage (Nouman et al., 2014). Anthocyanins as an antioxidant play a vital role in ROS scavenging and reducing oxidative stress (Cheah et al., 2015; Khan et al., 2020) and proline is a kind of amino acid and the most abundant endogenous osmolyte act as a protector of the plants against various stresses. It's accumulation increases under various abiotic stresses including salinity (Slama et al., 2015). Malondialdehyde (MDA), which is one of the final products of lipid peroxidation, is employed as a marker of oxidative stress. Assessment of MDA, is used as a method in determining degree of susceptibility of plants by researchers (Singh et al., 2014) and increase of MDA concentration in leaves indicate increased susceptibility.

Eggplant belongs to Solanaceae family and cultivated mostly around the South East Asia, Middle East and Mediterranean countries. It is known as moderately sensitive to abiotic stresses (Unlukara et al., 2010; Díaz-Pérez and Eaton, 2015; Brenes et al., 2020b). Environmental changes in recent years lead to an enthusiasm for crop wild relatives (CWRs) in agronomically important crop breeding programs (Knapp et al., 2013). Moreover, the inclusion of crop wild relatives in breeding programs may broaden the genetic base of the germplasm. Although, eggplant has many crop wild relatives (CRWs) that are believed to be more tolerant to abiotic stresses and it is essential to exploit their potential against salinity through interspecific hybridization studies. Cultivated eggplant can be crossed successfully with several wild relatives (Plazas et al., 2016). *Solanum incanum* L. which was defined as cross-compatible with *Solanum melongena* L., has desirable properties in development of cultivar eggplant breeding schemes (Gramazio et al., 2016). After interspecific hybridization, backcrossing with *S. melongena* for introgression breeding can cause addition of some traits of wild relatives into the eggplant gene pool (Kouassi et al., 2016; Garcia-Fortea et al., 2019; Brenes et al., 2020a).

The aim of this study was to improve ideal and high potential salt tolerant eggplant pure lines. Therefore, F_2 population derived from interspecific cross between inbred eggplant line (BATEM-TDC47) having desirable features and *S. incanum* L. was evaluated under the dose of 150 mM NaCl based salinity stress.

2. Material and Methods

2.1. Plant material

A total of 256 F_2 seedlings derived from a segregating population from an interspecific hybridization between the inbred line BATEM-TDC47 (sensitive parent) and the crop wild relative *Solanum incanum* L. (tolerant parent) were used as plant material. The inbred line "BATEM-TDC47" is distinguished with its agro morphological features and high marketable capacity. Tolerant parent *S. incanum* L. was provided by INRAE (French National Research Institute for Agriculture, Food and Environment) France. In addition, 36 seedlings from the F_1 hybrid and 36 seedlings from each parent were kept as negative and positive controls under non-saline and saline stress conditions.

2.2. Method

Seeds were sown on 13 August 2021 and seedlings were grown in trays containing a 1:1 mixture of peat moss and perlite. They were evenly watered with Hoagland solution (Hoagland and Arnon, 1950) until they reached the stage of 2-3 true leaves. They were transferred to 1-liter pots containing a 1:1 mixture of peat moss and perlite on

September 13, 2021, 31 days after sowing (DAS). Following transplantation, all plants were irrigated equally with Hoagland solution (Hoagland and Arnon, 1950) for 2 weeks to ensure proper root development. When seedlings reached to 4-5 true leaves stage, the experiment was initiated using 0 mM NaCl (only the control group of F_1 and parent seedlings) and 150 mM NaCl doses. In this study dose of 150 mM NaCl was applied to the 256 F_2 seedlings to distinguish salt-tolerant ones. Considering the previous studies (Akinci et al., 2004; Assaha et al., 2013; Hannachi and van Labeke, 2018; Brenes et al., 2020a; Alkhatib et al., 2021), the dose of 150 mM NaCl was used to define salt-tolerant plants. This amount did not kill the plants completely but enabled them to determine of tolerance in a short time. Before applying salt stress, a sufficient volume of 50 mM NaCl solution was prepared using distilled water and NaCl. When the seedlings reached the stage of 4-5 true leaves, pod treatment was initiated on September 28, 2021, with a concentration of 50 mM NaCl per day. This was followed by two consecutive days of 50 mM NaCl treatment until September 30, 2021, when a final concentration of 150 mM NaCl was reached. At the same time, a trial was set with parents and F_1 seedlings as the negative and positive control under non-saline and salt-stressed conditions. The positive control group was treated with 150 mM NaCl, similar to the F_2 seedlings. Meanwhile, the negative control group consisted of an equal number of F_1 and parent seedlings, which were kept in non-saline conditions and irrigated with salt-free water. A few days after the last treatment, damage symptoms began to develop on salt-treated plants. During this stage, irrigation was done manually when required. Although the salt-treated plants didn't require much watering, control plants were irrigated with regular water as needed. According to our earlier research from the ongoing project titled "Development of Tolerant Inbred Lines to Salt and Drought Stresses in Eggplant through Interspecific Hybridization" (Project number: TAGEM/BBAD/B/20/A1/P1/1476), and considering previous studies on eggplant (Assaha et al., 2013; Alkhatib et al., 2021), observations were made on the 12th day after the last salt treatment. On the 12th day, symptoms' severity was assessed using a 0-5 visual damage scale as suggested by Kıran et al. (2016), 0 indicating no effect, 1 indicating local yellowing and curling of leaves with slow growth, 2 indicating necrosis and chlorosis on 25% of the leaf, 3 indicating necrotic spots on the leaves and defoliation by 25-50%, 4 indicating necrosis by 50-75% and death of several plants, and 5 indicating severe necrosis on leaves by 75-100% and/or predominant plant deaths. After this, all plants were phenotypically observed on the same day. While shoot length and stem diameter were measured using a ruler and digital caliper, final leaf numbers were recorded for each plant, and observations for anthocyanin presence and prickliness features were conducted visually. Subsequently, leaf samples were collected from the selected 50 salt-tolerant F_2 seedlings, as well as from the F_1 and parent plants, for MDA and proline analysis. For leaf sampling, the top third vigorous leaf of each plant was collected, wrapped individually in aluminum foil, and stored in a freezer at -20°C until laboratory analysis. The MDA level was determined according to the method outlined by Lutts et al. (1996), while the proline content was evaluated using the method described by Bates et al. (1973). After the observations, 50 F_2 plants that scored "0" on the 0-5 scale, indicating the highest level of salt tolerance, were transferred to the greenhouse on October 12, 2021, for the inbreeding of tolerant individuals. Each 50 F_2 plants was self-pollinated to produce fruits for seeds of the F_3 generation. After fruits ripening 65-70 days later, mature fruits were harvested. Following, seeds were separated from fruit flesh, washed and dried under controlled room conditions at +25ºC. They were then stored at +4ºC with 5% humidity until further studies.

2.3. Electrical conductivity of the substrate

At the end of the salt treatment, after removing the plants from the pots, the remaining substrate was dried and a soil/water (1:5) suspension was prepared in deionized water and stirred well for an hour at a shaker. Electrical conductivity (EC) was measured using a portable conductivity meter (model mw - 302, Milwaukee Instruments, USA) and expressed in dS m^{-1}.

2.4. Data analysis

For the evaluation of MDA, proline, stem diameter, leaf number and shoot length data, percent rate of change was calculated using the following equation.

$$Percentage\ change = \left[\frac{Control - Salt\ treatment}{Control}\right] \times 100$$

The percent rate of change was based on the data collected from salt stressed and control plants. This equation didn't employ on F_2 population, because F_2 population was tested without control group. Each F_2 individual was treated as a separate breeding line in the study. The extent of the salt stress effect on F_1 and parents were based on the percent rate of change ratios. When evaluating morphological data, it was hypothesized that a lower percent rate of change indicated a higher tolerance level to salt stress.

3. Results and Discussion

3.1. Analysis of morphological parameters

In this study, plants in early growth stage were subjected to test under salt effect. At the end of the experiment, the electrical conductivity (EC) of the substrate was determined as 0.65 dS m^{-1} for the control and 7.90 dS m^{-1} for the salt-treated samples**.** This proved that, salt stress was created successfully around the plant root zone. The sensitive plants exposed to 150 mM NaCl displayed symptoms such as necrosis, chlorosis and defoliation (Figure 1) increasing progressively after the salt treatment.

The effects of salinity stress on shoot length, stem diameter and leaf number of 256 F_2 segregating population and negative/positive control seedlings were investigated and presented in Table 1. According to the findings, while shoot length and stem diameter decreased dramatically, number of leaves per plant was not affected much however decreases on leaf size under salt effect could be visually observed during the experiment. Moreover, it was estimated that, if the stress condition would be prolonged number of leaves could be affected negatively and decreased. It was clear from the study, salt stress induced reduction of all growth parameters in plants (Table 1). However, this reduction in growth was greater in *S. melongena* than in *S. incanum* L. (Figure 2) whereas F_1 and F_2 plants showed heterosis.

The rate of change ratios were calculated for negative and positive control group using equation 1 and presented as figure (Figure 3). Shoot length, stem diameter and leaf number values were reduced under salt effect compared to their control plants (for F_1 and parents). Additionally, change ratios of BATEM TDC47 is defined as higher than the *S. incanum* L. that means inbred line BATEM TDC47 was more affected from salt stress. This result proved that, even in early growth stage, genotypes could be differentiated with their tolerance level using basic morphological measurements.

A total of 256 seedlings from F_2 segregating population were classified using 0-5 visual scale (Kıran et al., 2016), considering damages caused by salt stress and presented in Table 2 and Figure 4. According to data collected on the 12th day (unpublished project data; Assaha et al., 2013; Alkhatib et al., 2021) after final salt application, 50 seedlings responded well to the salt stress, showed “no effect” and scored as “0” (Table 2). Brenes et al. (2020a) suggested that the survival percentage is one of the most effective criteria and generally used to assessing of the salt tolerance degree of tested plants.

Seedlings from F_2 segregating population examined in this study were classified into six groups based on 0-5 scale. According to scoring, 50 seedlings from group “0” with desirable features were defined as salt tolerant. Thus, 50 seedlings amongst the 256 F_2 individuals were selected to develop new salt tolerant pure lines.

Anthocyanin and prickliness of the genotypes were also observed (Table 3). While selection was making among the salt treated individuals, beside salt tolerance capacity, spineless, hairless and strong individuals were preferred for the desired line

Figure 1. F_2 seedlings responded to 150 mM NaCl stress as visual deterioration.

Table 1. Minimum, maximum, and average values of shoot length, stem diameter, and leaf numbers of *S. incanum* L., BATEM TDC47, BATEM TDC47 × *S. incanum* L., and F_2 population under 150 mM salt stress on the 12th day of salt application.

Accessions	Shoot length (cm)			Stem diameter (mm)			Leaf number		
	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
S. incanum L.	5.5±0.2	8.0±0.5	7.3±0.9	1.8±0.1	2.4±0.1	2.1±0.2	4.0±0.5	6.0±0.5	5.0±0.7
BATEM TDC47	8.0±0.5	11.0±0.3	9.6±0.9	2.1±0.1	2.9±0.1	2.6±0.2	5.0±0.4	7.0±0.5	6.2±0.5
F_1	7.0±1.0	14.0±1.3	10.3±1.8	2.0±0.2	4.6±0.5	2.8±0.5	3.0±0.5	6.0±0.4	5.0±0.7
F_2	9.0±1.3	15.0±0.9	10.8±1.9	2.0±0.2	3.7±0.3	2.8±0.3	3.0±0.3	6.0±0.2	5.0±0.5

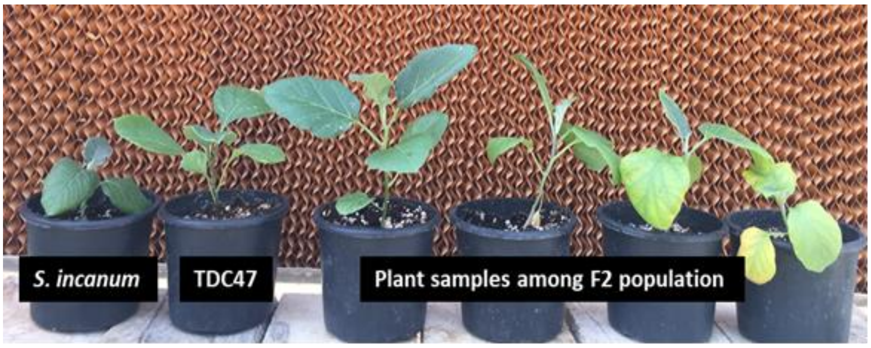

Figure 2. Parents and F_2 segregating population in pots under salt stress effect.

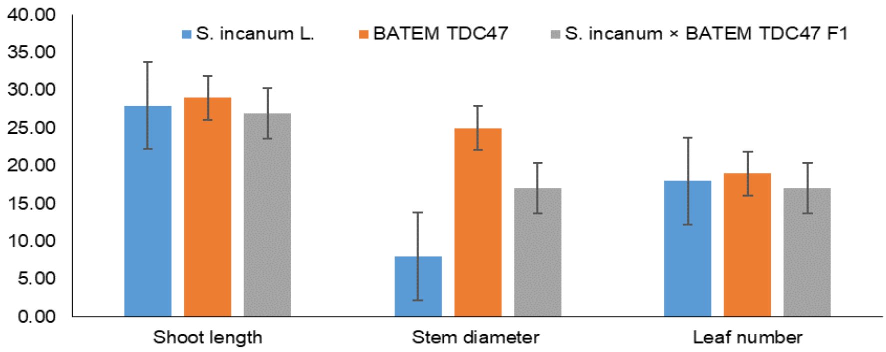

Figure 3. Rate of change (%) of *S. incanum* L., BATEM TDC47 and BATEM TDC47 × *S. incanum* L. hybrid seedlings in terms of shoot length, stem diameter and leaf number.

Table 2. Classifying of F_2 segregating population using 0-5 visual scale.

Scale	Description	Number of F_2 plant
0	No effect	50
1	Local yellowing and curling of leaves, slow growth	88
2	Necrosis and chlorosis in 25% of the leaf	62
3	Necrotic spots on the leaves and defoliation by 25-50%	36
4	Necrosis by 50-75% and death of several plants	18
5	Formation of severe necrosis in leaves by 75-100% and/or predominant deaths in plants	2

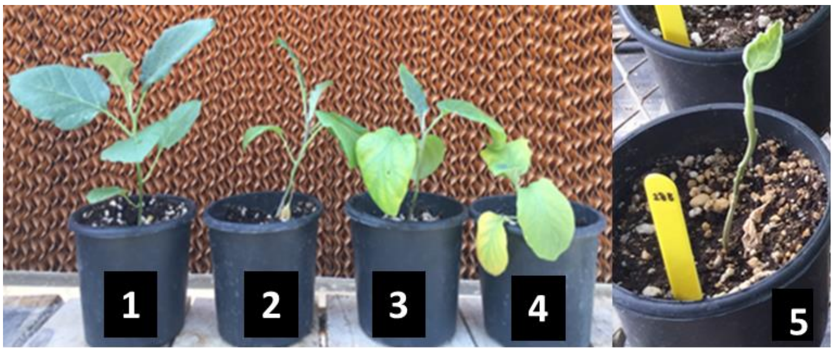

Figure 4. Samples from F_2 population, from left to the right, Pot 1: No effect (0), Pot 2: Local yellowing and curling of leaves, slow growth (1), Pot 3: Necrosis and chlorosis in 25% of the leaf (2), Pot 4: Necrosis by 50-75% and death of several plants (3), Pot 5: Necrosis by 50-75% and death of several plants (5).

Table 3. Anthocyanin and prickliness features of the salt stressed BATEM TDC47, *S. incanum*, BATEM TDC47 × *S. incanum* L. F_1 seedlings, and F_2 segregating population.

Accessions	Anthocyanin	Prickliness
BATEM TDÇ47 (Control)	+	-
BATEM TDÇ47 (Salt treated)	+	-
S. incanum (Control)	-	+
S. incanum (Salt treated)	-	+
F_1 (Control)	+	+
F_1 (Salt treated)	+	+
F_2 (Salt treated)	145 of 256 F_2 plants show anthocyanin presence	-

Table 4. Mean malondialdehyde (MDA) and proline values of *S. incanum* L., BATEM TDC47 and BATEM TDC47 × *S. incanum* L. F_1 and selected 50 F_2 seedlings as salt tolerant under salt stress.

Accessions	MDA (μ mol g^{-1} FW)			Proline (μ mol g^{-1} FW)		
	Minimum	Maximum	Average	Minimum	Maximum	Average
S. incanum L.	6.2±0.8	8.2±0.5	7.3±0.7	10.9±1.3	14.9±1.6	12.9±1.8
BATEM TDC47	7.8±0.7	9.7±0.5	8.7±0.6	7.3±1.0	9.7±1.0	8.8±1.0
F_1	15.3±0.5	17.3±0.9	16.0±0.7	6.3±1.1	10.1±1.5	8.1±1.3
F_2	2.1±1.7	21.4±3.0	10.9±4.9	4.2±0.9	12.1±1.2	8.4±1.6

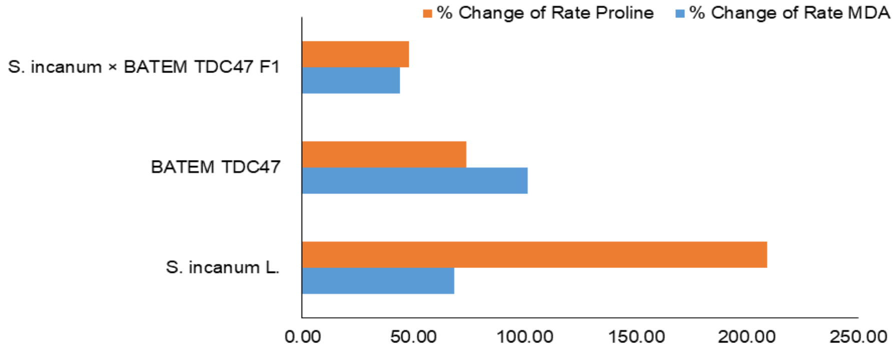

Figure 5. Rate of change (%) of *S. incanum*, BATEM TDC47 and BATEM TDC47 × *S. incanum* L. hybrid seedlings in terms of MDA and proline accumulation.

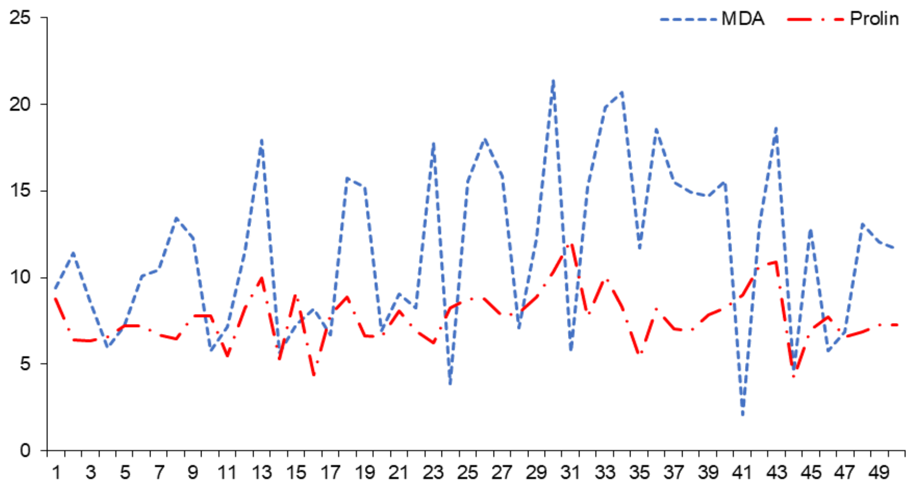

Figure 6. Graphic shows MDA and proline alteration in selected 50 F_2 plants.

development. Because it is known that these characters are undesirable for cultivars. While the tolerant parent lacks anthocyanin but possesses spines, the sensitive parent exhibits anthocyanin but lacks spines. F_1 seedlings have both anthocyanin and spine on body parts. A total of 145 F_2 plants show anthocyanin presence but no prickliness (Table 3).

3.2. Analysis of biochemical parameters

In the research, considering the visual scale results, MDA and proline amounts of selected 50 F_2 plants were analyzed together with F_1 and parent leaf samples and presented in Table 4. As an indicator to lipid peroxidation, MDA increased in all samples under salt stress. Segregating population's MDA level ranked between 2.1- 21.4 μ mol g^{-1} FW. Salt stress resulted in increases of proline accumulation thus, F_2 population's proline amount ranked between 4.2-12.1 μ mol g^{-1} FW.

Rate of change ratios were calculated for negative and positive control plants using equation 1 and presented as Figure 5. MDA and proline amounts showed increases in different degrees under salt effect compared to the control plants (for F_1 and parent plants). While the most increase of proline was obtained from *S. incanum* L., the most increase of MDA was obtained from BATEM TDC47. Changes in F_1 seedlings under salt, stayed between the parents' values. At the plant selection stage, these habits should be considered by the plant breeders.

In this study, considering the visual scale results, MDA and proline amounts of selected F_2 plants were analyzed together with F_1 and parent leaf samples. MDA and proline contents in F_2 plants subjected to the salt stress displayed the high levels of variation. While the highest MDA level was determined as 21.40 μ mol g^{-1} FW, the lowest MDA level was determined as 2.11 μ mol g^{-1} FW. Whereas, proline content was recorded in between 12.14 μ mol g^{-1} FW and 4.21 μ mol g^{-1} FW. Alterations in proline and MDA amounts among the selected 50 salt-tolerant individuals are presented as a graphic in Figure 6.

This study aimed to improve cultivar eggplant tolerance to salt stress. Although many research has been done to reveal resistance and tolerance of eggplant wild relatives to abiotic and biotic stresses (Rotino et al., 2014; Plazas et al., 2016; Gramazio et al., 2017; García-Fortea et al., 2019; Caliskan et al., 2023), efforts on transferring these skills to the cultivar eggplant were limited (Toppino et al., 2008; Liu et al., 2015). From the secondary gene pool, *S. incanum* L. is a close relative of eggplant, and its hybrids and backcrosses with cultivar eggplant are found mostly fertile by the researchers (Knapp et al., 2013; Kouassi et al., 2016; Plazas et al., 2016; Gramazio et al., 2017) and in agreement with previous studies, obtained seeds from the hybridization gave highly fertile individuals in this study. Although, drought tolerance of *S. incanum* L. has been described previously in many studies (Gramazio et al., 2017; Plazas et al., 2022; Cebeci et al., 2022; Cebeci et al., 2023), salt tolerance of this wild relative studied first in the present breeding project. Studies on salt tolerance levels of some commercial or local eggplant varieties (Akinci et al., 2004; Unlukara et al., 2010; Hannachi et al., 2014; Hannachi et al., 2018; Suarez et al., 2021) and some wild relatives, such as, *S. torvum* (Brenes et al., 2020a), *S. insanum* (Brenes et al., 2020b) were studied before however, responses of F_2 segregating population were observed first under the salt effect in this study. However, salt tolerance of *S. incanum* L. has been studied in molecular level and scientists have found unigenes related to the drought and salt tolerance in *S. incanum* L. (Gramazio et al., 2016).

In the present study, plant growth characteristics and biochemical parameters were assessed to determine responses of segregating population at seedling stage to 150 mM NaCl stress. Previous researches were reported that final plant performance heavily depends on seedling features under stress conditions (Bybordi and Tabatabaei, 2009). Because of disrupted a number of physiological mechanisms such as photosynthetic efficiency and water uptake (Evelin et al., 2019), plants respond first as retardation or stopping growth, yellowing on leaves and at the further stage, necrotic spots on leaves, loss of leaves and death of whole plant under salt stress. The effects of salinity on plant growth may vary depend on plant species and even on different genotypes of a species (Bhati et al., 2020).

Analysis of salt effects on growth parameters can be useful for the formation of stress tolerance scale when comparing different species, varieties or inbred lines (Al Hassan et al., 2016). The results indicated that *S. incanum* L. is a good candidate for improving salt tolerance in eggplant germplasm through breeding and introgression programs. Compared to the cultivated species, wild relatives of eggplant show higher stress tolerance, since they habitually found in arid/semiarid regions and in saline environments (Knapp et al., 2013; Ranil et al., 2016).

A total of 50 seedlings responded well to the salt stress, showed “no effect” and scored as “0” (Table 2). Brenes et al. (2020a) suggested that the survival percentage is one of the most effective criteria and generally used to assessing of the salt tolerance degree of tested plants. Different visual damage scales were used on different plants by researchers in previous studies such as Kıran et al. (2016) and Bhati et al. (2020) for eggplant, Kusvuran (2010) and Ekincialp (2019) for melon, Fidan and Ekincialp (2017) for bean and Shaheenuzzamn (2014) for chickpea.

Plant breeders are currently focusing their effort on genotype × environment interactions with variation in morpho-physiological variables to identify breeding methods to create more stress tolerant crops (Toppino et al., 2022). Plants under stress showed MDA content increases as stress increase. The more these increase the more plant sensitive. Previous studies on eggplant reported that, plants with high proline accumulation showed greater tolerance to stress conditions (Kıran et al., 2016; Plazas et al., 2019). Consistent with previous studies, in the present study proved that *S. incanum* L. as a crop wild relative has greater tolerance to salt than the susceptible parent.

It was stated that progress has been made in breeding in the study conducted on rice to improve salinity tolerance and that selection is important in early generations. The selection of early generation (F_2-F_5) breeding materials, especially in severe salinity conditions, according to good plant type and seedling tolerance, brings success in the development of salinity tolerance lines in the later generations (F_6-F_7) (Gregorio et al., 2002). In this study, 50 seedlings selected as having desirable attributes and tolerance in severe salinity conditions were transferred to the greenhouse to obtain the next generation.

4. Conclusion

This study aimed to contribute to development of new eggplant inbred lines and varieties tolerant to salt stress by understanding the salt tolerance level within this eggplant F_2 segregating population. In accordance with the purpose, qualified inbreed eggplant line (BATEM TDC47) and *Solanum incanum* L*.* were crossed successfully, F_2 segregating population was tested under salt stress conditions comparatively with parents and F_1 plants. Selected as salt tolerant 50 F_2 lines were transferred to the greenhouse for selfing and progressed to the F_3 level. Parameters that utilized in the study were found highly useful for the selection of salt tolerant individuals. Studies on the salinity tolerant inbreed line development are still processing under the project of "Development of Tolerant Inbred Lines to Salt and Drought Stresses in Eggplant through Interspecific Hybridization" (Project No: TAGEM/BBAD/B/20/A1/P1/1476)".

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RESEARCH PAPER

HORTICULTURAL STUDIES

The Determination Mealybug Species and Natural Enemies in Pitaya Greenhouses in the Mediterranean Region

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Abstract

Pitaya, *Hylocereus* spp. (Caryophyllales: Cactaceae) has been one of the new species cultivated in Türkiye in recent years. There are many limiting factors, including pests, diseases and weeds, to decrease the yield and fruit quality in pitaya (dragon fruit) areas in Türkiye. In addition, the pests of pitaya is not fully studied therefore this research has been conducted to determine mealybug species in pitaya greenhouses in Türkiye. The present study aimed to determine mealybug species and its natural enemies (parasitoids, and predator insects) in pitaya greenhouses in Adana, Mersin, and Antalya between 2021 and 2022. According to results of this study, 2 different mealybug species was determined; *Phenacoccus solenopsis* Tinsley 1898, *Phenacoccus madeirensis* Green, 1923 (Hemiptera: Pseudococcidae) in pitaya greenhouses. Moreover one parasitoid (*Aenasius arizonensis* Girault (Hymenoptera: Encyrtidae), and two different predatory insects from 2 different orders and 2 different families (*Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), *Nephus includens* Kirsch (Coleoptera: Coccinellidae) were detected within this research. These two invasive mealybug species have wide spectrum host plants and may cause economically important damages to pitaya, if natural enemies cannot work successfully or control strategies do not applied properly in pitaya greenhouses.

1. Introduction

Pitaya or dragon fruit (*Hylocereus* spp.) is known as a new species in the world and studies about this plant have been conducted since the mid-'90s. Pitaya (Dragon fruit) is known as a new species for Türkiye as well. Especially, it has been planted in greenhouses on the coast of the Mediterranean region (Antalya and Mersin) in recent years. In addition, pitaya plantations have been started in the other districts of the Mediterranean region such as Adana, and Muğla in greenhouses (Soydal et al., 2019). Many factors affect negatively and cause yield loss in pitaya production. The pest and diseases cause yield loss and affect the quality of the product. Limited studies were conducted about the pests and diseases of dragon fruit. Eusebio and Alaban (2018) reported the common pests of dragon fruit in the Philippines. According to results of this research, ants, scale insects, mealybugs, borers, and fruit flies caused damage on dragon fruit and all plant parts were affected by these pests. In addition, Duncan et al. (2021) reported the pests and beneficial insects of dragon fruit in Southern Florida (USA) and found that thrips, leaf-footed bugs, aphids, and mealybugs were detected as major pests in pitaya plantation Florida. Moreover, Choi et al. (2013) reported pests list and damage to mango, dragon fruit, and atemoya between 2008 and 2011 in Korea. Rezzeki et al. (2021) observed mealybug species on dragon fruits in Indonesia and they found 4 different species: *Ferrisia virgata*

(Cockerell), *Phenacoccus solenopsis* Tinsley, *Planococcus minor* (Maskell) and *Pseudococcus jackbeardsleyi* Gimpel & Miller. Mealybugs induce damage characterized by dry skin, fruit contraction, wrinkling of the skin, and the secretion of honeydew, leading to the development of sooty molds (Mani and Shivaraju 2014; Nurhafizhah et al. 2020). Major pests are determined as thrips, aphids, mealybug, and Lepidoptera species. *Aphis gossypii* and *Spodoptera litura* were determined as important pests on dragon fruit, ants and aphids generally cause damage on stems, flowers and fruits. Sartiami et al. (2019) conducted a study about mealybug species on dragon fruit in India and 4 mealybug species (*Ferrisia virgata, Planococcus minor, Phenacoccus solenopsis* and *Pseudococcus jackbeardsleyi*) were found on dragon fruit and dragon fruit was recorded as a new host for *P. solenopsis* within this study.

As can be explained above, there were limited studies conducted about the pests of dragon fruit. This study was carried out to determine the mealybug species and its natural enemies in pitaya greenhouses in Adana, Mersin and Antalya provinces in Türkiye between 2021 and 2022. In addition, mealybug species, which were detected on pitaya with the surveys, were identified with the morphological within this study. The results of this study will help to develop control strategies against mealybug species in pitaya plantations in Türkiye.

2. Material and Methods

This research was carried out in pitaya greenhouses in Adana, Mersin and Antalya between July and December in 2021 and 2022. Total pitaya greenhouses were 138.6 ha in Adana, Mersin, and Antalya. Surveys were done according to Bora and Karaca (1970) and it was used to periodic and non-periodic ways to collect mealybug materials from pitaya greenhouses between July and December in three different provinces (Antalya, Mersin, and Adana). Size of pitaya greenhouses was between 0.1-1.8 ha in Adana, 0.05-1.0 ha in Mersin, and 0.1-2.0 ha in Antalya. Table 1 showed the details of surveys in our research. Mealybug samples were collected with plant parts, placed into a paper bag and recorded with location and date. Samples were brought to a laboratory and prepared for identification accordin to Kosztarab and Kozár (1988) method.

Mealybugs were identified morphologically by Associate Prof. Asime Filiz Çalışkan Keçe with a stereo microscope. Synonyms, host plants data were prepared according to ScaleNet (Garcia Morales et al., 2016).

The natural enemies of the mealybug species were determined in this study as well. Mealybug-infected plant parts were taken to a laboratory, parasitized mealybugs mumies were placed into parasitoid box and obtained parasitoids were preserved in 70% alcohol solution for identification (Çalışkan Keçe et al., 2018). In addition, mealybug-infected plant parts with predator insects were cultured to obtain adult individuals for identification (Kahya, 2020). Detected predator species belonging Coccinellidae family were preserved and identified (Uygun, 1981). The identification of predator and parasitoids were also done by Assoc. Prof. Asime Filiz Çalışkan Keçe.

3. Results and Discussion

According to results, 2 different mealybug species were detected *Phenacoccus solenopsis* and *Phenacoccus madeirensis*, belonging the *Phencoccus* genus. These mealybug species and its natural enemies was also reported in Türkiye and Eastern Mediterranean region on different host plants by different researchers (Kaydan et al., 2012; Kaydan et al., 2013; Çalışkan, 2015; Kaydan et al., 2016; Çalışkan et al., 2016; Çalışkan Keçe et al., 2018). The determined mealybug species and its parasitoids and predators during this study were given below:

3.1. *Phenacoccus solenopsis* (Tinsley, 1898)

3.1.1. Synonyms

Phenacoccus cevalliae Cockerell 1902, *Phenacoccus gossypiphilous* Abbas, Arif & Saeed 2005, *Phenacoccus gossypiphilous* Arif, Abbas & Saeed 2007, *Phenacoccus gossypiphilous* Abbas, Arif, Saeed & Karar 2008, *Phenacoccus solenopsis* Tinsley, 1898, *Phenacoccus solenopsis* Weintraub, et al. 2017, *Phenaccocus solenopsis*; Nawaz & Freed 2022.

3.1.2. Examined materials

Türkiye, Mersin, Akdeniz, 13.08.2021, 5 ♀♀, *Hylocereus* spp. (Cactaceae) Collector: M. Yayla**;** Mersin, Erdemli, 13.08.2021, 2 ♀♀, *Hylocereus* pp. Collector: M. Yayla; Adana, Karaisalı, 20.08.2021, 5 ♀♀, *Hylocereus* spp. Collector: M. Yayla; Antalya, Aksu, 25.08.2021, 4 ♀♀, *Hylocereus* spp. Collector: M. Yayla; Antalya, Gazipaşa, 25.08.2021, 6 ♀♀,

Table 1. Survey areas of pitaya greenhouses in Antalya, Adana, and Mersin.

Surveyed provinces	Surveyed greenhouses areas (ha)	Total greenhouses (ha)	The number of surveyed greenhouses
Antalya	2.75	111.5	22
Adana	2.24	11.2	17
Mersin	3.18	15.9	26

Hylocereus spp. Collector: M. Yayla; Mersin, Silifke, 08.09.2021,7 ♀♀, *Hylocereus* spp. Collector: M. Yayla; Adana, Ceyhan, 10.09.2021, 5 ♀♀, *Hylocereus* spp. Collector: M. Yayla; Mersin, Akdeniz, 15.09.2021, 6 ♀♀, *Hylocereus* spp. Collector: M. Yayla; Adana, Çukurova, 17.09.2022, 4 ♀♀, *Hylocereus* spp. Collector: M. Yayla; Mersin, Akdeniz, 13.10.2021, 8 ♀♀, *Hylocereus* spp. Collector: M. Yayla; Mersin, Anamur, 20.10.2022, 4 ♀♀, *Hylocereus* spp. Collector: M. Yayla; Adana, Karaisalı, 21.x10.2021, 9 ♀♀, *Hylocereus* spp. Collector: M. Yayla; Mersin, Akdeniz, 03.11.2022, 3 ♀♀, *Hylocereus* spp. Collector: M. Yayla; Adana, Karataş, 04.11.2021, 2 ♀♀, *Hylocereus* spp. Collector: M. Yayla.

Hylocereus spp. was determined as a host plant for *Phenacoccus solenopsis* during this study. This invasive pest is widespread in the world and determined in 210 genus from 64 families of host plants (Garcia Morales et al., 2016). *Phenacoccus solenopsis* was determined as a pest in pitaya greenhouses in Adana, Mersin, and Antalya. Moreover, *P. solenopsis* were detected in pitaya other parts of the world (Rezeki et al., 2021; Sartiami et al., 2019) In addition, parasitized mealybug mummies were found during survey (Figure 1).

3.2. *Phenacoccus madeirensis* (Green, 1923)

3.2.1. Synonyms

Phenacoccus grenadensis Green & Laing, *Phenacoccus harbisoni* Peterson, *Phenacoccus gossypii* Tranfaglia.

3.2.2. Examined materials

Türkiye, Adana, Karaisalı, 20.08.2021, 2 ♀♀, Hylocereus spp. Collector: M. Yayla; Antalya, Kepez, 03.09.2021, 2 ♀♀, *Hylocereus* spp. Collector: M. Yayla; Antalya, Alanya, 03.09.2021, 4 ♀♀, *Hylocereus* spp. Collector: M. Yayla; Mersin, Silifke, 08.09.2021, 3 ♀♀, Hylocereus spp. Collector: M. Yayla; Mersin, Akdeniz, 15.09.2022, 2 ♀♀, Hylocereus spp. Collector: M. Yayla; Mersin, Akdeniz, 13.10.2021, 2 ♀♀, Hylocereus spp. Collector: M. Yayla; Adana, Karaisalı, 21.10.2022, 2 ♀♀, Hylocereus spp. Collector: M. Yayla. *Hylocereus* spp. was determined as a host plant for *Phenacoccus madeirensis* during this study. Maderia mealybug is known as one of the most widespread invasive mealybug species and is found on 210 genus from 64 families of the host plant (Garcia Morales et al., 2016). *P. madeirensis* was

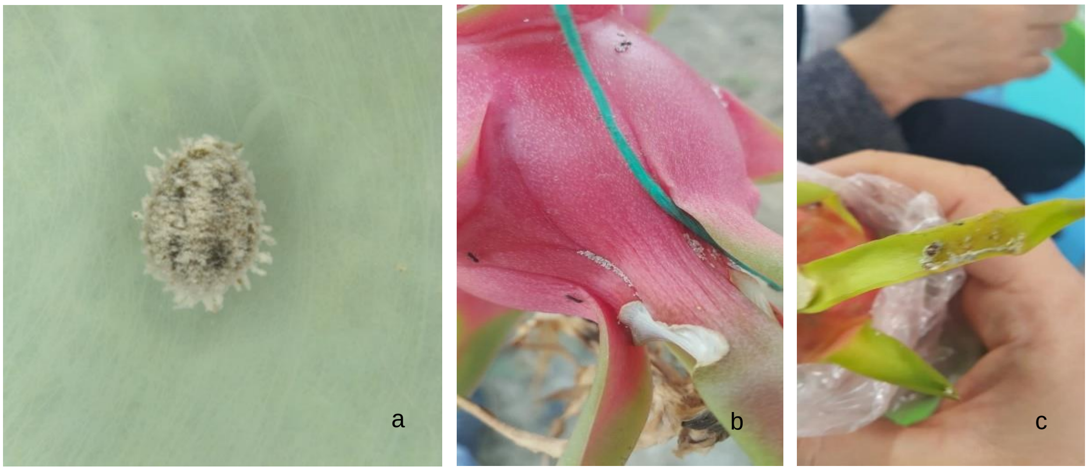

Figure 1. Adult female of *Phenacoccus solenopsis* (a), the adult and nypmphal stages of *Phenacoccus solenopsis* on pitaya fruit (b), parasitized adult mealybug mummy (c).

Figure 2. Adult female of *Phenacoccus madeirensis.*

Table 2. Detected parasitoids and predator species of *Phenacoccus solenopsis* in pitaya greenhouses in Adana, Antalya, and Mersin.

Detected predatory insects			
Order	Family	Species	Collected locations
Neuroptera	Chrysopidae	*Chrysoperla carnea* Stephens	Adana, Mersin, Antalya
Coleoptera	Coccinellidae	*Nephus includens* Kirsch	Adana, Mersin, Antalya
Detected parasitoid insects			
Order	Family	Species	Collected locations
Hymenoptera	Encyrtidae	*Aenasius arizonensis* Girault	Adana, Mersin

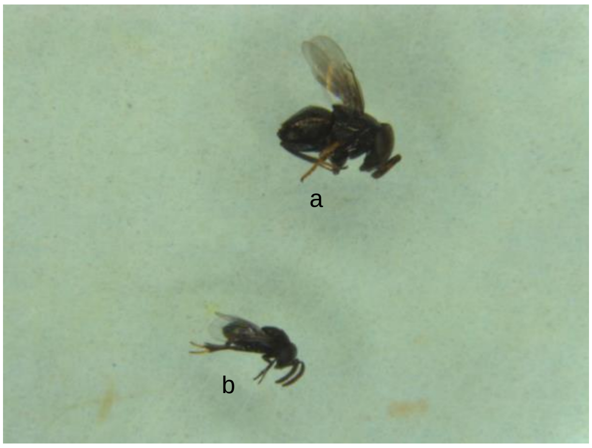

Figure 3. Female (a) and male (b) of *Aenasius arizonesis*.

detected as a pest in pitaya greenhouses in Adana, Mersin and Antalya (Figure 2).

In addition, parasitoids and predators of mealybugs were determined during this research. According to the results, one parasitoid and two different predator species were detected in pitaya greenhouses infected with *Phenacoccus solenopsis.* The list of predators and parasitoids were given in Table 2 with locations. In addition, Figure 3 demonstrated adult (a) female and (b) male of *A. arizonensis* (parasitoid). There were many studies conducted about the predators and parasitoids of *P. solenopsis* and *P. madeirensis.* According to result of these studies *A. arizonensis* was found most common and important parasitoids in Türkiye and other countries in the world (Hayat, 2009; Chen et al., 2011; Tanwar et al. 2011; Suroshe et al., 2013; Çalışkan Keçe et al., 2018). This parasitoid is a solitary endoparasitoid of this mealybug species, and the parasitism rate of *A. arizonensis* was varied between from 30% to 80% in field surveys in India and Türkiye (Aga et al., 2016; Kahya et al., 2021). Moreover, predator species of *P. solenopsis* has been determined. Different Coccinellidae and Chrysopidae species were detected within some survey studies in Türkiye and other countries (Hanchinal et al., 2010; Attia and Awadallah, 2016; Kahya et al., 2018). In addition, some laboratory studies were conducted about some Coccinellid and Chrysopid predators. Khan et al. (2012) Khan et al. (2012) conducted a study to assess the predatory potential of *C. carnea* and *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) against the first nymphal stage of *P. solenopsis*. Both predators were observed to have the capacity to feed on this stage. In addition, Ibrahim (2018) observed feeding potential of *C. carnea* against *P. solenopsis* on cotton under semi-field condition and results showed that 5 larvae of *C. carnea* can feed with 100 nymphs of *P. solenopsis.* As can be seen above studies, *A. arizonensis* had potential to use against *P. solenopsis* successfully. The main advantage of this parasitoid is solitary and widespread in Türkiye. Further studies may be conducted against *P. solenopsis* in pitaya greenhouses. In addition, *C. carnea* and *N. includens* is known as predator insects and both may feed with *P. solenopsis* and *P. madeirensis*. Moreover, more studies may be done for both mealybugs with the use of these detected predators under semi-field and field conditions within biological control and Integrated Pest management programs.

4. Conclusion

Pitaya, *Hylocereus* spp, will become one of the most economically important agricultural products and plantation areas will increase in the Mediterranean Region of Türkiye. In addition, dragon fruit has the potential to increase polyculture in Adana, Antalya, and Mersin provinces. Pests and diseases of pitaya should be determined in our region and further studies should be conducted to prevent yield loss due to pests and diseases. This research may be the first step to determine pests of pitaya in Türkiye. As a result of this study, 2 different invasive mealybug species (*P. solenopsis*, and *P. madeirensis*) were determined as a pest on pitaya fruit. Moreover, parasitoid (*A. arizonensis*) and predator species (*N. includens*, and *C. carnea*) of these mealybug species were also detected in this research. The effectiveness of these natural enemies and other pests and diseases of pitaya should be considered in further studies.

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HORTICULTURAL STUDIES

RESEARCH PAPER

Variety Breeding Studies on *Hesperis isatidea* (Boiss.) D.A. German & Al-Shehbaz

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Abstract

Hesperis isatidea (Boiss.) D.A.German & Al-Shehbaz, a member of the Brassicaceae family, represents a distinctive natural species characterized by its fragrant, conspicuous inflorescences, unique flower coloration, and distinct feather-like foliage. The Turkish name of the species is known as 'Allı Gelin'. This species is native, exhibiting resilience to drought conditions and requiring minimal care. Cultivation studies on this species were carried out at Erzincan Horticultural Research Institute (Türkiye) with project number 106G022 within the scope of TÜBİTAK (Turkish Scientific and Technological Research Council). The research aimed to determine optimal production techniques through generative and vegetative propagation methods. The project, initiated in 2013 and concluded in 2022, focused on developing a cultivar of the 'Allı Gelin' plant using single selection breeding techniques. Thirteen populations of 'Allı Gelin' from institute's gene pool were utilized. Employing the single selection method, four distinct lines were identified: compact, tightly spread compact, pyramid-1 and pyramid-2 shaped. Subsequent baby plant tests revealed stability in the tightly spread compact and pyramid-1 lines, leading to their selection for further breeding. Although breeding efforts continued on the compact line, the pyramid-2 line was discontinued due to an inability to attain the desired stability. The successfully tested variants, namely the pyramid-1 and tightly spread compact forms, have been earmarked as candidates for outdoor ornamental plant varieties. Registration procedures for these candidates are underway. These new varieties promise to enrich diversity within the ornamental plants sector, potentially enhancing competitiveness in foreign markets.

1. Introduction

Türkiye, distinguished for its geographical positioning, geomorphological structure, and diverse ecology, holds significant prominence as one of the world's key genetic centers. The country's flora encompasses approximately 12.000 plant taxa, with around 35% exhibiting endemism (Anonymous, 2021). Among these, the 'Allı Gelin' plant, exclusively found in limited locales, notably along roadside slopes, carries substantial economic prospects. However, the recent intensification of road construction has resulted in the loss or reduction of many of these habitats.

The 'Allı Gelin' plant, scientifically known as *Hesperis isatidea* (Boiss.) D.A. German & Al-Shehbaz belonging to the Brassicaceae family, represents a distinctive biennial species. Its distinct appearance, characterized by fragrant and flamboyant flowers, unique flower colors, and distinctive feather cover, is endemic to various locations in Erzincan (Baytop, 1994; Kandemir et al., 2022). Notably, it exhibits resilience to drought and thrives under limited maintenance conditions.

This species showcases considerable potential in the ornamental plants sector, particularly in landscaping along highways, roadsides, parks, and gardens, where it can serve as seasonal flower.

The 2020 report from the Ornamental Plants Producers Sub-Union underscores the necessity of prioritizing the development of drought-resistant species and varieties, particularly in grass seeds, within the ornamental plants sector. Moreover, it emphasizes establishing a gene pool to facilitate the long-term breeding of crucial species and implementing measures to prevent the introduction of invasive foreign species that could endanger the native flora (SÜSBİR, 2020).

Policy recommendations from the Ornamental Plants Sector Document Workshop for the 2020-2024 period primarily advocate supporting research and development endeavors aimed at creating new varieties adaptable to evolving climate conditions, specifically focusing on drought-resistant species requiring minimal maintenance. The proposed approach entails leveraging high-value plant species from the indigenous flora through collaborative efforts involving governmental bodies, universities, and the private sector. This initiative aims to tackle a key challenge faced by the sector: the developping of new varieties capable of thriving under changing climate conditions and limited maintenance, utilizing species from the local flora (Anonymous, 2021).

Republic of Türkiye Ministry of Agriculture and Forestry General Directorate of Agricultural Research and Policies (TAGEM) 2021-2025 Agricultural Research Master Plan outlines objectives within its ornamental plants research program, including the development of outdoor ornamental plant varieties resilient to drought and low water consumption, cultivation of economically viable indigenous species for export, and ensuring sustainable utilization. The research program's strategy in the short and medium term aims to develop high-yield, high-performance varieties for both indoor and outdoor ornamental plants adaptable to shifting climate dynamics. This strategic direction aims to curtail water usage in landscaping while meeting market demands for new varieties suited for drought conditions. In the long run, emphasis will be placed on integrating economically valuable species into breeding programs to align with market expectations and develop new drought-resistant varieties utilizing local genetic resources in response to climate change (Anonymous, 2021).

A major hurdle faced by the ornamental plants sector lies in the scarcity of indigenous and nationally recognized varieties (Kaya et al., 2015). The study on the *H. isatidea*, an integral component of our diverse biodiversity and one of the most sought-after outdoor ornamental plants has been specifically designed to address these objectives.

In line with the aforementioned rationale, Erzincan Horticulture Research Institute has concluded cultivation studies on the *H. isatidea* and aims to introduce a selection of candidates derived from a selective breeding (single selection) method into the ornamental plants sector. Initiated in 2013, the project involved 13 ‘Allı Gelin’ populations from our institute's genetic pool. Over the period from 2013 to 2022, purpose-driven self-pollination techniques were employed (compact, pyramid, tightly spread compact), resulting in the selection of three distinct lines. Baby plant trials conducted in the tightly spread compact and pyramid forms in 2022 exhibited a 98% stability rate. These lines in these two forms have been identified as candidates for outdoor ornamental plant varieties, and necessary registration procedures have been set in motion.

2. Material and Method

2.1. Materials

The materials for our study were constituted by individuals from a collection garden, which represented 13 different 'Allı Gelin’ populations. These were gathered as part of the TÜBİTAK-supported project "Cultivation of Some Perennial Plant Species in the Eastern Anatolia Region" (Project No: 106G022) between 2006 and 2009 (Aslay et al., 2009).

2.2. Method

2.2.1. Selection breeding

The most suitable production technique for the species was identified by applying generative and vegetative propagation techniques (Aslay et al., 2013). The "Single-Selection Breeding Method" was utilized in this study aimed at standard variety development (Demir, 1975). The following stages were followed in a single selection:

2.2.2. Population inspection (1-4 Years)

The ‘Allı Gelin’ plant produces seeds biennially, so each generation requires two years. Samples from 13 populations, collected as part of the “Cultivation of ‘Allı Gelin’ Plant in Eastern Anatolia Region” project, were present in the institute's gene pool. Seed sowing of these 13 populations was conducted, and plants possessing desired traits (Figure 1) were marked for self-pollination.

2.2.3. Elite inspection (4-8 Years)

Selected elite plants were numbered and grown in separate rows without repetition. Self-pollination was conducted on plants exhibiting desired forms (compact, tightly spread compact, and pyramid form). This self-pollination process continued until pure lines were obtained.

Figure 1. Preferred plant forms (1: Compact, 2: Pyramid, 3: Tightly spread compact).

Table 1. Morphological measurement and observation criteria for Allı Gelin plant.

No	Morphological measurement and observation criteria	Descriptions
1	Plant height (cm)	Distance from the soil surface to the furthest point of the plant
2	Branching status of stem	Presence or absence of branching on the stem and if present, the number of branches
3	Leaf status on stem	Presence or absence of leaves on the stem
4	Flowering date	Date when the first flowering begins in the plant
5	Flower color	Identification of flower colors of the plant was done using the RHS color catalogue
6	Flower cluster diameter (cm)	Distance between the two farthest points of the flower when evaluated in its full bloom
7	Flower cluster type	Form of the flowers on the plant: compact, pyramid, tightly spread compact
8	Flower longevity (days)	Duration for which flowers remain vibrant on the plant, in days
9	Petal width (mm)	Distance between the two widest points of the petals in the flower
10	Petal length (mm)	Distance between the two furthest points of the petals in the flower
11	Number of petals	Number of petals in the flower
12	Fragrance	Scent of the flowers in the plant was determined sensorially
13	Fuzziness	Presence or absence of fuzziness on the stem and leaves of the plant
14	Number of leaves	Number of leaves on the plant
15	Leaf color	Color of the leaves on the plant was determined using the RHS color catalogue
16	Leaf shape	Shape of the leaves at the base of the plant was determined
17	Leaf width (cm)	Distance between the two widest points of the leaf
18	Leaf length (cm)	Distance between the two furthest points of the leaf
19	Seed maturation time	Date when all the seeds in the plant have matured

2.2.4. *Baby plant testing and selection of suitable variety candidates (9-10 Years)*

Stability checks were performed on plants in the selected lines, and off-target plants were removed. Selection of individuals suitable for outdoor and potted floriculture was done from the baby plants, followed by seed production. Morphological measurements and observations of the obtained pure lines were conducted according to the criteria listed in Table 1.

3. Results and Discussion

3.1. 2013-2014 (Population inspection)

In 2013, seed sowing of the existing 13 populations in the institute's gene pool was carried out, resulting in the production of seedlings. When these seedlings had reached the first true-leaf stage, they were transplanted into 45-cell trays and grown in peat+perlite (3/1) mixture medium (Aslay et al., 2010). The matured seedlings in the trays were then planted in the field using a triangular planting pattern with spacing of 50×50×50 cm between and along the rows. The 'Allı Gelin' plants that matured during this period and went into dormancy in winter, flowered/bloomed in 2014. Among these flowering plants, those with desired traits such as compact, pyramid, and tightly spread compact forms (Figure 1) were numbered, and seeds were obtained by selecting self-pollinated ones and 9 lines.

3.2. 2015-2016 (Elite inspection)

In 2015, the selected elite plants were cultivated in separate rows without repetition. Based on plant observations conducted in 2016, self-pollination was carried out on plants meeting the desired criteria (compact, pyramid, tightly spread compact), and 5 lines possessing the targeted features were selected. Seed planting for these selected lines was performed in the autumn.

3.3. 2017-2018 (Elite inspection)

Seedlings were obtained from seeds planted in the autumn when they germinated in March. These seedlings were then transplanted into trays. Seedlings that matured in the trays were planted in the field. The following year, in April, among the plants of the five ‘Allı Gelin’ lines that began forming flower buds, 35 plants possessing the desired traits were isolated in iron cages covered with fly netting (9×6, 30 mesh) for self-pollination (Figure 2). Among these caged plants, 4 lines that formed seeds and met the desired form (compact, pyramid, tightly spread compact) were selected and named.

For the plants of the four selected elite lines (Figure 3), the duration of flower retention varied between approximately 30 to 40 days. The plants transitioned to seed formation by the end of May and matured their seeds in June. The ‘Allı Gelin’ seeds from these four purposefully selected lines were collected and dried under laboratory conditions. The seeds from these lines were then sown in trays on October 17th for seedling production, with the intention of planting them in the field the following year.

3.4. 2019-2020 (Examination of elite lines)

Our study commenced in March 2019 with the germination of seeds from elite plants, which had been sown in trays in October 2018. During this period, the germinating seeds were transferred to cell packs for seedling development, and necessary care was provided until they reached 6-7 leaves by May. In May, the seedlings were planted in the field.

In April 2020, the plants of the four ‘Allı Gelin’ lines, which began forming flower buds, were individually isolated within their respective lines using insect mesh (9×6, 30 mesh) covered iron cages (Figure 4). In the ‘Allı Gelin’ lines, inbreeding depression was observed in the tightly spread compact line, characterized by a reduction in flower diameter and seed size. Gökçora (1969) reported that in cross-pollinating plants, self-pollination of a single plant could lead to a regression in various characteristics in the offspring. To prevent inbreeding depression, each line was subjected to collective isolation within itself. From the plants

Figure 2. Self-pollination study.

Figure 3. Images of the selected four lines (1: Compact, 2: Tightly spread compact, 3: Pyramid, 4: Pyramid 2).

Figure 4. Collective isolation within the lines.

Table 2. Morphological measurements and observations for the tightly spread compact-1 line.

No	Morphological measurement and observation criteria	Descriptions
1	Plant height (cm)	25.6-32.5 (Min.-Max.)
2	Branching condition of stem	20-40 (Min.-Max.)
3	Stem leaf condition	Present
4	Flowering date	09.05.2022-02.06.2022
5	Flower color	RHS 85B-82C-76B-77B-155B-155D
6	Flower cluster diameter (cm)	41-60 (Min.-Max.)
7	Type of flower cluster	Tightly spread compact
8	Flower longevity (days)	35-40 (Min.-Max.)
9	Petal width (mm)	0.3-0.4 (Min.-Max.)
10	Petal length (mm)	0.5-0.7 (Min.-Max.)
11	Number of petals	4
12	Fragrance	Fragrant
13	Hairiness	Present
14	Number of leaves	392-812 (Min.-Max.)
15	Leaf color	RHS 137A-137C
16	Leaf shape	Between reverse lanceolate and spoon-shaped
17	Leaf width (cm)	1.2-3.0(Min.-Max.)
18	Leaf length (cm)	5.4-10.5 (Min.-Max.)
19	Seed maturation time	19.08.2022

caged within the 'Allı Gelin' lines, four lines forming seeds in the desired forms (compact, pyramid, tightly spread compact) were selected and named.

3.5. 2021-2022 (Baby plant testing)

The study in 2021 focused on growing seedlings of elite plants and planting them in the field. In 2022, the project began with the plants emerging from dormancy in March, following the winter of 2021, and the plants were monitored daily. In April, observations were made on the plants of the four 'Allı Gelin' lines as they started forming flower buds. The Pyramid 2 line failed to achieve stability and was therefore eliminated from the project. In the Tightly Spread Compact-1 and Pyramid-1 forms, pure lines were established, and non-target individuals were removed from the field, achieving a 98% rate of stability, and these lines were designated as variety candidates. For the Compact line, selections of individual plants in the desired form were made and isolated in iron cages covered with insect mesh (9×6, 30 mesh). The morphological measurements and observations of the promising variety candidates from the Tightly Spread Compact-1 and Pyramid-1 forms are presented in Table 2 and Table 3.

The plant height of the tightly spread compact variety candidate ranges from 25.6 to 32.5 cm. Among the three forms, only the tightly spread compact form exhibits branching, with 20-40 branches emerging from the stem. The diameter of the flower cluster varies between 41-60 cm, and the flowers remain blooming for 35-40 days. The flowers are fragrant. The number of leaves is quite high, ranging from 392 to 812 leaves (Figure 5).

Among the tightly spread compact, compact, and pyramid forms, individuals of the pyramid form were taller. The height of the plants in the pyramid variety candidate ranged between 38.5-43.5 cm. No branching was observed on the stem in the pyramid form. The diameter of the flower cluster varied between 20-50 cm, and the duration of flower retention was between 49-53 days. The flowers were fragrant. The number of leaves ranged between 50-123 (Figure 6).

Table 3. Morphological measurements and observations for pyramid-1 line.

No	Morphological measurement and observation criteria	Descriptions
1	Plant height (cm)	38.5-43.5 (Min.-Max.)
2	Branching condition of the stem	None
3	Stem leaf condition	Present
4	Flowering date	28.04.2022
5	Flower color	RHS N82C-RHSN81B-RHS76B
6	Flower cluster diameter (cm)	20-50 (Min.-Max.)
7	Flower cluster type	Pyramid
8	Duration of flower retention (days)	49-53 (Min.-Max.)
9	Petal width (mm)	0.3-0.5 (Min.-Max.)
10	Petal length (mm)	0.8-0.9 (Min.-Max.)
11	Number of petals	4
12	Fragrance	Fragrant
13	Hairiness	Present
14	Number of leaves	50-123 (Min.-Max.)
15	Leaf color	RHS137C
16	Leaf shape	Between inverted lanceolate and spoon-shaped
17	Leaf width (cm)	2.2-3.0 (Min.-Max.)
18	Leaf length (cm)	13-15.4 (Min.-Max.)
19	Seed maturation time	19.08.2022

Figure 5. Tightly spread compact variety candidate.

Figure 6. Visuals of the pyramid-1 line.

Both forms of promising variety candidates have wide flower forms, are fragrant, and also possess showy flower clusters. In light of these characteristics, trials for the registration process of these outdoor ornamental plant variety candidates have been set up within 2023, and applications for registration have been made.

Among 2000 Paeonia varieties collected from various countries around the world, a relative evaluation was made considering their general appearances, initially selecting 81 varieties. From these varieties, based on criteria such as leaf type, color and shape, flower cluster type and size, petal size, number and color, stem height, branching number, and flowering date, ultimately 9 varieties were selected for use. These were classified according to their usage possibilities as cut flowers, perennial garden flowers, and pot flowers (Choi, 1994). This study shows parallels with our research.

In the breeding research on the *Anemone coronaria* L. (poppy anemone) species, Kostak and Köse (1998) identified 8 clones of yield and quality suitable for use as cut flowers, based on phenological and morphological observations on samples collected from various regions. However, since the breeding studies were not continued, the variety stage could not be reached. Our study has reached the stage of variety registration by following the appropriate stages for breeding.

In the study conducted by Kaya et al. (2009) all regions of the Turkish Flora were surveyed; 1166 populations encompassing 177 geophyte species, 20 dunes, and 44 outdoor ornamental plant species, totally 241 species, were identified. Plant samples were selected through preliminary selection, transferred to culture conditions, and preserved in responsible institutions. Of these collected plants, 70 species with economic potential were selected and introduced to the ornamental plants sector. The results of this study align with ours.

Similarly, in the study "Cultivation and Breeding of Turkish Peonies" conducted by Kaya (2010) based on selection breeding, 6 varieties suitable for use as outdoor ornamental plants, 2 as pot plants, and 3 as cut flowers were identified, considering criteria like flower color, numerous flower clusters, double or semi-double, flower type, leafy multiple branching, relatively short height, flowering vegetation period, thick flower stem, vase life, etc.

In the project conducted by Kaya et al. (2015) aimed at developing varieties of Turkish *Fritillaria* species, 22 varieties were developed from natural populations using selection and mutation breeding methods. Important selection criteria such as plant propagation speed, plant growth strength, application area, flowering date, number of flowers, flower life, flowering vegetation period, flower shape, flower diameter, tepal size, color, position, leaf shape, number, color, and position were considered in single selection of flowering types, and among the developed varieties, Vuslat (*F. imperialis*), Doğu Güneşi (*F. aurea*), and Aslay (*F. michailovskyi*) were protected for breeder's rights (Aslay, 2015; Yıldız et al., 2022; Aslay et al., 2023).

Our selection study in the 'Allı Gelin' plant differs from other conducted studies in that it develops varieties of a special species capable of tolerating the effects of drought, one of the negative impacts of global climate change, and thriving under limited maintenance conditions, thereby making these developed varieties unique in this aspect.

4. Conclusion

The ornamental plant sector's key objectives involve prioritizing the development of drought-resistant species and varieties that require minimal maintenance. Furthermore, establishing a gene pool for the long-term breeding of crucial species within the sector is crucial. Incorporating native plants into landscaping projects not only boosts success rates and decreases expenses but also bolsters the national economy. To this end, our study focused on the 'Allı Gelin' plant, an endemic species possessing these desired characteristics. We utilized the single selection breeding method, resulting in stable compact and pyramid-1 forms. We have also initiated registration procedures for the newly developed variety.

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HORTICULTURAL STUDIES

RESEARCH PAPER

Determination of Phenolic, Flavonoid Content and Antioxidant Activity of Oil Rose Products

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Abstract

Rose and rose products are used as raw materials in many sectors including cosmetics, food and medicine. The *Rosa damascena* Mill. rose genotype, which is intensively cultivated in the Isparta region of Türkiye, is an important export product. The flower of the plant is main source of raw materials for rose oil, herbal teas and cosmetic products due to its high essential oil and polyphenols contents. In this study, *Rosa damascena*, *Rosa centifolia, Rosa alba* L., *Rosa alba* 'Semiplena' genotypes were grown in Yalova, which is an alternative region in terms of agro-climatic conditions from Isparta and its surroundings where rose oil cultivation is carried out in Türkiye. Within the scope of the study, total phenolic matter, total antioxidant activity (DPPH and CUPRAC) and total flavonoid contents of flowers (whole flowers), rose water and wastewater of the genotypes were determined. It is important to identify alternative rose genotypes to *R. damascena* in Türkiye and their potential for cultivation under different climatic conditions. The highest phenolic content (4115 mg gallic acid equivalent GAE 100 g^{-1}) and DPPH antioxidant activity (4893 mg trolox equivalent TE 100 g^{-1}) values were obtained in *R. damascena* genotype at the first harvest period. The highest CUPRAC antioxidant activity (34237 mg TE 100 g^{-1}) values were obtained in *R. centifolia* genotype at the first harvest period. In the second harvest period, the highest phenolic content, DPPH antioxidant activity and CUPRAC antioxidant activity values were determined in *R. centifolia* genotype. Antioxidant activity, phenolic and flavonoid contents of dried rose flowers were higher than in rose water and wastewater. Rose petals were found to have the potential to be used as an important antioxidant source, while total phenolic matter and antioxidant activity values were found to be low in rose water and wastewater. The harvest period was found to be major factor in oil rose flowers, which can be an important polyphenol source.

1. Introduction

Roses have been one of the most valuable flowers for centuries. In addition to its socio-cultural importance and use as an ornamental plant, roses are also a rich source of biologically active substances. Although the genus Rosa includes 200 species and more than 18000 varieties (Gudin, 2000), only a few (*R. damascena*, *R. alba*, *R gallica* L. subsp. *eriastila* Kell. var. Austriaca Grantz f. panonica Br, *R. francofurtana* var. Agatha, *R. centifolia*, *R. rugose*, *R. moschata*) are used industrially in the production of rose oil and its products (Georgiev and Stoyanova, 2006; Kovacheva et al., 2010; Rusanov et al., 2012; Baydar, 2016). *R. damascena* is the most commonly used species in oil rose production in Türkiye and in the world.

The most important products of roses are essential oil, concretes, absolutes and rose water (Kumar et al., 2013). All these products are used in the food, cosmetics and perfumer industry. Due to the antioxidant and phenolic contents in phytochemicals, it has also effect on the prevention of many diseases. Recently, the production of products derived from natural plants has been increasing due to the disadvantages of synthetic antioxidants. For this reason, interest in phytochemicals is increasing. Rose species have been reported to have anti-human immunodeficiency virus, antidepressant and anti-inflammatory properties (Nowak and Gawlik-Dziki, 2007). Each of the oil rose genotypes can be a powerful source of antioxidants such as phenolics, flavonoids, carotenoids and anthocyanins. Today, natural plant sources such as oil rose antioxidants are used in the treatment of diseases, nutrition, health care and inhibition of collagenase enzyme (Liu et al., 2020; Mohsen et al., 2020). There are several industrial demands that need to identify new chemotypes with beneficial effects (Moein et al., 2016). Since artificial antioxidants are suspected of toxicity and risks to human health, the demand for the use of natural antioxidants is increasing (Weisburger, 1999). Traditionally, it has been used for chest pain, constipation, depression, gastrointestinal disorders, inflammation, respiratory problems and menstruation (Moein et al., 2016). Rose petals are a natural and powerful antioxidant. Moreover, natural compounds from leaf extracts, such as polyphenols and flavonoids, have anti-aging properties and antioxidant activity (Mohsen et al., 2020). Anthocyanins, as water-soluble vacuolar pigments, are responsible for the color in flowers and fruits of plants and have strong antioxidant properties. The amount of anthocyanins, like other metabolites, depends on environmental factors (temperature, light intensity, nutrition, pH) (Shameh et al., 2019). Rose oil cultivation in Türkiye has focused on *R. damascena* in a single region (Isparta and its surroundings), and the current research results represent the products grown in this region.

This study was conducted in Yalova, as an alternative region whose agro-climatic conditions such as altitude, temperature and precipitation are completely different from Isparta. Total phenolic matter, antioxidant activities and total flavonoid contents of rose petals and rose water of two different periods of *R. damascena* and other oil rose genotypes (*R. alba*, *R. alba* 'semiplena', *R. centifolia*) about which there is limited information were investigated. In addition, the phenolic contents and antioxidant activity potential of the wastewater, which is formed as a residue in the rose oil process, were determined.

2. Material and Methods

2.1. Metarial

In this study, *R. damascena*, *R. centifolia*, *R. alba*, *R. alba* 'Semiplena' oil rose genotypes grown in Atatürk Horticultural Central Research Institute (Yalova) were used. The flowers of the oil rose genotypes were collected in the early morning hours (between 07:00 and 08:00) in two different periods on the dates which were specified in Table 1. The performances of rose genotypes under Yalova conditions were investigated during two different periods. Meteorological data covering these two periods in Yalova conditions were given in Figure 1.

Table 1. Harvest periods of the samples used in the study.

Genotypes	First period	Second period
Rosa damascena	23.05.2022	08.06.2022
Rosa centifolia	23.05.2022	08.06.2022
Rosa alba	01.06.2022	15.06.2022
Rosa alba 'semiplena'	01.06.2022	15.06.2022

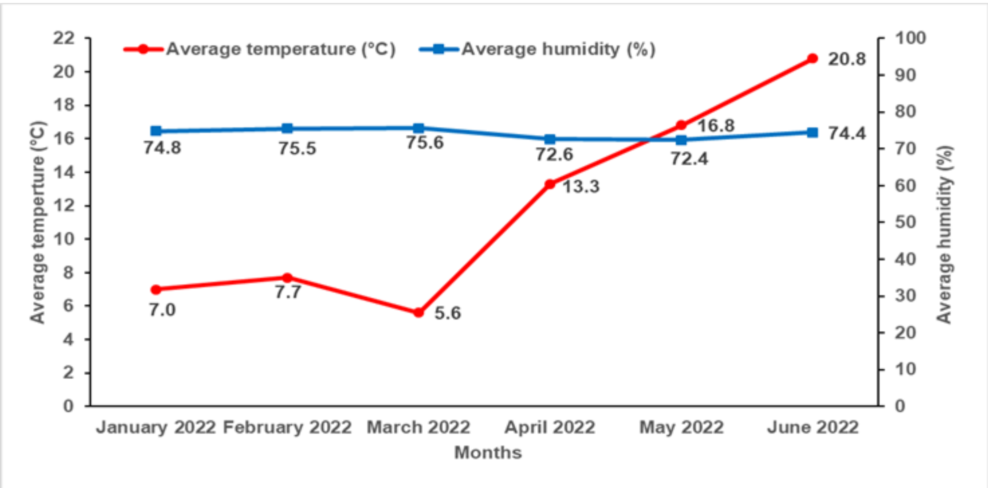

Figure 1. Meteorological data between January and June 2022 in Yalova.

2.2. Methods

In this study, phenolic content and antioxidant activity of the products (flowers, rose water and wastewater) of four different oil rose genotypes were investigated. After drying and powdering the whole rose flowers, phenolic content, antioxidant activity and total flavonoid content were determined by methanol extraction and spectroscopic methods. Rose water and wastewater were obtained by hydrodistillation method, phenolic contents were obtained by solvent extraction and total phenolic matter and antioxidant activity contents were analyzed (Figure 2).

2.2.1. Obtaining rose water and wastewater

200 g of fresh rose flowers were filled into the 5 L balloon of the Clevenger hydro-distillation unit and 2 L of distilled water was added into the mixture. The hydro-distillation process was continued for 3 hours after the water in the distillation flask started to boil with a jacketed heater. At the end of distillation, essential oil (rose oil) and aromatic water (hydrosol) accumulated under the oil were obtained as rose water. The wastewater was obtained by squeezing the remaining pulp in the hydro-distillation flask (Baydar and Baydar, 2017).

2.2.2. Extraction of rose flowers

Rose flowers were dried in a tray dryer (at 40°C) until the water content decreased below 5%, and 3 g were taken from the powdered samples, homogenized with 100 ml of pure methanol for 2 minutes and kept at room temperature for 24 hours. The solvent extract mixture was filtered through Toyo N. 2 filter paper, the solvent was removed in a rotary evaporator at 37°C until 50 ml of methanol remained. The resulting extract was stored at -18°C until analysis (Li et al., 2014).

2.2.3. Phenolic extraction of rose water and rose wastewater

60 mL of rose water and rose wastewater were mixed separately with 30 ml of 5% sodium bicarbonate and 60 mL of ethyl acetate in a vortex for 2 minutes. After separating the ethyl acetate phase, ethyl acetate was evaporated in a rotary evaporator and the remaining residue was dissolved in methanol and then filtered and used in the analysis (Baydar and Baydar, 2013).

2.2.4. Total phenolic analysis

It was measured spectrophotometrically by the Folin-Ciocalteu method 2400 µL of distilled water and 150 µL of 0.25 N Folin-Ciocalteu solution were added to 150 µL of extract and mixed in vortex for 3-4 minutes. 300 µL of sodium carbonate (Na_2CO_3) (1 N) was added to this mixture and left for 2 hours at room temperature, then the absorbance of the samples was measured at 725 nm wavelength in a spectrophotometer (Hitachi, model). Total phenolic content (mg GAE 100 g^{-1}) was calculated from the

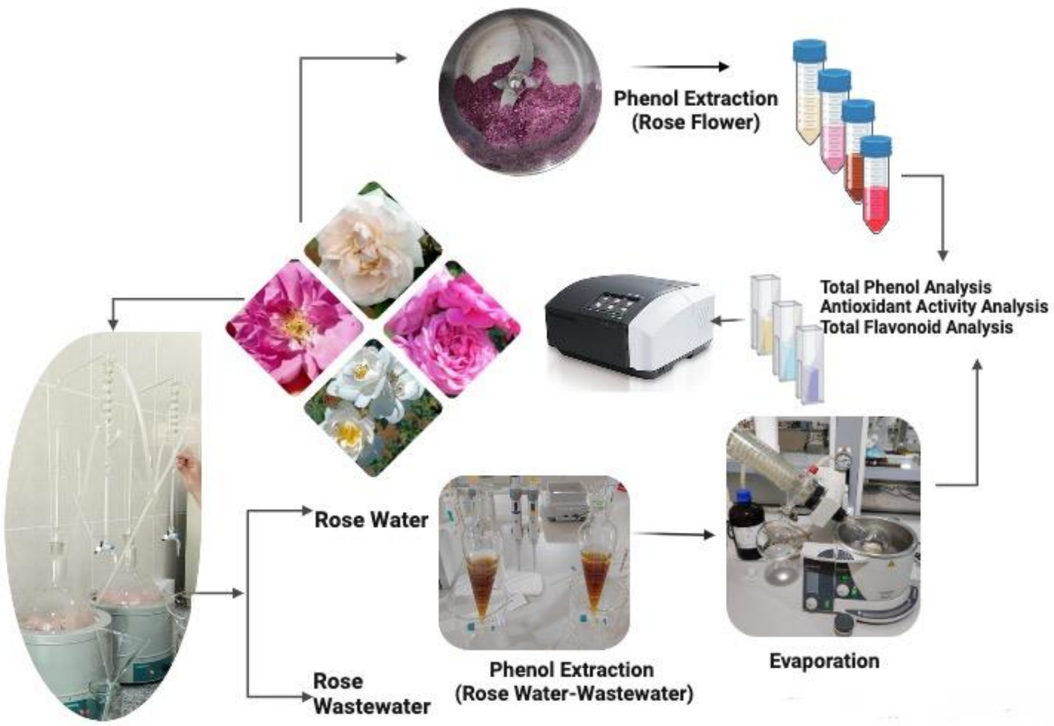

Figure 2. Flow chart examining the phenolic flavonoid and antioxidant properties of rose flowers rose water and rose wastewater.

calibration curve using a standard of gallic acid prepared at different concentrations (Thaipong et al., 2006).

2.2.5. DPPH antioxidant activity

The antioxidant activities of the samples were analyzed by applying the DPPH (2.2-diphenyl-1-picrylhydrazil) method. Stock solution; 0.12 mg of DPPH was weighed and dissolved in a 50 mL flask and stored at -20°C. Working solution; by adding 45 mL of methanol to 10 L of stock solution, an absorbance value of 1.1±0.02 was obtained at 515 nm wavelength in the spectrophotometer. 2850 µl of DPPH solution was added to 150 µL of extract taken from the samples prepared before, stored at -20°C and kept in the dark for 24 hours. Measurements were made with a spectrophotometer at a wavelength of 515 nm. DPPH was calculated from the calibration curve obtained with the trolox standard (Thaipong et al., 2006).

2.2.6. Copper Reducing Antioxidant Capacity (CUPRAC)

Copper Reducing Antioxidant Capacity (CUPRAC) analysis was performed using the method of Apak et al. (2004). To 100 µL of the extract, 1 mL 10 mM $CuCl_2$, 1 mL 7.5 mM neocuproin and 1 mL 1M NH4 Ac (pH:7) were added respectively. 1 mL of distilled water was added immediately to the mixture to give a final volume of 4.1 mL. After incubation at room temperature for 60 min, absorbance values at 450 nm were determined. The CUPRAC antioxidant activity value was determined according to the calibration curve against a blank reagent with trolox standard prepared at different concentrations.

2.2.7. Total flavonoid analysis

4 mL of distilled water and 0.3 mL of 5% $NaNO_2$ were added to 1 ml of extract and then mixed. After 5 minutes, 0.6 mL of 10% $AlCl_36H_2O$ was added, after 5 minutes, 2 mL of 1 mol L^{-1} NaOH is added, the total volume was made up to 10 mL with distilled water. Readings were made at a wavelength of 510 nm. Total flavonoid contents were calculated using (+)-catechin standard calibration curve (Karadeniz et al., 2005).

2.2.8. Statistical analysis

All of the trials and analyses were repeated three times for each sample. Random plots design was used in the study. Analyzes were made using the JMP statistical package program. The significance value was taken as $p<0.05$. And, results were given as mean±standard deviation.

3. Results and Discussion

The values of total phenolic matter and antioxidant activities (DPPH and CUPRAC) determined in rose flowers are given in Table 2. The highest total phenolic matter in first period harvested oil rose flowers was determined as 4115 mg GAE 100 g^{-1} in *R. damascena* genotype. In the other genotypes, it was 3529 mg GAE 100 g^{-1} in *R. centifolia*, while there was no statistical difference between *R. alba* 'Semiplena' (2064 mg GAE 100 g^{-1}) and *R. alba* (1888 mg GAE 100 g^{-1}). In the first harvest period, the highest DPPH antioxidant activity was found as 4893 mg TE 100 g^{-1} in *R. damascena* genotype, while there was no statistical difference between the other genotypes. As for CUPRAC antioxidant activity, the highest values were found in *R. centifolia* (34237 mg TE 100 g^{-1}) and *R. damascena* genotypes (27539 mg TE 100 g^{-1}), respectively. There was no statistical difference between *R. alba* 'Semiplena' (7422 mg TE 100 g^{-1}) and *R. alba* (6496 mg TE 100 g^{-1}). In the second harvest period, total phenolic content, DPPH and CUPRAC antioxidant activity was found in *R. centifolia* 3218 mg GAE 100 g^{-1}, 4860 mg GAE 100 g^{-1}, and

Table 2. Total phenolic content and antioxidant activity of flowers of rose genotypes harvested at different periods.

Periods	Genotypes	Total phenolic content (mg GAE 100 g^{-1})	DPPH* Antioxidant activity (mg TE 100 g^{-1})	CUPRAC Antioxidan capacity (mg TE 100 g^{-1})
Rose flower (First period)	*R. damascena*	4115±33 a	4893±56 a	27539±1257 b
	R. centifolia	3529±28 b	4147±76 b	34237±910 a
	R. alba	1888±95 c	4068±60 b	6496±408 c
	R. alba 'Semiplena'	2064±119 c	4068±60 b	7422±961 c
	CV	2.75	1.49	4.95
Rose flower (Second period)	*R. damascena*	2196± 22 b	4169±18 b	8576±881 b
	R. centifolia	3218±47 a	4860±19 a	14863±414 a
	R. alba	1991±53 c	4077±13 b	7288±370 b
	R. alba 'Semiplena'	2094±150 bc	3915±93 c	8008±1137 b
	CV	3.53	1.23	7.96

* DPPH: 2.2-diphenyl-1- picrylhydrazil CUPRAC: Copper Reducing Antioxidant Capacity.
Each value in the table was obtained by calculating the average of three analysis ± standard deviation.

14863 mg GAE 100 g^{-1}, respectively. In the second period, phenolic contents were 2196 mg GAE 100 g^{-1} in *R. damascena,* 2094 mg GAE 100 g^{-1}GAE in *R. alba* 'Semiplena', 1991 mg GAE 100 g^{-1} in *R. alba* for flower parts. There was no statistical difference between *R. damascena* (4169 mg GAE 100 g^{-1}) and *R. alba* (4077 mg GAE 100 g^{-1}) genotypes in terms of DPPH antioxidant activity. The lowest DPPH antioxidant activity (3915 mg GAE 100 g^{-1}) was detected for *R. alba* 'Semiplena' genotype. In terms of second period CUPRAC antioxidant activity, no statistical difference was detected between *R. damascena, R. alba, R. alba* 'Semiplena' genotypes. When the harvest period was compared, *R. damascena* genotype showed a significant decrease in total phenolic matter content and CUPRAC antioxidant activity content, while no significant changes were found in other genotypes.

Some research has focused on the antiradical and antioxidant activities of rose compared to other plants. Vinokur et al. (2006) analyzed hot water infusions (teas) of dried petals of twelve rose varieties for antioxidant activity. Phenolics, total anthocyanins and high antioxidant capacity of rose petal tea can be used as a caffeine-free beverage, consumed separately or in combination with other herbal ingredients. In a study investigating the total phenolic matter and antioxidants of fresh and dried flowers, flowers and green leaves of *R. damascena* genotype were dried and powdered, and then hot and cold extractions were carried out with methanol. While hot extractions yielded more extracts, cold extractions yielded higher total phenolic matter, flavanol and flavonol contents. The highest values for total phenolic matter were 478.34 mg GAE g^{-1} and 530.40 mg GAE g^{-1} in hot and cold extraction of leaves, respectively. The phenolic content of petals of 9 different rose genotypes were analyzed by Khurshid et al. (2018), and was found between 39.10-91.19 mg GAE g^{-1}. *Rosa moschata* was found to have the highest phenolic content, while *R. hybrida* (pink-yellow) had the lowest. In our study, total phenolic matter and antioxidant activities of rose flowers were found to be high by reported Khurshid et al. (2018) and Vinokur et al. (2006).

White rose petals (Rosa spp.) can be used in the treatment of allergic diseases due to their antioxidant effect. For this reason, the extraction of white rose petals was optimised for the independent variables of ethanol concentration, extraction temperature, and extraction time. Predicted response values for the phenolic and flavonoid contents were 243.50 mg gallic acid equivalent/g dry mass and 19.93 mg catechin equivalent, CE g^{-1} dry mass, respectively (Choi et al., 2015). Total phenolics values of white rose petals of *Rosa alba* and *R. alba* 'Semiplena' were found between 1888-2094 mg GAE 100 g^{-1}. Total flavonoid contents of *Rosa alba* and *R. alba* semiplena were 1401.0-1899.3 mg quercetin kg^{-1}. It was observed that the total phenolic substance contents agreed between similar values, but the difference between the total flavonoid contents is thought to be due to the fact that the same equivalent standard material was not used. In a similar study, they were collected early in the morning (from May to early July 2017, depending on the flowering time of each accession) from nine cities of Western and Eastern Azerbaijan. The percentage and composition of essential oil, total phenols, flavonoids, anthocyanins, anthocyanins, carotenoids and antioxidant capacity of 24 Damask rose accessions were investigated. The highest total phenolic content was 165 mg GAE g DW^{-1} and flavonoid content was 81 mg quercetin g DW^{-1}). The antioxidant activities of the samples were determined using DPPH free radical scavenging activity and ferric reducing antioxidant power (FRAP) test. All the accessions (4-12 µg ml^{-1}) had lower IC_{50} values than ascorbic acid (18 µg ml^{-1}). In the FRAP assay, they had high antioxidant activity in the range of 10-25 µmol $Fe^{+2/g}$ DW. Based on the high and valuable bioactive compound source of Damask rose, elite accessions have potential to be used in cultivation and food applications (Alizadeh and Fattahi, 2021).

It was observed that rose flowers have the potential to be used in many fields as a natural antioxidant source. Table 3 shows the flavonoid values determined in the rose flowers of oil roses, flavonoids were not detected in the studies conducted in rose water. The highest flavonoid content was obtained in *R. damascena* genotype 3062.05 mg quercetin kg^{-1} in the first harvest period, while *R. centifolia* 2805.45, *R. alba* 'Semiplena' 1899.30 and the lowest *R. alba* 1401 mg kg^{-1}. The second period flavonoid contents were highest in *R. centifolia* genotype, there was no statistical difference between the total flavonoid contents of other oil rose genotypes.

Khurshid et al. (2018) examined the total flavonoid content of nine different rose petals. They

Table 3. Total flavonoid content (mg quercetin equivalent kg^{-1}) of flowers of rose genotypes.

Genotypes	Rose flower (First period)	Rose flower (Second period)
Rosa damascena	3062.05±36.27 a	1247.50±35.23 b
Rosa centifolia	2805.45±9.54 b	1851.00±23.58 a
Rosa alba	1401.00±63.07 d	1413.13±88.07 b
Rosa alba 'Semiplena'	1899.30±18.36 c	1409.10±10.89 b
CV	3.01	4.47

Each value in the table was obtained by calculating the average of three-analysis ± standard deviation.

reported that the total flavonoid content varied between 3.91-8.04 mg g^{-1} quercetin equivalents. The highest total flavonoid content was determined in *R. moschata* genotype, while the lowest was determined in *R. hybrida* (pink yellow) genotype. In another study, the flavonoid content of methanolic rose extracts was found to vary between 3.6-23.7 mg g^{-1} (Li et al., 2014). It was observed that the total flavonoid content of rose genotypes in the literature was compatible with the results of our study.

In our study, the total phenolic matter and antioxidant activity (DPPH and CUPRAC) values of rose water and wastewater, which were obtained as by-products of essential oil extraction from oil roses by hydrodistillation method, in two harvest periods are given in Table 4. In rose water, the highest total phenolic matter in *R. damascena* genotype was 4.92 mg GAE L^{-1} in the 1st period, 4.82 mg GAE L^{-1} in the second period, DPPH antioxidant activity was 3.18 mg TE L^{-1} in the first period, 0.71 mg TE L^{-1} in the second period, CUPRAC antioxidant activity was 24.56 mg TE L^{-1} in the first period, 4.20 mg TE L^{-1} in the second period. Total phenolic matter, DPPH antioxidant activity and CUPRAC antioxidant activity values were found to be very low in the first and second period rose waters of other oil rose genotypes and no statistical difference was found. By-products or residues of agricultural industries have attracted more attention in recent years as they are valuable sources of natural antioxidants. Taif rose water by-product obtained after hydrodistillation of Taif rose (*Rosa damascena* trigintipetala Dieck) was investigated for its biological and phytochemical properties. According to these results, it was found to have SC50= 23.72±0.36 µg ml^{-1} against DPPH radical. Direct infusion ESI (-and)-MS analysis of Taif rose water by-product showed the presence of the following substances: phenolic compounds belonging to hydrolyzable tannins and flavonoids. Acute, sub-chronic and chronic toxicity studies of taif rose water by-product on mice have shown that it is safe and non-toxic (Abdel-HameEd et al., 2015). From this study, it can be concluded that rose water has antioxidant activity and has the potential to be used safely due to its non-toxicity. In another study, the activities of cream formulations obtained by using different concentrations of rose water were examined. In this study, it was stated that rose water was helpful in reducing inflammation due to its polyphenolic substance content. It was determined that increasing the concentration of rose water increases antioxidant and anti-inflammatory activities (Safia et al., 2019).

In rose wastewater, the highest total phenolic content was 112.36 mg GAE 100 g^{-1} in *R. alba* genotype and the lowest was 16.91 mg GAE 100 g^{-1} in *R. centifolia*. The highest DPPH antioxidant activity value was determined in *R. alba* (87.73 mg TE 100 g^{-1}) and *R. alba* 'Semiplena' (89.41 mg TE 100 g^{-1}), *R. damascena* (85.76 mg TE 100 g^{-1}).

In CUPRAC antioxidant activity, the highest value of *R. damascena* was 726.69 mg TE 100 g^{-1}. *R. alba* 'Semiplena' 686.66 mg TE 100 g^{-1}, *R. alba* 364.31 mg TE 100 g^{-1}, while the lowest values were found in *R. centifolia* 184.26 mg TE 100 g^{-1}. In rose wastewater, *R. centifolia* showed the highest total phenolic matter (178.58 mg GAE 100 g^{-1}), DPPH antioxidant activity (58.02 mg TE 100 g^{-1}) and CUPRAC antioxidant activity (1933 mg TE 100 g^{-1})

Table 4. Total phenolic and antioxidant content of the rose waters and wastewater of rose genotypes.

Rose water and wastewater	Genotypes	Total phenolics (mg GAE L^{-1})	DPPH* Antioxidant activity (mg TE L^{-1})	CUPRAC Antioxidant activity (mg TE L^{-1})
Rose water (First period)	*R. damascena*	4.92±0.60 a	3.18±0.78 a	24.56±3.12 a
	R. centifolia	0.09±0.04 b	1.11±0.00 b	2.07±0.98 b
	R. alba	0.00±0.00 b	0±0.00 b	0.01±0.00 b
	R. alba 'Semiplena'	0.00±0.00 b	1.11±0.00 b	0.00±0.01 b
	CV	2.30	4.80	2.40
Rose water (Second period)	*R. damascena*	4.82±0.49 a	0.71±0.53 a	4.20±0.25 a
	R. centifolia	0.13±0.00 b	0.00±0.00	1.50±0.76 b
	R. alba	0.086±0.00 b	0.00±0.00	1.01±0.43 b
	R. alba 'Semiplena'	0.00±0.05 b	0.00±0.00	0.84±1.31
	CV	9.04	10.04	1.75
Rose wastewater (First period)	*R. damascena*	86.71±0.92 b	85.76±6.80 a	726.69±0.00 a
	R. centifolia	16.91±0.04 c	3.71±0.62 b	184.26±4.06 d
	R. alba	112.36±11.82 a	87.73±10.15 a	364.31±22.36 c
	R. alba 'Semiplena'	68.45±15.97 b	89.41±1.24 a	686.66±13.20 b
	CV	9.97	9.21	3.17
Rose wastewater (Second period)	*R. damascena*	87.80±0.06 b	0±0.00	1542±33.49 b
	R. centifolia	178.58±0.13 a	58.02±2.76	1933±4.92 a
	R. alba	68.76±1.48 d	0±0.00	366±7.69 d
	R. alba 'Semiplena'	81.15±1.48 c	0±0.00	1265±14.19 c
	CV	0.91	9.80	1.50

* DPPH: 2.2-diphenyl-1- picrylhydrazil CUPRAC: Copper Reducing Antioxidant Capacity.
Each value in the table was obtained by calculating the average of three analysis ± standard deviation.

values in the second harvest period in contrast to the first harvest period. In the second harvest period, the total phenolic matter contents of the wastewater were 87.80 mg GAE 100 g^{-1} in *R. damascena,* 178.58 mg GAE 100 g^{-1} in *R.centifolia,* 81.15 mg GAE 100 g^{-1} in *R. alba* 'Semiplena', and 68.76 mg GAE 100 g^{-1} in *R. alba*, respectively. DPPH antioxidant activity values of second period wastewater were not detected in genotypes other than *R*. *centifolia.* CUPRAC antioxidant activity values were determined as *R. centifolia* 1933 mg TE 100 g^{-1}, *R. damascena* 1542 mg TE 100 g^{-1}, *R. alba* 'Semiplena' 1265 mg TE 100 g^{-1} and *R. alba* 366 mg TE 100 g^{-1}, respectively.

4. Conclusion

In our study, total phenolic matter, total antioxidant activity (DPPH and CUPRAC) and total flavonoid contents of whole flowers, rose water and wastewater of *R. damascena*, *R. centifolia*, *R. alba*, *R. alba* 'Semiplena' oil rose genotypes at two different harvest periods were determined in the climatic conditions in Yalova. There were differences in phenolic content and antioxidant activity content of oil rose genotypes according to harvest period. *R. damascena* showed higher total phenolic matter and antioxidant activity in the first harvest period, while *R. centifolia* showed an increase in total phenolic matter and antioxidant activity in the second harvest period.

In our study, total phenolic content and antioxidant activity values of dried flowers of rose genotypes were found to be higher than rose water and wastewater. While it was determined that rose flower has the potential to be used as an important antioxidant source, total phenolic matter and antioxidant activity values of rose water and wastewater were found to be low. Despite the low total phenolic matter and antioxidant activity values, the fact that rose water and wastewater can be supplied at lower costs compared to rose flower. Lower cost can still make them an important resource for the cosmetic industry.Total phenolic matter and antioxidant activity values of rose water were very low in both periods. Only in *R. damascena* genotype, a small amount of total phenolic matter and antioxidant activity values were detected. In wasterwater, harvest period had a significant effect on phenolic content. Phenolic contents of the wastewater were found to be the highest in the second period of *R. centifolia,* while the first period phenolic contents of *R. alba* genotype were found to be high. Rose wastewater has much higher phenolic content and antioxidant activity than rose water. The rose genotype with the highest phenolic content as an alternative to *R. damascena* was determined as *R. centifolia* genotype. Similar to this study, it is important to determine the contents of by-products such as rose water and wastewater, along with antioxidants in rose, with future studies. In this way, by-products of rose industry will be evaluated more and resource waste will be reduced.

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