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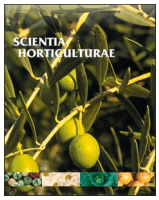

The Growth, Leaf Antioxidant Enzymes and Amino Acid Content of Tomato as Affected by Grafting on Wild Tomato Rootstocks 1 (*S. Pimpinellifolium* and *S. Habrochaites*) Under Salt Stress

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A R T I C L E I N F O

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A B S T R A C T

Salinity in soil or water is a serious threat to plant growth, which reduces yields and threatens food security. It is possible to reduce the negative effects of salinity on tomato scions through grafting. To evaluate the effects of salt stress on graft combinations in hydroponic conditions, SP3, SP4, SP5 (*S. pimpinellifolium*), SH1, SH3, SH5 (*S. habrochaites*) and two hybrids *L* × SP5 (*S. lycopersicum* L. × *S. pimpinellifolium*) and *L* × SH3 (*S. lycopersicum* L. × *S. habrochaites*) were used as rootstock and two varieties Galaxy and H2274 (*S. lycopersicum* L.) were used as scion. Plants were evaluated in terms of biomass parameters and antioxidant enzyme activities and amino acid contents in leaf tissues under control and salt stress conditions. Salt stress decreased the fresh weight of shoots and roots by 68.71 and 23.59, respectively, and increased H_2O_2 content (93.90 %) and MDA content (63.71 %). In addition, salt stress in leaf tissues POD (204.49 %), CAT (83.87 %), SOD (204.49 %), GR (88.23 %), GST (100.16 %), G6PD (32.85 %), 6GPD (46.19 %), APX (157.29 %) caused an increase in enzymes and total amino acid content (160.57 %). In salt stress conditions, SH5/Galaxy and *L* × SP5/Galaxy are the graft combinations with the highest fresh shoot and root weight, leaf number and root volume. These graft combinations contain the most isoleucine, proline lysine, alanine arginine, sucrosine leucine, methionine, hydroxyproline glutamate, glutamine and histidine amino acids in leaf tissues and have the lowest MDA content. Ungrafted H2274 and Galaxy plants have the highest MDA and content under salt stress conditions, these ungrafted plants have the least other amino acids and enzymes other than theonine amino acid in their leaf tissues. Furthermore, ungrafted plants under saline conditions had the lowest shoot and root fresh weight, leaf number and root volume. MDA content correlated negatively with shoot and root fresh weight, leaf number and root volume. H_2O_2 correlated positively with CAT, SOD, GST and peroxidase enzymes and negatively with APX, GR and G6PD enzymes. It seems that the antioxidant enzyme activity and amino acid content in leaf tissues influence salinity response and the appropriate rootstock/scion combination could be a viable method for tomato cultivation in saline conditions.

1. Introduction

In the world, salinity is a major abiotic threat to food security (Julkowska and Testerink, 2015). Salinity can reduce crop yields, the quality of food and the availability of fresh water for crop irrigation (Souri and Ahmadi, 2018). In addition, salinity can increase the risk of water-borne diseases (Colla et al., 2010; Julkowska and Testerink, 2015; Mahajan and Tuteja, 2005; Rao et al., 2013). Low rainfall reduces the amount of fresh water available to leach salts out of the soil and the high evaporation rate increases the concentration of salts in the soil. Intensive cultivation practices that relies on irrigation and heavy chemical fertilization frequently results in salinity building of the soil (Souri and Tohidloo, 2019; Souri and Hatamian, 2019). Salt stress affects plant development primarily because of reduced osmotic potential in the root environment and excessive Na^+ and Cl^- ions accumulation. As well as salt stress can contribute to the overproduction of reactive oxygen species (ROS), disrupting regular cell metabolism and causing damage to cells (Elkahoui et al., 2005; Gadelha et al., 2017; Mittler, 2017; Raja et al., 2017). As a consequence of salt stress, seed germination rate is reduced, photosynthesis is inhibited, ionic toxicity is present, water absorption is limited, chlorosis occurs and senescence occurs, resulting in oxidative stress, reduced growth and yield losses (Gadelha et al.,

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2017; Göçer et al., 2021; Jain et al., 1990; Singh et al., 2012; Tavakoli et al., 2019; Zhu et al., 2008). In many vegetable production areas of the world, salinity is becoming a major abiotic stress that reduces plant growth and crop productivity. Several strategies have been tested to reduce the negative impact of salt on crop yield. Among them are chemical amendments, drainage techniques to drain salt away from plant roots, exogenous application of plant growth regulators and grafting high-yielding cultivars onto salt-tolerant rootstocks (Balliu et al., 2015; Goreta et al., 2008; Jain et al., 1990; Mills and Tal, 2004; Rasel et al., 2021).

The benefits of grafting include reducing the use of chemicals (fumigants, pesticides and fertilisers), effectively controlling soil borne diseases, managing abiotic stresses such as high salinity, flooding, extreme soil temperatures, extending the growing season and increasing yield and quality (Davis et al., 2008; Keatinge et al., 2014; Rivero et al., 2003; Rouphael et al., 2010; H. Singh et al., 2020). In addition to controlling soil-borne diseases, grafting can increase tolerance to abiotic stresses and productivity while maintaining high fruit quality. But the degree of tolerance varies with the type of rootstock used. The benefits of grafting can only be obtained when the right scion and rootstock combination is used. Incompatible combinations can cause problems, such as delayed growth or poor fruit quality. For this reason, it is important to choose the right rootstock for the desired results (Rouphael et al., 2010; Yarşi and Sari, 2006; Yetişir et al., n.d.; Yetisir and Sari, 2003).

The tolerance of varieties to the salinity of up to 2.3–2.5 dS m^{-1} varies in cultivated tomatoes. Adaptive strategies are employed by grafted tomato plants to overcome the detrimental effects of salinity, including salt exclusion or retention, osmotic adjustment, activation of antioxidant defense system and nutrient homeostasis (Abdeldym et al., 2020; Singh et al., 2020). As a result of salt stress, a chain of events occurs resulting in reduced stomatal conductance, decreased photosynthetic electron transport and increased production of harmful reactive oxygen species (ROS). As evidenced by the significant increase in antioxidant activity in salt-treated (grafted) tomatoes, salt generally does not induce a significant increase in ROS production in grafted plants. This is likely due to the fact that the salt-treatment activates the defense mechanisms of the grafted plants, so they are better able to cope with the oxidative stress caused by the salt (He et al., 2009; Singh et al., 2020). Higher expression of CAT, SOD have been observed in salt stressed grafted cumcumber plants (Aydın and Yetişir, 2022; Gao et al., 2008). Under $Ca(NO_3)_2$ stress, grafted eggplant seedlings produced lower O_2, H_2O_2 and MDA levels than non-grafted seedlings. This indicated that the grafting process had a positive effect on the plants antioxidant activities. Plants accumulate amino acids in response to lowered water potential caused by salt This increases their tolerance of salt stress and helps them to survive in saline conditions. Salt stress in plants results in an increase in Aydın and Yetişir (2022) proline (cucumber), Akladious and Abbas (2013) aspartate (tomato), Sadak et al. (2015) glutamate (broad bean), Wang et al. (2013) lysine (maize) and Neto et al. (2009) methionine (maize).

Salt tolerance can be enhanced by selecting salt-tolerant rootstocks. Grafting requires testing and screening of available commercial and wild relatives under salt stress conditions. Grafting can effectively utilize natural genetic variation with specific root characters (Albacete et al., 2015). Various wild tomato/Solanum species including *S. cheesmaniae, S. chmielewskii, S. habrochaites, S. pennellii, S. pimpinellifolium* and *S. peruvianum* demonstrate salt tolerance (Rao et al., 2013; Xu et al., 2015). These species can accumulate higher levels of Na and Cl in their leaves. This allows the species to survive in salt-rich areas, where other plants would struggle to survive. The private and public sectors are focused on breeding rootstocks that are resistant to biotic and abiotic stresses (Colla et al., 2010; King et al., 2010; Xu et al., 2015). In the present study, the purpose was to investigate whether grafting with different wild tomato rootstocks could improve the salt tolerance of tomato scions and to establish the physiological and biochemical responses induced by the rootstocks. The research used a hydroponic system to simulate the effects of salt stress and aimed to understand the effects of rootstock on the amino acid content and antioxidant enzyme capacity performance of the tomato scions.

2. Material and method

Thisexperiment was conducted at the Agricultural Research and Application Greenhouse at Kırşehir Ahi Evran University in 2022–2023 during the growing season. Six wild tomatoes SP3 [LA1269], SP4 [LA2914], SP5 [LA1279] *(S. pimpinellifolium)*, SH1 [LA1764], SH3 [LA1378], SH5 [LA2650] (*S. habrochaites*) and two hybrid *L* × SP5 [*L* × LA1279] (*S. lycopersicum* L. × *S. pimpinellifolium*) and *L* × SH3 [*L* × LA1378] (*S. lycopersicum* L. × *S. habrochaites*) were used as rootstock and two cultivars Galaxy and H2274 (*S. lycopersicum* L.) as scions. Plant seeds were sown in multipots filled with a 2:1 mixture of peat (pH: 6.0–6.5) and perlite and suitable seedlings were selected for grafting using the "tube grafting" described by Lee and Oda (2010). Ungrafted tomato plants were used as control plants. Water was used to clean the seedlings from the growth substrate before transplanting them into 136 L plastic pots. Pumps continuously aerated the cultivation solution. Modified nutrient solution was used according to Hoagland and Arnon, 1950. The nutrient solution contained 1125 μM $Ca(N0_3)_2$, 375 μM $(NH_4)_2SO_4$ 750 μM K_2SO_4, 650 μM $MgSO_4$, 500 μM KH_2PO_4, 10 μM H_3BO_3, 0.5 μM $MnSO_4$, 0.5 μM $ZnSO_4$, 0.4 μM $CuSO_4$, 0.4 μM $MoNa_2O_4$ and 80 μM Fe EDDHA. During the experiment, two different EC levels were used (1.5 dS m^{-1} and 8 dS m^{-1}). The experiment was designed according to the randomized plot design with 3 replications and 3 plants in each replication (136 L pots of 1 m height are arranged in rows and the plants were planted 50×50 between rows and above). The study was continued for 30 days under controlled greenhouse conditions (22–24 °C day /16–18 °C night and 60 % relative humidity).

2.1. Plant growth measurements

After four weeks of growing, plants were harvested and separated into shoots and roots. Main stem diameter (mm) was measured using a caliper. Shoot and root fresh weight (g) was determined with a digital scale with an accuracy of 0.001. The root volume (cm^3) and diameter (mm), of the plants was determined by using the special software program WinRHIZO (Win/Mac RHIZO Pro-V. 2002c Regent Instruments Inc. Canada).

2.2. Lipid peroxidation analysis

Lipid peroxidation level was determined as the content of malondialdehyde (MDA) using the thiobarbituric acid reaction as described by (Madhava Rao and Sresty, 2000).

2.3. Antioxidant enzyme activity analysis

In brief, for peroxidase (POD), superoxide dismutase (SOD), glutathione peroxidases (GPX), glucose-6-phosphate dehydrogenase (G6PD), ascorbate peroxidase (APX) glutathione S-transferase (GST), glutathione reductase (GR) and catalase (CAT) activities the tomato leaves were homogenized into 5 mL of 100-mM phosphate buffer (pH 7.0) containing 1 % (*w/v*) of polyvinylpyrrolidone polymers (PVPP) and all processes proceeded at 4 °C (Breksa et al., 2010). The homogenate for the tomato leaf samples was centrifuged at 15,000 × g for 15 min and the supernatant fraction of the samples was directly examined for enzyme activities. CAT activity was analyzed based at the rate of hydrogen peroxide decomposition according to the method described by Abedi and Pakniyat (2010). The CAT activity in the samples was determined by a decrease in the reaction mixture absorbance at 240 nm that was caused by adding H_2O_2. The reaction mixture contained 50-mM phosphate buffer (pH 7.0), 100-μL extract and 10-mM H_2O_2. This reaction was run

at 25 °C for 2 min after the enzyme extract was added to the samples and the rate of decrease in the absorbance at 240 nm (E = 39.4 mM^{-1} cm^{-1}) was utilized to calculate the enzyme activity of the samples. The POD activity was measured to base its capability to turn guaiacol into tetra-guaiacol at 436 nm, according to the method described by Angelini et al. (1990). The SOD activity was defined based on the determination of the inhibition in the photochemical diminution of nitro-blue tetrazolium at 560 nm, according to the methodology exposed by Abedi and Pakniyat (2010). The total SOD activity was detected by monitoring the prevention of the depletion of p-nitro-blue tetrazolium chloride (NBT). Two hundred microliters of the reaction mixture (50-mM phosphate buffer (pH 7.8), 50-μM riboflavin, 0.1-mM ethylenediaminetetraacetic acid (EDTA), 63-μM NBT, 13-mM methionine and 50 μL of plant extract) were placed in the wells of a 96-well microplate under a 40 W fluorescent lamp. After 10 min of lightening, the absorbance was read at 560 nm. A nonilluminated reaction mixture, which is conducted in the same manner, was used as the blank. One unit of SOD was determined as the amount of the enzyme that produced a 50 % inhibition of the NBT reduction. Tomato leaf samples for glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44) and glutathione reductase (GR; EC 1.8.1.7) and glutathione S-transferase (GST; EC 2.5.1.18) were washed three times with 50-mM Tris–HCl + 0.1-M Na_2SO_4 (pH 8.0). Each sample was homogenized by liquid nitrogen, then transferred to 100-mM PVP + 10-mM NaN_3 + 50-mM Tris–HCl + 0.1-M Na_2SO_4 (pH 8.0) buffer and centrifuged at 4 °C, 15,000 × g for 60 min. G6PD and 6PGD activities were determined according to the methodology described by Minucci et al. (2009). The activities of glutathione reductase (GR; EC 1.8.1.7) and glutathione S-transferase (GST; EC 2.5.1.18) were assayed by the method of Chikezie et al. (2009) and Minucci et al. (2009) respectively. All reactions were initiated by the addition of the enzyme solution. All enzymatic activities were determined spectrophotometrically at 25 °C using a spectrophotometer Shimadzu 1208 UV (Shimadzu Corporation, Tokyo, Japan).

2.4. Amino acid analysis

After harvesting of plants, amino acid analyses were performed on leaf samples taken from plants as described by Aristoy and Toldrá (1991), Antoine et al. (1999) and Henderson et al., (1999).

2.5. Statistical analysis

Levels of significance are represented by $p< 0.05$, $p< 0.01$, $p< 0.001$, $p< 0.0001$ and ns means not significant. Differences between means were analyzed using the Duncan Multiple Test ($p< 0.05$). Classification of genotypes was achieved by principal component analysis (PCA) using XLSTAT software (XLSTAT, New York, USA). A heat map was drawn on GraphPad Prism 8 (GraphPad Software, Boston, USA).

3. Results and discussion

Shoot and root fresh weight were significantly affected by grafting in both control and saline conditions ($p<0.0001$) (Table 1). Regardless of the graft combinations, salt stress in hydroponic conditions had a negative effect on shoot and root growth, resulting in significantly lower fresh weights for tomato plants shoots (68.71 %) and roots (23.59 %) than under control conditions. The effects of salt stress on shoot growth were more pronounced than those on root growth (Fig. 1). This is because salt stress affects the uptake of water and nutrients by the roots, which can inhibit shoot growth more than root growth (Oliveira et al., 2013). Under salt stress, the highest shoot fresh weight was determined in SH1/Galaxy, SP5/Galaxy and SP4/Galaxy graft combinations, while the highest root fresh weight was determined in SP5/Galaxy and *L* × SP5/Galaxy graft combinations. Under salt stress, the lowest shoot fresh weight was determined in SH3/H2274 graft combination. The lowest root fresh weight was measured in ungrafted H2274 plants in saline conditions. It has been reported that different combinations of grafts respond differently to salt stress, similar to our study results (Aydın and Yetişir, 2022; Ulas et al., 2020). Our data suggests that the choice of grafting combination is an important factor to consider when assessing salt stress tolerance. This can help to improve crop yields in saline soils (Di Gioia et al., 2013; Martorana et al., 2007).

In all graft combinations, salt stress decreased the diameter of the main stem. Under salt stress, SH5/Galaxy, *L* × SP5/Galaxy, SP4/Galaxy, SH1/H2274, and ungrafted H2274 plants had the largest main stem diameters, while ungrafted Galaxy plants had the smallest. Under control conditions, there were mean 11.31 leaves per plant, while under salt stress, there were mean 8.41 leaves per plant for all graft combinations. Salt stress reduced the number of leaves per plant by 25.67 % compared to control conditions (Fig. 2). It is likely that the salt caused dehydration, which caused the leaves to dry out and fall off, leading to the reduction in the number of leaves per plant (Del Amor et al., 2001; Mohammad et al., 2008).

Except for *L* × SH3/H2274 graft combination, salt stress reduced root volume in other graft combinations. Among salt-treated plants, SP5/Glaxy graft combination had the highest root volume, while ungrafted H2274 plants had the lowest root volume. Except for *L* × SP5/H2274, SH3/Galaxy, *L* × SP5/Galaxy, *L* × SH3/Galaxy and non-grafted H2274 plants, salt stress increased the diameter of the roots in all other graft combinations (Fig. 3). The results indicate that grafting may be an effective way to improve root characteristics and plant performance (Aydın and Yetişir, 2022; Keatinge et al., 2014; Khankahdani et al., 2012).

Main stem diamater, shoot and root fresh weight, root volume and diameter were significantly affected by grafting in both control and saline conditions ($p<0.0001$). Considering the 95 % confidence interval difference of the measured biomass parameters, number of leaves per plant (2.51 to 3.31), main stem diameter (2.20 to 2.74), shoot fresh weight (78.16 to 83.84), root fresh weight (4.84 to 6.14), root volume (6.18 to 7.80) and root diameter (−0.03 to −0.01). We found that there was a statistically significant difference between the groups because the confidence interval did not contain a zero value for the biomass parameters (Table 1).

The results of GR, POD and SOD were presented in Fig. 4 and antioxidative enzyme activities were significantly affected by grafting under both control and salt stress conditions (Table 2). Under salt stress, SH1/H2274 and SP5/H2274 graft combinations showed higher levels of GR

Table 1
Biomass parameters of plants grown in control and saline conditions, means, decrease, standard error (SE) difference, 95 % confidence interval (CI) difference and P value.

Biomass Parameters	Difference between column means					Source of Variation P value		
	Mean of Control	Mean of NaCl	% Decrease	SE of difference	95 % CI of difference	Grafting	NaCl	Grafting × NaCl
Number of leaves per plant	11.31	8.41	−25.67	0.20	2.51 to 3.31	<0.0001	<0.0001	0.0025
Main stem diameter	7.90	5.43	−31.29	0.14	2.20 to 2.74	<0.0001	<0.0001	<0.0001
Shoot fresh weight	117.90	36.89	−68.71	1.42	78.16 to 83.84	<0.0001	<0.0001	<0.0001
Root fresh weight	23.27	17.78	−23.59	0.33	4.84 to 6.14	<0.0001	<0.0001	<0.0001
Root volume	23.45	16.46	−29.81	0.41	6.18 to 7.80	<0.0001	<0.0001	<0.0001
Root diameter	0.37	0.39	5.59	0.01	−0.03 to −0.01	<0.0001	<0.0001	<0.0001

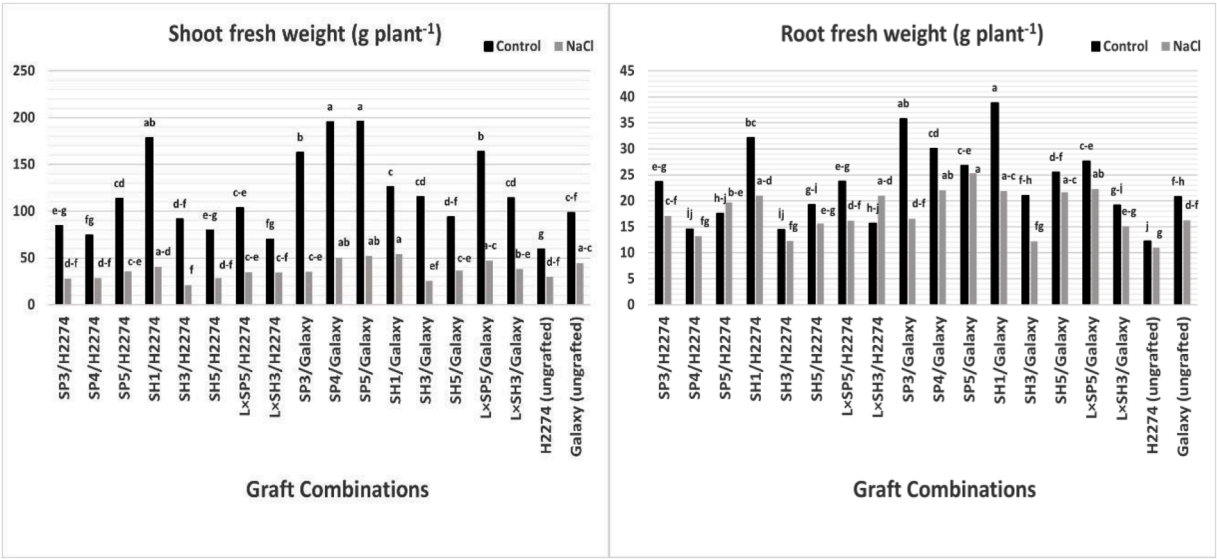

Fig. 1. The effects of graft combinations and different NaCl levels (1.5 dS $m^{-1}$and 8 dS m^{-1}) on shoot and root fresh weight of tomato plants. Means that do not share a letter are significantly different.

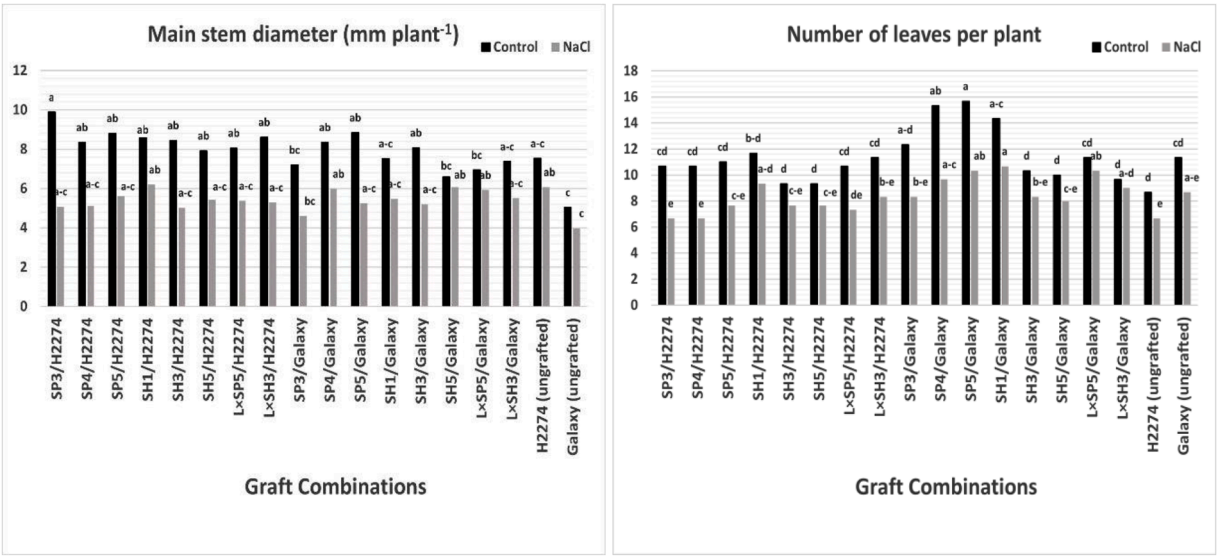

Fig. 2. The effects of graft combinations and different NaCl levels (1.5 dS $m^{-1}$and 8 dS m^{-1}) on main stem diameter and number of leaves per plant of tomato plants. Means that do not share a letter are significantly different.

activity than ungrafted plants. While the peroxidase enzyme activity was determined as 5996.00 EU g^{-1} leaf under control conditions, it was determined as 18,251.00 EU g^{-1} leaf under salt stress conditions. Salt stress increased peroxidase enzyme activity by 204.39 % compared with control conditions. This enzyme is known to play a role in plant defense mechanisms. It is an important enzyme in the plant's response to abiotic stress, such as salt stress(Kaur et al., 2019; Mittler, 2017; Raja et al., 2017). In saline conditions, the highest peroxidase enzyme activity was determined in SP4/Galaxy and SP3/Galaxy graft combinations, while the lowest peroxidase activity was determined in SH1/H227, SP5/H2274 and ungrafted Galaxy and H2274 plants. Compared with control conditions, salt stress increased leaf SOD activity by 88.87 %. In saline conditions, the highest leaf SOD activity was measured in the SP3/Galaxy and SP4/Galaxy graft combinations, while the lowest SOD activity was measured in ungrafted H2274 and Galaxy plants. This is likely due to the fact that the SP3/Galaxy and SP4/Galaxy graft combinations were able to better adapt to saline conditions than the ungrafted plants, resulting in higher SOD activity as a protective mechanism against oxidative stress. This substantial increase indicates the beneficial influence of salt stress on SOD activity in the leaf tissues of the grafted combinations (Aydın and Yetişir, 2022).

The results of H_2O_2, MDA and CAT were presented in Fig. 5 and H_2O_2, MDA and CAT were significantly affected by grafting under both control and salt stress conditions (Table 2). The amount of H_2O_2 in the leaves of plants grown under hydroponic conditions was 14.42 mmol/kg under the control conditions and 27.96 mmol/kg under the saline conditions. Salt stress increased the H_2O_2 content of leaves by 93.90 % compared to the control conditions. This increase in H_2O_2 can lead to oxidative damage to cellular components such as proteins, lipids and DNA (Abogadallah, 2010; Ransy et al., 2020). The highest amount of H_2O_2 in the leaf was determined in SP4/Galaxy, SP3H2274 and ungrafted H2274 tomato plants under salt stress conditions, while the lowest amount was determined in the $L \times$ SH3/Galaxy graft combination. The results suggest that grafting can reduce the negative effects of

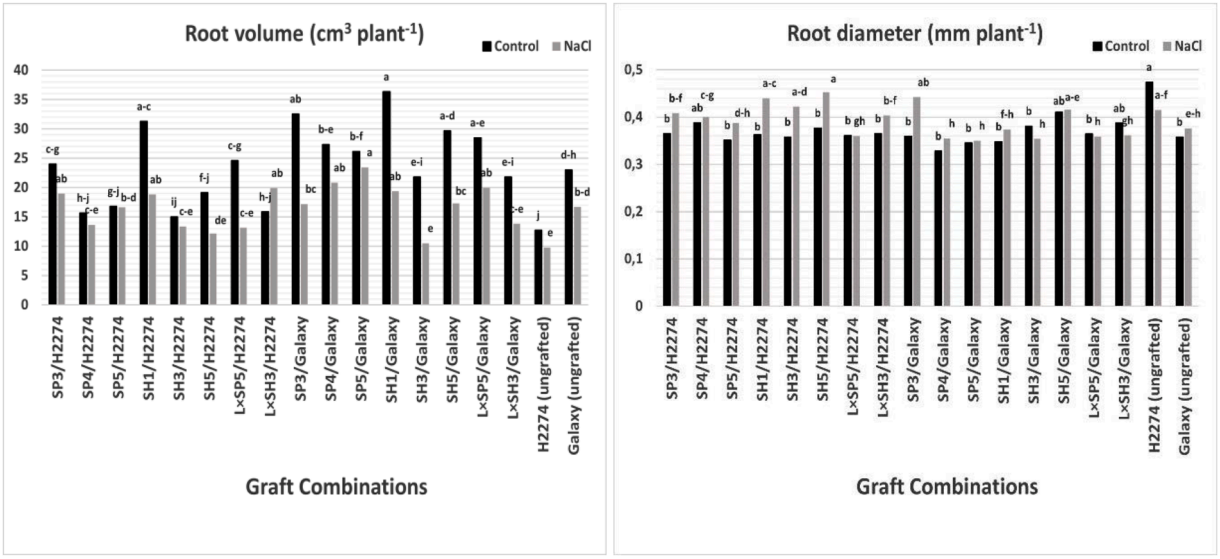

Fig. 3. The effects of graft combinations and different NaCl levels (1.5 dS $m^{-1}$and 8 dS m^{-1}) on root volüme and root diameter of tomato plants. Means that do not share a letter are significantly different.

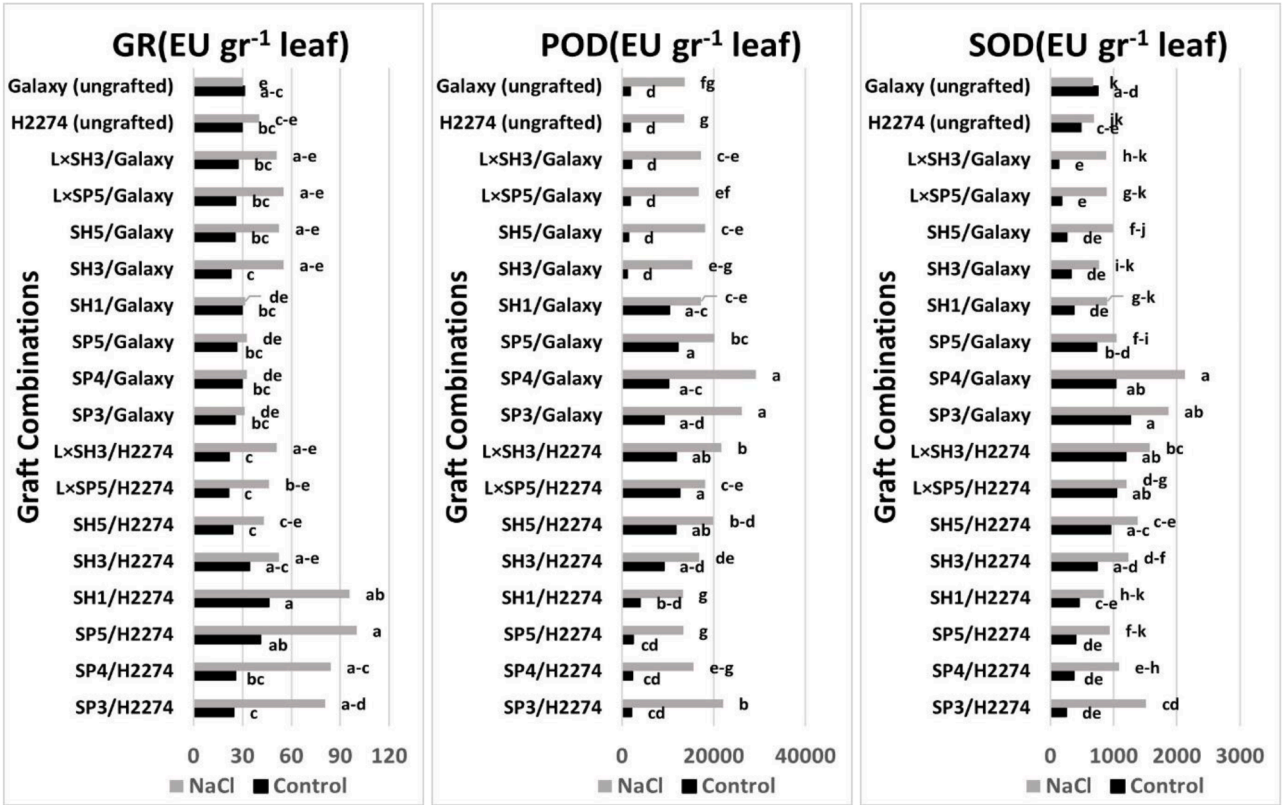

Fig. 4. Glutathione reductase (GR), peroxidase (POD) and superoxide dismutase (SOD) activities in leaf samples of tomatoes grafted grown under control (1.5 dS m-1) and NaCl (8 dS m-1) stress conditions. Means that do not share a letter are significantly different.

salt stress on tomato plants by decreasing the amount of H_2O_2. While salt stress increased the leaf MDA content by 63.71 % the highest leaf MDA content was determined in ungrafted Galaxy plants, while the lowest was measured in the SH3/Galaxy graft combination. These results demonstrate that the SH3/Galaxy graft combination was the most resilient to salt stress. Leaf CAT activity of plants grown under control conditions was 186.60 EU g^{-1} leaf, while CAT activity increased to 343.10 EU g^{-1} leaf in saline conditions. Salt stress increased leaf CAT activity by 83.87 %. This indicates that CAT activity is a mechanism employed by plants to cope with salt stress. Studies have shown that CAT activity contributes to the detoxification of reactive oxygen species and thus helps plants to combat salt stress (Aydın and Yetişir, 2022; Sudhakar et al., 2001).

The results of APX, G6PD, GST AND 6GPD were presented in Fig. 6 and antioxidative enzyme activities were significantly affected by grafting under both control and salt stress conditions (Table 2). Salt

Table 2
H_20_2, MDA and enyzme activities in leaf samples of plants grown in control and saline conditions, means, increase, standard error (SE) difference, 95 % confidence interval (CI) difference and P value.

Metabolites	Difference between column means					Source of Variation P value		
	Mean of Control	Mean of NaCl	Increase (%)	SE of difference	95 % CI of difference	Grafting	NaCl	Grafting × NaCl
H_2O_2	14.42	27.96	93.90	0.34	−14.22 to −12.88	<0.0001	<0.0001	<0.0001
MDA	9.65	15.80	63.71	0.22	−6.59 to −5.71	<0.0001	<0.0001	<0.0001
CAT	186.60	343.10	83.87	5.40	−167.30 to −145.70	<0.0001	<0.0001	<0.0001
POD	5996.00	18,251.00	204.39	389.80	−13,032.00 to −11,477.00	<0.0001	<0.0001	<0.0001
SOD	607.30	1147.00	88.87	27.14	−593.50 to −485.20	<0.0001	<0.0001	<0.0001
GR	28.55	53.74	88.23	2.37	−29.92 to −20.48	<0.0001	<0.0001	<0.0001
GST	369.80	740.20	100.16	26.46	−423.10 to −317.60	<0.0001	<0.0001	<0.0001
G6PD	266.70	354.30	32.85	11.04	−109.60 to −65.57	<0.0001	<0.0001	<0.0001
6GPD	373.90	546.60	46.19	15.03	−202.70 to −142.80	<0.0001	<0.0001	<0.0001
APX	35.87	92.29	157.29	5.55	−67.48 to −45.36	<0.0001	<0.0001	<0.0001

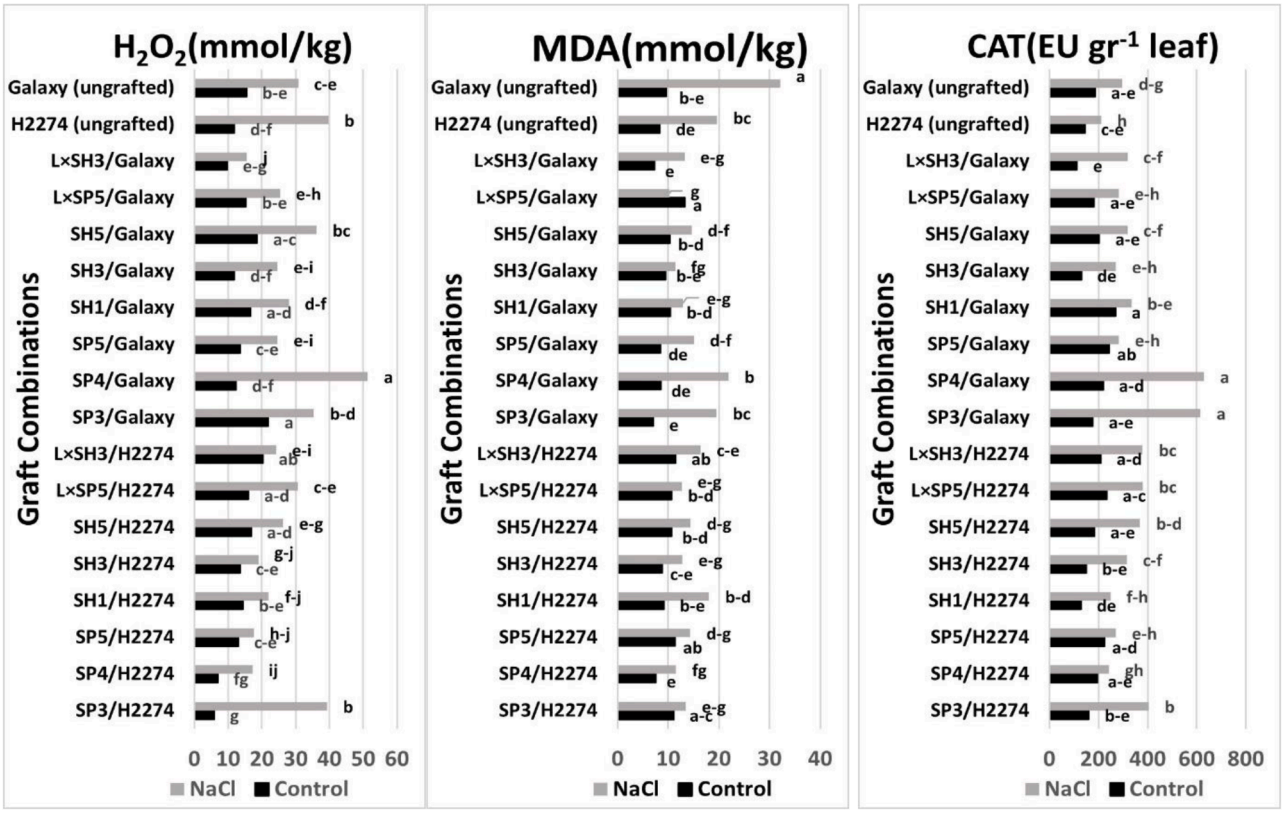

Fig. 5. Hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and catalase (CAT) activities in leaf samples of tomatoes grafted grown under control (1.5 dS m-1) and NaCl (8 dS m-1) stress conditions. Means that do not share a letter are significantly different.

stress application increased the enzyme activities of APX (157.29 %), G6PD (32.85 %), GST (100.16 %) and 6GPD (46.19 %) in leaf tissues compared with control conditions The APX activity of all graft combinations under control conditions was 35.87 EU g^{-1} leaf, while it was 92.29 EU g^{-1} leaf in saline conditions. The results suggest that the APX activity was significantly increased under saline conditions, compared to control conditions. The findings indicate that graft combinations may be an effective and efficient way to enhance antioxidant activity in response to salinity stress (Kuşvuran et al., 2021). The highest APX activity in saline conditions was determined in the graft combinations SP4/H2274, SP5/H2274 and SH1/H2274, while the lowest was determined in the ungrafted Galaxy plants. The higher activity of APX in the graft combinations indicates that the plants were able to withstand the salinity better than the ungrafted ones, suggesting that grafting is an effective way to help plants tolerate salinity (Gong et al., 2014; Singh et al., 2020; Singh and Soltan, 2016; Yan et al., 2018). The highest G6PD enzyme activity was measured in saline-treated SP4/H2274 and SP5/Galaxy graft combinations, while the G6PD enzyme activity was measured in ungrafted H2274 and *L* × SP5/H2274 graft combinations. In saline conditions, the highest GST enzyme activity was measured in the SH3/H2274 graft combination, while the lowest was in the SP3/H2274 graft combination. The difference between the highest and lowest GST activity was statistically significant. The results indicate that the graft combination significantly affects the expression of GST enzyme activity in saline conditions (Hossain and Fujita, 2010; Misra and Gupta, 2006). During control conditions, the 6GPD enzyme activity of all graft combinations was 373.90 EU g^{-1} leaf, whereas it was 546.60 EU g^{-1} leaf under saline conditions. This resulted in an increase of 46.19 % in enzyme activity under saline stress compared to control conditions. The highest increase in 6GPD enzyme activity was observed in graft combinations SH3/Galaxy and SH5/H2274. Further studies are needed to determine the exact mechanisms that make these graft combinations more successful.

H_2O_2, MDA, CAT, Peroxidase, SOD, GR, GST, G6PD, 6GPD and APX

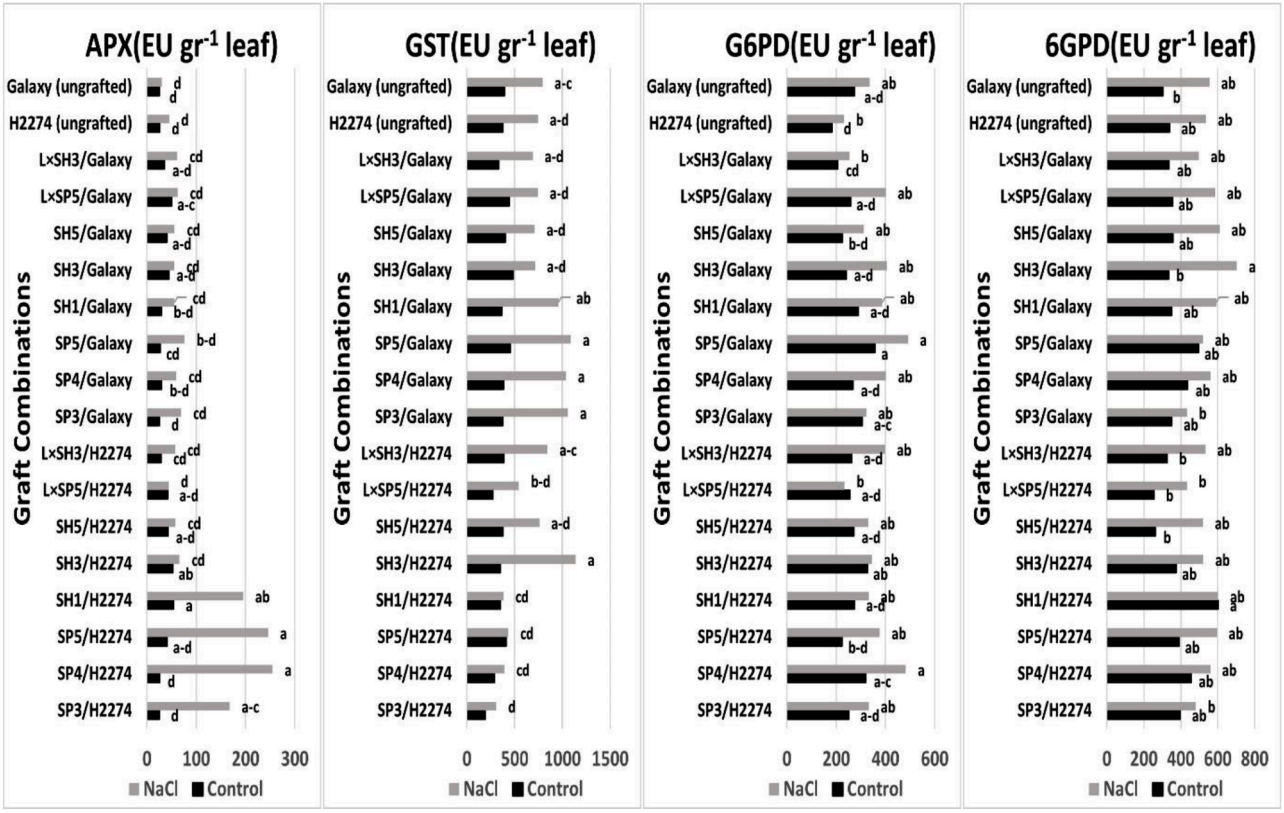

Fig. 6. Ascorbate peroxidase (APX), glutathione S-transferase (GST), glucose 6-phosphate dehydrogenase (G6PD), and 6-glucose phosphate dehydrogenase (6GPD) activities in leaf samples of tomatoes grafted grown under control (1.5 dS m^{-1}) and NaCl (8 dS m^{-1}) stress conditions. Means that do not share a letter are significantly different.

were significantly affected by grafting in both control and saline conditions ($p<0.0001$). Considering the 95 % confidence interval difference of the measured, H_2O_2 (−14.22 to −12.88), MDA (−6.59 to −5.71), CAT (−167.30 to −145.70), POD (−13,032.00 to −11,477.00), SOD (−593.50 to −485.20), GR (−29.92 to −20.48), GST (−423.10 to −317.60), G6PD (−109.60 to −65.57), 6GPD (−202.70 to −142.80) and APX (−67.48 to −45.36). We found that there was a statistically significant difference between the groups because the confidence interval did not contain a zero value for the parameters (Table 2).

A total of 32 metabolites were examinations in graft combinations under non-stress control and salt stress, including H_2O_2, MDA, antioxidant enzyme activities and amino acid contents. The heat map depicts metabolites with increased (fold change > 1 indicated up-regulation shown by yellow bars) content or decreased (fold change < 1 indicated down-regulation shown by blue bars) content in grafted plants in the control and salt stress conditions. Salt application significantly increased H_2O_2 and MDA and amino acid contents and antioxidant enzyme activities (Fig. 7).

The result of analysis of leaf aspartate, asparagine, glutamate and serine amino acid contents under control and saline conditions is shown in Fig. 8. Under control conditions, aspartate (158.20 pmol ml^{-1}), asparagine (81.25 pmol ml^{-1}), glutamate (127.10 pmol ml^{-1}) and serine (130.50 pmol ml^{-1}) were determined in leaf tissue of all graft combinations, whereas under salinity conditions, asparte (325.70 pmol ml^{-1}), asparagine (180.80 pmol ml^{-1}), glutamate (444.20 pmol ml^{-1}) and serine (323.50 pmol ml^{-1}) were determined. In comparison to control conditions, the levels of aspartate (108.88 %), asparagine (121.70 %), glutamate (210.28 %) and serine (147.89 %) increased during salt stress. In saline conditions, the graft combinations had the highest aspartate (SH1/Galaxy), asparagine (SP5/H2274 and SP4/H2274), glutamate and serine (SP3/Galaxy) contents. These findings suggest that salt stress stimulates the biosynthesis of amino acids, which in turn leads to increased tolerance of the plant to salt stress. The increased levels of amino acids under salt stress can be used to identify gene variants and develop crop varieties that are more tolerant to salt stress (Farooq et al., 2023; Patel et al., 2020). In saline and control conditions, the levels of aspartate, asparagine, glutamate and serine amino acids varied for all graft combinations.

The result of analysis of leaf glutamine, glycine, histidine and theonine amino acid contents under control and saline conditions is shown in Fig. 9. Except for the ungrafted H2274 plant, all plants grown in saline conditions had higher glutamine amino acid contents than those grown in control conditions. In control conditions, the glycine content of all graft combinations was determined as 48.92 pmol ml^{-1}, while in saline conditions it was determined as 162.80 pmol ml^{-1}. The glycine content increased by 232.74 %, indicating a significant difference between the two conditions. Graft combinations had no effect on the amino acid content of thenonine in plants subjected to salt stress ($p>0.05$). Plants grown under saline conditions had higher leaf histidine and theonine contents compared with control plants. These amino acids are known to play a role in the plants' ability to respond to stress and the higher concentrations of them indicate that the plants were able to better tolerate the saline conditions (Zanganeh et al., 2019; Zeid, 2009). A photosynthetic pigment in wheat has been found to be salt-stress-tolerant due to Glycine salt-stress-protective properties (Gupta and Thind, 2017).

The analysis results of arginine, cysteine, tyrosineand alanine amino acid contents of leaf tissues of plants grown in control and saline conditions are shown in Fig. 10. There was an increase in the levels of arginine (212.49 %), cysteine (150.14 %), tyrosine(72.18 %) and alanine (328.49 %) amino acids in plants grown in saline conditions compared to controls. Graft combinations had no effect on the amino acid content of arginine in plants subjected to salt stress ($p>0.05$). In saline conditions, the graft combinations had the highest cysteine,

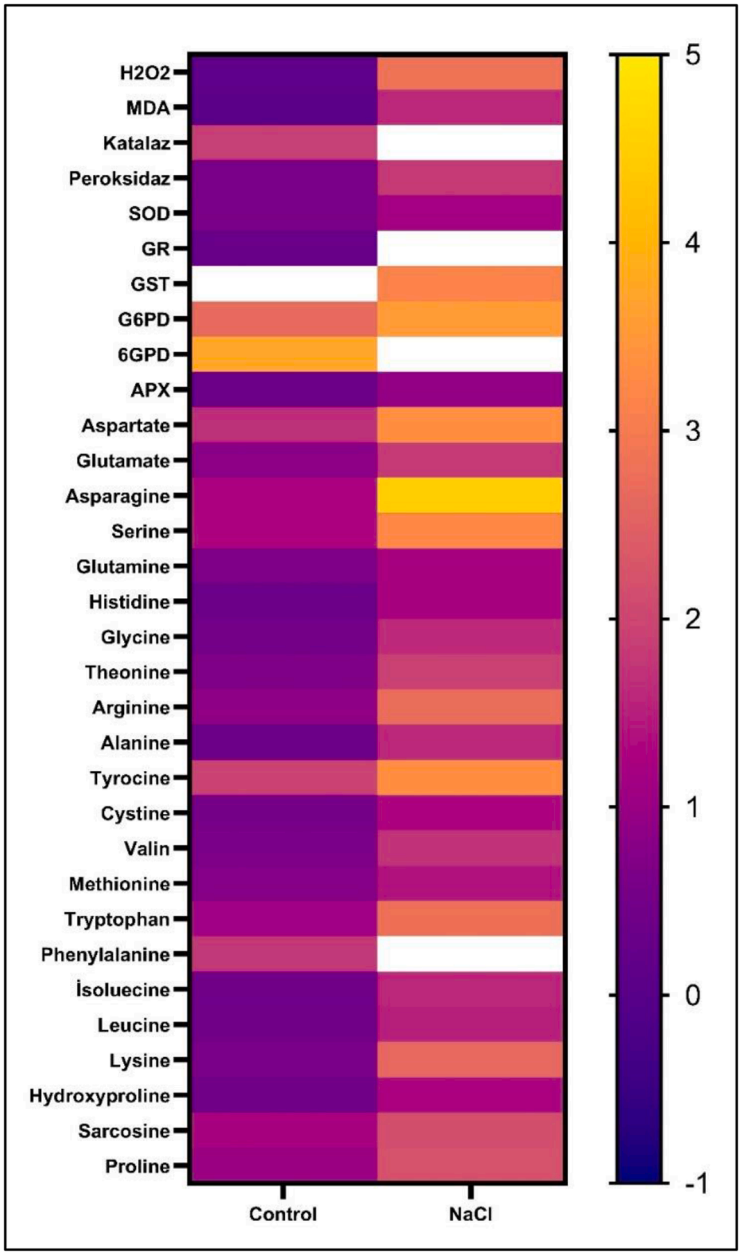

Fig. 7. Heat map showing fold-changes for metabolites responsive to salt stress with increases (up-regulation showing in yellow bars) or decreases (down-regulation showing in blue bars).

tyrosine (SP3/Galaxy) alanine (SH5/Galaxy) contents. Similar to our study results Farooq et al. (2023), reported that the total amino acid content in plants subjected to salt stress was affected by the cultivar and application and that the salt-sensitive cultivars had lower amino acid content.

The analysis results of valine, methionine, phenylalanine and tryptophan amino acid contents of leaf tissues of plants grown in control and saline conditions are shown in Fig. 11. Under control conditions, the valin (179.78 pmol ml^{-1}), methionine (73.23 pmol ml^{-1}), phenylalanine (176.60 pmol ml^{-1}) and tryptophan (113.60 pmol ml^{-1}) were determined in all graft combinations, whereas under saline conditions, the of valine (175.20 pmol ml^{-1}), methionine (139.90 pmol ml^{-1}), phenylalanine (514.80 pmol ml^{-1}) and tryptophan (276.90 pmol ml^{-1}) were determined. The highest valine, tryptophan and phenylalanine SP3/Galaxy and methionine SH5/Galaxy graft combinations were determined in saline conditions. The lowest tryptophan amino acid activity in saline conditions was determined in ungrafted Galaxy plants. These results demonstrate that the protection mechanisms against salt stress were triggered leading to the accumulation of amino acids in plants and providing them with protection against salinity (Molazem et al., 2010; Szabados and Savouré, 2010).

The analysis results of isoleucine, hydroxyproline, leucine and lysine amino acid contents of leaf tissues of plants grown in control and saline conditions are shown in Fig. 12. While the highest isoleucine content was measured in the ungrafted H2274 plant under control conditions, salt stress caused an increase in isoleucine content in other graft combinations, while the isoleucine content of ungrafted H2274 plants did not change. In saline conditions, the highest hydroxyproline was determined in the SH5/Galaxy graft combination, while the lowest was determined in the ungrafted H2274 plants. Based on all graft combinations tested, the leucine content was 44.74 pmol ml^{-1} in the control condition and 151.50 pmol ml^{-1} in saline condition. In saline conditions, the highest lysine content was measured in SH5/Galaxy, $L \times$ SP5/Galaxy and $L \times$ SH3/Galaxy graft combinations, while the lowest was

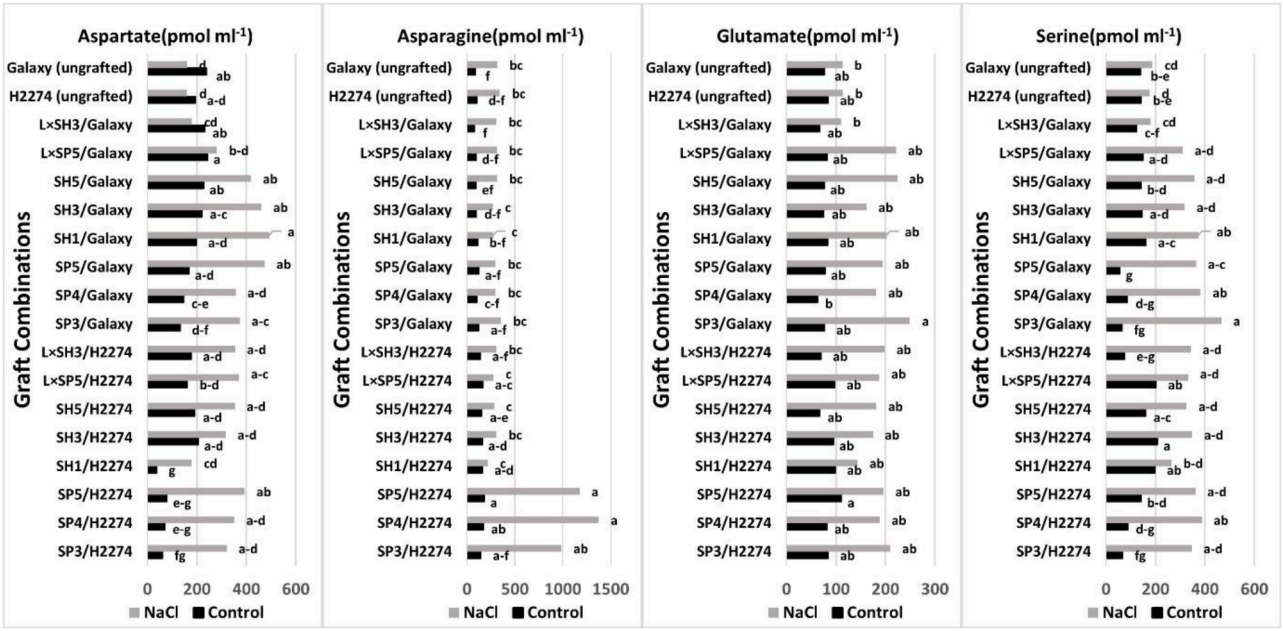

Fig. 8. Aspartate, asparagine, glutamate and serine amino acid contents in leaf samples of tomatoes grafted grown under control (1.5 dS m^{-1}) and NaCl (8 dS m^{-1}) stress conditions. Means that do not share a letter are significantly different.

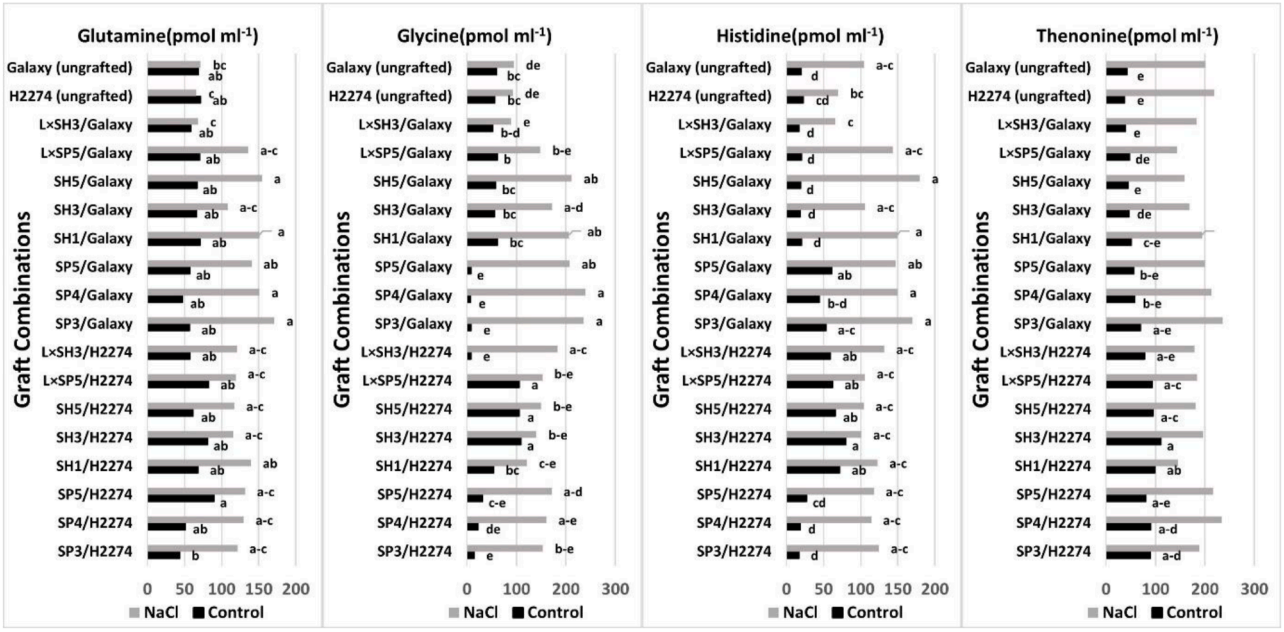

Fig. 9. Glutamine, glycine, histidine and theonine amino acid contents in leaf samples of tomatoes grafted grown under control (1.5 dS m^{-1}) and NaCl (8 dS m^{-1}) stress conditions. Means that do not share a letter are significantly different.

measured in ungrafted H2274 plants. In grafted watermelon plants, the contents of alanine, cysteine, methionine, leucine, tyrosine and phenylalanine were significantly increased, whereas arginine content was notably depressed in self-grafted seedlings under salt stress. In a study by Wang et al. (2013), the amount of lysine produced in maize rose significantly under salinity stress, which aided in salinity tolerance and the plants demonstrated substantial improvement under salt stress. In different wheat cultivars, Saeedipour and Moradi (2012) reported that lysine content increased significantly under salt stress, enhancing tolerance to salt stress and viability.

The analysis results of proline and sarcosine amino acid contents of leaf tissues of plants grown in control and saline conditions are shown in Fig. 13. In saline conditions, the highest proline content was measured in the graft combinations with the highest $L \times$ SP5/Galaxy and SH5/Galaxy content, while the lowest was measured in the ungrafted Galaxy plant. In the control condition, all graft combinations had a proline content of 0.10 pmol ml^{-1}, while in the saline condition, it was 0.22 pmol ml^{-1}. Salt stress increased the proline content by 120.00 % compared to control conditions. According to the results, the sarcosine content of all graft combinations was 121.50 pmol ml^{-1} in control conditions and 213.40 pmol ml^{-1} in saline conditions. There was an increase of 75.64 % in sarcosine content as a result of salt stress. Aydın and Yetişir (2022),observed increased proline content in grafted cucumber plants under salt stress. Ulas et al. (2020), reported that

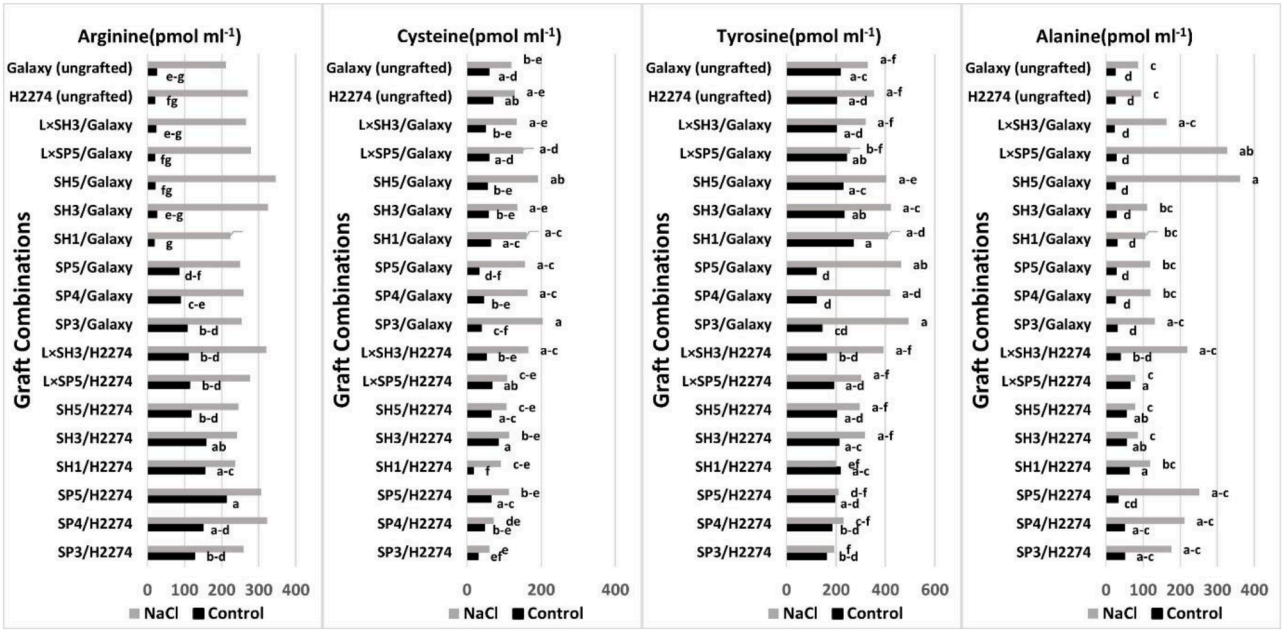

Fig. 10. Arginine, cysteine, tyrosineand alanine amino acid contents in leaf samples of tomatoes grafted grown under control (1.5 dS m^{-1}) and NaCl (8 dS m^{-1}) stress conditions. Means that do not share a letter are significantly different.

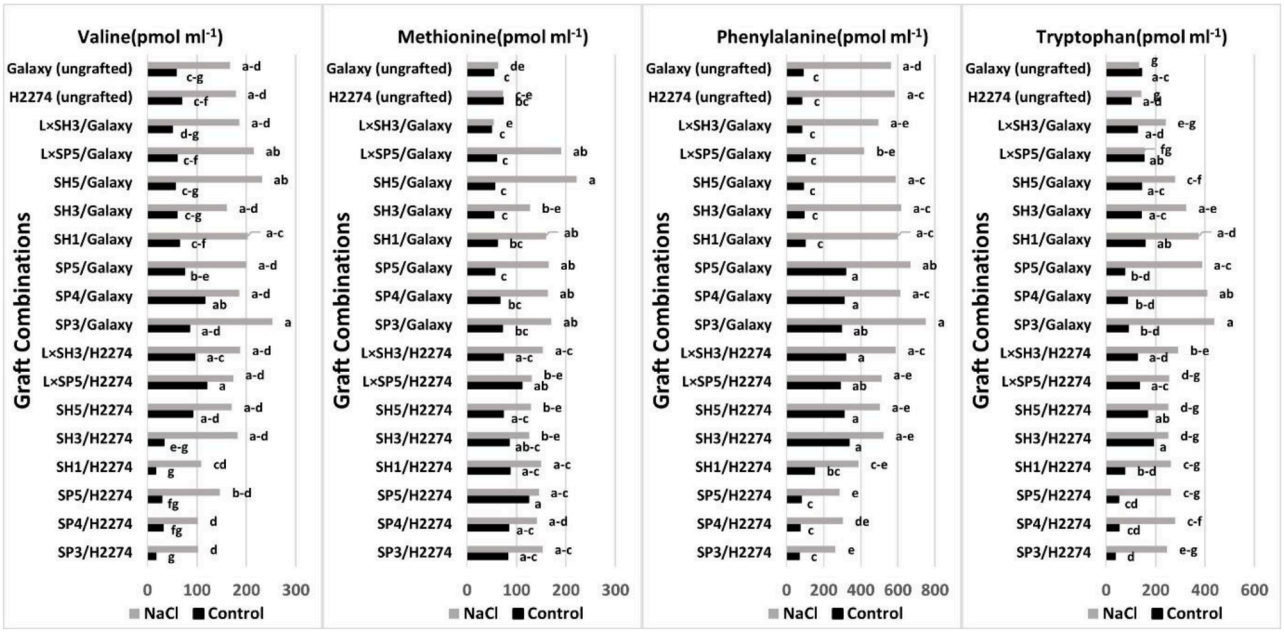

Fig. 11. Valin, methionine, phenylalanine and tryptophan serine amino acid contents in leaf samples of tomatoes grafted grown under control (1.5 dS m^{-1}) and NaCl (8 dS m^{-1}) stress conditions. Means that do not share a letter are significantly different.

regardless of the graft combination, salt stress caused a significant increase in proline content in leaf and root of salt-treated melon plants compared to control. The proline content was higher in the grafted plants and this increased tolerance was attributed to the higher proline content. This suggests that grafting could be used as a method to increase the salt tolerance of tomato plants (Keatinge et al., 2014; Nawaz et al., 2016). According to He et al. (2017), proline induces some salt-stress-responsive genes and improves salt tolerance in *Pancratium maritimum* by upregulating stress-protective proteins and protecting protein turnover machinery from stress damage.

While the total amino acid contents of graft combinations grown under control conditions were 1837.32, it was 4787.42 in saline conditions. While salt stress increased the total amino acid content by 160.57 %, the highest increase was determined for alanine (328.49 %) and lysine (310.27 %) amino acids. Aspartate, glutamate, asparagine, serine, glutamine, histidine, glycine, theonine, arginine, alanine, tyrocine, cysteine, methionine, tryptophan, phenylalanine, isoleucine, leucine, lysine, hydroxyproline and proline were significantly affected by grafting in both control and saline conditions ($p<0.0001$). Considering the 95 % confidence interval difference of the amino acid contents aspartate (−182.20 to −152.80), glutamate (−111.20 to −87.21), asparagine (−380.40 to −255.60), serine (−210.50 to −175.40), glutamine (−65.39 to −50.51), histidine (−91.47 to −76.36), glycine (−121.70 to −106.20), theonine (−135.40 to −110.70), arginine

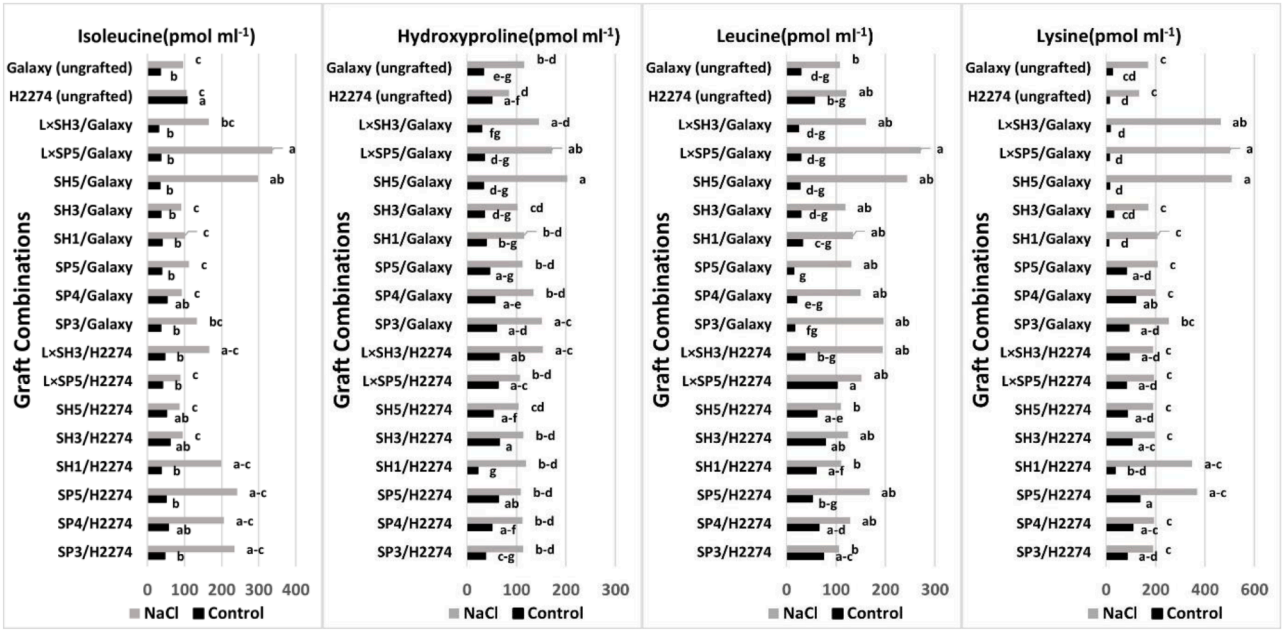

Fig. 12. Isoleucine, hydroxyproline, leucine and lysine amino acid contents in leaf samples of tomatoes grafted grown under control (1.5 dS m^{-1}) and NaCl (8 dS m^{-1}) stress conditions. Means that do not share a letter are significantly different.

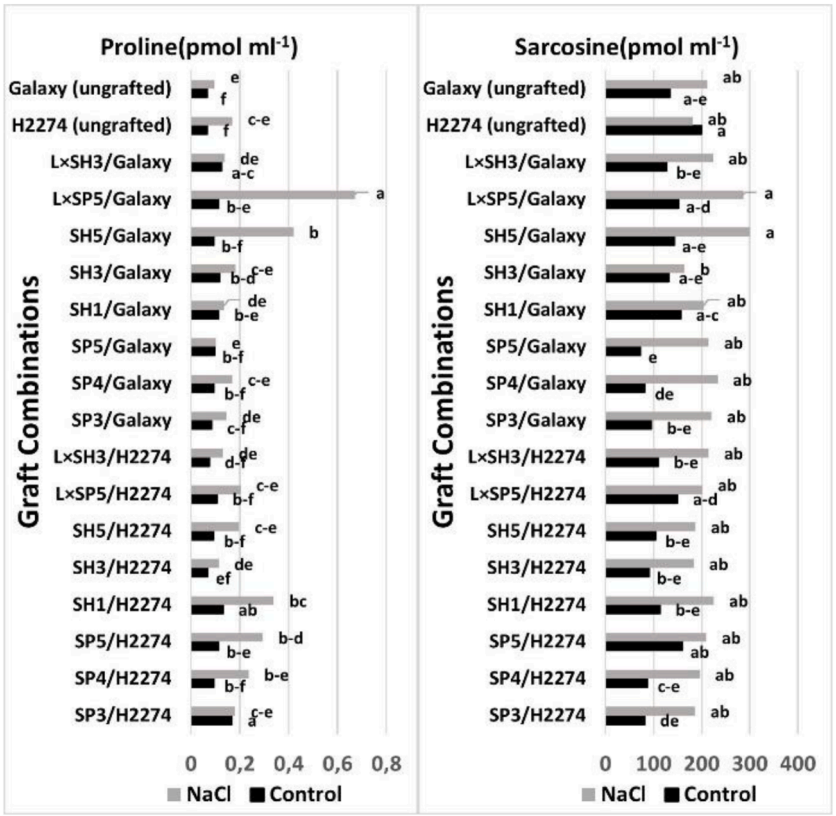

Fig. 13. Proline and sarcosine amino acid contents in leaf samples of tomatoes grafted grown under control (1.5 dS m^{-1}) and NaCl (8 dS m^{-1}) stress conditions. Means that do not share a letter are significantly different.

(−202.20 to −167.30), alanine (−141.30 to −100.40), tyrosine (−160.10 to −120.20), cysteine (−86.95 to −71.72), valin (−122.20 to −103.00), methionine (−75.21 to −58.09), tryptophan (−177.50 to −149.20), phenylalanine (−366.10 to −310.40), isoleucine (−128.20 to −96.11), leucine (−121.40 to −92.13), lysine (−220.00 to −173.90), hydroxyproline (−86.15 to −73.62), sarcosine (−104.40 to −79.42) and proline (−0.14 to −0.10). We found that there was a statistically significant difference between the groups because the confidence interval did not contain a zero value for the amino acid contents (Table 3).

PCA was used for classifying grafting combinations based on plant

Table 3
Amino acid contents in leaf samples of plants grown in control and saline conditions, means, increase, standard error (SE) difference, 95 % confidence interval (CI) difference and P value.

Amino acids	Difference between column means					Source of Variation P value		
	Mean of Control	Mean of NaCl	Increase (%)	SE of difference	95 % CI of difference	Grafting	NaCl	Grafting × NaCl
Aspartate	158.20	325.70	105.88	7.38	−182.20 to −152.80	<0.0001	<0.0001	<0.0001
Glutamate	81.55	180.80	121.70	6.02	−111.20 to −87.21	<0.0001	<0.0001	<0.0001
Asparagine	127.10	445.20	250.28	31.30	−380.40 to −255.60	<0.0001	<0.0001	<0.0001
Serine	130.50	323.50	147.89	8.81	−210.50 to −175.40	<0.0001	<0.0001	<0.0001
Glutamine	64.98	122.90	89.14	3.73	−65.39 to −50.51	<0.0001	<0.0001	<0.0001
Histidine	38.69	122.60	216.88	3.79	−91.47 to −76.36	<0.0001	<0.0001	<0.0001
Glycine	48.92	162.80	232.79	3.88	−121.70 to −106.20	<0.0001	<0.0001	<0.0001
Theonine	68.27	191.30	180.21	6.19	−135.40 to −110.70	<0.3450	<0.0001	<0.0001
Arginine	86.98	271.80	212.49	8.76	−202.20 to −167.30	<0.3420	<0.0001	<0.0001
Alanine	36.78	157.60	328.49	10.25	−141.30 to −100.40	<0.0001	<0.0001	<0.0001
Tyrocine	194.10	334.20	72.18	10.02	−160.10 to −120.20	<0.0001	<0.0001	<0.0001
Cysteine	52.81	132.10	150.14	3.82	−86.95 to −71.72	<0.0001	<0.0001	<0.0001
Valin	62.62	175.20	179.78	4.82	−122.20 to −103.00	<0.0001	<0.0001	0.0007
Methionine	73.23	139.90	91.04	4.29	−75.21 to −58.09	<0.0001	<0.0001	<0.0001
Tryptophan	113.60	276.90	143.75	7.11	−177.50 to −149.20	<0.0001	<0.0001	<0.0001
Phenylalanine	176.60	514.80	191.51	13.96	−366.10 to −310.40	<0.0001	<0.0001	<0.0001
Isoleucine	46.25	158.40	242.49	8.04	−128.20 to −96.11	<0.0001	<0.0001	<0.0001
Leucine	44.74	151.50	238.62	7.34	−121.40 to −92.13	0.0178	<0.0001	<0.0001
Lysine	63.47	260.40	310.27	11.54	−220.00 to −173.90	<0.0001	<0.0001	<0.0001
Hydroxyproline	46.33	126.20	172.39	3.14	−86.15 to −73.62	<0.0001	<0.0001	<0.0001
Sarcosine	121.50	213.40	75.64	6.27	−104.40 to −79.42	<0.0001	<0.0001	0.0007
Proline	0.10	0.22	120.00	0.01	−0.14 to −0.10	<0.0001	<0.0001	<0.0001
Total Amino Acids	1837.32	4787.42	160.57	170.47				

growth parameters, H202, MDA, enzyme and amino acid contents in leaf tissues in salt stress. According to the analysis, two principle components described 99 % of the total variation (98.55 % by PC1 and 0.85 % by PC2). When PCA charts were examined, it could be seen that graft combinations were separated into four different regions based on measured one feature (Fig. 14). While the SP3/Galaxy, SP4/Galaxy, SP5/Galaxy, SH1/Galaxy and *L* × SH3/H2274 graft combinations are in the I. region of the PCA graph, these graft combinations contain the most SOD, CAT, GST and peroxidase enzymes and phenylalanine, tyrocines tryptophan, valine, cysteine aspartate and glycine amino acids. In addition, combinations of grafts in region I and IV. SH5/Galaxy and *L* × SP5/Galaxy graft combinations in the region are the graft combinations with the highest plant shoot and root fresh weight leaf number and root volume. The most isoleucine, proline lysine, alanine arginine, sarcosine leucine, methionine, hydroxyproline glutamate, glutamine and histidine amino acids in leaf tissues under salt stress conditions IV. determined in SH5/Galaxy and *L* × SP5/Galaxy graft combinations in the region. In addition, the lowest MDA content was determined in leaf tissues of SH5/Galaxy and *L* × SP5/Galaxy graft combinations. While ungrafted H2274 and Galaxy plants in the II. region of the graph has the highest MDA and content under salt stress conditions, these ungrafted plants have the least other amino acids and enzymes except theonine amino acid in their leaf tissues. In addition, ungrafted plants had the lowest plant shoot and root fresh weight leaf number and root volume in saline conditions. GR APX and 6GPD enzymes are in III of the highest graph. While it is determined in the SP3/H2274, SH1/H2274, SP4/H2274 and SP5/

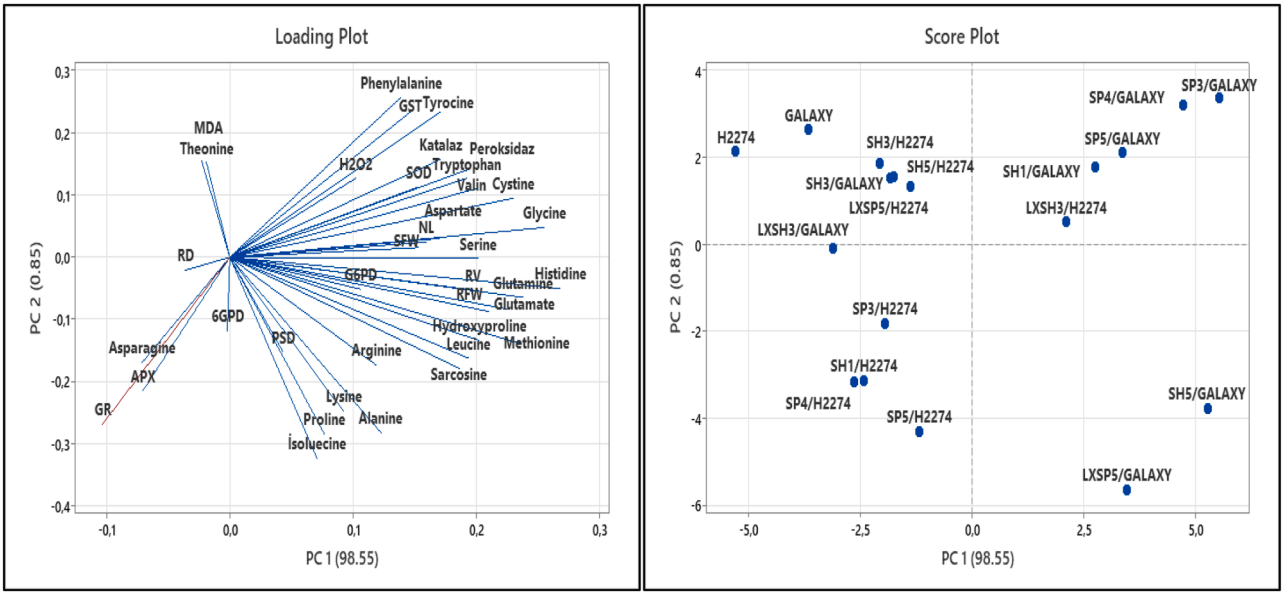

Fig. 14. PCA of graft combinations grown under salt stress conditions based on biomass parameters and metabolites in leaf tissues. PCA, principal component analysis; PSD, plant stem diameter; RD, root diameter; RFW, root fresh weight; SFW, shoot fresh weight; RD, root diameter; RV, root volume; NL, number of leaves; MDA, malondialdehyde; H_2O_2 hydrogen peroxide; SOD, superoxide dismutase, GST, Glutathione S-transferases; G6PD, glucose 6 phosphate dehydrogenase;; 6GPD, 6 glucose phosphate dehydrogenase; GR, glutathione reductase; APX, ascorbate peroxidase.

H2274 graft combinations in the region, these graft combinations are the graft combinations that contain the lowest amino acids in their tissues, except for the asparagine amino acid. While a positive correlation was observed between malondialdehyde (MDA) and theonine amino acid in the leaves of graft combinations grown in saline conditions, a negative correlation was observed with other amino acids. While salt stress increased the amount of MDA and theonine amino acids in the leaves of the plants, it caused a decrease in the amount of other amino acids. In addition, a negative correlation was obtained between MDA content and plant shoot and root fresh weight, number of leaves and root volume parameters. A highly positive correlation was determined between the amount of hydrogen peroxide (H_2O_2) in the leaves of plants subjected to salt stress and CAT, SOD, GST and peroxidase enzymes, while a negative correlation was determined between APX, GR and G6PD enzymes. A negative correlation was determined between plant shoot and root fresh weight, number of leaves, root volume and root diameter. A negative correlation was obtained between root diameter and other amino acids except for asparagine and tehonine amino acids. A positive correlation was obtained between plant shoot and root fresh weight, number of leaves, root volume and CAT, SOD, GST and peroxidase enzymes.

4. Conclusion

Salinity levels in soils are increasing due to climate change, water scarcity and human activities. Salinity in soil or water is a serious threat to plant growth, which reduces yields and threatens food security. Plant tolerance to salinitycan be enhanced and fruit quality and yield can be maintained by grafting tomato scions. Enhancing salt tolerance requires the selection of salt-tolerant rootstocks A successful graft requires testing and screening commercial cultivars, landraces, wild relatives and inbred lines or hybrids with a resistant parent under salt stress conditions. As a result of the study, rootstocks affected root and fresh weight in tomato plants grafted on different tomato rootstocks and the grafted plants had more root and shoot fresh weight compared to the ungrafted plants in appropriate rootstock/scion combinations. Ungrafted plants showed the highest increase in MDA and H_2O_2 in response to salt stress. Salt stress increased antioxidant enzyme activity and amino acid content in plants. When plants are subjected to salt stress, choosing the right rootstock/scion combination can contribute positively to antioxidant enzyme activity and amino acid content. Under saline conditions, tomato grafting can affect yield and fruit characteristics both positively and negatively. It is also important to select a suitable combination of scion and rootstock at the morphometric, physio-biochemical and molecular levels of plants to maximize the benefits of grafting, given that weather, salinity level, soil type, cultural practices and consumer preferences vary from location to location or country.

Compliance with ethical standards

The author declares that he has no known competing financial interests or personal relationships that could influence the work in this article.

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I work as a lecturer at Kırşehir Ahi Evran University, under the coordination of pilot projects.

CRediT author statement

Alim AYDIN: study concept and design: Alim AYDIN; data collection: Alim AYDIN; analysis and interpretation of results: Alim AYDIN; preparing a draft text: Alim AYDIN. Author reviewed the results and approved the final version of the article.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interestss: The contributions of author is defined as follows: study concept and design: A. Aydın; data collection: A. Aydın; analysis and interpretation of results: A. Aydın; preparing a draft text: A. Aydın. Author reviewed the results and approved the final version of the article.

Data availability

No data was used for the research described in the article.

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