



Effects of grafting with wild tomato (*Solanum pimpinellifolium* and *Solanum habrochaites*) rootstocks on growth and leaf mineral accumulation in salt stress

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Abstract

The positive response of grafting by tolerant rootstocks or scion-stock interactions on yield and fruit traits of tomatoes under saline conditions is attributed to several physiological and biochemical changes. In this study, we investigated some tolerance mechanisms by which grafting on wild rootstocks in tomatoes can prevent or minimize the effects of salt stress in plants under hydroponics conditions. Two tomato cultivars H2274 and Galaxy were grafted onto three *S. pimpinellifolium*, three *S. habrochaites*, *S. lycopersicum* L. × *S. pimpinellifolium* and *S. lycopersicum* L. × *S. Habrochaites* hybrid tomato genotypes. Plants were grown in hydroponic culture at two electrical conductivity (EC) levels (control at 1.5 dSm⁻¹ and salt at 8.0 dSm⁻¹). Salt stress led to a significant reduction in biomass growths of both grafted and nongrafted tomatoes. However, the plants that are least affected by salt stress are those grafted on wild tomato rootstocks. Leaf nutrient contents were significantly affected by rootstocks under both control and salt stress conditions. In this study, under saline conditions, plants grafted on wild rootstocks had higher N, P, K, Ca, Mg, S, Mn, Fe, Zn and B contents in leaf tissues and lower Na and Cl contents than ungrafted plants. Biochemical and physiological results revealed that *S. pimpinellifolium* and *S. habrochaites* have inherited salt tolerance from their genetic background. These wild tomato genotypes can be used as rootstocks in tomato breeding programs to develop salt-tolerant tomatoes or in grafting techniques under saline irrigation conditions.

Keywords Scion · Salt tolerant · Leaf nutrient contents · Tomato breeding

1 Introduction

In agricultural production worldwide, biotic and abiotic stress factors consistently lead to crop losses and reduced product quality. Nevertheless, abiotic stresses are considered to be the primary factor for yield loss in plants, up to about 70% (Bauchet et al. 2012; Ansari et al. 2019; Behera et al. 2022). Salinity is one of the most important abiotic stress factors that prevent the expected yield in crop production worldwide (Munns and Tester 2008). Salinity in soil or water is a serious threat to plant growth that prevents plants from achieving their genetic potential (Yamaguchi

and Blumwald 2005; Zhu et al. 2008) Salinity annually damages about 20% of the world's crops grown under irrigation (Arora 2019). Salinity has been reported to disrupt the physiological and biochemical processes of plants, causing changes in morphological characteristics that eventually lead to yield loss (Ali et al. 2021; Hasanuzzaman and Fujita 2022). Salt stress causes a reduction in shoot and root biomass, root length, root volume, stem diameter and leaf area in vegetables (Ulas et al. 2020; Göçer et al. 2021; Aydın and Yetişir 2022). Physiologically and biochemically, it decreases chlorophyll, carotenoid and polysaccharide levels, stomatal conductivity, photosynthetic activities and increases the accumulation of reactive oxygen species in plants (Brugnoli and Lauteri 1991; Rubaye et al. 2020; Aydın and Yetişir 2022; Kesawat et al. 2023). In addition to the difficulty of transporting water under salt stress, the plant has to deal with salt ions (Na⁺ and Cl⁻) that are toxic at high concentrations (Summart et al. 2012; Hou et al. 2022).

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Tomato (*Solanum lycopersicon* Mill.), a member of *Solanaceae* is one of the most-produced vegetables around the world and also is one of the most economically important vegetables, grown in many countries in the open and greenhouses using soil and soilless techniques. Salinity is a significant threat to tomato cultivation. It causes considerable reductions in tomato growth and yield (Kiferle et al. 2022; Raziq et al. 2022). Most tomato cultivars are considered moderately susceptible to salt stress, which affects seed germination and the vegetative and reproductive stages of growth (Ali et al. 2021). Since improving saline soils is very costly and difficult, salt-resistant or tolerant varieties/rootstocks need to be developed for these types of soils. One of the approaches to improve the performance of commercial cultivars susceptible to salinity in plants is the technique of grafting the target plant with other salt tolerant plants (Keatinge et al. 2014). The grafting of fruiting vegetables is now a common technique for developing salt-tolerant plants, in which both the rootstock and the scion influence the salt tolerance of the grafted plants (Etehadnia et al. 2008; Colla et al. 2013). Previous studies have reported that grafting tomatoes increases plant vigor, provides earlier maturity, and has a positive effect on resistance to stress factors, depending on which rootstock genotypes are used (Martorana et al. 2007; Di Gioia et al. 2013; İşeri et al. 2015).

In contrast to cultivated tomatoes (*S. lycopersicum* L.), which are moderately sensitive to salt stress, some wild species are reported to be resistant to salt (Foolad 2004; Rao et al. 2013). Tomato can be grafted onto intra/inter-species hybrids of other species in the *Solanaceae* family. Wild tomato species are a rich source of the genes and traits needed to increase resistance to various biotic and abiotic stresses. Major biotic and abiotic resistance genes for different stress factors such as viruses, fungi, bacteria, nematodes and salt and drought tolerant are mainly derived from wild tomato species and introgressed into cultivated tomatoes (Foolad 2004; Ji et al. 2009; Robbins et al. 2009; Hutton et

al. 2012; Ebert and Schafleitner 2015; Szymański et al. 2020; Conti et al. 2023). Genetic variability for salt tolerance traits is limited in domesticated tomatoes, whereas wild *Solanum* species, such as *S. pimpinellifolium*, *S. habrochaites*, *S. peruvianum*, *S. chilense* and *S. pennellii*, have been reported to be a source of salt tolerance (Frary et al. 2010; Gharbi et al. 2017; Kashyap et al. 2020; Ali et al. 2021). However, these wild species could be exploited as salt-resistant rootstocks for grafting susceptible but high yielding commercial tomato cultivars (Voutsela et al. 2012). Generally excludes the salt ions, most wild types accumulate higher concentrations of Na^+ and Cl^- in the leaves (Albaladejo et al. 2017). Using salt-tolerant species as rootstocks can protect sensitive species from the deleterious effects of salinity. Thus, the objective of the present study was to investigate whether grafting with different wild tomato rootstocks could improve the salt tolerance of tomato scions and to determine the physiological and nutritional responses induced by the rootstocks under different EC levels.

2 Materials and methods

2.1 Plant material, treatments, and experimental design

A hydroponic experiment was conducting during 2022–2023 growing season in a Kırşehir Ahi Evran University, Agricultural Research and Application Greenhouse, Kırşehir, Turkey. Two tomato cultivars Galaxy (Sakata Seed Southern Africa (Pty) Ltd.) and H2274 were used as scion and eight wild tomatoes were used as rootstock (Table 1). The hybrid tomato genotypes were developed in our tomato rootstock development project. The seeds were sown in multipots in a mixture of peat (pH: 6.0–6.5) and perlite in a 2:1 ratio and then the appropriate seedlings were selected for the grafting process using the procedure of “tube grafting” described by Lee and Oda (2010), while non-grafted tomato used as control plants. The seedlings were transplanted to 136 L plastic pots after cleaning from the growth substrate by washing them with tap water. The cultivation solution was constantly aerated with a pump. The experiment was conducted with two different EC levels (1.5 dSm^{-1} and 8 dSm^{-1}). The nutrient solution contained $1125 \mu\text{M Ca}(\text{NO}_3)_2$, $375 \mu\text{M} (\text{NH}_4)_2\text{SO}_4$, $750 \mu\text{M K}_2\text{SO}_4$, $650 \mu\text{M MgSO}_4$, $500 \mu\text{M KH}_2\text{PO}_4$, $10 \mu\text{M H}_3\text{BO}_3$, $0.5 \mu\text{M MnSO}_4$, $0.5 \mu\text{M ZnSO}_4$, $0.4 \mu\text{M CuSO}_4$, $0.4 \mu\text{M MoNa}_2\text{O}_4$ and $80 \mu\text{M Fe EDDHA}$ (Hoagland and Arnon 1950). The experiment was designed according to the randomized plot design with 3 replications and 3 plants in each replication. The study was continued for 30 days under controlled greenhouse conditions ($22\text{--}24 \text{ }^\circ\text{C}$ day / $16\text{--}18 \text{ }^\circ\text{C}$ night and 60% relative humidity).

Table 1 Genotype code, rootstock and scion list used in this study

Genotype Code	Rootstocks and scion genotype
SP3	LA1269 (<i>S. pimpinellifolium</i>) (Rootstock)
SP4	LA2914 (<i>S. pimpinellifolium</i>) (Rootstock)
SP5	LA1279 (<i>S. pimpinellifolium</i>) (Rootstock)
SH1	LA1764 (<i>S. habrochaites</i>) (Rootstock)
SH3	LA1378 (<i>S. habrochaites</i>) (Rootstock)
SH5	LA2650 (<i>S. habrochaites</i>) (Rootstock)
L×SP5	L×LA1279 (<i>S. lycopersicum</i> L. × <i>S. pimpinellifolium</i>) (Rootstock)
L×SH3	L×LA1378 (<i>S. lycopersicum</i> L. × <i>S. habrochaites</i>) (Rootstock)
H2274	(<i>S. lycopersicum</i> L.) (Standard variety indeterminate) (Scion)
Galaxy	(<i>S. lycopersicum</i> L.) (Hybrid variety determinate) (Scion)

2.2 Plant growth measurements

After four weeks of growing, plants were harvested and separated into shoots and roots. Main stem length (cm) was measured using a meter rule. To determine shoot and root dry weight (g), plant materials were dried in a forced-air oven for 48 h at 70 °C. The root length, of the plants was determined by using the special software program WinRHIZO (Win/Mac RHIZO Pro V. 2002c Regent Instruments Inc. Canada). Total leaf area (cm² plant⁻¹) and healthy and damaged leaf area (%) of a plant were measured by WinDIAS Leaf Image Analysis System (WinDIAS 3 Rapid System, Delta-T Devices, Cambridge, U. K.).

2.3 Determination of chlorophyll index

The Minolta SPAD-502 chlorophyll meter was used to take SPAD readings. During the growth period, fully expanded leaves of whole plants for each treatment were twice measured for SPAD.

2.4 Determination of ion leakage

Ion leakage (IL) of three replication was measured by using the method described by Flint et al. (2011). Samples were cut into equal size pieces (0.5 g per replication) and placed in a test tube containing 10 mL of distilled water, and at 45 °C for 30 min in a water bath. The initial conductance of the solution was measured using a conductivity meter (model-146, Systronics India Limited, Mumbai, India). The tubes were then kept in a boiling water bath for 10 min then cooled to room temperature, and their conductivity was measured once again. IL (%) was calculated by following formula. $IL = (\text{initial EC}/\text{final EC}) \times 100$.

2.5 Nutrient analysis

After harvest, fresh plant material was divided into two parts. One part was frozen in liquid nitrogen and stored at 80 °C for later use. The remaining fresh plant material was dried at 70 °C for 24 h. For determination of N, P, K, Ca, Mg, S, Mn, Fe, Zn, B, Cu, Na and Cl concentrations, 100 mg dried plant material was extracted by one hour boiling in 5 ml MilliQ. The solution was filtered through 0.2 mm filters (Whatman, England) and N, P, K, Ca, Mg, S, Mn, Fe, Zn, B, Cu, Na and Cl contents in the filtrate were analyzed using high-performance liquid chromatography (HPLC, Shimadzu Japan). The HPLC system was equipped with a ϕ 4.6 mm 6125 mm Shodex IC YS-50 column (Showa Denko). As an eluent, 4.0 mM methane sulfonic acid was used in HPLC graded H₂O (J.T. Baker, The Netherlands) with a flow rate of 1 mL

min⁻¹. Final ion concentrations in the filtrate were calculated according to a calibration curve.

2.6 Statistical analysis

The data were analyzed with the SAS Statistical Software (SAS 9.0, SAS Institute Inc., Cary, NC, USA). A two-factorial analysis of variance was performed to study the effects of salinity (NaCl), rootstock, scion, salt and interactions on the variables. Levels of significance are represented by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, 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).

3 Results and discussion

The results of the main stem length, shoot dry weight and root dry weight at the of graft combinations in different NaCl levels (1.5 and 8 dSm⁻¹) were shown in Table 2. Main stem length was significantly ($p < 0.001$) affected by scion, rootstock, NaCl levels, scion \times rootstock, scion \times NaCl levels and rootstock \times NaCl levels interactions while main stem length was not affected by rootstock \times scion \times NaCl levels interactions. Under control conditions, the longest main stem length was recorded in SP5/Galaxy, SP4/Galaxy and SH1/Galaxy graft combinations and SH3/H2274, SH5/H2274, SP4/H2274, SP3/H2274 and ungrafted H2274 graft combinations had the shortest main stem length. In saline conditions the highest main stem length is measured in SP4/Galaxy (39.33 cm plant⁻¹) and SP5/Galaxy (39.33 cm plant⁻¹) graft combinations. The lowest main stem length in saline conditions was measured in the graft combinations SH3/H2274 (17.67 cm plant⁻¹) and SH5/H2274 (18.67 cm plant⁻¹). All graft combinations in Group I (all graft combinations grafted with the H2274 scion), except the L \times SH3/H2274 and SH1/H2274 graft combinations, showed better tolerance than the ungrafted H2274. In the comparison of the control conditions and the salt application plants, the shoot lengths in Group II. (all graft combinations grafted with the Galaxy scion) decreased less than those of the ungrafted (Galaxy) plants. Shoot dry weight was significantly ($p < 0.001$) affected by rootstock, scion NaCl levels, rootstock \times scion, rootstock \times NaCl, scion \times NaCl and rootstock \times scion \times NaCl levels interactions. Under control conditions, SP4/Galaxy (19.96 g plant⁻¹) and SP5/Galaxy (19.94 g plant⁻¹) graft combinations produced significantly higher shoot dry weight, whereas non grafted H2274 (6.30 g plant⁻¹) significantly produced the minimum shoot dry weight. Under saline conditions, SH1/Galaxy

Table 2 The effects of graft combination and different NaCl levels (1.5 dSm⁻¹ and 8 dSm⁻¹) on main stem length, shoot dry weight and root dry weight of tomato plants

Graft Combinations	Main stem length (cm plant ⁻¹)			Shoot dry weight (g plant ⁻¹)			Root dry weight (g plant ⁻¹)		
	Control	NaCl	%R	Control	NaCl	%R	Control	NaCl	%R
SP3/H2274	38.68f	21.33f-h	-45	8.86e-g	3.00d-f	-66	2.57d-h	1.84b-f	-28
SP4/H2274	38.00f	20.33f-h	-46	7.82 fg	3.05d-f	-61	1.59jk	1.42 fg	-11
SP5/H2274	46.00d-f	22.67f-h	-51	11.76 cd	3.723c-e	-68	1.89ĭ-k	1.78d-g	-6
SH1/H2274	41.67ef	27.00d-h	-35	18.25ab	4.30a-d	-76	3.35bc	2.20a-e	-34
SH3/H2274	36.00f	17.67 h	-51	9.52d-f	3.09d-f	-68	1.58jk	1.26 g	-20
SH5/H2274	37.67f	18.67gh	-50	8.37e-g	3.06d-f	-63	2.09 h-j	1.66e-g	-20
L×SP5/H2274	44.33ef	23.67e-h	-47	10.68c-e	3.63c-e	-66	2.51e-h	1.79d-g	-29
L×SH3/H2274	41.00ef	28.00c-g	-32	7.40 fg	3.62c-e	-51	1.77ĭ-k	2.23a-d	26
Mean	40.42	22.42	-44	10.33	3.43	-65	2.17	1.77	-15
SP3/Galaxy	63.67ab	33.33a-e	-48	16.68b	3.69c-e	-78	3.71ab	1.82c-f	-51
SP4/Galaxy	67.67a	39.33a	-42	19.96a	5.28ab	-74	3.14b-d	2.30a-d	-27
SP5/Galaxy	68.00a	39.33a	-42	19.94a	5.33ab	-73	2.81c-f	2.70a	-4
SH1/Galaxy	67.67a	37.67a-c	-44	12.92c	5.57a	-57	4.02a	2.29a-d	-43
SH3/Galaxy	56.33b-d	32.67a-e	-42	11.91 cd	2.80ef	-76	2.24f-ĭ	1.36 fg	-39
SH5/Galaxy	57.67a-c	36.00a-d	-38	9.79d-f	3.77c-e	-61	2.75d-g	2.36ab	-14
L×SP5/Galaxy	68.00a	38.00ab	-44	16.78b	4.92a-c	-71	2.93c-e	2.33a-c	-21
L×SH3/Galaxy	50.33c-e	35.00a-d	-30	11.80 cd	4.01b-e	-66	2.02 h-j	1.68e-g	-17
Mean	62.42	36.42	-41	14.97	4.42	-70	2.95	2.00	-27
H2274 (ungrafted)	38.70f	23.67e-h	-39	6.30 g	2.22f	-65	1.39k	1.09 h	-22
Galaxy (ungrafted)	61.00ab	28.67b-f	-53	10.23c-f	4.70a-c	-54	2.21 g-ĭ	1.79d-g	-19
Rootstock	***			***			***		
Scion	***			***			***		
NaCl	***			***			***		
Rootstock×Scion	***			***			***		
Rootstock×NaCl	***			***			***		
Scion×NaCl	***			***			***		
Rootstock×Scion×NaCl	n.s.			***			**		

% I: Increase, %D: Decrease, IL: Ion leakage, ns: non-significant Values denoted by different letters are significantly different between graft combinations within both columns at $P < 0.05$. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

(5.57 g plant⁻¹), SP5/Galaxy (5.33 g plant⁻¹) and SP4/Galaxy (5.28 g plant⁻¹) graft combinations produced the highest shoot dry weight, respectively, while the ungrafted H2274 (2.24 g plant⁻¹) plant produced the lowest shoot dry weight, as in the control conditions (Fig. 1).

Root dry weight was significantly ($p < 0.001$) affected by rootstock, scion NaCl levels, rootstock × scion, rootstock × NaCl, scion × NaCl and rootstock × scion × NaCl levels interactions ($p < 0.01$). Under control and salinity conditions, the Galaxy scion had a strong root system compared to the H2274 scion. Under control conditions, the SH1/Galaxy (4.02 g plant⁻¹) graft combination produced the highest root dry weight, while the SP5/Galaxy (2.70 g plant⁻¹) graft combination produced the highest root dry weight under saline conditions. The lowest root dry weight under control (1.39 g plant⁻¹) and saline (1.09 g plant⁻¹) conditions were produced by ungrafted H2274 plants. The mean of main stem length, shoot dry weight and root dry weight parameters of all graft combinations using Galaxy scion in control and saline conditions were higher than the mean

of graft combinations using H2274 scion. Salt application reduced main stem length and shoot and root dry weight in both grafted and non-grafted plants. The decline in plant growth may be caused by osmotic stress (indirect negative effect of salinity), which limits water uptake and determines metabolic activities (Singh et al. 2012; Brdar et al. 2015). This problem is followed by ion toxicity (Na⁺ and Cl⁻), which can cause inhibition of enzymes or alteration of hormonal activities, leading to a decrease in vegetative growth (Gomes-Filho et al. 2008; Dehnavi et al. 2020). In our study, the degree to which plants were affected by salt stress varied depending on the rootstock/scion combination. Balliu et al. (2008), reported that plants grafted on ‘Cynthia’ rootstocks had a higher stem growth rate than ungrafted plants. Plant growth (e.g., stem growth rate) of plants grafted on Zhejiang, which is widely used in China for production of grafted tomatoes, was less affected by salt stress than non-grafted plants (He et al. 2009). Since roots are the primary organ exposed to salt stress, salt-induced inhibition of root growth is quite obvious. Salt stress reduced the root



Fig. 1 Control and saline conditions, (a) ungrafted H2274, (b) SH1/H2274, (c) ungrafted Galaxy and (d) SP5/Galaxy graft combinations

growth of tomato plants in all graft combinations, but the rate of root dry mass reduction was less in grafted plants than in ungrafted plants. The performance of grafted plants compared to non-grafted plants under stress conditions generally depends on the characteristics of the root system of the rootstock; a strong root system may be the most important criterion for increasing salt tolerance. In parallel with our results, He et al. (2009), reported that grafted plants under salt stress (100 and 150 mM NaCl) conditions had more root dry mass than ungrafted plants. In all graft combinations, the dry weight of the shoots decreased more than that of the roots under salt stress. This finding agrees with Foolad (1997) and Ali et al. (2021), who mentioned that salinity reduces shoot growth more than root growth. In the study, the tolerance to salinity of SP5 and SP4 (*S. pimpinellifolium*) wild rootstocks increased. Parallel to our results, Pailles et al. (2020), reported that salt-tolerant and wild tomato species were able to maintain growth (based on

dry mass) under salt stress conditions better than the cultivated tomato.

Plant growth was negatively affected by increasing the salinity level of the nutrient solution. The leaf area and root length of the grafted and non-grafted tomato plants decreased in response to the solution NaCl level. The result of leaf area and root length at the end of the growing period of graft combinations and tomato cultivars in different NaCl levels (1.5 and 8 dSm⁻¹) was shown in Table 3. Leaf area was significantly affected by rootstock, scion NaCl levels, rootstock × scion, rootstock × NaCl, scion × NaCl levels and rootstock × scion × NaCl levels interactions in both control and saline conditions ($p < 0.001$). In control conditions, all grafted plants using the H2274 scion produced higher leaf area than ungrafted H2274 plants. Under control conditions, the highest leaf area was determined in the SP5/Galaxy 3123.00 cm² plant⁻¹ and SP4/Galaxy (3111.00 cm² plant⁻¹) graft combinations, respectively and the lowest leaf area was determined in the ungrafted H2274 plants

Table 3 The effects of graft combination and different NaCl levels (1.5 dSm⁻¹ and 8 dSm⁻¹) on leaf area and root length of tomato plants

Graft Combinations	Leaf area (cm ² plant ⁻¹)			Root length (m plant ⁻¹)		
	Control	NaCl	%D	Control	NaCl	%D
SP3/H2274	1483.70c-f	538.10b-d	-64	23.18d-f	14.85d-f	-36
SP4/H2274	1088.40e-g	452.60c-e	-58	13.24 h	10.73f-h	-19
SP5/H2274	1972.40 cd	659.70b	-67	19.21f-h	14.97de	-22
SH1/H2274	1724.00c-e	571.90b-d	-67	30.24b-d	12.36e-g	-59
SH3/H2274	1397.30d-g	402.50de	-71	14.90 h	7.05 h	-53
SH5/H2274	944.13f-h	328.50e	-65	17.16f-h	7.54 h	-56
L×SP5/H2274	1902.00 cd	619.50bc	-67	24.23c-f	12.94e-g	-47
L×SH3/H2274	827.44f-h	445.40c-e	-46	15.21gh	15.03d-e	-1
Mean	1417.46	502.28	-63	19.67	11.93	-36
SP3/Galaxy	2203.20bc	526.68b-e	-76	32.08ab	12.02e-g	-63
SP4/Galaxy	3111.00a	911.10a	-71	30.51bc	21.11ab	-31
SP5/Galaxy	3123.00a	983.70a	-68	27.97b-e	24.41a	-13
SH1/Galaxy	2766.00ab	931.60a	-66	39.32a	17.69b-d	-55
SH3/Galaxy	2067.00b-d	484.10b-e	-77	19.17f-h	10.69f-h	-44
SH5/Galaxy	1138.08e-g	494.40b-e	-57	22.23e-g	12.32e-g	-45
L×SP5/Galaxy	2212.00bc	928.70a	-58	27.52b-e	19.64bc	-29
L×SH3/Galaxy	827.40f-h	446.50c-e	-46	18.48f-h	13.50d-g	-27
Mean	2180.90	713.36	-66	27.16	16.42	-38
H2274 (ungrafted)	648.10gh	381.85de	-41	12.29 h	9.92gh	-19
Galaxy (ungrafted)	1462.11c-f	604.84bc	-59	23.46c-f	15.03de	-36
Rootstock	***			***		
Scion	***			***		
NaCl	***			***		
Rootstock×Scion	***			***		
Rootstock×NaCl	***			***		
Scion×NaCl	***			**		
Rootstock×Scion×NaCl	***			***		

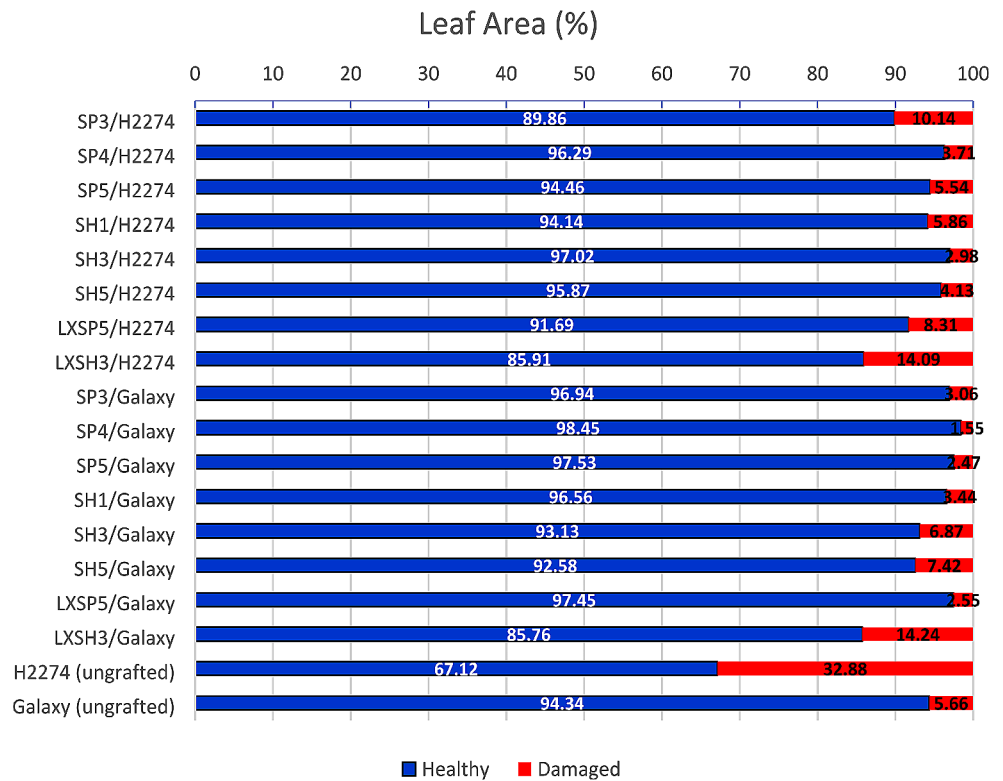
% I: Increase, %D: Decrease, IL: Ion leakage, ns: non-significant. Values denoted by different letters are significantly different between graft combinations within both columns at $P < 0.05$. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

(648.10 cm² plant⁻¹). With the salt application, there was a decrease in the leaf area varying between 41% (ungrafted H2274) and 77% (SH3/Galaxy) in all graft combinations. Under salt stress, all graft combinations produced more leaf area than the ungrafted H2274 plants except for the SH5/H2274 graft combination. Under saline conditions, the highest leaf area was determined in the SP5/Galaxy (983.70 cm² plant⁻¹), SH1/Galaxy (931.60 cm² plant⁻¹) and SP4/Galaxy (911.10 cm² plant⁻¹) graft combinations, respectively. Root length was significantly ($p < 0.001$) affected by scion, rootstock, NaCl levels, scion × rootstock, scion × NaCl levels ($p < 0.01$) and rootstock × NaCl levels interactions while main stem length was not affected by rootstock × scion × NaCl levels interactions. The root length was ranked between 12.29 m plant⁻¹ (ungrafted H2274) and 39.32 m plant⁻¹ (SH1/Galaxy) under control conditions. Under saline conditions, SP5/Galaxy graft combination produced significantly longer roots (24.41 m plant⁻¹) and the shorter roots produced SH3/H2274 (7.05 m plant⁻¹), SH5/H2274 (7.54 m plant⁻¹) and ungrafted H2274 (9.92 m plant⁻¹) plants, respectively. The mean of main of root length

parameter of all graft combinations using Galaxy scion in control and saline conditions was higher than the mean of graft combinations using H2274 scion. The performance of grafted plants compared to ungrafted plants under stress conditions generally depends on the characteristics of the root system of the rootstock. A vigorous root system (root length and volume) could be the most important criterion for increasing salt tolerance (Ulas et al. 2020; Göçer et al. 2021; Aydın and Yetişir 2022). In this study, the salt-tolerant graft combinations (SP4/Galaxy, SP5/Galaxy and SH1/Galaxy) were specified with increased leaf area and root length under saline conditions in comparison to nongrafted control plants (H2274 and Galaxy). In parallel with our study, Ulas (2021), reported that grafted plants under saline conditions had greater leaf area and root length than ungrafted plants.

Saline conditions, healthy and damaged leaf area (%) were determined with the WinDIAS 3 Rapid System (Fig. 2). The healthy leaf area varied between 67.12% and 98.45% under saline conditions, while the damaged leaf area varied between 1.55% and 32.88%. Under saline conditions, SP4/Galaxy (98.45%), SP5/Galaxy (97.53%) and

Fig. 2 Effect of salt stress on leaf area (%)



H2274 (ungrafted)

■ 67.12%
■ 33.88%

SP4/Galaxy

■ 98.45%
■ 1.55%

■ Healthy

■ Damaged

Fig. 3 Effect of salt stress on leaf area in ungrafted H2274 and SP4/Galaxy plants (%)

SH3/H2274 (97.02%) graft combinations produced the highest healthy leaf area, respectively, while the ungrafted H2274 (67.12%) plant produced the lowest healthy leaf area (Fig. 3). As a result of the salt application, significant

differences were found between graft combinations in both total leaf area and area of damaged leaves. Specifically, salt damage can reduce leaf expansion. Plants that reduce leaf area adapt to salt stress by reducing transpiration and

nutrient requirements (Bernstein et al. 1993; Greenway and Munns 2003). The present result for leaf area is in agreement with the results of Balibrea et al. (2000), who reported that leaf area of the salt-sensitive genotype of tomato was significantly affected by salinity. Rao et al. (2013) (*S. pimpinellifolium*), Albacete et al. (2009) (*S. cheesmaniae*) and Ali et al. (2021) (*S. habrochaites* and *S. pennellii*) reported that it reduced the negative effects of salt stress in tomato cultivation in areas with soil salinity problems from wild rootstocks.

Leaf chlorophyll content, which was significantly affected by rootstock, NaCl levels and rootstock \times NaCl levels interactions in both control and saline conditions ($p < 0.001$). Salt application caused decreases in leaf chlorophyll content in all applications and the highest decrease was observed in L \times SH3/Galaxy graft combination plants at 50% (Table 4). With the salt application, there was a decrease in the leaf chlorophyll content varying between 5% (ungrafted H2274) and 50% (L \times SH3/Galaxy) in all graft combinations. Under saline conditions, the highest leaf chlorophyll content was determined in the ungrafted Galaxy (54.10) and SH3/H2274

(52.37) graft combinations. There was no significant variation in ion leakage (root and leaf) under control conditions among graft conditions. Root ion leakage, which was significantly affected by scion ($p < 0.01$), NaCl levels ($p < 0.001$), rootstock \times scion ($p < 0.05$) and rootstock \times NaCl levels ($p < 0.05$) interactions in both control and saline conditions. The root ion leakage ranged from 7 to 31% under salt stress. Saline conditions the highest root ion leakage was determined in the ungrafted plants H2274 (96.75%) and the lowest root ion leakage was determined in the SP5/H2274 (88.11%) graft combination. Leaf ion leakage was significantly ($p < 0.001$) affected by rootstock, scion NaCl levels, rootstock \times NaCl, scion \times NaCl levels and rootstock \times scion \times NaCl levels interactions. The leaf leakage ranged from 22 to 129% under salt stress. The highest increase in leaf ion leakage was determined in the L \times SP5/H2274 (129%) and ungrafted H2274 (114%) plants. In saline conditions, the highest leaf ion leakage was determined in H2774 (68.95%) plants, as was root ion leakage. The results of this study show that the value of SPAD decreases under saline conditions in all graft combinations. The decrease in SPAD

Table 4 The effects of graft combinations and different NaCl levels (1.5 dSm⁻¹ and 8 dSm⁻¹) on leaf chlorophyll content, root ion leakage and leaf ion leakage of tomato plants

Graft Combinations	Leaf chlorophyll content (SPAD)			Root IL (%)			Leaf IL (%)		
	Control	NaCl	%D	Control	NaCl	%I	Control	NaCl	%I
SP3/H2274	53.97e	48.07b-e	-12	81.72	92.88a-d	14	26.44	48.95c-f	85
SP4/H2274	65.77a	46.60c-e	-41	84.28	90.31b-d	7	29.52	52.33b-e	77
SP5/H2274	63.00a-d	50.67a-d	-24	77.75	88.11d	13	28.168	46.00d-g	63
SH1/H2274	54.03e	45.63de	-18	75.66	92.20a-d	22	30.39	49.29c-f	62
SH3/H2274	58.00c-e	52.37ab	-11	78.81	92.78a-d	18	33.34	59.08b	77
SH5/H2274	60.47a-e	49.47a-d	-22	80.27	92.76a-d	16	26.88	56.27bc	109
L \times SP5/H2274	58.60b-e	48.13b-e	-22	84.16	92.38a-d	10	23.65	54.05b-d	129
L \times SH3/H2274	56.93de	46.87c-e	-21	73.24	94.88ab	30	27.35	51.05b-e	87
Mean	58.85	48.48	-22	79.49	92.04	16	28.22	52.13	86
SP3/Galaxy	55.53e	48.63b-d	-14	78.59	92.12a-d	17	30.65	46.76d-f	53
SP4/Galaxy	59.47a-e	48.60b-d	-22	67.87	88.59 cd	31	28.56	42.49f-h	49
SP5/Galaxy	60.37a-e	49.73a-d	-21	80.60	88.00d	9	28.41	45.06e-g	59
SH1/Galaxy	57.43de	49.63a-d	-16	82.66	88.42 cd	7	29.21	34.99 h	20
SH3/Galaxy	60.00a-e	48.07b-e	-25	74.95	93.36a-c	25	34.09	42.33f-h	24
SH5/Galaxy	63.63a-d	50.87a-c	-25	78.41	89.45 cd	14	26.19	45.93d-g	75
L \times SP5/Galaxy	64.63a-c	48.70b-d	-33	70.19	88.35 cd	26	27.60	47.37d-f	72
L \times SH3/Galaxy	65.07ab	43.27e	-50	69.97	91.57b-d	31	31.06	37.83gh	22
Mean	60.77	48.44	-25	75.41	89.98	20	29.47	42.84	47
H2274 (ungrafted)	59.37a-e	50.80a-c	-17	72.10	96.75a	34	32.22	68.95a	114
Galaxy (ungrafted)	56.83de	54.10a	-5	66.34	90.33b-d	36	32.63	51.00b-e	56
Rootstock	***			n. s.			***		
Scion	n.s.			**			***		
NaCl	***			***			***		
Rootstock \times Scion	*			*			n.s.		
Rootstock \times NaCl	***			*			***		
Scion \times NaCl	n.s.			n.s.			***		
Rootstock \times Scion \times NaCl	n.s.			n.s.			***		

% I: Increase, %D: Decrease, IL: Ion leakage, ns: non-significant Values denoted by different letters are significantly different between graft combinations within both columns at $P < 0.05$. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

value in response to abiotic stress leads to a decrease in net photosynthesis and thus energy, which is very important for metabolism and growth (Sousaraei et al. 2021). The current result on leaf chlorophyll content is consistent with the result by Ali et al. (2021), working on wild tomato rootstock who reported that leaf chlorophyll content was significantly affected by the salinity in salt sensitive genotype. Similar to our leaf chlorophyll content results, in the study conducted on 8 salt-resistant rootstocks, the leaf chlorophyll content of plants grafted on salt-resistant rootstocks were higher than non-grafted plants (Abdeldym et al. 2020). It is a well-known phenomenon that salt stress leads to an increase in IL in plants. Reduced translocation of NaCl to the shoot system is achieved either by exclusion or restricted absorption by the roots (Moya et al. 1999). In our study, ion leakage from roots of all graft combinations was higher than leaf ion leakage from leaves under salt stress. However, the increase in ion leakage in leaf tissue is higher than in root tissue with salt applications. Aydın and Yetişir (2022), obtained results similar conclusions to our study in their study on grafted cucumbers.

The leaf nitrogen (N), phosphorus (P) and potassium (K) contents of tomato plants grown under different salt concentrations are given in Table 5. Leaf N content was significantly affected by rootstocks, salt treatment, rootstock × scion, rootstock × salt and scion × salt interaction in saline conditions. There was a significant decrease (1-78%) in the N content of the leaves under salt stress. Under salt stress, the highest leaf N content was detected in plants grafted SH3/H2274 (1.49%), SP4/H227(1.47%), L×SH3/H2274 (1.38%) and SH5/Galaxy (1.28%) while the lowest leaf N content was determined in ungrafted Galaxy (0.42%) plants. Leaf P content was also significantly influenced by the rootstock, scion, NaCl, rootstock × scion, rootstock × NaCl and rootstock × scion × NaCl interactions in both control and saline conditions ($p < 0.001$). In saline conditions, the highest leaf P content was determined in SP5/H2274, SH3/H2274 and L×SH3/H2274 graft combinations, while the lowest was determined in L×SH3/Galaxy, L×SP5/Galaxy and Galaxy (ungrafted) plants. Leaf potassium (K) content was also significantly influenced by all applications and the interaction of the applications in both control and saline

Table 5 The effects of graft combinations and different NaCl levels (1.5 dSm⁻¹ and 8 dSm⁻¹) on leaf nitrogen (N), phosphorus (P) and potassium (K) contents of tomato plants

Graft Combinations	N (%)			P (%)			K (%)		
	Control	NaCl	%D	Control	NaCl	%D	Control	NaCl	%D
SP3/H2274	1.44	0.63 g-l	-56	0.16a-c	0.07ef	-54	0.90bc-f	0.46fgh	-50
SP4/H2274	1.59	1.47a	-8	0.23a	0.16a	-30	0.89b-f	0.72b-d	-18
SP5/H2274	1.27	0.94d	-25	0.14bc	0.10c	-25	0.80b-f	0.83bc	3
SH1/H2274	1.79	0.83de	-54	0.15bc	0.10 cd	-35	1.15ab	0.65c-f	-44
SH3/H2274	1.76	1.49a	-15	0.18ab	0.15a	-15	1.44a	1.16a	-19
SH5/H2274	1.23	0.80ef	-35	0.12bc	0.08de	-30	0.99a-e	0.67b-e	-33
L×SP5/H2274	1.26	0.68 fg	-46	0.13bc	0.07ef	-47	1.00a-e	0.53e-h	-47
L×SH3/H2274	1.39	1.38ab	-1	0.14bc	0.15a	9	1.06a-d	0.85b	-20
Mean	1.46	1.03	-29	0.15	0.11	-29	1.03	0.73	-28
SP3/Galaxy	1.77	0.64gh	-64	0.17a-c	0.07ef	-59	1.44a	0.55d-g	-62
SP4/Galaxy	1.40	0.54 h-k	-61	0.14bc	0.06 fg	-57	1.17ab	0.43gh	-63
SP5/Galaxy	1.56	1.20c	-23	0.11bc	0.13b	17	0.87b-f	0.69b-e	-21
SH1/Galaxy	1.70	1.16c	-32	0.09c	0.12b	31	1.09a-c	0.84bc	-23
SH3/Galaxy	1.92	0.53 h-k	-73	0.11bc	0.06 fg	-49	0.49ef	0.38gh	-21
SH5/Galaxy	1.64	1.28bc	-22	0.10bc	0.13b	25	0.42f	0.33 h	-21
L×SP5/Galaxy	1.66	0.50i-k	-70	0.11bc	0.05 g	-55	0.44f	0.40gh	-10
L×SH3/Galaxy	2.08	0.46jk	-78	0.15a-c	0.04 g	-71	0.59c-f	0.34 h	-43
Mean	1.72	0.79	-53	0.12	0.08	-27	0.81	0.49	-33
H2274 (ungrafted)	1.78	0.58 g-j	-67	0.14bc	0.06 fg	-58	0.54ef	0.46f-h	-16
Galaxy (ungrafted)	1.79	0.42k	-76	0.14bc	0.05 g	-68	0.57d-f	0.33 h	-41
Rootstock	***			***			***		
Scion	n.s.			***			***		
NaCl	***			***			***		
Rootstock×Scion	***			***			***		
Rootstock×NaCl	***			***			***		
Scion×NaCl	***			n.s.			***		
Rootstock×Scion×NaCl	n.s.			***			**		

% I:Increase, %D:Decrease, IL:Ion leakage, ns:non-significant Values denoted by different letters are significantly different between graft combinations within both columns at $P < 0.05$. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

conditions. In graft combinations under salt stress, the highest leaf K content was determined in SH1/H2274 (1.16%), SH3/Galaxy (0.84%) and SP5/H2274 (0.83%) plants, while the lowest was determined in SH5/Galaxy and ungrafted Galaxy plants with 0.33%. Significant negative correlations were found between leaf N, P and K content and Na, Cl, Cu, root ion leakage, leaf ion leakage and damaged leaf area. (Fig. 4). The accumulation of Na in the biomass may also be an indicator of salt tolerance. However, when Na accumulates in the cytosol of cells, it is toxic and leads to ionic imbalance (Hanin et al. 2016). In addition, Na reduces the availability of K binding sites for important metabolic processes in the cytoplasm. For the plant to protect itself from salt stress, it must either limit Na influx from the roots or control Na concentration and distribution after entry (Tester and Davenport 2003; Wei et al. 2017). A higher K, P and N content of plants seems to be related to the improvement in salt tolerance in grafted plants (Huang et al. 2009; Aydın and Yetişir 2022). In addition, high concentrations of Cl cause nitrogen or phosphorus deficiency (Wu and Li 2019). Accessions of the wild tomato species *S. pimpinellifolium*

were screened for salt tolerance and provided tolerance to an EC level of 40 dSm⁻¹ by Rao et al. (2013) who suggested that it could be a potential source of salt tolerance for the breeding of *S. pimpinellifolium* and results of the path analysis along with heritability and genetic advance showed that shoot dry weight and K/Na ratio are the two most critical component traits for survival, while fruit number is critical for yield per plant.

Leaf calcium (Ca), magnesium (Mg) and sulphur (S) contents of plants grown in control and saline conditions are given in Table 6. In control and saline conditions, leaf Ca content was significantly affected by rootstock, scion, NaCl, rootstock × scion, rootstock × NaCl and scion × NaCl interactions ($p < 0.001$). Salt application caused a decrease in Ca content in all graft combinations except SP4/H2274, SH5/Galaxy, SP5/Galaxy, L×SH3/H2274 and SP5/H2274 graft combinations. Under saline conditions, the highest leaf Ca content was determined in SP4/H2274 and SH3/H2274 grafted plants, while the lowest was determined in ungrafted H2274 plants. Under control and saline conditions, leaf Mg content was significantly affected by all treatments

Table 6 The effects of graft combinations and different NaCl levels (1.5 dSm⁻¹ and 8 dSm⁻¹) on leaf calcium (Ca), magnesium (Mg) and sulphur (S) contents of tomato plants

Graft Combinations	Ca (%)			Mg (%)			S (%)		
	Control	NaCl	%D	Control	NaCl	%D	Control	NaCl	%D
SP3/H2274	0.54ab	0.31h ₁	-43	0.10 cd	0.06e-h	-44	0.07c	0.04e-g	-40
SP4/H2274	0.56ab	0.95a	69	0.11 cd	0.06e-h	-45	0.07c	0.15a	125
SP5/H2274	0.46ab	0.47de	2	0.11 cd	0.09d	-15	0.07c	0.06c-e	-8
SH1/H2274	0.60ab	0.43ef	-28	0.11 cd	0.08de	-32	0.09c	0.05d-f	-36
SH3/H2274	0.87a	0.66b	-23	0.17a-d	0.16a	-10	0.13c	0.11b	-14
SH5/H2274	0.61ab	0.38 fg	-38	0.11 cd	0.08d-f	-29	0.08c	0.05e-g	-38
L×SP5/H2274	0.59ab	0.35gh	-42	0.13b-d	0.06f-h	-58	0.09c	0.04e-g	-52
L×SH3/H2274	0.60ab	0.61b	3	0.15b-d	0.14ab	-1	0.11c	0.08bc	-21
Mean	0.60	0.52	-12	0.12	0.09	-29	0.09	0.08	-11
SP3/Galaxy	0.82ab	0.30h ₁	-63	0.17a-d	0.06e-g	-62	0.55a	0.08 cd	-86
SP4/Galaxy	0.64ab	0.28ij	-57	0.14bcd	0.05gh	-66	0.11c	0.04 fg	-67
SP5/Galaxy	0.50ab	0.52 cd	4	0.09d	0.13bc	43	0.07c	0.07c-e	-5
SH1/Galaxy	0.47ab	0.27ij	-44	0.18a-d	0.06f-h	-68	0.13c	0.04 fg	-72
SH3/Galaxy	0.56ab	0.26ij	-54	0.34ab	0.05gh	-85	0.24bc	0.03 fg	-86
SH5/Galaxy	0.46b	0.55c	21	0.25a-d	0.13bc	-45	0.24bc	0.09bc	-64
L×SP5/Galaxy	0.49ab	0.23jk	-53	0.28a-d	0.05gh	-83	0.24bc	0.03 fg	-87
L×SH3/Galaxy	0.62ab	0.23jk	-63	0.37a	0.04 h	-90	0.41ab	0.03 fg	-93
Mean	0.57	0.33	-39	0.23	0.07	-57	0.25	0.05	-70
H2274 (ungrafted)	0.50ab	0.19k	-61	0.27a-d	0.13c	-53	0.58a	0.02 g	-96
Galaxy (ungrafted)	0.56ab	0.50 cd	-10	0.31a-c	0.04gh	-86	0.12c	0.04e-g	-65
Rootstock	***			***			***		
Scion	***			***			***		
NaCl	***			***			***		
Rootstock×Scion	***			n.s.			***		
Rootstock×NaCl	***			***			***		
Scion×NaCl	***			***			***		
Rootstock×Scion×NaCl	n.s.			***			***		

% I: Increase, %D: Decrease, IL: Ion leakage, ns: non-significant Values denoted by different letters are significantly different between graft combinations within both columns at $P < 0.05$. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

and interactions except rootstock \times scion interaction. Salt application caused a decrease in leaf Mg content in all graft combinations except SP5/Galaxy (43%) graft combination, while the highest decrease was determined in L \times SH3/Galaxy (90%) and ungrafted Galaxy (86%) plants. Leaf S content was significantly affected by all applications and interactions ($p < 0.001$). In plants grown under salt stress, leaf S content decreased in all graft combinations except SP4/H2274. Under saline conditions, the highest leaf S content was determined in the SP4/H2274 (0.15%) graft combination, which did not decrease (125%) with salt application, while the lowest was determined in the ungrafted H2274 (0.02%) plants, which showed the highest decrease (-96%). Leaf calcium (Ca), magnesium (Mg) and sulphur (S) contents were significantly positively correlated with N, P, K, Zn, Ca, Fe, SPAD and healthy leaf area (Fig. 4). The root system of grafted plants is stronger and more efficient in water and nutrient uptake. In addition, grafted tomato plants improve salt tolerance by reducing ionic stress, increasing K, Ca and Mg transfer to shoots and leaves (Singh et al. 2012; Kolečka et al. 2018). Al-Harbi et al. (2017), reported that under saline conditions, grafted plants accumulated more Ca and K in the leaves and had lower Na and Cl levels. Salinity with a predominance of Na⁺ salts not only reduces the availability of Ca²⁺, but also reduces Ca²⁺ transport and mobility to the growing parts of the plant, which affects the quality of both vegetative and reproductive organs (Navarro et al. 2000; Aydın and Yetişir 2022). In our study, leaf S content decreased under salt stress. Similarly, Mor and Manchanda (2009), reported that S content decreased in tomato shoots under salt stress. P, Mg, Fe, Mn, Zn and Cu contents of plants generally decreased under salt stress (Kipçak et al. 2019).

Leaf manganese (Mn), iron (Fe) and zinc (Zn) contents of tomato plants grown under control and saline conditions are given in Table 7. In all graft combinations, salt application significantly decreased leaf Mn (19–90%) and Fe (4–61%) contents. Under saline conditions, the highest leaf Mn content was determined in SH1/Galaxy (21.38 mg kg⁻¹) graft combination, while the lowest was determined in L \times SH3/Galaxy (4.49 mg kg⁻¹) and ungrafted Galaxy (4.60 mg kg⁻¹) plants. In salt application plants, the highest leaf Fe content was determined in SH3/H2274 (48.92 mg kg⁻¹), SP5/Galaxy (38.20 mg kg⁻¹) and SP3/Galaxy (37.89 mg kg⁻¹) grafted plants, while the lowest was determined in ungrafted H2274 (13.23 mg kg⁻¹) plants. Leaf Zn content was significantly affected by all applications and interactions ($p < 0.001$). Under saline conditions, leaf Zn content decreased in all graft combinations except SP4/H2274 graft combination, while the highest decrease was obtained in ungrafted H2274 plants with 96%. The solubility and mobility of Cu, Fe and Zn ions are further reduced in saline soils. With increasing

salinity, leaf Cu, Zn and Fe levels decrease proportionally. Similar to our results, NaCl-induced Mn deficiency has been previously observed in tomato (Balliu et al. 2015).

Leaf boron (B), copper (Cu), sodium (Na) and chlorine (Cl) contents of tomato plants grown under control and saline conditions are given in Table 8. Salt application caused an increase in leaf boron content in some graft combinations, while it caused a decrease in some graft combinations. The highest increase was determined in SP4/H2274 (204%) graft combination, while the highest decrease was determined in SP4/Galaxy (74%) graft combination. While the leaf Cu content of plants under salt stress decreased between 77 and 98%, the highest leaf Cu content was determined in ungrafted Galaxy (0.33 mg kg⁻¹) and H2274 (0.32 mg kg⁻¹) plants under saline conditions. Leaf Na and Cl contents of tomato plants grown under control and saline conditions were significantly affected by all applications and interactions ($p < 0.001$). Salt stress application increased leaf Na content between 375 and 8937% and Cl content between 389 and 5875% in all graft combinations. Under saline conditions, the lowest Na content was determined in SP4/Galaxy (8.34 mg kg⁻¹) and SP3/Galaxy (9.16 mg kg⁻¹) graft combinations, while the highest was determined in ungrafted H2274 (21.25 mg kg⁻¹) and Galaxy (21.24 mg kg⁻¹) plants. In NaCl application, the highest leaf Cl content was determined in H2274 (119.80 mg kg⁻¹) and Galaxy (101.87 mg kg⁻¹) ungrafted plants, while the lowest was determined in SH1/Galaxy (12.51 mg kg⁻¹) graft combination. Leaf Na and Cl content were positively correlated with leaf Cu content, root ion leakage, leaf ion leakage and damaged leaf area, while SPAD, healthy leaf area, leaf area, root length, main stem length, shoot dry weight and root dry weight were negatively correlated (Fig. 4). In general, the main factor inhibiting growth of salt-stressed plants is elevated levels of Na and Cl with roots remaining the primary sites for stress perception and the subsequent responses at the cell, organ or whole plant levels (Rajaei et al. 2009). He et al. (2009) found that Na⁺ levels in leaves and roots increased significantly with increasing NaCl concentration. In parallel with our results, Al-Harbi et al. (2017), reported that leaf Na and Cl accumulation of grafted tomato plants were lower than ungrafted tomato plants under salt stress conditions. The detrimental effects of Cl are caused by interference with the uptake or metabolism of other essential ions such as N, P and K (Al-Harbi et al. 2017; Zhao et al. 2020). Parallel to our results, tomato cultivars Fanny and Goldmar grafted onto rootstock AR-9704 showed differential accumulation of Na and Cl, with Cl and Na concentrations being significantly higher in non-grafted than in grafted plants (Fernández-García et al. 2004). Di Gioia et al. (2013), reported that the leaf K/Na, Ca/Na and Mg/Na ratios

Table 7 The effects of graft combinations and different NaCl levels (1.5 dSm⁻¹ and 8 dSm⁻¹) on leaf manganese (Mn), iron (Fe) and zinc (Zn) contents of tomato plants

Graft Combinations	Mn (mg kg ⁻¹)			Fe (mg kg ⁻¹)			Zn (mg kg ⁻¹)		
	Control	NaCl	%D	Control	NaCl	%D	Control	NaCl	%D
SP3/H2274	13.80b	6.12f-h	-56	31.74b-f	20.09d	-37	0.58e	1.24 g	-40
SP4/H2274	10.82b	6.29f-h	-42	23.96c-f	21.13d	-12	0.60e	20.77ab	125
SP5/H2274	15.15b	10.60de	-30	21.64d-f	17.94d	-17	0.45e	9.24d-f	-8
SH1/H2274	11.34b	8.04e-g	-29	33.60b-f	23.06 cd	-31	4.50b-e	7.26ef	-36
SH3/H2274	18.99b	15.42b	-19	57.20a	48.92a	-14	12.66ab	19.42b	-14
SH5/H2274	13.52b	8.65ef	-36	39.03a-f	24.6 cd	-37	10.39a-d	9.98de	-38
L×SP5/H2274	13.50b	6.63f-h	-51	41.09a-d	21.13d	-49	9.53a-d	7.84d-f	-52
L×SH3/H2274	20.02b	14.23bc	-29	48.00ab	34.38bc	-28	11.31a-c	23.14a	-21
Mean	14.64	9.50	-36	37.03	26.41	-28	6.25	12.36	-11
SP3/Galaxy	18.93b	12.00 cd	-37	56.80a	37.89ab	-33	13.76a	10.78d	-86
SP4/Galaxy	15.63b	5.30gh	-66	46.23a-c	17.94d	-61	12.13ab	8.47d-f	-67
SP5/Galaxy	38.30a	12.42 cd	-68	39.67a-f	38.20ab	-4	7.42a-e	14.89c	-5
SH1/Galaxy	43.18a	21.38a	-50	24.77c-f	17.37d	-30	5.09b-e	6.94ef	-72
SH3/Galaxy	43.71a	5.13 h	-88	20.02d-f	15.86d	-21	3.31c-e	6.36f	-86
SH5/Galaxy	36.38a	13.18b-d	-64	40.57a-e	16.33d	-60	2.88de	19.29b	-64
L×SP5/Galaxy	40.89a	5.45gh	-87	17.26f	13.79d	-20	3.03c-e	7.49ef	-87
L×SH3/Galaxy	47.21a	4.49 h	-90	22.02d-f	13.89d	-37	3.57c-e	6.87ef	-93
Mean	35.53	9.92	-69	33.42	21.41	-33	6.40	10.13	-70
H2274 (ungrafted)	39.29a	6.98f-h	-82	17.97ef	13.23d	-26	3.11c-e	21.65ab	-96
Galaxy (ungrafted)	44.16a	4.60 h	-90	26.90b-f	20.67d	-23	3.27c-e	8.09d-f	-65
Rootstock	***			***			***		
Scion	***			***			***		
NaCl	***			***			***		
Rootstock×Scion	***			n.s.			***		
Rootstock×NaCl	***			***			***		
Scion×NaCl	***			***			***		
Rootstock×Scion×NaCl	n.s.			***			***		

% I: Increase, %D: Decrease, IL: Ion leakage, ns: non-significant Values denoted by different letters are significantly different between graft combinations within both columns at $P < 0.05$. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

of grafted tomato plants grown under salt stress were higher than those without grafting.

3.1 Principal component analysis

PCA was used for classifying graft combinations based on plant growth parameters and leaf ion content under salt conditions. According to the PCA analysis, the two principal components formed 99% of the total variation (99.89% by PC1 and 0.10% by PC2). When PCA charts were examined, it could be seen that graft combinations were separated into five different groups based on measured features (Fig. 4). The first group is the group containing L×SH3/Galaxy graft combination in the region I of the graph and is indicated by the black circle. The L×SH3/Galaxy graft combination in this circle has the highest leaf Cu content, while the healthy leaf area is the lowest. The ungrafted H2274 graft combination in the yellow circle contains the most Na and Cl in the leaf tissues, while it is negatively correlated with biomass parameters. The graft combinations in the green circle are

the graft combinations that contain the most nutrients in the leaves except Na, Cl and Cu. The graft combinations in the red circle show positive correlation with biomass parameters and negative correlation with leaf Na, Cl, and leaf and root ion leakage parameters. Principal component analysis (PCA) was used in different crops such as *Brassica napus* L. (Shuvo 2021), *Zea mays* L. (Andrade et al. 2020), *Glycine max* (Azam et al. 2020), *Triticum aestivum* (Uzair et al. 2022) and *Oryza sativa* (Das et al. 2019) to identify salt tolerant plants.

4 Conclusion

Salinity, one of the most common abiotic stressors, often has detrimental effects on crop production capacity by reducing yield and quality, especially in arid and semi-arid regions of the world. Salt stress causes a decrease in plant height, shoot and root biomass root length, plant height, leaf area, and the overall development process in tomato plants. To overcome

Table 8 The effects of graft combinations and different NaCl levels (1.5 dSm⁻¹ and 8 dSm⁻¹) on leaf boron (B), copper (Cu), sodium (Na) and chlorine (Cl) contents of tomato plants

Graft Combinations	B (mg kg ⁻¹)			Cu (mg kg ⁻¹)			%D			Na (mg kg ⁻¹)			Cl (mg kg ⁻¹)		
	Control	NaCl	%D	Control	NaCl	%D	Control	NaCl	%D	Control	NaCl	%D	Control	NaCl	%D
	RootStock/Scion	Control	NaCl	%D	Control	NaCl	%D	Control	NaCl	%D	Control	NaCl	%D	Control	NaCl
SP3/H2274	2.61a-c	1.56ef	-40	1.15	0.04e	-97	0.87ab	16.34b	1784	4.19a-e	75.36bc	1700	4.19a-e	75.36bc	1700
SP4/H2274	2.69a-c	8.16a	204	1.21	0.03e	-98	2.1ab	17.69ab	742	4.72a-d	82.48bc	1649	4.72a-d	82.48bc	1649
SP5/H2274	2.05bc	2.86de	39	1.40	0.03e	-98	1.23ab	16.37b	1230	3.91a-e	81.95bc	1995	3.91a-e	81.95bc	1995
SH1/H2274	2.45a-c	2.19ef	-11	1.34	0.04e	-97	1.82ab	17.31ab	852	2.44b-f	29.79de	1121	2.44b-f	29.79de	1121
SH3/H2274	5.25a	5.52b	5	1.16	0.03e	-97	1.37ab	15.66b	1040	2.06c-f	49.12 cd	2286	2.06c-f	49.12 cd	2286
SH5/H2274	3.00a-c	2.28ef	-24	0.85	0.04e	-95	1.67ab	15.88b	853	0.87ef	26.18de	2918	0.87ef	26.18de	2918
L×SP5/H2274	4.66a-c	1.55ef	-67	0.78	0.05e	-94	1.75ab	17.39ab	894	1.60c-f	48.40 cd	2920	1.60c-f	48.40 cd	2920
L×SH3/H2274	4.39a-c	5.32bc	21	1.08	0.04e	-96	1.77ab	17.62ab	894	1.57c-f	37.51de	2289	1.57c-f	37.51de	2289
Mean	3.39	3.68	16	1.12	0.04	-96	1.57	16.78	1036	2.67	53.85	2110	2.67	53.85	2110
SP3/Galaxy	4.95ab	1.95ef	-61	0.84	0.05e	-94	1.68ab	9.16c	446	1.26ef	21.95de	1644	1.26ef	21.95de	1644
SP4/Galaxy	5.09ab	1.34f	-74	0.74	0.06e	-92	1.76ab	8.34c	375	1.50d-f	20.72de	1280	1.50d-f	20.72de	1280
SP5/Galaxy	2.44a-c	4.41bc	81	1.09	0.05e	-95	2.13ab	13.05bc	513	0.57f	34.21de	5875	0.57f	34.21de	5875
SH1/Galaxy	2.98a-c	1.57ef	-47	1.08	0.08de	-93	2.56a	13.12bc	412	1.16ef	12.51e	977	1.16ef	12.51e	977
SH3/Galaxy	2.97a-c	1.30f	-56	1.11	0.16 cd	-86	2.23ab	15.09b	578	3.39a-f	18.29de	440	3.39a-f	18.29de	440
SH5/Galaxy	1.74c	4.44bc	155	1.26	0.23bc	-82	1.38ab	17.73ab	1187	5.57ab	49.33 cd	785	5.57ab	49.33 cd	785
L×SP5/Galaxy	1.66c	1.36f	-18	1.08	0.26ab	-76	0.21b	16.55ab	711	4.17a-e	20.41de	389	4.17a-e	20.41de	389
L×SH3/Galaxy	3.09a-c	0.99f	-68	1.16	0.28ab	-76	0.25b	16.6ab	6586	3.66a-f	27.84de	661	3.66a-f	27.84de	661
Mean	3.11	2.17	-11	1.05	0.15	-87	1.52	13.71	2226	2.66	25.66	1507	2.66	25.66	1507
H2274 (ungrafted)	2.48a-c	4.15 cd	67	1.41	0.32a	-77	0.24b	21.25a	8937	6.19a	119.80a	1834	6.19a	119.80a	1834
Galaxy (ungrafted)	2.46a-c	1.19f	-52	1.44	0.33a	-77	0.28ab	21.24a	7397	4.86a-c	101.87ab	1998	4.86a-c	101.87ab	1998
Rootstock	***			***			***			***			***		
Scion	***			n.s.			***			***			***		
NaCl	n.s.			***			***			***			***		
Rootstock×Scion	***			**			***			***			***		
Rootstock×NaCl	***			***			***			***			***		
Scion×NaCl	***			*			***			***			***		
Rootstock×Scion×NaCl	n.s.			n.s.			***			***			***		

% I:Increase, %D:Decrease, IL:Ion leakage, ns:non-significant Values denoted by different letters are significantly different between graft combinations within both columns at $P < 0.05$. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

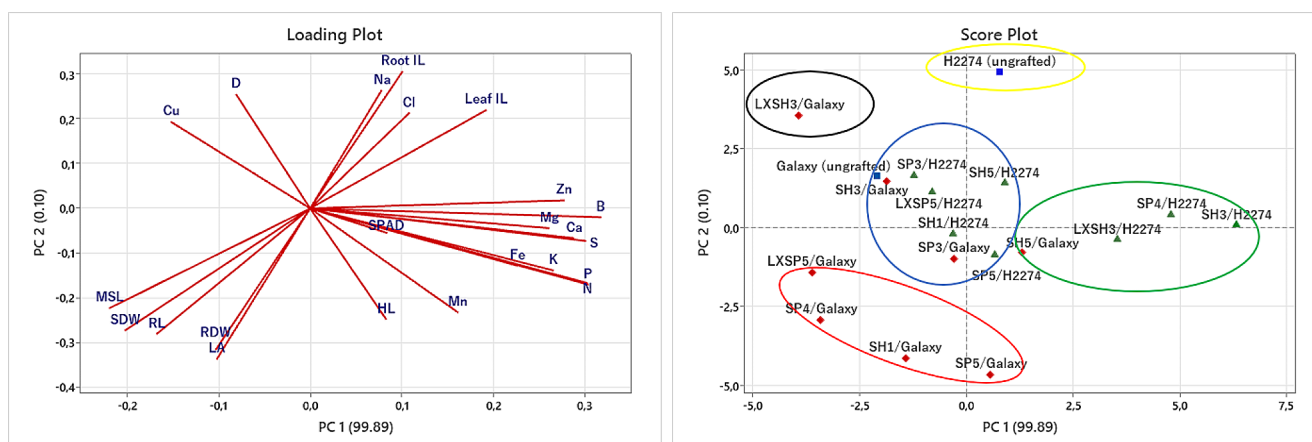


Fig. 4 PCA formed by physiological and biochemical contents of plants under salt stress. PCA:principal component analysis, RL:root length, RDW:root dry weight,SDW:shoot dry weight, MSL:main stem length, LA:leaf area, HL: healthy leaf, D:damaged leaf, Root IL:

this problem grafting with salt-tolerant rootstocks can be an effective management strategy to improve the salt tolerance of crop plants. The selection of salt-tolerant rootstocks is an important strategy to improve salt tolerance. Testing and screening of existing commercial and wild relatives under salt stress conditions is a prerequisite for grafting. Due to incompatibility in crossing and linkage drag, it is cumbersome to transfer salt tolerant genes from wild to cultivated tomato cultivars. However, these wild species could be exploited as salt-resistant rootstocks for grafting susceptible but high yielding commercial tomato cultivars. Plants grafted on wild tomato rootstocks (*S. habrochaites* and *S. pimpinellifolium*) used in our study showed more tolerance to salt stress than ungrafted plants. Plants grafted on wild rootstocks were less affected in terms of biomass development by having less Na and Cl in the leaf than ungrafted plants. Biotic and abiotic stress factors vary in different locations or countries, so the combination of scion and rootstock should be carefully selected to obtain maximum benefit from grafting.

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root ion leakage, Leaf IL:leaf ion leakage, N:nitrogen, P:phosphorus, K:potassium, Ca:calcium, Mg:magnesium, S:sulphur, Mn:manganese, Fe:iron, Zn:zinc, B:boron, Cu:copper, Na:sodium and Cl:chlorine

Declarations

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

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

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