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Salt Tolerant Eggplant Rootstocks Modulate Sodium Partitioning in Tomato Scion and Improve Performance under Saline Conditions

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Abstract: Grafting on salt tolerant eggplant rootstocks can be a promising approach for enhancing the salinity tolerance of tomato. In this study, the performance of tomato cv. Kashi Aman grafted on two salt tolerant eggplant rootstocks (IC-111056 and IC-354557) was evaluated against non-grafted control under saline (EC_{iw} 6 and 9 dS m^{-1}) and non-saline (EC_{iw} ~1 dS m^{-1}) irrigation for 2 years. Grafting improved tomato plant performance under salt stress. Moreover, rootstock IC-111056 outperformed IC-354557. An increase in the average fruit yield of grafted plants compared with non-grafted control at 6 and 9 dS m^{-1} was 24.41% and 55.84%, respectively with rootstock IC-111056 and 20.25% and 49.08%, respectively with IC-354557. Grafted plants maintained a superior water status under saline irrigation, evidenced with the relative water content and chlorophyll SPAD index, along with higher proline and antioxidant enzyme activities (superoxide dismutase, catalase, and ascorbate peroxidase). Rootstocks mediated the partitioning of toxic saline ions in the scions by promoting higher Na^+ accumulation (14% of mean accumulation) in the older leaves and lower (24%) in the younger leaves of grafted plants. This resulted in higher K^+/Na^+ ratios within the younger (active) leaves of the grafted plants. Our study demonstrates that grafting tomato seedlings on selected salt tolerant eggplant rootstocks is a viable alternative for improving plant physiological status and fruit yield under salt stress, through favorable modulation of salt ion partitioning in the scions.

Keywords: tomato grafting; Na^+ partitioning; salinity tolerance; antioxidant enzymes; fruit yield

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1. Introduction

Among abiotic stresses, salinity is one of the critical stresses inhibiting plant growth and crop yields. Globally, salt-affected soils represent 7% of the total area, where saline and alkaline soils constitute about more than 1100 million hectares of land [1]. Salinity has affected approximately 20–33% of agricultural land across the world [2].

Soil salinity often occurs concomitantly with saline ground water in arid and semi-arid regions, exacerbating the effect on crop growth. Higher levels of salt in soil reduce the productivity of most of the agricultural crops, including vegetables, with the latter as more salt sensitive. The salinity-induced stress can be triggered by the excessive use of poor-quality ground water for irrigation, along with climate change and excessive

irrigation associated with intensive farming [3]. The salinity threshold (EC_t) of most of the vegetable crops is very low, generally between EC_t 1 to 2.5 dS m^{-1} [4].

Tomato (*Solanum lycopersicum* L.) is cultivated and consumed as fresh and processed food, and ranks second after potato. Tomatoes are reported as moderately sensitive (threshold limit up to 2.5 dS m^{-1}) to salt stress, and thus high salinity can substantially limit the productivity [5,6] through decreased plant height, shoot–root biomass [7], oxidative stress, and inhibition of photosynthesis [8]. The high salinity affects several physiological and biochemical processes due to ion toxicity, which is caused by the high accumulation of Na^+ and Cl^- ions combined with low levels of K^+, Ca^{2+}, SO_4^{2-}, and NO^{3-} ions, in addition to osmotic stress [9,10].

Improvement in tomato salt tolerance through modern breeding and biotechnological approaches has been limited since salt tolerance is a complex trait involving several quantitative and environmental factors [11,12]. Although advanced genetic mapping strategies and QTL analysis improved the understanding of the genetics of salt tolerance and related traits, limited success was achieved through marker-assisted selection. The dynamic nature of salinity with respect to time and space, as well as limited experimental designs restrict the complete study of genotype–environment interactions [13]. Therefore, the crop breeding program can be complemented with a suitable management option, such as grafting tomato on appropriate salt tolerant rootstocks [14].

Grafting has been reported as a rapid method for enhancing salt tolerance in vegetable crops [10]. Although grafting was initially used for improving crop tolerance against biotic stress, additional evidence proved the association of grafting with yield improvement under abiotic stresses (salinity, temperature, flooding), and better water and nutrient use efficiency [15,16]. Grafting counteracts the salinity effects by maintaining low Na^+/K^+ ratios in the shoot and improves leaf stomatal conductance [17]. The behavior of the rootstock in different plant species influences the metabolic processes of the scion leading to tolerance [18].

Most of the Solanaceous crops have been used as a rootstock for tomato cultivation to manage abiotic stress [19]. *Solanum habrochaites* and other wild species provide a broad spectrum of tolerance [20]. Previously, a tomato scion was grafted on a tomato rootstock for salt stress tolerance [21–23]. However, only a small amount of information is available for salt tolerance of tomato grafted on an eggplant rootstock. Therefore, this study was planned to explore (i) the agronomic performance of high yielding tomato cultivars grafted on two eggplant rootstocks, and (ii) assess the biochemical and physiological changes resulting from scion–rootstock interactions under saline water irrigation.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

The seeds of eggplant rootstocks IC-354557 and IC-111056 (indigenously collected and registered at the National Bureau of Plant Genetic Resources, New Delhi, India) were sown during the winter season (October) of 2017 and 2018. One week later, tomato seeds of cv. Kashi Aman used as a scion, were sown. These two eggplant rootstocks were reported as tolerant to abiotic stress, specifically waterlogging stress [24] and salinity stress (EC_{iw}) of 9 dS m^{-1} (our unpublished data). Kashi Aman is a high yielding round-fruited tomato cultivar that is salt sensitive. Single seeds of rootstocks were sown in small disposable 100 mL plastic cups, while tomato seeds were sown in standard 20 cm pots. The potting mixture for both species comprised of soil, coco peat, vermiculite, and perlite in 3:1:1:1 ratio. Light irrigations were provided daily and the seedlings of rootstocks and scions were raised for 30 and 23 days, respectively. At this stage, the plants attained the stem thickness of 1.5–3.0 mm and each plant had at least 2–3 true leaves.

The splice grafting technique was used to graft 23-day-old tomato scions on 30-day-old eggplant rootstocks. About 7 mm of slanting cut was made in the rootstock and scion to allow for a perfect union. Grafting union was supported with grafting clips

and grafted plants were immediately transferred to a grafting chamber with very low light, high humidity (more than 85%), and moderate temperature (24–30 °C). After 5–7 days, the grafted plants were shifted to a polyhouse covered with a shading net for acclimatization. Sprouts from rootstocks were removed at regular intervals. Grafted plants were transplanted to pots 17–18 days after grafting.

The grafted and non-grafted tomato plants were transplanted in 24 cm diameter pots filled with 16 kg topsoil (sandy loam with 0.45% organic carbon) during the winter season (20 December) of 2017–2018 and (22 December) of 2018–2019. An estimated dose of fertilizer, i.e., 3.75, 2.0, 2.5 g of N, P, and K was applied. However, half of the quantity of N and a full dose of P and K were added at the time of pot filling. In addition, the remaining quantities of N were applied in an equal dose at 30 and 60 days after transplanting. Each replicate consisted of nine plants, i.e., three non-grafted tomato plants, and three each of grafted on eggplant rootstocks IC-111056 and IC-354557. Natural saline ground water (EC_{iw}~18 dS m^{-1}) available at the Nain experimental farm of the Institute situated at Panipat (Haryana), India was used to prepare the saline water of desired salinity (EC_{iw} 6 and 9 dS m^{-1}) by diluting with good quality water, while for control treatment, the best available water of EC_{iw}~1 dS m^{-1} was used. Saline ground water of Nain farm had neutral pH with a dominance of Na^+, Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2-} ions. Saline irrigation for different treatments was first applied 10 days after transplanting. Further irrigation was scheduled based on 100% evapotranspiration (ET) and 21 irrigations were provided during the whole crop period. At the time of final harvesting, soil samples were collected to measure the build-up of soil salinity in each treatment (Table 1).

Table 1. Soil status: Before and after the experiment.

Parameters	Initial Soil Status	Control	EC_{iw} 6 dS m^{-1}	EC_{iw} 9 dS m^{-1}
		2017		
EC_e	0.35	0.42	6.14	8.86
pH_s	7.05	7.18	7.25	7.29
		2018		
EC_e	0.30	0.44	6.21	8.94
pH_s	7.08	7.24	7.31	7.38

2.2. Fruit Yield and Quality Parameters

The plant height of the three plants from each replicate was measured before the last picking date. When the fruits turned slightly pink or red, they were harvested manually every 3–5 days and the total yield/plant (g) was calculated. The average fruit weight (g) was calculated using the data of 10 fruits from each replicate. Total soluble solids (TSS) of a representative sample size (four fruits per treatment) were measured on a portable hand refractometer (Erma Inc., Tokyo, Japan) as °Brix at 20 °C.

2.3. Physiological and Biochemical Traits

All of the physiological and biochemical parameters were determined at the onset of flowering. The leaf greenness SPAD index was measured between 09:00 to 11:00 h using SPAD-502 (Konica Minolta Corp., Solna, Sweden) on the intact top of three fully opened leaves. The relative water content (RWC) was measured in detached third and fourth leaves from the top at 10:00–12:00 h [25].

$$RWC = (FW - DW)/(FW - TW) \times 100$$

where FW is the leaf fresh weight, DW is the leaf dry weight, and TW is the turgid leaf weight.

The proline content of fresh leaves was estimated using the ninhydrin reagent [26] and quantified as mg g^{-1} fresh weight. Antioxidant enzymes, superoxide dismutase (SOD), and ascorbate peroxidase (APX) were extracted from leaves in a 0.1 M phosphate

buffer (pH 7.5) consisting of 5% (*w*/*v*) polyvinylpolypyrrolidone, 1 mM EDTA, and 10 mM β-mercapto-ethanol, according to the modified method [27]. Peroxidase (POX) was extracted in a 0.01 M phosphate buffer (pH 7.5) with 3% (*w*/*v*) polyvinylpolypyrrolidone. The SOD enzyme activity was estimated as its ability to inhibit light-induced conversion of nitroblue tetrazolium (NBT) to formazan [28]. APX was quantified as one unit of APX corresponding to 1.0 O.D. change per min [29]. The POX activity was calculated as 1.0 μmol of H_2O_2 utilized per min [30]. The catalase (CAT) activity was measured for 1 min based on the decomposition of H_2O_2 at 240 nm [31].

2.4. Ionic Content

Na^+ and K^+ contents of leaves and roots were determined at the harvest stage. Properly oven dried and ground fine samples were digested in di-acid mixture for estimation of Na^+ and K^+ contents using the flame photometer (PFP7, Jenway, Bibby Scientific, Stone, UK).

2.5. Statistical Analysis

The experiment was conducted in a randomized complete block design replicated five times with three plants per replicate. Morphological and biochemical observations were tested for normality and variance homogeneity through the Shapiro–Wilk test and Levene's test. Additionally, if necessary, appropriate transformations were applied. All of the means of morphological and biochemical traits were compared using the two-way ANOVA (grafted plants × salinity levels) and repeated measures analysis, by the Type III sum of squares of GLM procedure on SAS (version 9.3, SAS Institute Inc., Cary, NC, USA). ANOVA tables for different parameters are provided in Supplementary Tables S1–S3. To discriminate significant differences between the grafted and non-grafted combinations, the least significant difference (LSD) test was used at probability levels of 0.05 and 0.01. The R program was used for the correlation matrix [32] and data were analyzed using Corrplot [33] package.

3. Results

3.1. Yield and Quality Traits

The fruit yield per plant, average fruit weight, and plant height decreased significantly with gradient salinity levels in both non-grafted and grafted plants, with a pronounced effect at EC_{iw} 9 dS m^{-1} during the two years (Table 2). The interaction effect was significant for fruit yield per plant and average fruit weight between the saline treatment and different rootstocks, indicating the differential response of each rootstock to increasing salinity levels. For plant height, the salinity–rootstock interaction was non-significant. During 2017, under control conditions, the yield was at par in plants grafted on rootstock IC-111056 and non-grafted plants, whereas it was significantly reduced (7.35%) in plants grafted on rootstock IC-354557. On the contrary, at an increased salinity level of 6 dS m^{-1}, plants grafted on rootstocks IC-111056 and IC-354557 produced 24.07% and 21.08% higher yield than non-grafted pants. Furthermore, the difference in the fruit yield per plant for both rootstocks was non-significant. As the salinity level further increased to 9 dS m^{-1}, the yield considerably reduced in comparison with control and 6 dS m^{-1} salinity level. However, the fruit yield produced by plants grafted on rootstocks IC-111056 (58.68%) and IC-354557 (50.37%) was significantly more in comparison with non-grafted plants. The fruit yield was 16.74% more with rootstock IC-111056 than IC-354557.

Table 2. Effect of salinity and rootstock combinations on yield components and physiological parameters of tomato (cv. Kashi Aman).

Salinity Treatment (dS m^{-1})	Rootstock	Plant Height (cm)	Fruit Yield (g plant^{-1})	Av. Fruit wt. (g)	TSS (°Brix)	SPAD Index	RWC (%)
			2017				
Control	Non-grafted	64.33a	2577.3a	64.25a	4.35a	60.62b	83.30 a
	IC-111056	69.23a	2518.0a	63.12a	4.40a	62.63a	82.31 a
	IC-354557	62.90a	2388.0b	63.83a	4.38a	62.39ab	83.06 a
EC_{iw} 6	Non-grafted	58.33a	1420.8b	56.10b	5.10a	58.79c	70.91 a
	IC-111056	60.66a	1871.3a	59.23a	5.14a	60.47a	76.63 a
	IC-354557	57.66a	1800.4a	60.22a	5.16a	59.51b	76.12 a
EC_{iw} 9	Non-grafted	48.55a	522.8c	41.37b	4.45a	50.77c	64.82 a
	IC-111056	55.42a	1265.3a	48.63a	4.32b	57.19a	71.20 a
	IC-354557	51.88a	1053.4b	47.62a	4.36b	54.07b	70.03 a
			Significance				
Salinity		**	**	**	**	**	**
Rootstock		**	**	**	ns	**	ns
Salinity x rootstock		ns	**	**	**	**	ns
			2018				
Control	Non-grafted	64.88 a	2243.10b	60.15b	4.46a	58.21b	83.44a
	IC-111056	67.35a	2301.87a	61.87a	4.42a	59.35a	82.63 a
	IC-354557	63.97a	2227.15b	62.05a	4.35a	58.54a	84.68 a
EC_{iw} 6	Non-grafted	56.48a	1340.77c	54.30a	5.16a	55.09b	72.54 a
	IC-111056	58.62a	1781.70a	55.82a	5.08a	57.47a	77.10 a
	IC-354557	56.33a	1664.05b	54.53a	5.14a	56.17b	77.78 a
EC_{iw} 9	Non-grafted	48.37a	532.18c	39.00c	4.37a	49.42c	66.45 a
	IC-111056	52.82a	1132.25a	46.53a	4.25a	54.98a	72.86 a
	IC-354557	50.55a	1019.10b	43.52b	4.31a	52.67b	71.35 a
			Significance				
Salinity		**	**	**	**	**	**
Rootstock		**	**	**	ns	**	ns
Salinity x rootstock		ns	**	**	ns	**	ns

Means followed by different letters within a column and under a specific treatment effect are significantly different at $p = 0.05$ by the least significant difference (LSD) test; ns, ** non-significant or significant at $p = 0.01$, respectively.

During 2018, at salinity levels of 6 and 9 dS m^{-1}, grafted plants on rootstock IC-111056 produced higher yield of 24.75% and 53%, respectively, whereas on rootstock IC-354557 the grafted plants produced higher yield of 19.43 % and 47.78%, respectively than non-grafted plants. The yield was higher by 6.6% and 10%, respectively with rootstock IC-111056 than IC-354557 at the salinity level of 6 and 9 dS m^{-1} (Table 2). Similarly, the plants grafted on rootstock IC-111056 performed better for the average fruit weight at different salinity levels. However, total soluble solids were observed as significantly higher in the year 2017 only at different salinity treatments.

3.2. Physiological Traits

The SPAD index and RWC were significantly affected by different levels of salinity, while a significant interaction effect with rootstock alone was observed for SPAD index only (Table 2). The SPAD index and RWC were highest in plants under control condition followed by plants stressed with saline water at 6 and 9 dS m^{-1} salt concentrations. Grafted plants on rootstocks IC-111056 and IC-354557 had more leaf greenness SPAD index than non-grafted rootstocks (Kashi Aman) under control, as well as at EC_{iw} 6 and 9 dS m^{-1}. The SPAD index values of 1.58–2.26% and 4.20–5.46%, respectively were sig-

nificantly higher for rootstock IC-111056 than IC-354557 at 6 and 9 dS m^{-1}, during the two years.

3.3. Biochemical Traits

The relative concentrations of proline and the activities of CAT, APX, SOD, and POX enzymes were significantly affected by gradient salinity treatments (Table 3). The grafted and non-grafted plants showed higher proline content, as well as CAT, APX, SOD, and POX activities under salinity treatments, although they were significantly enhanced in grafted plants only. For proline content, differences between the two rootstocks were significant for 9 dS m^{-1} treatment only. At 9 dS m^{-1}, higher APX and CAT activities of 11–14% and 13–14% were observed in rootstock IC-111056 grafted plants than non-grafted plants in both years. No significant differences were seen in POX and SOD activities between the grafted and non-grafted plants in control condition. However, under salt treatment, the grafted plants showed significantly higher SOD and POX activities than non-grafted plants (Table 3).

Table 3. Effect of salinity and rootstock combinations on biochemical parameters of tomato (cv. Kashi Aman).

Salinity Treatment	Rootstock	Proline	CAT	APX	SOD	POX
(dS m^{-1})		(μg g^{-1} FW)		(Units g^{-1} FW)		
			2017			
Control	Non-grafted	387.63a	12.86b	75.50b	185.83a	25.23a
	IC-111056	364.38b	13.68ab	77.64a	173.40a	26.07a
	IC-354557	337.51c	14.38a	75.96b	181.73a	23.83a
EC_{iw} 6	Non-grafted	754.17b	13.32c	145.80b	246.87b	32.07b
	IC-111056	943.73a	16.13a	160.20a	271.03a	39.67a
	IC-354557	936.53a	15.42b	156.40a	236.13a	38.70a
EC_{iw} 9	Non-grafted	991.25c	15.36c	157.24c	294.13c	46.13b
	IC-111056	1324.80a	17.84a	176.30a	333.33a	56.57a
	IC-354557	1110.03b	16.61b	168.45b	315.43b	54.70a
			Significance			
Salinity		**	**	**	**	**
Rootstock		**	**	**	**	**
Salinity x rootstock		**	**	**	**	**
			2018			
Control	Non-grafted	346.40a	14.27b	68.30b	196.03a	22.25a
	IC-111056	351.39a	14.07b	70.90a	190.90a	21.05a
	IC-354557	353.90a	15.13a	70.30a	188.60a	20.47a
EC_{iw} 6	Non-grafted	709.03b	15.22b	136.95b	245.13b	31.30b
	IC-111056	920.40a	17.46a	148.32a	266.07a	41.57a
	IC-354557	898.45a	17.25a	145.70a	258.73a	39.12b
EC_{iw} 9	Non-grafted	1009.15c	16.51c	144.30c	295.57b	44.27b
	IC-111056	1343.61a	19.32a	167.50a	266.30a	59.48a
	IC-354557	1123.75b	18.81b	160.30b	314.57a	55.18a
			Significance			
Salinity		**	**	**	**	**
Rootstock		**	**	**	**	**
Salinity x rootstock		**	**	**	*	*

Means followed by different letters within a column and under a specific treatment effect are significantly different at $p = 0.05$ by the least significant difference (LSD) test; *, ** significant at $p = 0.05$ or 0.01, respectively.

3.4. Ionic Content and Ion Partitioning

Salinity and rootstock combinations significantly affected Na^+ and K^+ contents and Na^+/K^+ ratios in both roots and shoots along with the salinity–rootstock interaction (Table 4). With the increase in salinity, a significant increase in Na^+ content in the roots and shoots, while a decrease in K^+ content and K^+/Na^+ ratio was observed in both grafted and non-grafted plants (Table 4). In 2017, for the 6 dS m^{-1} salt treatment, the Na^+ content in shoots was lower by 9.98% and 14.61%, respectively in plants grafted on rootstocks IC-111056 and IC-354557, whereas the concentration in roots was lower by 5.65% and 5.05%, respectively. Similarly, during 2018, the Na^+ content was lower by 7.91% and 10.79%, respectively in shoots and lower by 10.62% and 5.01%, respectively in roots of IC-111056 and IC-354557 grafted plants than non-grafted plants. In comparison, at the 9 dS m^{-1} salinity level, the Na^+ content in shoots of IC-111056 and IC-354557 grafted plants was lower by 3.40% and 2.61%, respectively than non-grafted plants in 2017 and lower by 2.43% and 2.19%, respectively in 2018. Whereas, the Na^+ content in roots of IC-111056 and IC-354557 grafted plants was lower by 12.03% and 5.84%, respectively than non-grafted plants in 2017 and lower by 10.03% and 3.05%, respectively in 2018.

The roots and shoots of tomato plants, grafted on rootstock IC-111056 showed a significantly higher amount of K^+ content than the plants grafted on IC-354557 and non-grafted plants under salt treatment (EC_{iw} 6 and 9 dS m^{-1}) (Table 4). Although, in grafted plants, at salinity of 6 and 9 dS m^{-1}, higher K^+/Na^+ ratios were found in roots and shoots, except in roots at EC_{iw} 9 dS m^{-1}.

Table 4. Effect of salinity treatments on Na^+ and K^+ contents as well as K^+/Na^+ ratios of root and shoot parts of grafted and non-grafted tomato plant (cv. Kashi Aman).

Salinity Treatment		Element (mg g^{-1} DW)					
	Rootstock	Na^+		K^+		K^+/Na^+	
(dS m^{-1})		Root	Shoot	Root	Shoot	Root	Shoot
		2017					
Control	Non-grafted	4.75b	4.20a	17.02b	13.32a	3.58b	3.17a
	IC-111056	4.85a	4.17a	18.05a	13.56a	3.72a	3.25a
	IC-354557	4.65c	4.16a	17.24b	12.22b	3.71a	2.94b
EC_{iw} 6	Non-grafted	8.32a	5.61a	15.31b	11.77b	1.84c	2.10b
	IC-111056	7.85b	5.05b	15.86a	12.44a	2.02a	2.46a
	IC-354557	7.90b	4.79c	15.23b	11.89b	1.93b	2.48a
EC_{iw} 9	Non-grafted	11.64a	8.81a	10.72b	9.89b	0.92c	1.12b
	IC-111056	10.24c	8.51b	11.27a	10.20a	1.10a	1.20a
	IC-354557	10.96b	8.58b	11.25a	9.77b	1.03b	1.14ab
		Significance					
Salinity		**	**	**	**	**	**
Rootstock		**	**	**	**	**	**
Salinity x rootstock		**	**	**	**	**	**
		2018					
Control	Non-grafted	4.82a	4.18b	16.66c	13.40a	3.21a	3.46b
	IC-111056	4.93a	4.26a	17.84a	13.46a	3.16b	3.62a
	IC-354557	4.86a	4.30a	17.46b	13.12b	3.05c	3.59a
EC_{iw} 6	Non-grafted	8.19a	5.56a	15.12c	11.58c	2.08b	1.85c
	IC-111056	7.32c	5.12b	15.48a	12.28a	2.40a	2.11a
	IC-354557	7.78b	4.96b	15.32b	12.05b	2.43a	1.97b
EC_{iw} 9	Non-grafted	11.16a	8.64b	10.56b	9.78c	1.13b	0.95c
	IC-111056	10.04c	8.43a	11.24a	10.08a	1.20a	1.12a
	IC-354557	10.82b	8.45a	11.18a	9.92b	1.17ab	1.03b
		Significance					
Salinity		**	**	**	**	**	**
Rootstock		**	**	**	**	**	*
Salinity x rootstock		**	**	**	**	**	**

Means followed by different letters within a column and under a specific treatment effect are significantly different at $p = 0.05$ by the least significant difference (LSD) test; *, ** significant at $p = 0.05$ or 0.01, respectively.

The Na^+ and K^+ contents as well as the K^+/Na^+ ratios in leaves were significantly affected by salinity level, rootstock combinations, leaf orientation, salinity × rootstock, salinity × leaf orientation, rootstock × leaf orientation, year × leaf orientation, year ×

salinity × leaf orientation during the two seasons. As the salinity level increased, a respective increase in Na^+ content as well as a decrease in K^+ content and K^+/Na^+ ratios in bottom (BL), middle (ML), and upper (UL) leaves were observed during the two seasons (Table 5).

Leaves of grafted plants had lower Na^+ content in younger leaves and higher in older leaves at salinity levels of 6 and 9 dS m^{-1}. The average Na^+ content of young leaves of plants grafted on rootstock IC-111056 was lower by 19.87% and 27.50%, respectively than non-grafted plants at EC_{iw} 6 and 9 dS m^{-1}. On the contrary, the Na^+ content of older leaves of plants grafted on rootstock IC-111056 was higher by 14.89% and 13.82%, respectively than non-grafted plants at both salinity levels (Table 5). In grafted plants, the K^+ content was high in upper and middle leaves and lower in bottom leaves under different salt treatments. Therefore, K^+/Na^+ ratios of grafted plants were significantly high in upper and middle leaves and low in bottom leaves than non-grafted plants. The plants grafted on rootstock IC-111056 showed significantly higher K^+/Na^+ ratios in upper and middle leaves compared to non-grafted plants as well as plants grafted on rootstock IC-354557.

Table 5. Effect of salinity and rootstock on ion partitioning in leaves orientation.

Salinity Level (dS m^{-1})	Rootstock	Na^+ (mg g^{-1} DW)			K^+ (mg g^{-1} DW)			K^+/Na^+		
		BL	ML	UL	BL	ML	UL	BL	ML	UL
2017										
Control	Non-grafted	4.24aB	4.06aB	3.88aA	10.37bC	16.56bB	20.54cA	2.45aC	4.08cB	5.29cA
	IC-111056	4.30aC	3.91aB	3.35bA	10.44bC	16.19cB	29.01aA	2.43aC	4.14bB	6.57aA
	IC-354557	4.47aC	3.98aAB	3.69aA	10.68aC	17.13aB	28.29bA	2.39aC	4.30aB	5.77bA
EC_{iw} 6	Non-grafted	6.22cC	5.38aB	4.47aA	9.74aC	14.49bB	18.61cA	1.57aC	2.69bB	3.72cA
	IC-111056	7.29aC	5.17bB	3.80cA	9.27bC	14.91aB	22.08aA	1.27cC	2.88aB	4.76aA
	IC-354557	6.89bC	5.50aAB	3.96bA	9.75aC	15.12aB	20.40bA	1.42bC	2.75bB	4.56bA
EC_{iw} 9	Non-grafted	8.06cC	7.64aB	6.84aA	8.93aC	12.01cB	13.55cA	1.11aC	1.31bB	1.69cA
	IC-111056	9.88aC	7.04bB	5.06cA	8.29cC	13.19aB	17.77aA	0.84abC	1.59aB	2.72aA
	IC-354557	9.21bC	7.72aB	5.69bA	8.58bC	12.39bB	15.93bA	0.93aC	1.60aB	2.27bA
2018										
Control	Non-grafted	4.38bC	4.04bB	3.76aA	11.06aC	16.21aB	19.42cA	2.53aC	4.01aB	5.16cA
	IC-111056	4.62aC	4.16aB	3.29cA	10.94aC	15.97bB	26.30aA	2.37abC	3.84bB	6.47aA
	IC-354557	4.48bC	4.03bB	3.42bA	10.88aC	16.27aB	25.52bA	2.43aC	4.04aB	6.00bA
EC_{iw} 6	Non-grafted	6.30cC	5.24aB	4.93aA	9.95aC	14.69aB	16.34cA	1.58aC	2.80bB	3.31cA
	IC-111056	7.42aC	5.32aB	3.71cA	9.44cC	14.89aB	23.10aA	1.27bC	2.80bB	4.85aA
	IC-354557	7.02bC	5.16aB	4.02bA	9.66bC	14.74aB	21.33bA	1.38bC	2.86aB	4.31bA
EC_{iw} 9	Non-grafted	7.98cC	7.54aB	7.28aA	8.74aB	12.26cA	12.08cA	1.10aC	1.36bB	1.66cA
	IC-111056	8.79aC	6.92cB	5.17cA	8.42bC	13.08aB	17.32aA	0.96bC	1.75aB	2.58aA
	IC-354557	8.24bC	7.22bB	6.14bA	8.54abC	12.48bB	16.14bA	1.04abC	1.68aB	2.14bA
ANOVA										
Salinity			***			***			***	
Rootstock			***			***			***	
Leaf orient			***			***			***	
Salinity x Rootstock			*			***			***	
Salinity x Leaf orient			***			***			***	
Rootstock x leaf orient			***			***			***	
Year x Salinity			ns			***			***	
Year x Rootstock			*			ns			*	
Year x leaf orient			*			***			***	
Salinity x Rootstock x leaf orient			***			***			***	
Year x Salinity x Rootstock			ns			***			***	
Year x Salinity x leaf orient			*			***			***	
Year x Rootstock x leaf orient			ns			***			ns	

Year x Salinity x Rootstock x leaf orient	ns	***	***

BL: Bottom leaves; ML: Middle leaves; UL: Upper leaves; the small letter is for comparing rootstocks grafting and the capital letter is for comparing leaf orientation; values are the means of three replicate samples. Means followed by different letters within a column and row and under a specific treatment effect are significantly different and separated using the least significant difference (LSD) test; ns, *, *** non-significant or significant at $p < 0.05$, 0.001, respectively.

3.5. Trait Association

Trait association revealed through Pearson's correlation coefficients indicated that horticultural traits, such as plant height, fruit weight, and fruit yield were significantly positively correlated ($p < 0.01$) with SPAD, RWC, K^+ concentration, and K^+/Na^+ ratios in different plant parts. Conversely, these three traits were significantly negatively associated with antioxidant enzymes (CAT, APX, SOD, and POX), organic osmolyte (proline), and Na^+ content in root, shoot, and leaves. This suggested that K^+ partitioning may assist in survival under salinity stress. Furthermore, the total soluble sugar (TSS) showed a negative association with APX and Na^+ concentration in plant shoots (Figure 1). Proline accumulation showed a strong positive association with antioxidant enzymes (CAT, APX, SOD, and POX) and Na^+ compartmentation in organs and a strong negative association with K^+ concentration and K^+/Na^+ ratios in different organs. However, the SPAD index and RWC showed a reverse trend, i.e., they were negatively associated with antioxidant enzymes (CAT, APX, SOD, and POX) and Na^+ compartmentation and positively associated with K^+ concentration and K^+/Na^+ ratios in different organs.

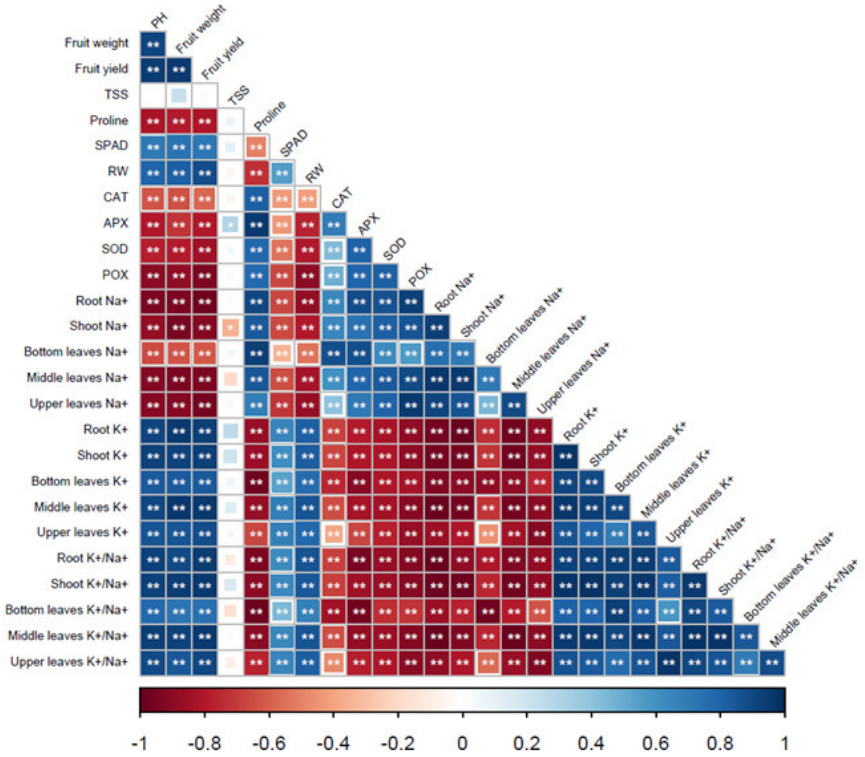

Figure 1. Association between horticultural and biochemical traits of tomato under saline environment.

4. Discussion

It is a well-documented fact that plant growth and yield decrease with the increasing salt concentrations. Grafting of salt sensitive plants on tolerant rootstocks provides an alternate and/or complementary mechanism to improve stress tolerance and

economic yield. In the present study, tomato plants grafted on two different eggplant rootstocks produced more fruit weight and yield per plant than non-grafted plants under saline water irrigation (EC_{iw} 6 and 9 dS m^{-1}). The grafting of salt sensitive tomato plants on salt tolerant eggplant rootstocks improved the salt tolerance of tomato plants through a combination of physiological and biochemical factors. In this study, the average fruit weight and TSS content in tomato under saline environment were determined by scion–rootstock interactions. Moreover, these changes correlated with morphological adaptations that allow survival under the higher salt concentrations.

Semiz et al. [34] also reported the enhanced tomato yield under elevated salinity levels in grafted plants. The salt tolerance of grafted plants among various rootstock–scion combinations was attributed towards the ionic tolerance at 50 and 75 mM NaCl in comparison with the lower salinity of 25 mM NaCl [22,23]. The grafted cucumber on bottle gourd rootstock showed less decrease in yield than non-grafted plants with increasing salinity [35]. The plants grafted on rootstock IC-111056 produced more fruit and yield than plants grafted on IC-354557, indicating that the response of grafting combinations on the fruit yield of tomato also depends on the rootstock genotype. Moreover, the effect of both the rootstock genotype and salinity levels on the yield of grafted tomato plants was reported by Savvas et al. [36]. Numerous reports are available that show the enhanced tolerance of grafted Solanaceae crops under saline conditions than self-rooted plants [37–39].

In grafted plants, total soluble solids (TSS) in fruits were higher in plants treated with 6 dS m^{-1} saline irrigation, but decreased at 9 dS m^{-1} treatment compared to control. Savvas et al. [36] and Di Gioia et al. [21] observed no effect of grafting combinations on the TSS content, while Rouphael et al. [40] and Turhan et al. [41] reported a reduction in TSS content in grafted tomato plants than non-grafted plants. Several other studies reported decreased soluble solids in plants grafted on different rootstocks [42–45].

In the present study, the relative water content (RWC) and SPAD index were significantly affected by different salinity levels. Although the RWC was generally lower under salinity, the grafted plants displayed higher RWC than non-grafted plants, indicating that the rootstocks contributed to the maintenance of water uptake under salt stress. Herein, we observed 27.08% reduction in leaf RWC under salinity. Similarly, Tanveer et al. reported that salinity negatively affected the RWC of tomato leaves [46]. However, no significant effect of salinity on leaf RWC was observed in tomato and cucumber grafted on different rootstocks [22,35], which is probably due to the osmotic adjustment [35]. On the contrary, Santa-Cruz et al. [47] observed 35% increased leaf water content under saline conditions in grafted plants, where scion had a salt-induced character.

Saline toxicity caused a significant decline in chlorophyll content, measured as the SPAD index in non-grafted plants than grafted plants. The SPAD index was higher in plants grafted on the two rootstocks than non-grafted plants, where plants grafted on rootstock IC-111056 displayed more leaf greenness. Colla et al. [48] observed that cucumber plants grafted on Affyne rootstock had high chlorophyll content (SPAD index) than non-grafted plants under salinity stress. A consistent decrease in chlorophyll content and RWC was observed in three cultivars of walnut under saline water irrigation [49]. This reduction in chlorophyll content may be due to the ion accumulation and functional distress of the chlorophyll synthesizing machinery [50,51].

Salinity stress negatively affects various physiological and metabolic processes, leading to the generation of reactive oxygen species (ROS), which could seriously disrupt cellular homeostasis and plant metabolism [52]. To avoid or tolerate these effects, plant cells over synthesize certain osmolytes, especially proline which mainly regulates osmoticum in addition to the stabilization of proteins/membranes [53]. Furthermore, antioxidant enzymes prevent the accumulation of the toxic ROS or detoxify them to minimize the oxidative damage. In this study, proline accumulation

increased significantly with the increase in salinity level of both rootstocks of grafted plants compared to non-grafted plants. Generally, the osmolytes, such as proline, sucrose, and glycine betaine increase under salt stress to protect the plants by maintaining cell-homeostasis [54,55]. Grafted plants of cucumber [56,57] and tomato [58] have better salt tolerance due to the high amount of soluble sugar and proline content under salinity.

The antioxidant enzymes help the plants overcome the salt-induced oxidative stress [59]. In the present study, the activities of antioxidant enzymes CAT, APX, SOD, and POX increased with the salinity level in rootstock-grafted plants. These enzymes, CAT, and SOD, in rootstock-grafted plants might detoxify the generated ROS since these two enzymes are the first to control the generation of reactive species, and thus protect the cells [60]. Grafted cucumber plants have lower H_2O_2 content along with higher activity of CAT, SOD, and POD [61] under salt stress. Similarly, at higher levels of $Ca(NO_3)_2$, grafted tomato plants had lower O^{2-}, H_2O_2, MDA contents and high POD, CAT, and SOD activities than self-rooted plants [62]. Therefore, the higher expression of antioxidant enzymes in rootstocks IC-111056 and IC-354557 of grafted tomato plants may be responsible for their enhanced salt tolerance.

The ability of plants to inhibit the translocation of ions between the root and shoot is the main factor for the enhanced salt tolerance [63], which in grafted vegetables has often been correlated with lower ionic ratio in the shoots. In tomato, we observed that the Na^+ content was lower in the grafted plant's root and shoot than non-grafted plants. This indicates that both rootstocks enhanced the plant's capacity to exclude Na^+ with rootstock IC-111056 found to be superior to rootstock IC-354557. Colla et al. [48] reported less aerial Na^+ content in grafted plants than non-grafted cucumber, suggesting the higher Na^+ exclusion capacity of the grafted plants. The lower Na^+ content in the upper parts of grafted tomato plants was also reported by Estan et al. [22] and Martinez-Rodriguez et al. [23].

In contrast to Na^+, the root and shoot of grafted plants have high K^+ concentration than non-grafted plants. Interestingly, in both types of plants, shoots had higher K^+/Na^+ ratios than roots under both saline treatments. Comparatively, no effect was seen in K^+ level of leaves by Savvas et al. [36] and He et al. [64]. The K^+ homeostasis is also genotype and species dependent in defining salinity stress tolerance [65]. The high K^+/Na^+ ratios in the grafted tomato plants may indicate an increased level of salinity tolerance through K^+ homeostasis in the grafted plants [57,66].

Ion partitioning in different leaf orientations, i.e., bottom (older), medium, and upper (young) leaves was analyzed. Ion accumulation and subsequent partitioning are part of the salt tolerance mechanisms, in which all of the plants greatly employ to ease the toxic effect of salt [59]. In our study, the Na^+ content was lower in the middle and upper leaves, but high in bottom leaves of the rootstock grafted tomato plants compared with non-grafted plants, indicating the role of rootstock in salt exclusion. Furthermore, the uptake of K^+ and K^+/Na^+ ratios was higher in upper leaves followed by the middle and bottom leaves in both non-grafted and grafted plants. However, the grafted plants had better K^+ uptake with high K^+/Na^+ ratios than non-grafted plants, particularly in the upper leaves, indicating the potential of grafted plants to limit the ion imbalances under salt stress condition. This revealed Na^+ partitioning within the shoot tissue of grafted plant by the dint of the lowering Na^+ movement towards the younger leaves and inclusion of Na^+ in the bottom leaves for tackling excess Na^+ toxicity, as has been reported in previous studies [67]. Due to this partitioning, grafted plants were able to maintain favorable K^+/Na^+ ratios in the actively growing leaves enhancing their salt tolerance. Earlier studies also reported higher K^+/Na^+ ratios in the upper leaves or aerial parts of the grafted plants than non-grafted plants [21,36]. In saline environments, the equilibrium of high K^+/Na^+ ratios in grafted plants are generally due to the enhanced uptake of K^+ in rootstocks [68]. The maintenance of high K^+/Na^+ ratios in plant tissue and cytosols is the best strategy to adapt under salt stress, through the regulation of uptake and transfer of

Na^+ [69]. In addition, limiting ion accumulation in young tissues is important for salt tolerance [70,71]. Salt stress alters the K^+ efflux in both roots and shoots due to the salt stress -induced high Na^+ influx through membrane depolarization [72]. Briefly, the capacity of plants to maintain the cytosolic K^+/Na^+ ratios through K^+ accumulation or restricting Na^+ in leaves, helps in balancing the threshold level of K^+, and thus better plant performance under salt stress. As K^+ has an important role in osmoregulation through the accumulation of solutes and osmolytes [73], this in turn lowers the osmotic potential of the cell, and thus the water status of cell is maintained against turgor pressure, finally, enabling the plants to overcome the stress effects.

The available reports demonstrate the correlation of tomato fruit yield to grafting per se [74]. This positive correlation may be due to the improved water use efficiency of the rootstocks used for grafting [75,76] or enhanced scion vigor [77,78]. The combination of any or all of the mentioned mechanisms could contribute towards increasing the crop yield of grafted tomato plants under salt stress.

5. Conclusions

Soil salinity reduces tomato productivity to a large extent. In this study, tomato plants grafted on rootstocks IC-111056 and IC-354557 demonstrated better salt stress tolerance in comparison with non-grafted plants. The grafted plants maintained higher relative water content and antioxidant enzyme activities, along with the accumulation of osmolyte proline to balance the reduced damage caused by oxidative stress and desiccation. Furthermore, the grafted plants had more K^+ ions and high K^+/Na^+ ratios in younger leaves than older leaves, demonstrating that the rootstock may confer Na^+ exclusion and K^+ retention properties to the tomato scion, thereby enhancing the salt tolerance ability of grafted plants. This may be one of the key mechanisms of salt tolerance in the grafted tomato plants. From these results, it could be summarized that the use of appropriate salt tolerant rootstock for vegetable grafting could provide an alternate approach to increase the yield of high performing, salt-sensitive variety in salt affected soils.

Supplementary Materials: The following are available online at www.mdpi.com/article/10.3390/agriculture12020183/s1. Tables S1–S3: Analysis of variance (ANOVA) for yield components and physiological parameters of tomato.

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