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*Article*

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

**Satish Kumar Sanwal [1,*], Anita Mann [1], Arvind Kumar [1], Hari Kesh [1], Gurpreet Kaur [1], Arvind Kumar Rai [1], Raj Kumar [1], Parbodh C. Sharma [1], Ashwani Kumar [1], Anant Bahadur [2], Bijendra Singh [2] and Pradeep Kumar [3,*]**

1 ICAR-Central Soil Salinity Research Institute, Karnal 132001, Haryana, India; anita.mann@icar.gov.in (A.M.); arvind.kumar2@icar.gov.in (A.K.); harikeshkaul55@gmail.com (H.K.); gurpreetkaurcssri@gmail.com (G.K.); ak.rai@icar.gov.in (A.K.R.); rajkumar2@icar.gov.in (R.K.); Parbodh.Chander@icar.gov.in (P.C.S.); Ashwani.Kumar1@icar.gov.in (A.K.)
2 ICAR-Indian Institute of Vegetable Research, Varanasi 221305, Uttar Pradesh, India; anant.bahadur@icar.gov.in (A.B.); bsinghiivr@gmail.com (B.S.)
3 ICAR-Central Arid Zone Research Institute, Jodhpur 342003, Rajasthan, India
* Correspondence: satish.sanwal@icar.gov.in (S.K.S.); pradeep.kumar4@icar.gov.in (P.K.)

**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.

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.

**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 × 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 × 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.

### *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 significantly 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 × 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 × 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.

**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 | Rootstock | Element (mg $g^{-1}$ DW) | | | | | |
|---|---|---|---|---|---|---|---|
| | | $Na^+$ | | $K^+$ | | $K^+/Na^+$ | |
| (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 × 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 | 1.97 b |
| $EC_{iw}$ 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 | 1.03 b |
| | | | | Significance | | | |
| Salinity | | ** | ** | ** | ** | ** | ** |
| Rootstock | | ** | ** | ** | ** | ** | * |
| Salinity × 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 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 $m^{-1}$.

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).

**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 × Rootstock | | | * | | | *** | | | *** | |
| Salinity × Leaf orient | | | *** | | | *** | | | *** | |
| Rootstock × leaf orient | | | *** | | | *** | | | *** | |
| Year × Salinity | | | ns | | | *** | | | *** | |
| Year × Rootstock | | | * | | | ns | | | * | |
| Year × leaf orient | | | * | | | *** | | | *** | |
| Salinity × Rootstock × leaf orient | | | *** | | | *** | | | *** | |
| Year × Salinity × Rootstock | | | ns | | | *** | | | *** | |
| Year × Salinity × leaf orient | | | * | | | *** | | | *** | |
| Year × Rootstock × leaf orient | | | ns | | | *** | | | ns | |
| Year × Salinity × Rootstock × 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.

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.

### *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.

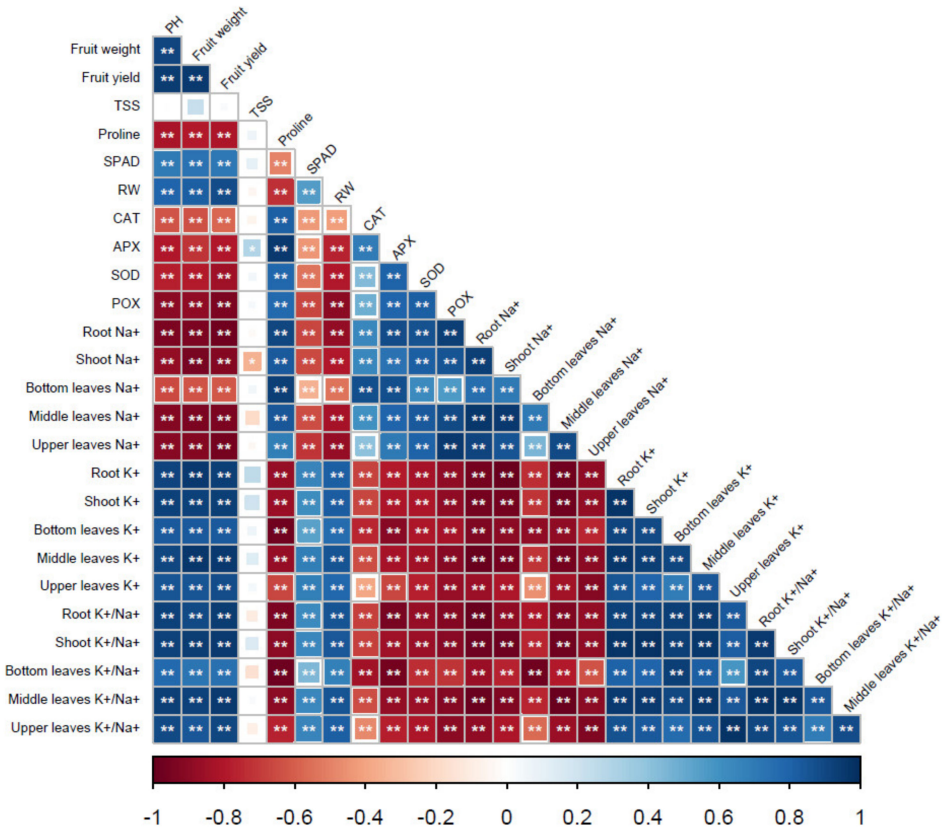


**Figure 1.** Association between horticultural and biochemical traits of tomato under saline environment.

## 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 https://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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