

Do rootstock-scion combinations and irrigation water salinity affect the growth and yield of grafted tomatoes under root-restricted conditions?

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Abstract

The experiment evaluated the impact of several commercial rootstocks on the growth and yield of a selected tomato cultivar under restricted volume conditions. The experiment included six different rootstocks and a single tomato cultivar (*Solanum lycopersicum* cv. Izmir). The respective rootstock-scion combination and self-grafted tomato plants were tested in a randomized complete block design. The plants, transplanted into 5 L plastic pots, were irrigated daily with equal amounts of a nutrient solution and were subject to two different levels of salinity, 0- or 50-mM NaCl solution, respectively. The relative stem elongation rate and new leaf emergence rate were calculated from day 1 to day 42 after transplanting, and nutrient concentrations of mature leaves were analyzed by the end of this period. Fruits were harvested at full maturity, and Brix (°Bx) and fruit acidity were measured using a digital refractometer. While the Na concentration of mature leaves significantly increased, the rise of salinity in the nutrient solution significantly reduced the plants' relative growth rate, K and P concentration of mature leaves, and the yield of tomato plants. Although the grafted plants demonstrated improved shoot growth and increased new leaf emergence rate, indicating enhanced capabilities to alleviate salinity stress, most of the rootstocks did not contribute to any increase in tomato yield. Since only carefully selected rootstock-scion combinations might contribute to higher yields and better quality, the introduction of new rootstocks should be followed by continuous testing of their compatibility with specific, well-recognized tomato cultivars under diverse growing conditions.

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Introduction

Salinity is one of the most detrimental environmental factors affecting the growth and productivity of cultivated crops. High salt concentrations in the soil and irrigation water lead to a progressive build-up of sodium (Na⁺) and chlorine (Cl⁻) in the plant cells. This causes dehydration, nutrient imbalances, and oxidative stresses^[1,2] which ultimately restricts the growth and yield of cultivated crops^[3–5]. Tomato (*Solanum lycopersicum* L.) is among the most widely produced and consumed vegetables. Although it is considered a moderately salt-sensitive crop^[6,7], a 10% decrease in yield has been reported for each unit of soil electrical conductivity (EC) above a threshold value of 2.5 dS·m⁻¹^[8].

Unfortunately, excess soil salinity is an increasing constraint for crop production worldwide. Excessive use of chemical fertilizers, the high salt content of irrigation water, and climate change are considered the main factors deteriorating the situation. Thus, 25% of irrigated agricultural land in the Mediterranean regions is significantly affected by high salinity^[9]. This significantly limits their production capacities and farming effectiveness.

Replacing highly saline soils with substrates, or simply growing plants in artificially prepared nutrient solutions, is considered a reliable alternative. Soilless culture systems have been recently developed and are gradually expanding^[10]. However, the spatial confinement of roots into a specific, well-defined root zone is a big constraint for these production systems^[11]. In container-grown plants, roots grow very dense to compensate for limited root zone volume^[12] and are much more exposed to environmental changes and human-imposed mistakes that can impact their morphology and nutrient uptake capabilities^[13]. Furthermore, the poor quality of irrigation water remains a primary concern for soilless cultivation systems^[14,15].

To relieve salinity pressure, different agricultural practices are applied in both soil-based and soilless production systems. In addition to the development of salt-tolerant cultivars^[16], plant inoculation with arbuscular mycorrhizal fungi^[17,18]—alone, or in combination with plant growth-promoting bacteria^[19]—and plant growth enhancement through high nitrogen doses^[20,21] are among commonly used agricultural practices.

In addition, grafting is considered one of the most effective agronomic interventions to alleviate the negative effects of salinity on cultivated crops^[22–24]. Although the published results are often contradictory, grafting tomatoes is often considered a powerful tool to increase the yield^[25,26]. However, the result depends on the characteristics of the scion and rootstock, their interaction, and stress intensity^[6].

As increased market demands and climate changes expose vegetable producers to new challenges, breeding new high-yielding, resilient, resource-use efficient, and high-quality cultivars is a common attitude of the industry^[27]. In addition, new rootstocks may help introduce other sources of genotypic and phenotypic variability into the crop^[28]. Testing and evaluating their effectiveness under commercial production systems and finding the most appropriate rootstock-scion combination under diverse production systems and growing conditions is a continuously ongoing task.

Although numerous studies have been conducted, the underlying mechanisms by which grafting improves the salt tolerance of vegetable crops remain largely unknown^[29]. Recently, we reported that root-restricted tomato plants benefit from appropriate rootstock-scion combinations. Increased root density, steadier growth under fluctuating salinity levels in the irrigation water due to a more stable uptake of nutrient elements, and higher sodium compartmentalization capabilities of the rootstocks were the most distinguished benefits^[30]. The question that follows is whether these

advantages will be reflected in a higher yield or improved fruit quality of the grafted tomato plants under root restriction saline conditions. Hence, this research aimed to evaluate the effectiveness of several tomato rootstocks, most recently introduced, on the growth and yield of a specific tomato cultivar under root-restricted, saline conditions.

Materials and methods

Plant materials and growing conditions

The experiment was conducted in a plastic greenhouse in Tirana, Albania, in the spring (March 24–June 23) of 2022. Six different rootstocks (Arnold, Dohkko, Emperador, He-man, Kaiser, and Suzuka; details are presented in [Supplementary Table S1](#)) and a tomato cultivar (Izmir) were included in the study. The grafted and self-grafted seedlings were produced by a commercial seedling producer nursery. Grafting was performed using the splice grafting method. Immediately after grafting, all plants were placed in a growth chamber. In the first 3 d, the air temperature was maintained at 26 °C, whereas the relative humidity was 100% at the start and then gradually decreased to 90%. A photosynthetic photon flux density (PPFD) of 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (white, fluorescent lamps) was maintained, with a photoperiod of 12 h. On the fourth day after grafting, plants were moved to a heated greenhouse (air temperatures 18–24 °C, relative humidity 80%, natural lighting) until the experiment started. 65 d after sowing, the grafted and self-grafted seedlings were arranged in a factorial experiment conducted in a non-heated greenhouse. For that purpose, the plants were transplanted in 5 L plastic pots, one plant per pot, filled up with a mixture of peat (Klasmann-Deilmann GmbH, Geeste, Germany) and vermiculite (Dupré Minerals Ltd, Staffordshire, England) (2:1, v/v). The total number of plants of each graft combination was split in half and treated, respectively with the standard nutrient solution (EC 2.2 $\text{mS}\cdot\text{cm}^{-1}$), or a 50 mM (EC 8 $\text{mS}\cdot\text{cm}^{-1}$) saline solution obtained by adding sodium chloride (NaCl, 3 $\text{g}\cdot\text{L}^{-1}$) to the nutrient solution. A mixture of commercial chemical fertilizers containing N, P_2O_5 , K_2O , S, and micronutrients was diluted in tap water (pH 7.6, EC 1.2 $\text{mS}\cdot\text{cm}^{-1}$) to prepare the nutrient solution (N: 100 $\text{mg}\cdot\text{L}^{-1}$, P_2O_5 25 $\text{mg}\cdot\text{L}^{-1}$, K_2O 140 $\text{mg}\cdot\text{L}^{-1}$). Since the water already contained considerable amounts of Ca (80 $\text{mg}\cdot\text{L}^{-1}$) and Mg (35 $\text{mg}\cdot\text{L}^{-1}$), no extra amounts of these elements were added to the nutrient solution. The pH of nutrient solutions was 7.6 and 8, respectively; no acidification was performed. A total of eight plants were used in each experimental plot-treatment. The plants were irrigated daily with equal amounts, equivalent to ETp values (3 $\text{mm}\cdot\text{day}^{-1}$ at the start of the experiment, 5 $\text{mm}\cdot\text{day}^{-1}$ during the harvesting time) corrected with the respective crop coefficient factors, of the normal or saline nutrient solution. To avoid salinity build-up in the root zone, a 20%–25% drainage rate coefficient was applied to all variants. At the beginning of the experiment, pH and EC values measured in the drainage water were similar to the respective values of the fresh nutrient solution. Later, pH of the drainage water remained almost constant, but slightly increased EC values (2.4 $\text{mS}\cdot\text{cm}^{-1}$ for the non-saline, and 8.3 $\text{mS}\cdot\text{cm}^{-1}$ for saline variants) were measured.

All plants grew up with a single (primary) stem, and plant height and leaf number were measured and recorded sequentially during the experiment. Further, the plant relative growth rate concept^[31] was adapted to calculate the relative stem elongation rate (RSER, $\text{cm}\cdot\text{cm}^{-1}\cdot\text{d}^{-1}$) and relative new leaf emerging rate (RLER, $\text{leaf}\cdot\text{leaf}^{-1}\cdot\text{d}^{-1}$) in the period from day 1 after transplanting, until day 42 after transplanting.

Three randomly selected plants were harvested 42 d after transplanting for biomass assessment and nutrient analyses. The roots were dissected from the shoots and washed off adhering soil particles, dried (65 °C, 72 h), and the dry matter of roots and shoots of each plant was determined separately to an accuracy of ± 1 mg (TP 303; Denver Instruments GmbH, Göttingen, Germany). Dried shoots (stems + leaves) of the self-grafted plants and two hetero-graft combinations (Dohkko/Izmir, and Suzuka/Izmir) were separately ground with a planetary mill (Pulverisette5; Fritsch, Idar-Oberstein, Germany) for mineral analysis, and nutrient contents (Ca, K, Mg, Na, and P) were analyzed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Optima 8300; Perkin Elmer, Waltham, USA). For that purpose, the samples were first dissolved in nitrohydrochloric acid to ensure that all minerals were transferred in a liquid solution. The liquid sample was transferred into an argon plasma at a high temperature (6,000–10,000 K), which excites the atoms and ions in the sample, causing them to emit light at characteristic wavelengths. The emitted light is passed through a spectrometer, which separates it into component wavelengths.

The remaining (5) plants were topped up after the fifth cluster was established. Fruits were harvested at full maturity and weighed separately for each experimental unit. The number of fruits was counted for each harvesting date, and the average fruit weight was calculated by dividing the weight of harvested fruits by the respective fruit number. Five randomly selected fruits at the third harvesting date were used to measure soluble solids content (SSC, Brix) and fruit acidity using a digital refractometer (Atago Co. Ltd., Tokyo, Japan).

Statistical analyses

A factorial arrangement of 14 treatments (seven rootstock-scion combinations \times two levels of salinity), eight replicates each, was employed in a factorial randomized complete block design.

The contribution of different growth and yield parameters under non-saline and saline conditions to the diversity of different rootstock-scion combinations was assessed by the principal component analysis (PCA). To produce a graphical evaluation of their relationships, the respective heat map was generated via the ClustVis (<https://biit.cs.ut.ee/clustvis/>, accessed on 20 October 2024) online program package.

Furthermore, to determine the influence of growth rate on plant yield, the respective correlograms of RSER and LRLER with yield were accomplished via STHDA (www.sthda.com), separately for saline and non-saline conditions.

Residuals of all variables were tested for equality of variances and normality using Brown-Forsythe and Shapiro-Wilk tests, respectively. Differences in DM, RSER, RLRLER, shoot nutrient concentration, yield, and yield quality parameters were tested by two-way ANOVA using the PC program SigmaPlot 13 (Systat Software Inc., San Jose, CA, USA). Each significant ANOVA result ($p < 0.05$) was followed by a Holm-Sidak test at $p < 0.05$ as a post hoc test. Values given throughout the text are means \pm standard errors (SE).

Results

Rootstock-scion combinations and salinity effects on plant growth

Only slight differences were found among the self-grafted and hetero-grafted variants regarding plant height under normal, non-saline conditions. Although the self-grafted variant (Izmir/Izmir) was among the tallest, the difference with most of the hetero-grafted variants was not significant ([Table 1](#)). Significantly fewer leaves (LN) were found in Arnold/Izmir vs the self-grafted variant, which led to a

significantly higher internode length (IL). Some variations regarding LN and IL did exist among other grafted variants, but were less important. No differences were found among the self-grafted and hetero-grafted variants under non-saline conditions regarding relative stem elongation rate (RSER), although some variation was found regarding relative new leaves emerging rate (RLER).

Raised salinity has significantly reduced growth parameters; plant height ($p < 0.001$), leaf number ($p < 0.001$), internode length ($p < 0.04$), relative stem elongation rate ($p < 0.001$), and relative new leaves emerging rate ($p < 0.004$) to all variants (Table 1). Under such conditions, almost all hetero-graft combinations recorded higher RSER than the self-grafted variant. Kaiser/Izmir and Suzuka/Izmir recorded significantly higher RLER values than the self-grafted plants. Interestingly, hetero-grafted variants were able to sustain RSER despite the rise of salinity, whereas the self-grafted variant did not. In addition, despite the overall reduction in the number of leaves per plant, Suzuka/Izmir recorded the highest value of RLER (Table 1).

Under non-saline conditions, the self-grafted seedlings (Izmir/Izmir) showed a significantly higher root dry matter (DM_{root}) than the hetero-grafted variants. Suzuka/Izmir was the only exception. However, except for Emperador/Izmir, no differences were found between the self-grafted and hetero-grafted variants regarding the dry matter of shoots (DM_{shoot}) (Table 2). Consequently, the self-grafted plants had a higher ratio of root to shoot dry matter ($DM_{root} : DM_{shoot}$) than most of the hetero-grafted variants (Arnold/Izmir, Dohkko/Izmir, Emperador/Izmir, and He-man/Izmir). The rest—Kaiser/Izmir and Suzuka/Izmir—were included in the same statistical group as Izmir/Izmir (Table 2). The rise of salinity in the irrigation water reduced DM_{root} and DM_{shoot} to all variants, but the self-grafted

variant, together with Suzuka/Izmir and Kaiser/Izmir, recorded the highest DM_{root} . Almost no differences were found between the self-grafted and hetero-grafted plants regarding DM_{shoot} (Table 2).

Rootstock-scion combinations and salinity effects on plant's nutrient concentration

Large variations in nutrient concentrations were found among the grafted plants. Lower Ca concentrations, but significantly higher P concentrations were found in the self-grafted plants compared with hetero-grafted variants under non-saline conditions. No difference was found regarding Na concentration. Although the rise of salinity in the irrigation water multiplied shoot Na concentration several times, there was no difference between the self-grafted and hetero-grafted variants (Table 3). The increase of salinity in the irrigation water did not affect Ca concentration, but significantly reduced the shoot concentration of K and P. On the contrary, Mg concentration was significantly increased alongside an immense increase in Na concentration. Ca, K, Mg, and P concentrations were also significantly affected by Rst/Sc combinations. Higher Mg and P concentrations were found in the shoots of the self-grafted variant compared to both hetero-grafted ones. However, the hetero-grafted variants were able to maintain higher K (Suzuka/Izmir, Dohkko/Izmir) and Ca (Dohkko/Izmir) shoot concentrations under saline conditions.

Rootstock-scion combinations and salinity effects on yield

Hetero-grafting did not provide any increase in the yield versus the self-grafted variants. No difference in the number of fruits per plant between the self-grafted and hetero-grafted variants was

Table 1. Plant growth parameters*.

Factors		PH (cm)	LN	IL (cm)	RSER (cm·cm ⁻¹ ·d ⁻¹)	RLER (leaf·leaf ⁻¹ ·d ⁻¹)
Salinity (S)	0 mM	103.1 ± 1.31A	25.6 ± 0.58A	4.08 ± 0.11A	0.039 ± 0.00A	0.029 ± 0.00A
	50 mM	89.8 ± 0.91B	23.7 ± 0.52B	3.84 ± 0.09B	0.036 ± 0.00B	0.027 ± 0.00B
Graft combination (Rst/Sc)	Arnold/Izmir	95.6 ± 2.78AB	20.8 ± 0.68C	4.63 ± 0.19A	0.040 ± 0.00A	0.023 ± 0.00B
	Dohkko/Izmir	92.1 ± 2.94B	22.6 ± 0.58BC	4.09 ± 0.14AB	0.037 ± 0.00AB	0.026 ± 0.00B
	Emperador/Izmir	95.2 ± 2.23AB	23.0 ± 1.04BC	4.20 ± 0.18AB	0.038 ± 0.00AB	0.026 ± 0.00B
	He-man/Izmir	92.5 ± 2.55B	25.1 ± 0.78B	3.72 ± 0.18B	0.038 ± 0.00AB	0.029 ± 0.00AB
	Kaiser/Izmir	102.4 ± 2.67A	27.6 ± 0.77A	3.71 ± 0.06B	0.039 ± 0.00A	0.031 ± 0.00A
	Suzuka/Izmir	98.9 ± 3.05A	27.8 ± 0.57A	3.56 ± 0.12B	0.039 ± 0.00A	0.031 ± 0.00A
	Izmir/Izmir	98.6 ± 3.74A	26.2 ± 0.81AB	3.79 ± 0.18B	0.035 ± 0.00B	0.029 ± 0.00AB
Graft combination performance under different salinity conditions (Rst/Sc × S)	Non-saline					
	Arnold/Izmir	103.0 ± 2.66ab	21.2 ± 1.24b	4.92 ± 0.32a	0.041 ± 0.000a	0.024 ± 0.001b
	Dohkko/Izmir	99.0 ± 3.01b	23.2 ± 0.97ab	4.28 ± 0.15a	0.039 ± 0.001a	0.027 ± 0.000ab
	Emperador/Izmir	100.0 ± 2.70ab	25.0 ± 1.22a	4.04 ± 0.25a	0.038 ± 0.001a	0.028 ± 0.000ab
	He-man/Izmir	95.6 ± 4.41b	26.0 ± 0.89a	3.71 ± 0.28b	0.039 ± 0.001a	0.029 ± 0.000ab
	Kaiser/Izmir	108.2 ± 3.39a	29.4 ± 0.74a	3.68 ± 0.08b	0.040 ± 0.000a	0.033 ± 0.001a
	Suzuka/Izmir	107.8 ± 1.39a	28.4 ± 0.81a	3.81 ± 0.15b	0.040 ± 0.000a	0.032 ± 0.001a
	Izmir/Izmir	108.2 ± 2.37a	26.6 ± 1.36a	4.10 ± 0.21a	0.039 ± 0.000a	0.029 ± 0.000ab
	Saline					
	Arnold/Izmir	88.2 ± 0.66b	20.4 ± 0.6b	4.34 ± 0.14a	0.039 ± 0.000a	0.023 ± 0.001b
	Dohkko/Izmir	85.2 ± 2.47b	22.0 ± 0.6b	3.89 ± 0.21ab	0.035 ± 0.001ab	0.025 ± 0.001b
	Emperador/Izmir	90.4 ± 1.91b	21.0 ± 1.1b	4.36 ± 0.27a	0.037 ± 0.001a	0.024 ± 0.000b
	He-man/Izmir	89.4 ± 2.24b	24.2 ± 1.2ab	3.74 ± 0.25ab	0.037 ± 0.000a	0.029 ± 0.001ab
	Kaiser/Izmir	96.6 ± 1.96b	25.8 ± 0.7a	3.75 ± 0.08ab	0.038 ± 0.000a	0.029 ± 0.001ab
	Suzuka/Izmir	90.0 ± 0.70b	27.2 ± 0.8a	3.32 ± 0.12b	0.038 ± 0.000a	0.031 ± 0.001a
	Izmir/Izmir	89.0 ± 3.36b	25.8 ± 1.0a	3.48 ± 0.23ab	0.032 ± 0.000b	0.029 ± 0.001ab
Significance						
	Rst/Sc	0.002	< 0.001	< 0.001	< 0.001	< 0.001
	Salinity (S)	< 0.001	< 0.001	0.040	< 0.001	0.004
	Rst/Sc × S	0.175	0.501	0.187	0.129	0.271

* Salinity (0, 50 mM), Graft combinations (Arnold/Izmir, Dohkko/Izmir, Emperador/Izmir, He-man/Izmir, Kaiser/Izmir, Suzuka/Izmir, Izmir/Izmir). PH Plant height, LN leaf number, IL internode length, RSER relative stem elongation rate, RLER relative new leaf emerging rate. Different letters (capital letters for main factors, small letters for Rst/Sc × S interactions) indicate significant differences within parameters (Holm-Sidak test, $p < 0.05$; mean ± SE); significant p -values of a two-way ANOVA are indicated in bold.

Table 2. Plant dry matter parameters*.

Factors		Dry matter (g)		
		DM _{root}	DM _{shoot}	DM _{root} : DM _{shoot}
Salinity (S)	0 mM	5.80 ± 0.28A	30.21 ± 0.59A	0.19 ± 0.00
	50 mM	4.36 ± 0.17B	24.23 ± 0.44B	0.18 ± 0.00
Graft combinations (Rst/Sc)	Arnold/Izmir	4.35 ± 0.26B	25.02 ± 1.49B	0.17 ± 0.00B
	Dohkko/Izmir	4.35 ± 0.14B	26.62 ± 1.58B	0.16 ± 0.00B
	Emperador/Izmir	4.17 ± 0.34B	30.41 ± 1.82A	0.14 ± 0.01B
	He-man/Izmir	4.85 ± 0.35AB	26.22 ± 1.43B	0.18 ± 0.00AB
	Kaiser/Izmir	5.42 ± 0.45AB	26.72 ± 1.12B	0.20 ± 0.00AB
	Suzuka/Izmir	6.62 ± 0.64A	29.67 ± 1.32A	0.22 ± 0.01A
	Izmir/Izmir	5.82 ± 0.54AB	26.12 ± 1.49B	0.22 ± 0.01A
	Graft combination performance under different salinity conditions (Rst/Sc × S)		Non-saline	
	Arnold/Izmir	4.90 ± 0.17bc	28.15 ± 1.06b	0.17 ± 0.01b
	Dohkko/Izmir	4.40 ± 0.23c	29.65 ± 1.47b	0.15 ± 0.01b
	Emperador/Izmir	4.90 ± 0.11bc	34.45 ± 0.43a	0.14 ± 0.00b
	He-man/Izmir	5.50 ± 0.23bc	29.30 ± 0.86b	0.18 ± 0.00b
	Kaiser/Izmir	6.15 ± 0.60b	29.10 ± 0.57b	0.21 ± 0.01a
	Suzuka/Izmir	7.80 ± 0.63a	32.50 ± 0.86ab	0.19 ± 0.01a
	Izmir/Izmir	7.00 ± 0.28ab	28.45 ± 1.70b	0.24 ± 0.00a
		Saline		
	Arnold/Izmir	3.80 ± 0.11c	21.90 ± 0.57c	0.17 ± 0.00b
	Dohkko/Izmir	4.30 ± 0.23c	23.60 ± 1.09bc	0.18 ± 0.01b
	Emperador/Izmir	3.45 ± 0.20c	26.35 ± 0.37b	0.13 ± 0.00c
	He-man/Izmir	4.20 ± 0.40c	23.15 ± 0.14bc	0.18 ± 0.01b
	Kaiser/Izmir	4.70 ± 0.34bc	24.35 ± 0.54b	0.23 ± 0.00 a
	Suzuka/Izmir	5.40 ± 0.54b	26.85 ± 0.20b	0.20 ± 0.02ab
	Izmir/Izmir	4.65 ± 0.20bc	23.80 ± 1.67bc	0.19 ± 0.00b
		Significance		
	Rst/Sc	< 0.001	< 0.001	< 0.001
	Salinity (S)	< 0.001	< 0.001	0.067
	Rst/Sc × S	0.053	0.643	0.062

* Salinity (0, 50 mM), Graft combinations (Arnold/Izmir, Dohkko/Izmir, Emperador/Izmir, He-man/Izmir, Kaiser/Izmir, Suzuka/Izmir, Izmir/Izmir). DM_{root} dry matter weight of roots, DM_{shoot} dry matter of shoots, DM_{root} : DM_{shoot} the ratio of root dry matter with shoot dry matter. Different letters (capital letters for main factors, small letters for Rst/Sc × S interactions) indicate significant differences within parameters (Holm-Sidak test, $p < 0.05$; mean ± SE); significant p -values of a two-way ANOVA are indicated in bold.

Table 3. Plant macronutrient (Ca, K, Mg, Na, P, and S) concentration*.

Factors	Rs/Sc combination	Ca	K	Mg	Na	P
Salinity (S)	0 mM	29.25 ± 0.77	23.57 ± 0.38A	8.93 ± 0.31B	1,189 ± 33B	4,602 ± 287A
	50 mM	29.31 ± 0.77	21.35 ± 0.38B	9.73 ± 0.39A	4,011 ± 47A	4,560 ± 244B
Graft combinations (Rst/Sc)	Izmir/Izmir	29.25 ± 0.35B	22.07 ± 1.11B	10.12 ± 0.32A	2,598 ± 762	5,201 ± 139A
	Dohkko/Izmir	27.24 ± 0.24C	22.17 ± 0.16B	8.28 ± 0.17B	2,560 ± 835	4,708 ± 126B
	Suzuka/Izmir	31.33 ± 0.24A	23.14 ± 0.69A	9.58 ± 0.21A	2,643 ± 848	3,833 ± 22C
Graft combination performance under different salinity conditions (Rst/Sc × S)	Non-saline					
	Izmir/Izmir	28.721 ± 0.07b	23.953 ± 0.48a	9.572 ± 0.02b	1,279 ± 37b	5,429 ± 8.35a
	Dohkko/Izmir	27.505 ± 0.48c	22.438 ± 0.09b	7.982 ± 0.03d	1,114 ± 29b	4,507 ± 91.0c
	Suzuka/Izmir	31.544 ± 0.52a	24.329 ± 0.01a	9.238 ± 0.08bc	1,174 ± 11b	3,869 ± 20.5d
	Saline					
	Izmir/Izmir	29.791 ± 0.39b	20.186 ± 0.26c	10.678 ± 0.19a	3,917 ± 60.9a	4,974 ± 118.1b
	Dohkko/Izmir	26.992 ± 0.03c	21.910 ± 0.12b	8.590 ± 0.01c	4,006 ± 97.1a	4,909 ± 84.4b
	Suzuka/Izmir	31.122 ± 0.01a	21.959 ± 0.28b	9.936 ± 0.03b	4,112 ± 42.2a	3,798 ± 3.26d
	Significance					
	Rst/Sc	< 0.001	0.011	< 0.001	0.366	< 0.001
Salinity (S)	0.875	< 0.001	< 0.001	< 0.001	0.05	
Rst/Sc × S	0.098	0.002	0.065	0.063	0.003	

* Salinity (0, 50 mM), Graft combinations (Arnold/Izmir, Dohkko/Izmir, Emperador/Izmir, He-man/Izmir, Kaiser/Izmir, Suzuka/Izmir, Izmir/Izmir). Different letters (capital letters for main factors, small letters for Rst/Sc × S interactions) indicate significant differences within parameters (Holm-Sidak test, $p < 0.05$; mean ± SE); significant p -values of a two-way ANOVA are indicated in bold.

observed under either non-saline or saline conditions. The average fruit size of the self-grafted variant was generally higher under non-saline conditions, but no differences existed under saline conditions (Table 4). There were cases of hetero-grafted variants (Arnold/Izmir under non-saline conditions) with a lower yield than the self-grafted variant. No correlations were found between RSER and RLER with

yield in non-saline conditions, but some mild positive correlations were found between RLER and yield under saline conditions (Fig. 1a, b).

To all variants, the yield was drastically reduced due to the rise of the salinity level in the irrigation water (Table 4). Mostly, the yield reduction was a consequence of significantly reduced (more than

halved) average fruit weight. However, a significant reduction in the number of fruits per plant due to raised salinity was observed in several hetero-grafted variants (Arnold/Izmir, Dohkko/Izmir, He-man/Izmir) (Table 4). No effects of grafting were found regarding soluble solids content (Brix) and acidity under non-saline conditions, whereas some variations were found regarding acidity

under saline conditions. Under such conditions, the self-grafted variant (Izmir/Izmir) appeared with the lowest acidity level (1.277), significantly lower than the rest of the grafted variants (Table 4).

The principal component analyses (PCA) have shown that the most contributing factors to the variability were the plant height (PH, −8.97) and average fruit weight (−6.15). The rest of the factors

Table 4. Yield (harvested yield, kg·var^{−1}; fruit number; average fruit weight, g), and fruit quality (Brix; acidity) parameters*.

Factors		Total yield (kg·plant ^{−1})	Fruit number	Average fruit weight (g)	Brix	Acidity
Salinity (S)	0 mM	3.75 ± 0.06A	35.02 ± 0.66	108.06 ± 2.35	5.38 ± 0.07B	0.91 ± 0.02B
	50 mM	1.51 ± 0.03B	32.31 ± 0.59	47.07 ± 1.08	9.28 ± 0.11A	1.67 ± 0.04A
Graft combinations (Rst/Sc)	Arnold/Izmir	2.37 ± 0.37	32.0 ± 1.06	71.86 ± 9.46B	7.33 ± 0.74	1.34 ± 0.16A
	Dohkko/Izmir	2.50 ± 0.40	34.2 ± 1.41	70.82 ± 9.66B	7.53 ± 0.84	1.43 ± 0.17A
	Emperador/Izmir	2.64 ± 0.42	34.1 ± 1.33	77.26 ± 11.96AB	7.01 ± 0.72	1.30 ± 0.14AB
	He-man/Izmir	2.70 ± 0.36	34.6 ± 1.20	82.31 ± 10.36AB	7.12 ± 0.66	1.35 ± 0.18A
	Kaiser/Izmir	2.66 ± 0.34	34.1 ± 1.03	78.76 ± 10.56AB	7.60 ± 0.83	1.29 ± 0.17AB
	Suzuka/Izmir	2.66 ± 0.36	35.2 ± 1.63	75.05 ± 9.11AB	7.72 ± 0.74	1.27 ± 0.13AB
	Izmir/Izmir	2.88 ± 0.41	33.5 ± 1.05	86.91 ± 12.72A	7.03 ± 0.69	1.04 ± 0.09B
Graft combination performance under different salinity conditions (Rst/Sc × S)						
			Non-saline			
	Arnold/Izmir	3.46 ± 0.18b	34.60 ± 0.81a	99.89 ± 3.16b	5.375 ± 0.19b	0.915 ± 0.04c
	Dohkko/Izmir	3.71 ± 0.09ab	37.40 ± 1.03a	99.37 ± 2.85b	5.375 ± 0.14b	1.008 ± 0.12c
	Emperador/Izmir	3.91 ± 0.12ab	35.00 ± 2.21a	112.7 ± 3.52ab	5.125 ± 0.22b	0.938 ± 0.07c
	He-man/Izmir	3.68 ± 0.31ab	34.00 ± 2.16a	109.3 ± 10.4ab	5.375 ± 0.08b	0.920 ± 0.09c
	Kaiser/Izmir	3.67 ± 0.12ab	33.80 ± 1.28a	109.4 ± 5.34ab	5.450 ± 0.27b	0.843 ± 0.03c
	Suzuka/Izmir	3.72 ± 0.19ab	37.20 ± 2.65a	101.12 ± 4.97b	5.800 ± 0.22b	0.94 ± 0.05bc
	Izmir/Izmir	4.11 ± 0.08a	33.20 ± 1.59a	124.59 ± 3.85a	5.225 ± 0.16b	0.818 ± 0.09c
			Saline			
	Arnold/Izmir	1.28 ± 0.04c	29.40 ± 1.03b	43.82 ± 0.63c	9.300 ± 0.08a	1.773 ± 0.06a
	Dohkko/Izmir	1.29 ± 0.02c	31.00 ± 1.67b	42.27 ± 2.13c	9.700 ± 0.39a	1.867 ± 0.10a
	Emperador/Izmir	1.38 ± 0.06c	33.20 ± 1.65a	41.77 ± 1.58c	8.900 ± 0.23a	1.670 ± 0.06a
	He-man/Izmir	1.71 ± 0.07c	31.20 ± 0.91b	55.31 ± 3.12c	8.875 ± 0.11a	1.788 ± 0.15a
	Kaiser/Izmir	1.64 ± 0.03c	34.40 ± 1.77a	48.12 ± 2.03c	9.750 ± 0.31a	1.738 ± 0.07a
	Suzuka/Izmir	1.60 ± 0.03c	33.20 ± 1.74a	48.98 ± 3.04c	9.650 ± 0.31a	1.615 ± 0.08a
	Izmir/Izmir	1.65 ± 0.05c	33.80 ± 1.56a	49.23 ± 1.98c	8.850 ± 0.20a	1.277 ± 0.04b
			Significance			
	Rst/Sc	0.983	0.544	0.003	0.989	0.003
	Salinity (S)	< 0.001	0.003	< 0.001	< 0.001	< 0.001
	Rst/Sc × S	0.204	0.257	0.050	0.474	0.158

*Salinity (0, 50 mM), Graft combinations (Arnold/Izmir, Dohkko/Izmir, Emperador/Izmir, He-man/Izmir, Kaiser/Izmir, Suzuka/Izmir, Izmir/Izmir). Different letters (capital letters for main factors, small letters for Rst/Sc × S interactions) indicate significant differences within parameters (Holm-Sidak test, $p < 0.05$; mean ± SE); significant p -values of a two-way ANOVA are indicated in bold.

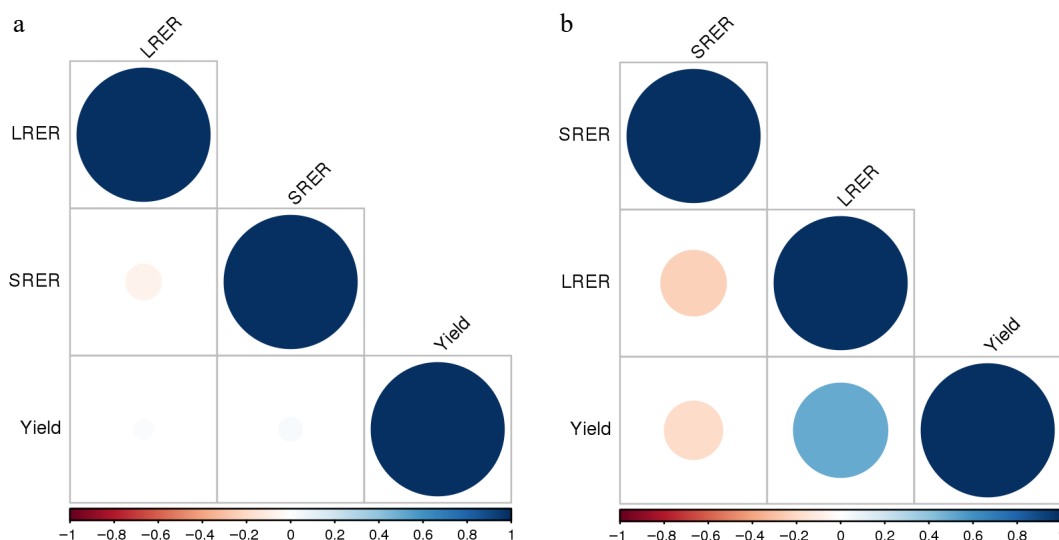


Fig. 1 (a) Yield-RSER-LRER correlograms of hetero-grafted and self-grafted tomato plants (cv. Izmir F1), under non-saline conditions. RSER (relative stem elongation rate), LRER (relative new leaf emerging rate). (b) Yield-RSER-LRER correlograms of hetero-grafted and self-grafted tomato plants (cv. Izmir F1), under saline conditions. RSER (relative stem elongation rate), LRER (relative new leaf emerging rate).

were grouped but still split into two subgroups with either positive (RSER (2.52), RLER (2.53), $DM_{root} : DM_{shoot}$ (2.51), fruit acidity (2.36), yield (2.24), DM_{root} (1.93), Brix (1.6)), or negative (fruit number (−1.51), DM_{shoot} (−0.7), LN (−0.43)) values (Table 5). Most of the variability was represented by the first principal component (PC1, 96%), and the next principal component (PC2) accounted for nearly 4%. The non-saline and saline variants were clearly distinguished (Fig. 2), whereas the plant traits most affected by raised salinity were stem length and average fresh fruit weight.

Interesting conclusions can be drawn from the heatmap chart regarding the variability of different rootstock-scion combinations under both non-saline and saline conditions. The non-grafted (Izmir/Izmir) variant was the most distinguished under non-saline conditions. Closely followed were Emperador/Izmir and He-man/Izmir.

The remaining variants (Arnold/Izmir, Dohkko/Izmir, Kaiser/Izmir, Suzuka/Izmir) were grouped. On the contrary, He-man/Izmir was the most distinguished variant under saline conditions. Closely followed were Suzuka/Izmir and Izmir/Izmir. All remaining variants were grouped (Fig. 2).

Discussion

It is commonly reported that grafting enhances growth and produces more vigorous plants^[23,26]. Although often seen as an advantage of grafted plants, this might be a concern for greenhouse tomatoes. The vegetative plants increase the chances of foliar fungi diseases^[32], induce assimilate partitioning imbalances between fruits and vegetative organs in the plants^[33], and delay

Table 5. The principal components scores of growth, yield, and fruit quality traits*.

Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
DMroot	1.93	0.06	0.02	0.05	−0.02	0.04	0.03	0	0
DMshoot	−0.7	−0.17	0.04	−0.19	−0.08	0	0	0	0
$DM_{root} : DM_{shoot}$	2.51	0.08	−0.03	0.02	0	0.01	−0.01	0	0
PH	−8.97	−1.26	−0.08	0.07	−0.02	0.01	0	0	0
LN	−0.43	−0.33	0.23	0.08	0	−0.01	−0.01	0	0
IL	2.05	0.01	−0.08	0	0	−0.01	0	0.01	0
RSER	2.52	0.08	−0.03	0.02	0	0.01	−0.01	0	0
RLER	2.53	0.08	−0.03	0.02	0	0.01	−0.01	0	0
Total yield	2.24	0.16	−0.03	0.01	0.01	0.02	−0.01	0	0
Fruit number	−1.51	−0.5	0.03	−0.12	0.1	0.01	0.01	0	0
Avg. fruit weight	−6.15	2.11	0	0.01	0.01	−0.01	0	0	0
Brix	1.6	−0.31	−0.01	0.03	0	−0.06	0.02	0	0
Acidity	2.36	0.01	−0.04	0.02	0	−0.01	0	−0.01	−0.01

* The self-grafted (Izmir/Izmir) and heterografted (Arnold/Izmir, Dohkko/Izmir, Emperador/Izmir, He-man/Izmir, Kaiser/Izmir, Suzuka/Izmir) tomato variants under non-saline (0 mM NaCl) and saline (50 mM NaCl) conditions.

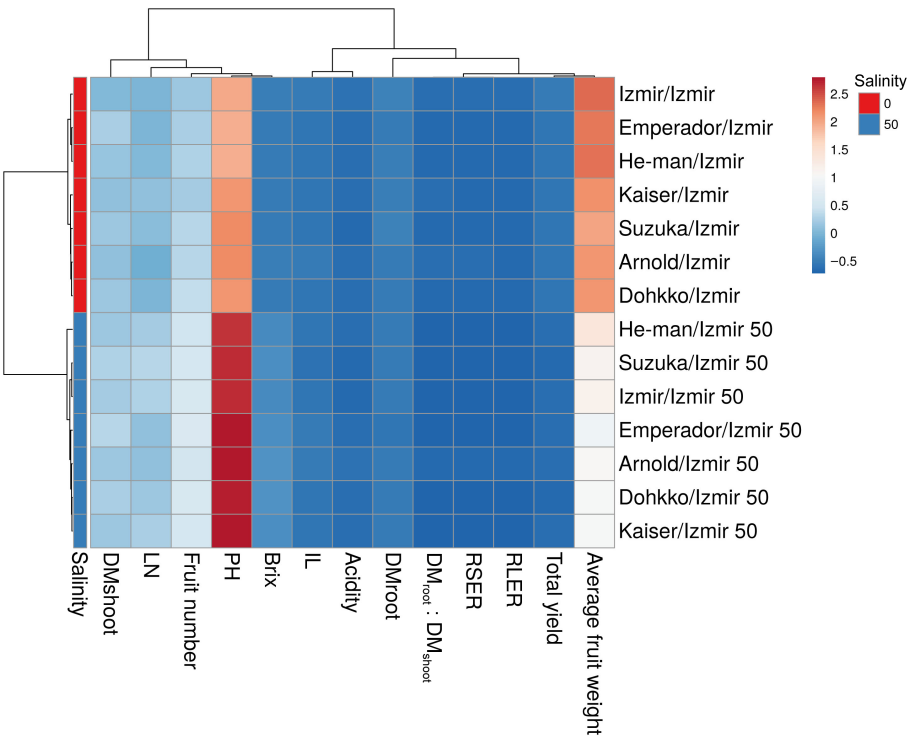


Fig. 2 The heatmap of growth, yield, and fruit quality traits of self-grafted (Izmir/Izmir) and heterografted tomato variants (Arnold/Izmir, Dohkko/Izmir, Emperador/Izmir, He-man/Izmir, Kaiser/Izmir, Suzuka/Izmir) under non-saline (0 mM NaCl) and saline (50 mM NaCl) conditions. Rows are centered; unit variance scaling is applied to rows. PH Plant height, LN leaf number, IL internode length, RSER relative stem elongation rate, RLER relative new leaf emerging rate, DM_{root} dry matter weight of roots, DM_{shoot} dry matter of shoots, $DM_{root} : DM_{shoot}$ the ratio of root dry matter with shoot dry matter. Both rows and columns are clustered using correlation distance and average linkage. Fourteen rows, 13 columns.

fruit setting and maturity^[34]. Therefore, the rootstocks that produce less vigorous, compact plants fit better into a greenhouse environment^[27] and might potentially allocate more carbohydrates toward fruit formation^[27,35]. No significant differences between the self-grafted and hetero-grafted plants was found regarding stem height. Some of the rootstocks were able to shorten the length of plant internodes which satisfies the demand for compact plants in greenhouse production. No difference exists between the self-grafted and hetero-grafted plants regarding dry matter of shoots (DM_{shoot}). Conversely, the self-grafted plants had a higher dry matter of roots (DM_{root}) than most of the grafted variants which grants them the highest $DM_{root} : DM_{shoot}$ ratio. Previous reports confirm that the reduced growth of the root system due to grafting is compensated by an enhancement in root enzyme activity^[36].

Although plant response to salinity is highly variable^[37], commonly, salinity reduces plant growth^[19] by inhibiting both cell division and elongation^[38]. Yet, little is known about salt-induced changes in shoot growth rate^[4]. Interestingly, the study found significant differences between self-grafted and hetero-grafted plants regarding relative stem elongation rate (RSER) and relative new leaf emerging rate (RLER). The enhanced shoot growth and the increased ability of certain rootstock-scion combinations to produce new leaves indicate that they are more capable of alleviating the salinity stress by improving the osmotic tolerance of grafted plants^[37]. Maintaining an appropriate stem growth rate was considered essential to achieving a good yield in cucumber plants^[39]. In contrast, the study did not find any significant correlation between relative stem elongation rate, or relative new leaf emerging rate with tomato yield in non-saline conditions, but good correlations were found between the relative new leaf emerging rate and yield under saline conditions.

Grafting is often considered a tool to increase yield in several vegetable crops^[17,22,40]. On the contrary, the rootstocks involved in this experiment were not able to increase tomato yield in the condition of reduced root volume, either in non-saline or saline conditions. No advantage of grafted variants was found versus the non-grafted plants, either regarding the number of harvested fruits per plant, or the average fruit weight. In contrast, in a previous publication of Djidonou et al.^[40] an increase in the yield of grafted tomatoes was supported by a higher number of larger fruits. Still, similar to us, di Gioia et al.^[25] did not report any significant change in yield components of a heirloom tomato variety grafted onto two interspecific (*S. lycopersicum* × *S. habrochaites*) rootstocks. More recently, Parisi et al.^[41] reported an increase in harvested yield by only three out of seven rootstocks used in grafting with an Italian tomato landrace, and Gong et al.^[42] found varying results depending on specific rootstock-scion combinations and planting season. Considering a large number of different, even contradictory, evidence, the study concludes that the wide range of rootstock-scion combinations leads to mixed results. Huge differences regarding specific production systems and the interactions of specific genotypes with production systems make the performance of grafted tomato plants even more complicated^[43].

The higher yield in grafted plants is often linked with a more vigorous rootstock root system, its enhanced root hydraulic conductance, and nutrient uptake^[44]. However, if the growing system can maintain a constant concentration of nutrient elements at the root surface, the restricted root system is no longer a limiting factor^[11]. Under these circumstances, frequent fertigation fulfills plants' requirements through the continuous replenishment of nutrients in the vicinity of the root interface^[45]. Indeed, despite the differences between self-grafted and hetero-grafted plants, shoot concentrations of Ca, K, Mg, and P to all variants varied within the range of

respective recommended optimum concentrations^[46]. We discussed in detail the differences between the self-grafted and heterografted variants regarding the root and shoot nutrient concentrations in a previous publication^[30]. Even under salinity conditions where the shoot concentration of some elements (K, Mg, P) falls, it remained within the recommended range of optimum concentration. Therefore, improved nutrient uptake in several rootstocks was not enough to distinguish the grafted and non-grafted plants regarding the harvested yield. To increase the efficiency of grafted plants under such production systems, further studies regarding the appropriate management of limited root systems to ensure adequate nutrition, optimum irrigation frequency, proper root zone temperature, and oxygenation level are needed^[13].

In both the self-grafted and hetero-grafted plants, yield was drastically reduced by increased salinity. A slight reduction in fruit number was noticed, but the real factor that led to severe yield reduction was the drop in average fruit weight by more than half. No difference was found between the self-grafted and heterografted variants regarding soluble solids content (SSC) and fruit acidity under non-saline conditions. In line with the study, Gong et al.^[47] concluded that scion cultivars have a much more pronounced impact on fruit quality attributes than rootstocks. On the contrary, SSC and acidity were significantly increased by the rise of salinity in the irrigation water. Under such conditions, no differences were found between the self-grafted and hetero-grafted variants regarding SSC, but a distinctive difference was found regarding fruit acidity. The self-grafted plants showed a significantly lower acidity level than each of the hetero-grafted variants. As such, the results conform with a previous report by Turhan et al.^[48] who reported that grafting tomatoes with interspecific rootstocks (*S. lycopersicum* × *S. habrochaites*) increased titratable acidity relative to the non-grafted controls. Contrary to the study, Djidonou et al.^[40,49] and Parisi et al.^[41] concluded that either beefsteak or cherry tomatoes grafted with interspecific rootstocks did not affect fruit SSC, acidity, and SSC/TA ratio. A clear answer regarding this issue is further complicated by the fact that rootstocks with similar genetic backgrounds could exert different impacts on tomato fruit sensory attributes depending on the scion genotype and growing conditions^[48].

Overall, the results of this experiment prove that there is not always a clear distinction between the non-grafted and grafted tomato plants under root-restricted conditions. Only carefully selected rootstock-scion combinations might make a distinction. Therefore, the effects of grafting itself and the role of specific rootstock-scion combinations on tomato growth, yield, and the fruit quality remain an open question for further elaboration.

Conclusions

Grafted plants demonstrated improved shoot growth and new leaf emerging rate, indicating enhanced capabilities to alleviate the salinity stress. Good correlations were found between the new leaf emerging rate and yield under saline conditions. Considering the low variability found between the self-grafted and grafted tomato variants under root-restricted volume conditions, only carefully selected rootstock-scion combinations might make a distinction. The study's result suggest that He-man can be considered an appropriate rootstock for root-restricted grafted tomatoes under salinity conditions.

Author contributions

The authors confirm their contribution to the paper as follows: study conception and design, analysis and interpretation of results,

and draft manuscript preparation: Balliu A; data collection: Pellumbi G; analysis, and interpretation of results: Sallaku G. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflict of interest

The authors declare that they have no conflict of interest.

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