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The Effective Role of Nano-Silicon Application in Improving the Productivity and Quality of Grafted Tomato Grown under Salinity Stress

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Abstract: This study aims to determine the influence of grafting and nano-silicon fertilizer on the growth and production of tomatoes (*Solanumlycopersicum* L.) under salinity conditions. A commercial tomato hybrid (cv. Strain B) was used as a scion and two tomato phenotypes were used as rootstocks: *S. pimpinellifolium* and Edkawy. The rootstock effect was evaluated by growing plants at two NaCl concentrations plus the control (0, 4000, and 8000 ppm NaCl). Nano-silicon foliar application (0.5 ppm) after 20, 28, and 36 days from transplanting was also used to mitigate salinity stress. Antioxidants, hormones, and proline were evaluated for a better understanding of the physiological changes induced by salinity and grafting. The results showed that grafting either on *S. pimpinellifolium* or Edkawy combined with nano-silicon application enhanced shoot and root growth, fruit yield, and fruit quality. The Edkawy rootstock was more effective than the *S. pimpinellifolium* rootstock in terms of counteracting the negative effect of salinity. Higher levels of mineral contents, GA3, ABA, and proline were detected in shoots that were subjected to grafting and nano-silicon application compared to the control treatment. This study indicates that grafting and nano-silicon application hold potential as alternative techniques to mitigate salt stress in commercial tomato cultivars.

Keywords: Solanum lycopersicum L.; salinity; quality; nano-silicon; plant hormones



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1. Introduction

The tomato plant (*Solanum lycopersicum* L.) is nowadays a very popular crop as a means of preventing many human diseases [1,2] and its fruit is well known for carotenoids like lycopene that seem to be active in cancer prevention, cardiovascular risk, and cellular aging [3]. Tomato is one of the most common and widely consumed vegetable crops in the world, and in Egypt a high-quality yield is a must for commercial success. Salinity is one of the most important abiotic stress factors limiting crop growth and productivity worldwide [4]. High salinity leads to a decrease in plant growth, biomass, yield, photosynthesis, and water use efficiency [5], as salinity stress negatively impacts the morphological, biochemical, and physiological processes of plants [6,7]. Salt stress reduces the growth and yield of grafted and non-grafted tomato plants, but the appropriate combination of scion and rootstocks can help mitigate the negative effects of salinity. An effective and sustainable method of improving the performance of commercial cultivars that are susceptible to abiotic stresses is to use resistant genotypes as rootstocks [8]. The local Egyptian cultivar Edkawy exhibits greater salinity tolerance by exhibiting greater growth stability as salinity

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increases [9]. In addition, S. pimpinellifolium is identified as an important source of genes that can help mature tomato plants cope with stress [10]. Conversely, it has been demonstrated that salinity eustress may contribute to augmenting organoleptic components of fruit quality, such as soluble carbohydrates and health-promoting phytochemicals [6,11]. Moreover, numerous experiments have indicated the beneficial role of silicon (Si) against different stresses, including salinity in tomatoes [12]. Exogenous Si spraying has been a recent eco-friendly strategy to improving plant salinity stress response [13] as it acts by increasing plant biomass [14] by reducing Na⁺ and Cl⁻ ion uptake into plants [15]. Silicon is found in nature as crystalline, amorphous, or weakly crystalline complex silicate minerals [16,17]. It also aids plant growth in a variety of ways by improving antioxidant activity, mineral absorption, organic acid anion and phenolic compound exudation, photosynthetic rates, the accumulation of suitable solutes, hydration status, and the control of plant growth regulators [18–28], as well as considerably lowering the deleterious impact of salinity on chlorophyll levels [29]. Nanotechnologies and plant biotechnology have attracted a lot of attention in agriculture in recent years [30,31] on account of their potential to increase plant productivity, improve plant tolerance to environmental stress conditions, improve nutrient use efficiency, and decrease hazardous environmental consequences [31–33]. Crop improvement experiments have been carried out with silicon nano-particles (n-Si) and many studies have found that increasing n-Si concentrations improves plant development and tolerance to hydroponic conditions [34]. Tomato plants treated with 1 mM n-Si displayed increased germination rates and seedling dry weight, thereby showing higher salinity tolerance at 50 mM NaCl compared with the controls [35,36]. A similar effect was shown in lentil seeds [37]. Nano-silicon sprays increased the amount of chlorophyll in stressed plants and enhanced physiological parameters, such as transpiration rate, photosynthetic rate, stomatal conductance, and photochemical efficiency, in *Indocalamus barbatus* [38]. Moreover, (n-Si) enhanced the nutritional quality of potatoes that had been exposed to salt [39], and n-Si spray significantly improved plant height, stem diameter, ground cover, canopy spread, and other growth characteristics in safflower [40]. Based on this body of evidence, the purpose of this study is to assess whether grafting and foliar application of n-Si have a positive effect in terms of improving the yield and quality of a tomato hybrid by evaluating plant growth characteristics, yield, and fruit quality traits.

2. Materials and Methods

2.1. Plant Material

In this study, the hybrid cv. Strain B tomato obtained from Ferry-Morse seed CO USA was employed as a scion. The seeds of a tomato rootstock with documented salinity tolerance features, *S. pimpinellifolium* L. (line AusTRCF31212), were obtained from the International Tomato Genetic Resource Center in the United States. In addition, Edkawy (obtained from Haraz company, Cairo, Egypt) was used in this study as a local cultivar with recognized tolerance qualities.

2.2. Grafting of Test Plants

Seeds of the scion and rootstock were sown on 21 March 2020 and 17 March 2021 in seedling trays filled with peat moss and vermiculite (1:1 v:v). Scion seedlings were grafted on rootstock manually via tongue grafting on 20 and 15 April in 2020 and 2021, respectively. Both rootstock and scion grafts were sliced obliquely at a 40° angle to the perpendicular axis at a sufficient depth to allow for greater vascular bundle overlap. Clips were applied at the grafting site to secure the graft in place, and the grafted plants were maintained under a clear polyethylene tunnel cover for 5–7 days under 90–95% RH and 50% shading at a temperature of 27.9 °C. The polyethylene cover was gradually opened to acclimatize the grafted plants to the greenhouse environment. Three sets of grafted plants were produced using the commercial tomato hybrid (cv. Strain B) as a scion grafted on one of two tomato rootstocks, namely, Edkawy and *S. pimpinellifolium*, respectively, or on the same hybrid (cv. Strain B) to serve as a basis for the control treatment.

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2.3. Greenhouse Experiment

2.3.1. Growth Conditions

Transplantation of grafted plants was carried out in the greenhouse at the Eastern Farm of the Faculty of Agriculture, Cairo University, Giza, Egypt on 28 April in 2020 and on 23 April in 2021. The three groups of successfully grafted tomato plants were transplanted in pots 60 cm² in diameter, each one filled with a 1:1:1 mixture of peat moss, vermiculite, and perlite and containing two seedlings, and were each subjected to (i) three salinity levels—0 ppm, 4000 ppm, and 8000 ppm of NaCl solution—and (ii) treatment with or without nano-silicon (n-Si) foliar application (0.5 ppm) three times after 20 and 28 and 36 days from transplanting. The reported design resulted in 18 treatment combinations. Physiological and biochemical investigations were performed 60 days after transplantation.

2.3.2. Salinity Treatments

Thirty days after transplantation, the NaCl solution was applied to the levels of saline treatments, which contained 0, 4000, and 8000 ppm NaCl (Technogene chemical company, Dokki, Egypt). The saline treatments were continued until the experiment was completed (190 days after seedling transplantation). A completely randomized design was used for the treatments and each treatment was replicated six times.

2.3.3. Preparation of Nano-Silicon

Nano-silicon in the form of silicon tetrachloride (SiCl₄) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The nano-silicon (Figure 1) was synthesized using the method described by Zhu and Gong [41] and published elsewhere [42]. The morphologies and sizes of the nano-particles (n-Si) were investigated using a JEOL 1010 transmission electron microscope at 80 kV (JEOL, Tokyo, Japan). One drop of the nano-particle solution was spread onto a carbon-coated copper grid for transmission electron microscopy (TEM) analysis. TEM imaging was carried out in the TEM lab of the Faculty of Agriculture, Cairo University Research Park (FA-CURP) to determine the nano-size of the silicon particles.

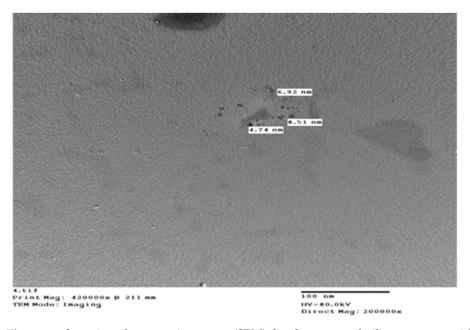


Figure 1. Scanning electron microscopy (SEM) for the prepared silicon nano-particles. Silicon nano-particle size from 4.75 to 6.92 nm.

2.3.4. Plant Growth and Yield

Plant growth was measured as a function of shoot length, plant fresh weight, leaf number, and leaf dry weight. Leaf chlorophyll content was recorded using a SPAD Meter.

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The fruits of each plot were picked during harvesting, and the number of fruits per plant, fruit fresh weight, and total yield per plant were recorded.

2.3.5. Characteristics of Fruit Quality

At the maturity stage, ten mature fruits per experimental unit were chosen to measure the following data. Total soluble solids percentage (TSS%) was measured using a Zeiss laboratory refractometer, and the ascorbic acid (AA) content of ripe fruits was evaluated via the titration method using 2,6-dichlorophenol indophenol according to the Society for Analytical Chemistry's Official Method 967.21. Five fruits were selected in each replicate to determine their firmness. The firmness of ripe fruits was determined using a Force Gauge Mode M4-200 (ELECTROMATIC Equipment Co., Inc., Cedarhurst, NY, USA) with a 1 mm diameter flat probe. The firmness readings of tomato fruits were taken at two opposing points of the equatorial region and expressed in newtons.

2.3.6. Physiological Parameters

To evaluate photosynthesis and leaf stomatal conductance, analysis was conducted via an infrared gas analyzer, the LICOR 6400 Portable Photosynthesis System (IRGA, Licor Inc., Lincoln, NE, USA), on the fifth leaves of twenty plants chosen from each treatment with six replications. Measurements were taken from 9 a.m. to 2 p.m., with a light intensity of around 1300 mol m $^{-2}$ s $^{-1}$ and 80% RH. The leaf chamber's temperature ranged from 25.2 to 27.9 °C, and the volume gas flow rate was 400 mL/min. The CO $_2$ content in the air was 398 μ mol mol $^{-1}$.

2.3.7. Activity of Antioxidant Enzymes, Gibberellic Acid, Abscisic Acid Content, and Leaf Proline

The leaf samples used to measure the activity of peroxidase (POD) were prepared according to Bates et al. [43]. The free proline content was analyzed as described by Bates et al. [43]. After homogenisation, 500 mg of freeze-dried materials in 5 mL of sulphosalicylic acid at 3% (w/v) was filtered using Whatman No. 1 filter paper. The filtrate was combined with acetic acid and a ninhydrin acid reagent ($2\% \ v/v$). Then, for 45 min, the mixture was immersed in a boiling water bath at $100\ ^{\circ}$ C. The tubes were then filled with 4 mL of toluene and soaked for 20 s, after which the reaction was quenched by submerging the tubes in an ice-bath. The free proline was measured spectrophotometrically against a blank reagent at 520 nm. The contents of gibberellic acid (GA3) and abscisic acid (ABA) in tomato leaves were analyzed using the method described by Fales et al. [44]. A 15 mL aliquot of a methanol/butylated hydroxytoluene ($80\% \ v/v$) solution was mixed with the homogenized freeze-dried samples, and GA3 and ABA were extracted and quantified according to AOAC guidelines [45].

2.3.8. Mineral Composition in Tomato Plants

The tomato leaf samples were dried for two days at 75 °C in a forced air oven and then coarsely pulverized for the determination of endogenous nutrients. Sulfuric acid (5 mL) and perchloric acid were used to digest a 0.2 g dried sample. The mixture was then heated for ten minutes and 0.5 mL of perchloric acid was added while continuing to heat the mixture until clear [46,47]. The total nitrogen (N) content of the dried leaf samples was analyzed using the AOAC-recommended modified micro-Kjeldahl method described by Singh et al. [46] The phosphorus (P) content was determined colorimetrically using the chlorostannous molybdophosphoric blue color method in sulfuric acid, according to [46]. A flame photometer (CORNINGM410, Halstead, UK) was used to test the content of potassium (K), magnesium (Mg), calcium (Ca), and sodium (Na). An atomic absorption spectrophotometer with air-acetylene fuel (PyeUnicam Company, model SP-1900, Ventura, CA, USA) was used to examine the iron (Fe) and zinc (Zn) concentrations.

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2.3.9. Statistical Analysis

A randomized complete block design with two factors was used to analyze all the data obtained from six replicates during two growing seasons using the computer application "MSTATC" [48]. The LSD test was used to assess changes across treatment modalities at a 5% probability level [49].

3. Results

3.1. Plant Growth Parameters

Plant growth parameters were affected by salinity levels and grafting (Table 1). The results of the present experiment indicated that among the tested water salinity concentrations, plant growth parameters decreased with increasing salinity.

Table 1. Effects of water salinity, grafting, foliar application of nano-silicon (n-Si), and their interactions on the vegetative growth of tomato plants (combined 2020 and 2021 seasons).

Salinity Levels	Rootstocks + (n-Si)	Plant Height (cm)	Number of Leaves/Plant	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Root Fresh Weight (g)	Root Dry Weight (g)
	Strain B hybrid	161.7 ^{ij}	24.7 ^e	306.8 ^d	49.37 ^e	63.2 ^e	17.6 ^{ef}
0 ppm	Edkawy	239.0 ab	35.3 a	440.7 a	62.50 ^c	81 ^{ab}	21.7 ^b
	S. pimpinellifolium	221.0 b-d	25.7 ^{de}	320.7 ^{cd}	65.37 ^b	75.3 ^c	18.8 ^{de}
	Strain B hybrid + (n-Si)	199.3 e-g	30.0 c	343.7 °	53.87 ^d	78.0 bc	20.3 b-d
	Edkawy + (n-Si)	248.0 a	37.0 a	463.3 a	66.43 ^b	84.0 a	25.7 a
	S. pimpinellifolium + (n-Si)	228.3 bc	34.0 ab	368.3 ^b	69.77 ^a	84.0 a	21.2 bc
4000 ppm	Strain B hybrid	117.7 ¹	18 ^{gh}	195.8 f-h	29.33 ⁱ	43.2 h	16.8 ^f
	Edkawy	194.7 ^{f-h}	29 ^{cd}	314.3 ^d	46.70 ^f	56.6 ^f	19.3 ^{c–e}
	S. pimpinellifolium	178.7 hi	25.3 ^e	252.7 ^e	39.37 ^g	56.0 ^f	18.2 ^{ef}
	Strain B hybrid + (n-Si)	182.0 ^{gh}	24. 7 ^e	198.3 ^{fg}	36.87 ^h	50.67 g	14.8 gh
	Edkawy + (n-Si)	213.7 ^{c–e}	31.3 bc	320.0 ^{cd}	55.27 ^d	70.60 ^d	21.3 ^b
	S. pimpinellifolium + (n-Si)	196.0 e-h	31.0 bc	256.3 ^e	49.43 ^e	63.67 ^e	17.6 ^{ef}
8000 ppm	Strain B hybrid	82.67 ^m	14.3 ⁱ	133.7 ⁱ	20.171 g	33.90 ⁱ	7.2 ⁱ
	Edkawy	158.0 ^{jk}	22.3 ef	191.7 ^{gh}	35.53 ^h	43.27 h	14.7 ^h
	S. pimpinellifolium	178.7 hi	17.7 ^{g-i}	188.3 gh	22.87 ^k	49.33 g	13.2 ^h
	Strain B hybrid + (n-Si)	142.0 ^k	15.3 hi	171.7 ^h	26.63 ^j	40.67 ^h	13.5 ^h
	Edkawy + (n-Si)	207.0 d-f	24.7 ^e	216.3 ^f	49.53 ^e	53.93 ^{fg}	18.5 ^{d-f}
	S. pimpinellifolium + (n-Si)	189.3 f-h	20.0 fg	191.7 ^{gh}	36.60 ^h	53.33 ^{fg}	16.7 ^{fg}
LSD value at 0.05:		18.5	3.7	24.24	2	4.6	1.97

Values followed by the same letter are not significant according to the LSD test ($p \le 0.05\%$).

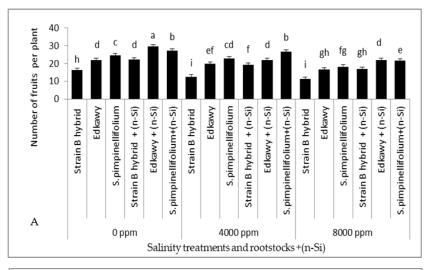
In general, it was observed that plant height was inversely proportional to salinity concentration. In the Edkawy cultivar, the n-Si treatment had a positive influence on plant height under no salinity (248.0 cm with 0 ppm) or moderate stress (213.7 cm with 4000 ppm). The leaf count per plant was slightly affected by moderate salinity and the results were comparable to those obtained in the no-salinity treatment (31.3 with 4000 ppm, 35.3 with 0 ppm) with interaction between grafting and nano-silicon (Table 1). Additionally, in the Edkawy rootstock, it was observed that the fresh weights of shoots and roots were higher when the grafting and n-Si treatments were combined. A slight reduction in fresh root weight was observed (70.60 g) under 4000 ppm salinity and n-Si treatment when compared with the control of the same rootstock (81 g) with unstressed plants without nano-silicon application; a similar trend was observed for the dry weight parameter. As can be seen, plant growth parameters decreased with increasing salinity levels, but the combination of grafting and n-Si foliar application mitigated the effects of salinity (Table 1).

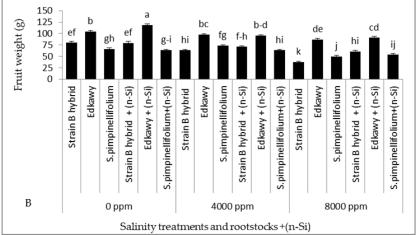
3.2. Tomato Fruit Yield and Its Components

Fruit number and fruit weight as well as total fruit yield per plant were significantly reduced with increasing salt levels. The self-grafted Strain B hybrid was the most negatively affected, particularly at 8000 ppm concentration. Grafted tomato plants subjected to the n-Si treatment registered greater fruit numbers. Similarly, significantly higher fruit number,

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fruit weight, and total yield per plant values were recorded for the Edkawy rootstock combined with n-Si treatment when compared with the untreated control under all three salinity levels. Notably, the n-Si treated Edkawy at 4000 ppm and 8000 ppm yielded fruit numbers comparable to the untreated Edkawy plants at 0 ppm (Figure 2A).





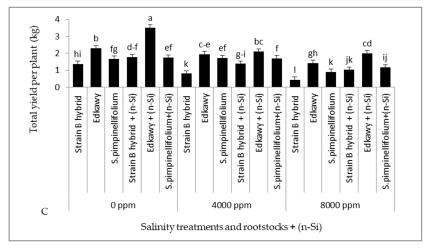


Figure 2. Effects of water salinity levels \times rootstocks and foliar spray with (n-Si) interaction on (**A**) average number of fruits, (**B**) average fruit weight (g), and (**C**) average total yield per plant (kg). Vertical bars represent standard errors of the mean; in each bar, values followed by different letters differ significantly at p = 0.05 according to the LSD test.

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The highest mean fruit weight was recorded using Edkawy combined with nano-silicon under 0 and 4000 ppm of salt concentration (Figure 2B). Plants grafted and combined with n-Si application had higher yields per plant (Figure 2C), although, at 4000 and 8000 ppm, all treatments had a higher value than self-grafted plants. Interaction between Edkawy and n-Si showed higher fruit weight at 0 ppm. Plants grafted on Edkawy and combined with nano-silicon had higher total yields per plant under non-saline water treatment.

3.3. Physiological Traits of Tomato Plants

Transpiration rate, stomatal conductance, photosynthesis, and chlorophyll reading (SPAD) were significantly influenced by salinity levels, grafting, foliar application of n-Si, and the interactions between them (Figure 3). The values obtained for the physiological traits were higher at 0 ppm salinity; however, no statistically significant differences were observed between n-Si and untreated counterparts of the same cultivars. Overall, all grafting combinations, with the exception of the self-grafted Strain B hybrid, showed similar levels of transpiration rates, stomatal conductance, photosynthesis, and SPAD readings, with only minor statistically significant differences. In this case, also, the self-grafted Strain B hybrid had the lowest transpiration rate, stomatal conductance, photosynthesis, and SPAD reading, and, notably, the negative impact of salinity was reduced for its grafted counterpart. Edkawy with nano-silicon and *S. pimpinellifolium* with nano-silicon exhibited better stomatal conductance and photosynthesis under 4000 ppm levels. SPAD showed no significant change between the control and 4000 ppm using Edkawy combined with the nano-silicon application.

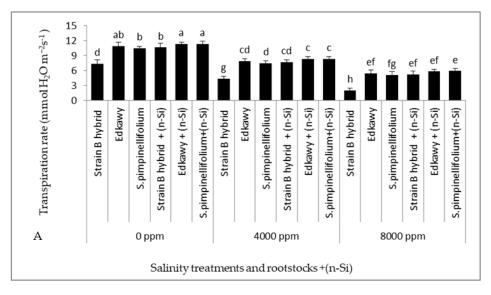
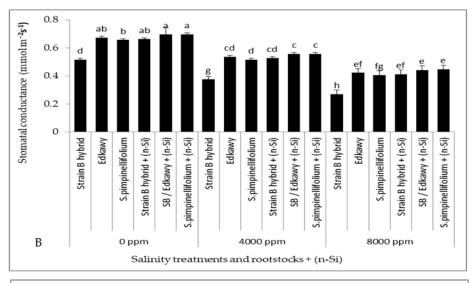
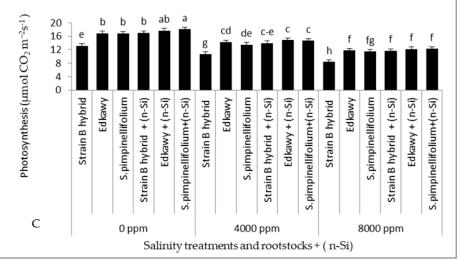


Figure 3. Cont.

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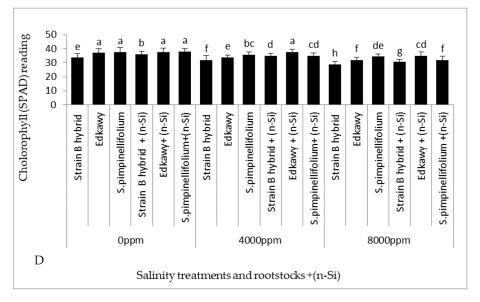


Figure 3. Effects of water salinity levels, rootstocks, foliar spray with (n-Si), and their interactions on (**A**) transpiration rates, (**B**) stomatal conductance, (**C**) photosynthesis and (**D**) SPAD readings. Vertical bars represent standard errors of the mean; in each bar, values followed by different letters differ significantly at p = 0.05 according to the LSD test.

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3.4. Quality Parameters of Tomato Fruits

Total soluble solids (TSS), firmness, and vitamin C values for tomato fruits were significantly influenced by salinity levels, grafting, foliar application of nano-silicon, and their interactions (Table 2). TSS, firmness, and vitamin C values for tomato fruits were increased considerably as a result of increasing salinity. Tomato fruits from the Edkawy rootstock with nano-silicon and from the *S. pimpinellifolium* rootstock with n-Si showed significantly greater TSS percentages compared to other treatments. Harvested fruits from the self-grafted Strain B hybrid had lower quality parameters than fruits from tomato plants subjected to the 8000 ppm concentration treatment.

Table 2. Effect of water salinity, grafting, foliar application of nano-silicon (n-Si), and their interactions on the quality parameters of tomato fruits (combined 2020 and 2021 seasons).

Salinity Levels	Rootstocks + (n-Si)	TSS% (Brix°)	Firmness (n)	Vitamin C (mg/100 g FW)
	Strain B hybrid	4.43 ^h	1.467 ^j	14.67 ^j
	Edkawy	5.60 ^{fg}	2.150 hi	18.27 ^{hi}
0 ppm	S. pimpinellifolium	6.20 ^{ef}	2.08 ⁱ	16.33 ^{ij}
оррии	Strain B hybrid + (n-Si)	4.33 ^h	1.67 ^j	16.33 ^{ij}
	Edkawy + (n-Si)	5.67 ^f	2.43 ^{e–h}	19.27 ^{f-h}
	S. pimpinellifolium + (n-Si)	6.23 ^{ef}	2.37 ^{f-i}	18.93 ^{gh}
	Strain Bhybrid	5.10 gh	2.22 ^{g-i}	17.67 ^{hi}
	Edkawy	6.43 ^{ef}	3.017 ab	20.60 ^{e–g}
4000 ppm	S. pimpinellifolium	6.37 ^{ef}	2.62 ^{d-f}	20.33 ^{e–g}
4000 ppm	Strain B hybrid + (n-Si)	6.07 ^{ef}	2.48 e-g	18.67 ^{gh}
	Edkawy + (n-Si)	6.53 ^e	3.017 ab	23.33 ^{b-d}
	S.pimpinellifolium + (n-Si)	$6.43 ^{\mathrm{ef}}$	3.02 ^{ab}	21.33 ^{de}
	Strain B hybrid	7.87 ^d	2.78 b-d	21.07 ^{ef}
	Edkawy	9.53 ^c	3.18 ^a	23.67 bc
8000 ppm	S. pimpinellifolium	10.43 ^b	2.98 ^{a–c}	23.67 bc
оооо ррш	Strain B hybrid + (n-Si)	8.23 ^d	2.72 ^{c–e}	22.0 ^{de}
	Edkawy + (n-Si)	11.30 ^a	3.12 ^a	26.67 ^a
	S. pimpinellifolium + (n-Si)	11.47 ^a	3.08 ^a	25.33 ab
L	SD value at 0.05:	0.86	0.28	2.03

Values followed by the same letter are not significant according to the LSD test ($p \le 0.05\%$).

3.5. Mineral Content in Tomato Shoots

Measured mineral traits in tomato shoots were significantly influenced by salinity, grafting, foliar application of nano-silicon, and their interactions (Table 3). Increasing salinity concentration significantly reduced N, P, K, Ca, and Mg contents in tomato shoots. All treatments accumulated minerals more than the self-grafted strain B hybrid. At 4000 ppm levels, plants grafted and combined with foliar nano-silicon had better shoot mineral concentrations, although the self-grafted strain B hybrid showed the lowest concertation of N, P, K, Ca, and Mg under 8000 ppm levels.

Tested Na, Fe, and Zn in tomato shoots were also considerably affected by salinity, grafting, foliar application of nano-silicon, and their interactions (Table 4). Increasing salinity concentration significantly reduced Fe and Zn contents in tomato shoots, while Na increased with increasing salinity.

Edkawy with nano-silicon exhibited the lowest content of Na and the highest contents of Fe and Zn. On the other hand, the self-grafted strain B hybrid recorded the higher content of Na. Nevertheless, the self-grafted strain B hybrid had the lowest concentrations for Fe and Zn. Salinity, grafting, and foliar application of nano-silicon interaction showed various meaningful influences. The highest Na content appeared with the self-grafted strain B hybrid at 8000 ppm salt levels. Despite this, the lowest Na content appeared using a

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combination of grafting with nano-silicon under control. *S. pimpinellifolium* combined with nano-silicon had a high Zn content under 4000 ppm levels compared with other treatments.

Table 3. Effect of water salinity, grafting, and foliar application of nano-silicon (n-Si) interactions on the mineral contents of tomato shoots (combined 2020 and 2021 seasons).

Salinity Levels	Rootstocks + (n-Si)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
0 ppm	Strain B hybrid	3.70 ^{de}	0.51 ^f	4.23 ^b	0.38 ^h	0.49 ^d
	Edkawy	4.29 ^c	0.64 ^c	4.72 ^a	1.11 ^c	0.58 ^c
	S. pimpinellifolium	3.83 d	0.57 ^d	3.93 ^c	0.99 ^{de}	0.48 ^d
	Strain B hybrid + (n-Si)	4.53 bc	0.66 ^c	4.49 ab	1.13 ^c	0.64 ^b
	Edkawy + (n-Si)	4.87 ^a	0.89 ^a	4.71 a	1.45 ^a	0.67 ^a
	S. pimpinellifolium + (n-Si)	4.617 ^{ab}	0.76 ^b	4.70 ^a	1.1 ^c	0.59 ^c
	Strain B hybrid	2.58 ^{jk}	0.45 g	2.42 ^g	0.34 ^h	0.33 h
	Edkawy	2.96 ^{g-i}	0.54 ^{ef}	3.21 ^d	1.08 ^{cd}	0.37 ^{fg}
4000 ppm	S. pimpinellifolium	2.860 h-j	0.55 ^{de}	2.85 ^{ef}	0.93 ^e	0.36 f-h
4000 ppiii	Strain B hybrid + (n-Si)	3.260 fg	0.56 ^{de}	3.11 ^{de}	1.06 ^{cd}	0.41 ^e
	Edkawy + (n-Si)	3.52 ^{d-f}	0.59 ^d	3.31 ^d	1.31 ^b	0.42 ^e
	S. pimpinellifolium + (n-Si)	3.45 ^{ef}	0.55 ^{de}	3.27 ^d	1.03 ^{cd}	0.40 ^e
8000 ppm	Strain B hybrid	1.987 1	0.41 ^h	1.90 ^h	0.6 g	0.19 ^j
	Edkawy	2.66 i-k	0.51 ^f	2.40 g	0.69 ^{fg}	0.28 i
	S. pimpinellifolium	2.170^{1}	$0.46 ^{\mathrm{g}}$	1.90 ^h	0.63 g	0.18 ^j
	Strain B hybrid + (n-Si)	2.52 ^k	0.44 ^{gh}	2.82 ^f	0.64 g	0.33 gh
	Edkawy + (n-Si)	3.44 ^{ef}	0.56 ^{de}	3.11 ^{de}	$0.74^{\rm f}$	0.37 ^f
	S. pimpinellifolium+ (n-Si)	2.97 ^{gh}	0.50 ^f	2.31 ^g	0.73 ^f	0.29 ⁱ
L	SD value at 0.05:	0.3	0.04	0.3	0.1	0.1

Values followed by the same letter are not significant according to the LSD test ($p \le 0.05\%$).

Table 4. Effect of water salinity, grafting, and foliar application of (n-Si) interactions on minerals of tomato shoots (combined 2020 and 2021 seasons).

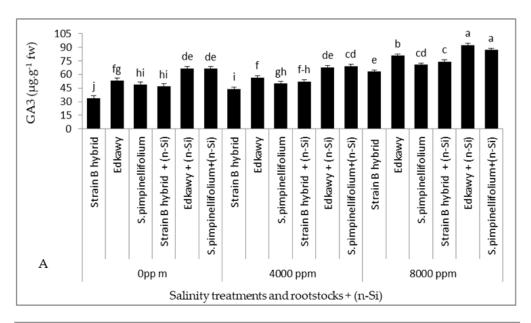
Salinity Levels	Rootstocks + (n-Si)	Na (%)	Fe (ppm)	Zn (ppm)
	Strain B hybrid	0.15 ^f	62.28 ^{cd}	44.55 ^d -g
0 ppm	Edkawy	0.11 ^{fg}	72.01 ^b	47.77 ^{de}
	S. pimpinellifolium	0.13 ^{fg}	65.18 ^c	45.5 ^{d–f}
орриг	Strain B hybrid + (n-Si)	$0.10^{{ m fg}}$	71.28 ^b	54.62 bc
	Edkawy + (n-Si)	0.09 g	85.30 a	64.15 a
	S. pimpinellifolium + (n-Si)	0.1 ^{fg}	82.33 a	58.61 ^{ab}
	Strain B hybrid	1.670 ^b	49.66 ^{jk}	26.43 ^{kl}
	Edkawy	1.102 ^{de}	52.34 ^{g-j}	42.31 ^{e–h}
4000 ppm	S. pimpinellifolium	1.108 ^{de}	50.28 ^{i–k}	41.53 f-h
4000 ppm	Strain B hybrid + (n-Si)	1.070 ^e	$53.47^{\text{ f-i}}$	39.71 ^{g-i}
	Edkawy + (n-Si)	1.085 ^e	59.4 ^{de}	48.60 ^d
	S. pimpinellifolium + (n-Si)	1.140 ^{cd}	55.87 ^{fg}	49.54 ^{cd}
	Strain B hybrid	1.740 ^a	42.19 ^m	20.91 ⁱ
	Edkawy	1.187 ^c	51.44 h-k	34.09 ^{ij}
8000 ppm	S. pimpinellifolium	1.188 ^c	48.50 ^{kl}	32.18 ^{jk}
оооо ррпп	Strain B hybrid + (n-Si)	1.17 ^c	44.94 ^{lm}	33.69 ^j
	Edkawy + (n-Si)	1.11 ^{de}	57.06 ef	41.08 f-h
	S. pimpinellifolium + (n-Si)	1.142 ^{cd}	54.20 fh	36.65 h-j
	SD value at 0.05:	0.05	3.58	5.7

Values followed by the same letter are not significant according to the LSD test ($p \le 0.05\%$).

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3.6. Plant Hormones, Antioxidant Enzymes, and Proline Content in Tomato Shoots

Salinity, grafting, foliar application of nano-silicon, and their interactions result in variations and changes in the activity of the most important hormones (GA3, ABA) and POD antioxidant enzymes as well as the amino acid proline. Increasing salinity concentrations increase the content of GA3, ABA, and proline (Figure 4A,B,D). Greater activity of POD was documented at 4000 and 8000 ppm compared with non-saline water (Figure 4C). The self-grafted Strain B hybrid recorded the lowest GA3, ABA, and proline content, as well as the lowest POD activity. The higher GA3, ABA, and proline contents were registered for Edkawy combined with nano-silicon under 8000 ppm. Moreover, Edkawy combined with nano-silicon registered the greatest POD activity at 8000 ppm (Figure 4C), while the self-grafted Strain B hybrid exhibited the lowest contents of GA3, ABA, and proline with 0 ppm. Correspondingly, the self-grafted Strain B hybrid had the lowest POD activity with non-saline water. The concentration of proline was significantly increased with increasing salinity levels (Figure 4D). A higher proline content was recorded for Edkawy combined with nano-silicon under 8000 ppm.



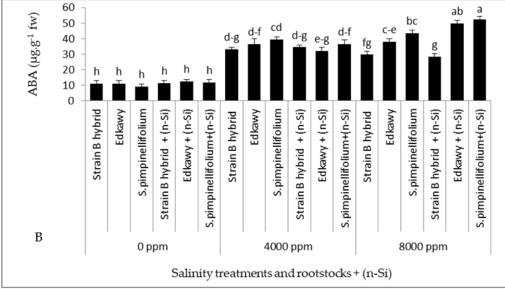
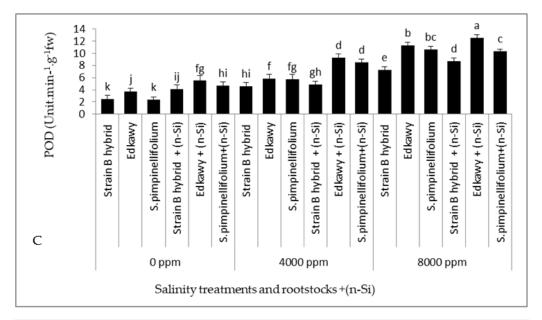


Figure 4. Cont.

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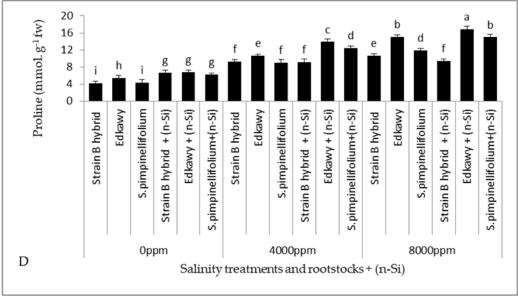


Figure 4. Effect of water salinity levels \times rootstocks and foliar spray with (n-Si) and their interactions on (**A**) GA₃, (**B**) ABA, (**C**) POD, and (**D**) proline. Vertical bars represent standard errors of the means; in each bar, values followed by different letters differ significantly at p = 0.05 according to the LSD test.

3.7. Clustering Analysis

Cluster analysis included all growth traits (i.e., plant height, the number of leaves per plant, fresh and dry weights of shoots and roots per plant), all yield components (i.e., the number of fruits per plant, average fruit weight, and fruit yield (kg) per plant), all mineral compositions in tomato plants (total nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), iron (Fe), and zinc (Zn) concentrations), physiological traits (i.e., transpiration rate, stomatal conductance, photosynthesis, and chlorophyll reading), and activity of antioxident enzymes, gibberellic acid, abscisic acid content, and leaf proline. Figure 5 illustrates a heatmap showing the relationships among the salinity levels and grafting combinations based on the tested parameters. Heatmap analysis clearly identified the overall variations among all treatments. The grafting treatment alone increased all studied traits compared with the non-salt-stressed control. However,

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foliar application of nano-silicon (n-Si) on the stressed grafted tomato exerted a positive role through decreasing oxidative injury by enhancing photosynthetic performance, increasing antioxidant enzyme concentrations, and significantly improving plant growth and yield.

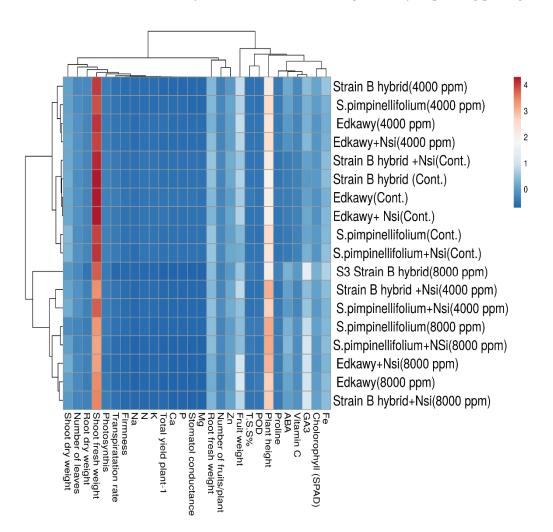


Figure 5. Heatmap of salinity stress, rootstocks, and measured parameters of tomato samples. The differences in the response variables between all studied treatments are visualized in the heatmap diagram. Columns represent the individual response variables, while rows represent the treatments. Lower numerical values are colored blue, whereas higher numerical values are colored red (see the scale at the bottom right corner of the heatmap).

4. Discussion

For the first time, this study introduced an approach to study the effect of grafting combined with foliar application of nano-silicon to improve salt tolerance in tomato. Recently, a few studies have aimed to improve salt tolerance in tomatoes utilizing grafting [50,51]. The tomato plant is a moderately salt-tolerant plant. Plant growth parameters are a perfect indicator for evaluating several abiotic stresses on plants (Table 1). The present data indicated that grafting with foliar application of nano-silicon is an effective approach for enhancing salt tolerance in tomato, as under saline conditions the plant growth parameters of most grafted combinations and nano-silicon combinations were significantly higher than their counterparts without nano-silicon application. Considerable reductions were observed in plant height, number of leaves, and shoot and root weights with higher salinity levels, confirming trends observed in other studies [50–52]. The combination of grafting and nano-silicon application could enhance the salt tolerance of tomato plants. The results also indicated that root growth was negatively affected by salt stress, which also affected

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shoot and plant height and lowered water and mineral uptake [50,52]. The trends observed were to be expected, since high salinity levels create water stress via osmotic imbalance at the soil–root interface and impair a plant's ability to take up water through the roots [53], thereby negatively impacting fresh and dry weights (Table 1). This could induce plasmolysis, which, in turn, might alter the plant's cellular and macromolecular metabolism and result in a slowing down or halting of plant growth [54]. Additionally, high salt concentrations might be damaging to both shoots and roots due to ionic toxicity brought about by excess Na+ ions, as they generate ionic imbalances within plants [50,54,55]. In this research, a high amount of Na⁺ documented in the shoot parts of tomato plants under salinity stress could be the result of Na+ accumulation in cellular vacuoles, whose function is to regulate osmotic balance inside the cell and maintain the photosynthetic rate at nominal levels [53]. It should be stated that the rootstock genotype had a prominent role in the performance of grafted tomato crops under stress conditions [56]. It is also worth mentioning that the data presented here suggest that the scion of the hybrid Strain B exhibited higher growth when grafted onto the rootstock of cvs Edkawy and S. pimpinellifolium as compared to self-grafted plants. However, there is a significant contribution made by the shoot genotype to growth [57]. Since cross-grafting involves tissue wounding and reunion, similar to what is observed in wound healing or self-grafting, it may be inferred that additional stress tolerance may be imparted by specific compatibility factors. These may include specific root/shoot signals [58], including RNA transport [59]. The foliar application of nano-silicon combined with grafting had a growth-promoting effect on unstressed tomato plants. The shoot and root fresh weights of grafted plants subjected to nano-silicon treatment increased significantly compared to the untreated grafted plants; the increase in shoot fresh weight might be described by the higher number of leaves (Table 1). The exogenous nano-silicon application may enhance the growth and yield of tomato plants [30–34], as was observed in various instances. In this study, the combination of grafting with nano-silicon application was associated with significantly higher fruit number, fruit weight, and total yield per plant values under non-saline conditions, although the smallest values were obtained at high salt levels (Figure 2). Tomato fruit yield might be described by the fruit weight and the number of fruits. Reduction in fruit weight or fruit number leads to reduced tomato fruit yield [55]. Salinity negatively impacts and decreases water and nutrient availability to the plant, causing a reduced photosynthetic rate. Such water stress is understood to result in a reduction in the number of fruits, total fruit yield, and fruit fresh weight [50,52,54]. In contrast, the data demonstrate that grafting with nano-silicon treatment counteracted salinity stress and resulted in higher fruit number, fruit fresh weight, and ultimately total fruit yield in the tomato plants compared with the controls. The appreciated effect of nano-silicon might be owed to its role in increasing RNA polymerase expression and the activity of ribosomal proteins, which stimulate stress tolerance and lower the transpiration rate and oxidative stress, thereby stabilizing the photosynthetic rate, and ultimately improving fruit yield [29,31,34]. The highest mean fruit weight was recorded for Edkawy combined with nano-silicon under 0 and 4000 ppm salt concentrations (Figure 2). Furthermore, the scion of hybrid Strain B produced a higher fruit crop when grafted onto the rootstock of cvs Edkawy and S. pimpinellifolium as compared to ungrafted plants, confirming the results obtained by [60]. It should be mentioned that *S. pimpinellifolium* is reputed to be rich in genes involved in biotic and abiotic stress responses in comparison with other varieties of cultivated tomato [10], which could potentially explain the trends observed. Chlorophyll is the major pigment involved in photosynthesis in plants [42]. The results indicated that under salt stress, chlorophyll readings (SPAD) were decreased, while nano-silicon addition increased chlorophyll readings (SPAD) (Figure 3C). The increase in chlorophyll readings (SPAD) with the application of nano-silicon under salinity stress might be associated with improved antioxidant defense and decreased oxidative damage due to the added nano-silicon, as observed in previous studies [31,35,47,51]. Potato plants treated with nano-silicon under salinity stress showed improved growth in terms of plant height, fresh and dry weights, and leaf chlorophyll contents [31]. In this study, transpiration rate, stomatal conductance, and photosynthesis

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were significantly decreased by increasing salinity levels, while this effect was significantly alleviated by grafting along with foliar application of nano-silicon alleviates (Figure 3A,B). Transpiration rate and photosynthesis are correlated with stomatal conductance [31,34,35]. According to the results obtained, stomatal conductance showed decreased values under salinity stress, resulting in decreased transpiration rates and photosynthesis, similar to what was observed by [61,62]. The application of nano-silicon in combination with grafting is known to enhance stomatal conductance, transpiration rate, and photosynthesis, in the latter case by improving the activity of enzymes involved in photosynthesis [33,35,36,61,62]. Furthermore, the benefits of silicon nano-particles for plants grown under salinity stress are linked to an increased photosynthetic rate, stomatal conductance, and water use efficiency, all of which improve crop plant tolerance to salinity [31]. Silicon increases the stomatal conductance of plants regarding improved water status in plants under water deficit due to increased water uptake by the roots [62–65], and this increases transpiration rates and photosynthesis. Edkawy with nano-silicon and *S. pimpinellifolium* with nano-silicon recorded better stomatal conductance and photosynthesis under 4000 ppm levels. According to the results (Table 2) and previous reports, measures of tomato fruit quality, such as TSS, vitamin C content, and firmness, are changed due to salinity [55,61]. TSS content in tomato fruits is an essential factor in manipulating tomato quality. The data generated in this study indicated that TSS increased with increasing salt levels. Previous research found that TSS in tomato increased as salinity increased [11,61,66]. Foliar application of nano-silicon combined with increasing salinity increased the TSS of tomato plants, which might be referred to higher accumulations of metabolites and the direct modification of starch into soluble sugar [31,33]. In this experiment we found a significant increase in TSS in tomato fruits with grafting under the 8000 ppm level. Tomato fruit firmness increased (Table 2) with increasing salinity. Similar results have been reported elsewhere [11,67,68], and it has been found that nano-silicon increases firmness and fruit quality [11,26,39]. Increasing fruit firmness could be referred to the powerful bonding of silica to the cellulose structure [39,69]. Vitamin C (ascorbic acid) is an antioxidant that protects the body from free radical damage. According to the data, vitamin C increased with increasing salinity levels and also with nano-silicon treatment. Similar data were obtained for tomatoes [26,31,39] using nano-silicon and salinity to enhance vitamin C. The results for tomato fruits from Edkawy (Tables 3 and 4) showed that increasing salinity concentration significantly reduced N, P, K, Ca, Mg, Fe, and Zn contents in tomato shoots. On the contrary, Na content was increased with rising salt levels in tomato plants. It was reported that N, P, K, Ca, Mg, Fe, and Zn contents were lowered at high salts levels [31,55]. The results showed a decrease in tomato growth parameters and yield due to less water uptake which led to decreased mineral absorption under salinity. The higher Na amount in plants under saline conditions could be referred to the accumulation of Na inside vacuoles. On the other hand, the data for nano-silicon combined with grafting showed increased absorption of minerals under saline conditions, except Na was decreased [26]. The self-grafted Strain B hybrid showed the lowest concentrations of N, P, K, Ca, Mg, Fe, and Zn under 8000 ppm levels. Phytohormones play a major part in plant development under stress and normal conditions. Hormones such as ABA and GA have been described that regulate plant growth and development [70]. In Figure 4, it can be seen from the data that GA_3 concentration reduced under salt stress but increased with the application of silicon combined with grafting. Nano-silicon mitigates the harmful effects of NaCl on plant growth by improving endogenous GA3 in soybean [31,71]. In this study (Figure 4B), ABA increased with increasing salinity levels, in a similar fashion to what has been reported in previous studies [68], and was reduced when no silicon treatment was applied, as was also observed in [72,73]. Furthermore, nano-silicon reduced Na absorbance and decreased ABA concentration in the leaves of salt-stressed plants, resulting in an increase in stomatal aperture and CO₂ supply from the stomatal cavity to the CO₂ fixation site [31]. In this research, POD increased with rising salt stress levels (Figure 4C). On the other hand, nano-silicon application alleviated this effect of salt. Using nano-silicon enhances antioxidant activity, hence it plays a protective function

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against salt stress [28,31,59]. Compatible solutes such as proline (non-enzymatic) act as ROS scavengers and increase resistance to drought, salinity, and cold stresses [45,47,69]. In this study, the concentration of proline was significantly increased with increasing salinity levels (Figure 4D). A previous study on tomatoes yielded a similar result [74]. Higher contents of GA3, ABA, and proline were registered by Edkawy combined with nano-silicon under 8000 ppm. This could be related to the beneficial effects of nano-silicon on lipid peroxidation, plasma membrane stability, and osmolyte accumulation, all of which result in increasing concentrations of scavenging reactive oxygen species, predominantly hydrogen peroxide and superoxide [31,75].

5. Conclusions

Based on our findings, yield improvement can be achieved for tomato plants with no known tolerance to salt stress by grafting their scions on rootstocks of genotypes possessing a salinity tolerance. However, outstanding crop gain would be obtained from shoot–root exchange between salt-adapted genotypes. According to the obtained results in this study, it may be concluded that grafting combined with foliar application of nano-silicon is a unique technique for improving salt tolerance and reducing salt damage in tomato plants. Plant growth, fruit yield, fruit quality, especially vitamin C content and TSS percentage, mineral contents, and GA3, ABA, and proline levels of grafted tomato combined with foliar application of nano-silicon were significantly higher than the self-grafted Strain B hybrid under saline stress conditions. Based on these findings, it is advisable to grow the cross-grafted hybrid Strain B/Edkawy or hybrid Strain B/Solanum pimpinellifolium combined with foliar spray application of nano-silicon when production of tomato under high salinity conditions cannot be avoided.

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