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Grafting improves growth and yield of *Cucumis sativus* plants grown under salinity stresses by modulating antioxidant enzymes, water status and nutrient uptake

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Abstract

This study aimed to assess the probability of using the tolerance of a new rootstock plant to salt stress to improve cucumber growth, fruit quantity, and quality under salt stress conditions. Greenhouse experiments were conducted for 2020 and 2021, with five salt stress-tolerant genotypes (rootstock) and salt-sensitive genotype of Luerans (scion). Grafts of these genotypes were subjected to distinct salinity levels of 0, 50, 100, and 150 mM (NaCl). The morphophysiological responses of these genotypes to salt stress were evaluated under normal and stressful conditions. The plant height, leaf area (LA), leaf water content (LWC), number of leaves, root dry matter, shoot dry matter, rates of leaf appearance and stem elongation, fruit yield, and quality increased significantly in grafted cucumber plants compared with non-grafted individuals (control). The physiological properties of antioxidant enzymes, proline content, and leaf nutrient concentration (N, P, K, Ca, Mg, Fe, Zn, and Na) showed similar findings. Grafting Luerans (scion) onto five rootstocks significantly raised the activity levels of antioxidant enzymes (catalase and peroxidase), boosted proline accumulation, and decreased leaf sodium (Na) content.

Key words: NaCl, grafting, cucumber, morphological and physiological properties, fruit quality

Introduction

Cucumber (*Cucumis sativus* L.) is one of the most important horticultural crops in the Cucurbitaceae family. Cucumber production in greenhouses faces some abiotic stresses, particularly in arid regions, due to overusing mineral fertilizers, the utilization of brackish water, soil salinization, and high summer temperatures (Wei *et al.,* 2019; Fan *et al.,* 2020; Phogat *et al.,* 2020). Cucumber is susceptible to salt stress (Wei *et al.,* 2019). Salinity stress is due to an increase in one or more salt ions in the soil containing sodium (Na), bicarbonate ($NaHCO_3$), magnesium (Mg), sulfate (SO_4^{2-}), potassium (K), chloride (Cl), calcium (Ca), and carbonate ($CaCO_3$). Therefore, salinity stress has a negative effect on seed germination, physiology, growth, productivity, and reproduction, sometimes causing plant death under severe conditions (Riyazuddin *et al.,* 2020). Furthermore, fruit length, fruit weight, fruit diameter, firmness, transpiration, and photosynthesis rate were reduced with salt stress (Farajimanesh and Haghighi, 2020). Salinization is one of the most dangerous problems that cause land degradation problems worldwide. Salinity has a negative effect on plant growth and low soil microbial activity because of toxic ions and osmotic stress (Yan *et al.,* 2015). Numerous agricultural practices have been used to mitigate the adverse effects of biotic and abiotic stresses (Abdelaziz *et al.,* 2019; Abuarab *et al.,* 2020; Atia *et al.,* 2020; Abdelaziz *et al.,* 2021; Cocozza *et al.,* 2021; EL-Bauome *et al.,* 2022; Mahmoud *et al.,* 2022; Shehata *et al.,* 2022). One of the approaches used in agriculture to overcome abiotic stresses, particularly salinity, is grafting (Abdelaziz and Abdeldaym, 2019). Grafting is one of the most innovative tools, which might save time and costs in breeding programs (El-Mogy *et al.,* 2022). The most common grafted vegetable crops are cucumber, tomato, melon, pepper, and eggplant (Reddy, 2016). Cucumber grafting is not only an effective method to control soil-borne diseases but also enhances abiotic stress tolerance as well as grafting may also modify plant growth and improve traits like numbers of lateral branches, nodes, flowering and harvest time (Guan *et al.,* 2015). Grafting onto some rootstocks enhances the photosynthesis rate of plants under salt stress. Grafting can also improve salt tolerance by improving grafted plants' water absorption (Bohm *et al.,* 2017). Likewise, rootstocks increased the activity of antioxidant enzymes and elimination of sodium in the cells due to salt stress. Moreover, the advantages of the graft are effectiveness in the usage of water and minerals, reflected in higher vegetative development and fruit output. Other characteristics correlated to rootstocks are the high absorption of important elements under salt stress, like potassium, calcium, and magnesium, and the enhanced metabolic action of nitrogen. The rootstock enhanced chlorophyll concentration and photosynthetic capacity (Suárez-Hernández *et al.,* 2019). Therefore, the present study aimed to investigate the impact of used rootstocks on the vegetative growth, physiological properties, fruit quantity, and quality of greenhouse cucumber plants grown under salt-stress conditions.

Materials and methods

Plant material and growth conditions: The experiments were conducted in the plastic greenhouse at the experimental farm of the vegetable department at the Faculty of Agriculture, Cairo University, Giza, Egypt (latitude 30.05N, longitude 31.21E, and mean altitude of 70 m above sea level). Cucumber (*C. sativus*) *cv.* Laurens was used as a scion (Enza Zaden Company, Enkhuizen, Netherlands). The scion was grafted onto five rootstocks: Super Green (squash hybrid, Modesto Seeds Company, Modesto, California 95357, U.S.A.), Just (squash hybrid, American Takii seeds, California, U.S.A.), Bottle Gourd 1 (*Legenaria siceraria,* PI 491352), Bottle Gourd 2 (*Legenaria siceraria*, PI 491365), Watermelon (*Citrullus lanatus* var. *Colocynthoides*), and Laurens (cucumber cultivar, Enza Zaden, Enkhuizen, Netherlands) using the splice grafting method, The grafting method was performed in a private nursery in Badr Center, EL-Beheira governorate, Egypt. After four weeks, the grafted plants were transferred and transplanted in a plastic pot containing a mixture of sand and silt at a 1:1 ratio.

Environmental conditions: The following environmental condition variables were recognized daily during separately cultivated seasons in the greenhouse: the maximum air temperature, minimum air temperature, average air temperature, relative air humidity, and a variety of sunshine hours throughout the season (from September to December). Every year, the total precipitation was negligible (20.0 mm) (Table 1).

Table 1. Monthly environmental condition variables in the greenhouse for the two cultivated seasons

Year	Climate parameter	Month		
		October	November	December
2019	T_{min}. (ºC)	19.1	16	10.2
	T_{max} (ºC)	32.6	28.2	22.4
	T_{ave} (0C)	25.5	22.1	16.3
	RH (%)	57.08	54.54	66.31
	WS (m sec^{-1})	2.2	1.9	2.3
	Solar radiation (W m^{-2})	211.2	178.5	140.4
2020	T_{min}. (ºC)	19.7	14.2	11.4
	T_{max} (ºC)	33	26.6	23.7
	T_{ave} (0C)	26.35	20.4	17.55
	RH (%)	57.28	63.52	60.63
	WS (m sec^{-1})	2.7	1.8	2.1
	Solar radiation (W m^{-2})	216	157.16	144.72

Experimental design and stress treatments: After seven days of transplanting, the grafted and non-grafted cucumber plants were exposed to the following four salt levels with a nutrient solution: 0 mM (control), 50 mM, 100 mM, and 150 mM NaCl (El-Nasr Pharmaceutical Chemical Company, Obour, Egypt) to evaluate how salinity and rootstocks affected the cucumber vegetative growth and yield characteristics. The application of saline treatments continued until the end of the experiment. Each treatment includes ten replicates.

Plant growth parameters: After 70 days of transplanting, we assessed vegetative growth by Biovis LA meter application to measure the leaf area (LA), count the number of leaves, and determine the maximum plant height. The calculation of leaf water content was performed using the following formula:

$$LWC = \frac{FW\text{-}DW}{FW} \times 100$$

Wherever FW refers to the plant's fresh weight and DW indicates the plant's dry weight. Root and shoot dry matters were assessed using the formula:

$$\text{Root and shoot dry matter} = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

The rate of leaf appearance (RLA) and the rate of stem elongation (RSE) were calculated using the following (Fullana-Pericàs *et al.,* 2018):

$$RLA = \frac{\text{Maximum leaves- Minimum leaves}}{t_2\text{-}t_1}$$

Where t_2 and t_1 refer to the maximum and minimum leaves at 70 and 35 days, respectively.

$$RSE = \frac{H_2\text{-}H_1}{t_2\text{-}t_1}$$

Where RSE is the root square error and t_2 and t_1 are the plant height at 70 and 35 days, respectively. The total yield, fruit diameter, length, fresh weight, fruit dry weight, and fruit dry matter were all calculated while the fruits were harvested. A digital refractometer (model PR101, Co. Ltd., Tokyo, Japan) was used to estimate cucumber fruits' total soluble solids (TSS).

SPAD index and chlorophyll fluorescence factors: At 70 days of transplant, the fourth leaf of six cucumber plants were chosen randomly from each treatment to measure SPAD (chlorophyll content) using a SPAD-meter (SPAD 502, Minolta Co, Osaka, Japan). Three SPAD readings were averaged and conducted around the third leaf of the cucumber.

Electrolyte leakage: Electrolyte leakage (EL) from the cucumber leaves was evaluated according to the method described by (Nanjo *et al.,* 1999). Cucumber leaves were sliced and bathed in a 0.4 M mannitol (Merck) solution for 3 h at 25°C. The primary conductivity (C1) was assessed using a conductivity meter (ECO 401, Adwa, Romania). The socked samples were heated for 15 min, and the electrical conductivity (C2) was assessed for the second time after cooling. The EL ratio was assessed using the equation: EL(%)= (C1/C2) x 100.

Leaf nutrient content: Six cucumber plants had their fully expanded leaves randomly chosen, crushed in liquid nitrogen, and frozen at -80°C for subsequent chemical analyses. The determination of essential minerals (N, P, K, Ca, Mg, Fe, Zn, and Na) in dried leaf samples was carried out. About 500 mg of cucumber leaves were treated with an acid mixture containing sulfuric and perchloric acids. Following a 10-minute heating of the solution at 50°C, an additional 0.5 mL of perchloric acid was added, and heating continued until a clear liquid was obtained. For assessing nitrogen (N) content in cucumber leaf tissues, the Kjeldahl (1990) method, was employed. To quantify phosphorus (P) in cucumber leaf tissues, the chlorostannous molybdophosphoric blue color method was applied. The concentration of potassium (K) was determined using a flame photometer (CORNING M410, Essex, UK). Moreover, the content of calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), and sodium (Na) was evaluated using an atomic absorption spectrophotometer equipped with air-acetylene fuel (Pye Unicam, model SP-1900, US).

Leaf proline content: The free proline content of cucumber leaves was determined using the methods proposed by Bates *et al.* (1973). Approximately 1 g of dried samples was homogenized in 10 mL 3% (w/v) sulfosalicylic acid. The mixture was filtered via filter paper (Whatman, No. 1). Then, 2 mL of ninhydrin acid agent and acetic acid were attached to a filtrated mixture. The tubes were heated in a water bath at 100°C for 60 min. Then, 4 mL of toluene was added and kept in tubes for 20 s. The colored fractions were read at a wavelength of 520 nm. Proline concentration was assessed using a standard curve and reported as µg. kg^{-1} FW.

Activity of antioxidant enzymes: A 50 mg leaf sample was mixed in liquid N and homologized in 5 mL potassium phosphate buffer (100 mM, pH 7.0). Homogenates were then centrifugated at 12,000 for 20 min at 4°C, and the supernatants were used to confirm the catalase (CAT, EC1.11.1.6) concentration following Aebi (El- Bedawy, 2014; Safaei Chaeikar *et al.,* 2020). All spectrophotometric analyses were performed as per Salehi-Lisar and Bakhshayeshan-Agdam, 2016).

The peroxidase (POD) content was determined following the method outlined by Aebi (El-Bedawy, 2014). The sample (0.5 g) was rapidly cooled using liquid nitrogen to halt the POD enzyme activity. The cooled samples were then crushed and subjected to centrifugation at 3930 rpm for 20 minutes, using 10 mL of extraction buffer [composed of 50 mM phosphate buffer, pH 7.0, containing 0.5 mM EDTA and 2 percent PVPP (w/v)]. The quantification of peroxidase content relied on a spectrophotometric approach that involves the formation of guaiacol in a reaction mixture (consisting of 450 µL of 25 mM guaiacol, 450 µL of 225 mM H2O2), and 100 µL of crude enzymes.

Statistical analyses: A complete randomized design with 10 replicates was employed to set up a factorial experiment. Subsequent to this, analysis of variance and mean comparisons were carried out using MSTAT C v.2.1 software. To identify significant differences, Duncan multiple range tests (at a significance level of $P \leq 0.05$) were performed.

Results

Plant growth characteristics: Table 2 illustrates the superior performance of grafted plants compared to non-grafted plants concerning their vegetative growth traits. Grafted plants exhibited greater excellence across parameters such as plant height, shoot dry matter, root dry matter, number of leaves, leaf area (LA), leaf water content (LWC), root length area (RLA), and root surface area (RSE) compared to non-grafted plants. Salinity treatments led to a decline in the vegetative growth of both grafted and non-grafted plants. However, grafted plants experienced a comparatively smaller reduction ratio than their non-grafted counterparts. This discrepancy can be attributed to the fact that heightened salinity had a more pronounced impact on diminishing growth parameters.

The best results for leaf appearance and the RSE were obtained by Gourd 1 on and watermelon rootstocks. The interaction between salinity treatments and the rootstocks showed that the best plant height was recorded when using Gourd 1 rootstock at a 50 mmol salinity level. At the same time, the best shoot dry matter was recorded when Gourd 2 was used at 50 and 100 mmol salinity levels. Regarding root dry matter, Gourd 1 rootstock reached the best measurements using a salinity level of 50 mmol. On the other hand, Super Green rootstock was the best among different rootstocks at the level of 50 mmol salinity, regarding the number

Table 2. Effect of interaction of salinity and rootstocks on vegetative growth

Rootstock	Treatment	Plant height (cm)	Shoot dry matter (g)	Root dry matter (g)	Number of leaves	Leaf area (cm^2)	Leaf water content (%)	Rate of Leaf appearance (Leaf tiller-. day^{-1})	Rate of stem elongation (cm tiller-. day^{-1})
Super green	Control	145.3 ab	68.20 a-c	59.69 a	30.65 a	23.63 a	86.74 a	0.287 a-c	1.567 a-c
	50 m mol NaCl	143.7 a-c	68.08 a-c	46.59 de	29.42 a	22.43 a-c	86.30 a-c	0.283 a-d	1.330 c-g
	100 m mol NaCl	138.0 a-d	46.36 d-f	42.38 e	25.54 a-c	19.82 a-e	83.66 b-g	0.270 a-e	1.047 f-i
	150 m mol NaCl	135.0 a-e	34.92 f	29.78 h	25.51 a-c	19.50 a-e	82.72 e-g	0.197 c-h	0.853 h-j
Just	Control	156.0 a	68.26 a-c	53.53 a-c	29.30 a	21.46 a-d	85.86 a-d	0.303 ab	1.487 b-d
	50 m mol NaCl	141.3 a-c	62.21 a-d	46.32 de	28.32 ab	19.81 a-e	85.06 a-f	0.257 a-e	1.360 c-f
	100 m mol NaCl	126.3 b-f	58.47 b-e	41.25 e	28.08 ab	18.30 c-f	84.09 a-g	0.223 b-h	1.280 c-g
	150 m mol NaCl	90.33 hi	49.46 d-f	36.74 f	25.50 a-c	16.96 d-g	83.05 d-g	0.157 gh	1.087 e-i
Guard 1	Control	162.3 a	72.42 ab	57.01 ab	30.44 a	20.64 a-d	85.43 a-e	0.310 ab	1.897 a
	50 m mol NaCl	145.0 ab	68.74 ab	55.13 ab	24.94 a-c	19.80 a-e	85.00 a-f	0.233 b-h	1.247 c-g
	100 m mol NaCl	120.0 b-g	59.25 a-e	49.71 cd	24.30 a-d	17.23 d-g	84.05 a-g	0.233 b-h	1.193 d-h
	150 m mol NaCl	110.3 d-h	46.78 d-f	41.47 e	20.06 cd	14.06 fg	82.31 fg	0.163 f-h	1.007 f-i
Guard 2	Control	145.0 ab	77.78 a	52.02 b-d	25.85 a-c	18.07 c-f	86.30 a-c	0.250 a-f	1.440 c-e
	50 m mol NaCl	125.7 b-f	74.61 ab	46.52 de	21.73 b-d	17.95 c-f	84.75 a-f	0.207 c-h	1.253 c-g
	100 m mol NaCl	121.5 b-g	71.69 ab	35.39 fg	21.17 b-d	15.70 e-g	84.45 a-g	0.190 e-h	1.113 e-i
	150 m mol NaCl	106.3 e-h	59.28 a-e	24.55 gi	18.83 cd	14.19 fg	78.41 h	0.180 e-h	0.980 g-j
C.L. Colocynthoides	Control	134.0 a-e	72.02 ab	52.83 b-d	25.87 a-c	23.22 ab	86.71 a	0.337 a	1.890 a
	50 m mol NaCl	122.3 b-g	68.79 ab	45.39 ef	24.19 a-d	18.83 b-e	86.55 ab	0.267 a-e	1.833 ab
	100 m mol NaCl	102.7 f	56.51 b-e	40.27 f	21.85 b-d	17.85 c-f	86.14 a-c	0.263 a-e	1.210 c-h
	150 m mol NaCl	93.67 gh	44.56 d-f	34.52 fg	21.64 b-d	16.84 d-g	83.42 c-g	0.247 a-g	0.760 ij
Luarens	Control	121.3 b-g	49.80 c-f	48.26 c-e	21.74 b-d	17.75 d-f	85.32 a-e	0.220 b-h	1.353 c-f
	50 m mol NaCl	115.7 c-h	45.20 d-f	39.78 f	20.51 cd	17.48 d-g	84.01 a-g	0.193 d-h	1.240 c-g
	100 m mol NaCl	98.00 f-h	41.34 ef	31.28 g	19.62 cd	15.95 e-g	81.74 g	0.190 e-h	1.000 f-i
	150 m mol NaCl	62.00 i	37.71 f	23.57 i	17.72 d	13.06 g	50.26 i	0.147 h	0.627 j

Different letters indicate significant differences between treatments (Duncan's multiple range test at $P < 0.05$)

of leaves, LA, and LWC. Watermelon rootstock recorded the highest stem elongation rate at 50 mmol salinity level.

Leaf nutrient content: The impact of different salinity levels and rootstocks on leaf nutrient content and the concentration of mineral elements within plants exhibited variations due to the diversity of rootstocks employed (Table 3). The highest nitrogen (N) concentration was discerned in the Gourd 2 rootstock, whereas the preeminent phosphorus (P) concentration was observed in the Super Green, Gourd 1, Gourd 2, watermelon, and Just rootstocks, respectively.

Concerning leaf potassium (K) concentration, the Gourd 2 and Gourd 1 rootstocks displayed the highest values when contrasted with the remaining rootstocks. Conversely, magnesium (Mg), calcium (Ca), and iron (Fe) concentrations showcased an increase in the Gourd 1 rootstock. As for zinc (Zn), the most notable concentration was achieved through employment of the Super Green rootstock, surpassing all other rootstocks.

The concentration of leaf elements exhibited a progressive decrease with the incremental elevation of salinity levels. In terms of the interplay between rootstocks and salinity treatments, the Watermelon rootstock at 50 mmol and 100 mmol salinity levels exhibited the highest N concentration. In contrast, the Super Green rootstock at 50 mmol salinity levels and the Watermelon rootstock at both 50 mmol and 100 mmol salinity levels showcased the highest P concentration. For K concentration, the 50 mmol salinity level elicited the highest values using the Just, Gourd 1, and Gourd 2 rootstocks. Similarly, the Gourd 2, Super Green, and Watermelon rootstocks demonstrated elevated Mg concentration at the 50 mmol salinity level.

Regarding calcium (Ca) concentration, the Super Green, Gourd 1, Super Green, and watermelon rootstocks yielded the highest values at the 50 mmol salinity level. Conversely, iron (Fe) concentration peaked at the same salinity level in the watermelon rootstock. The zinc (Zn) concentration decreased in the Super Green rootstock under 50 mmol salinity. The outcomes indicated an escalation in sodium (Na) concentration with the rise in salinity concentrations, albeit the percentage of increase was less pronounced in grafted plants compared to non-grafted ones. The lowest Na concentration was identified in the control treatment utilizing the Super Green, Just, and Watermelon rootstocks. Moreover, the Gourd 1, Super Green, and watermelon rootstocks at 50 mmol salinity levels exhibited the minimal Na concentration.

Photosynthetic pigment, EL, and total yield: The interaction between salinity levels and rootstocks had a noticeable impact on SPAD values (Fig. 1A), with an inverse relationship as salinity concentration increased. Interestingly, grafted plants exhibited a smaller decrease percentage compared to non-grafted plants. Among rootstocks, the Super Green rootstock displayed the highest SPAD value, even surpassing the control treatment. Notably, at the 50 mmol salinity level, the Super Green rootstock exhibited the highest leaf chlorophyll content.

EL (%) results (Fig. 1B) indicated higher values in non-grafted plants than in their grafted counterparts. The control treatment, particularly when using the Super Green and watermelon rootstocks, had the lowest EL, which increased with higher salinity levels. However, at the 50 mmol salinity level, the lowest

Table 3. Effect of interaction of salinity and rootstocks on leaf nutrient content

Root stock	Treatment	N (%)	P (%)	K (%)	Mg (%)	Ca (%)	Fe (ppm)	Zn (ppm)	Na (%)
Super green	Control	4.133 d	0.523 a	3.610 b	0.620 bc	2.510 c	198.1 d	92.13 a	0.02 k
	50 m mol NaCl	3.420 gh	0.390 b-f	3.440 ef	0.560 ef	1.390 fg	100.3 j	88.25 d	0.05 hi
	100 m mol NaCl	3.280 kl	0.310 e-i	3.220 hi	0.510 h	1.320 kl	79.82 p	58.43 q	0.09 f
	150 m mol NaCl	3.240 m	0.280 g-j	3.030 m	0.410 k	1.260 m	59.57 s	52.17 v	0.11 e
Just	Control	4.190 b	0.410 b-e	3.580 c	0.630 b	2.500 c	200.1 c	84.38 g	0.02 k
	50 m mol NaCl	3.420 gh	0.360 c-h	3.450 de	0.550 fg	1.400 f	97.92 l	82.31 h	0.06 gh
	100 m mol NaCl	3.290 jk	0.310 e-i	3.230 h	0.490 i	1.330 jk	79.78 p	58.23 r	0.10 ef
	150 m mol NaCl	3.230 mn	0.250 ij	3.100 k	0.410 k	1.200 o	55.64 t	53.28 u	0.17 ab
Guard 1	Control	4.150 c	0.470 ab	3.620 ab	0.650 a	2.550 a	204.3 a	82.16 i	0.04 ij
	50 m mol NaCl	3.410 h	0.330 d-i	3.450 de	0.540 g	1.400 f	101.1 i	86.27 e	0.04 ij
	100 m mol NaCl	3.310 i	0.290 f-j	3.220 hi	0.460 j	1.340 j	88.81 n	84.54 f	0.09 f
	150 m mol NaCl	3.240 m	0.280 g-j	3.050 l	0.410 k	1.250 mn	59.52 s	56.47 t	0.13 d
Guard 2	Control	4.207 a	0.460 a-c	3.630 a	0.590 d	2.530 b	203.3 b	88.86 c	0.03 jk
	50 m mol NaCl	3.420 gh	0.350 d-i	3.460 d	0.570 e	1.370 hi	98.62 k	79.63 l	0.06 gh
	100 m mol NaCl	3.300 ij	0.300 f-j	3.220 hi	0.520 h	1.340 j	87.94 o	59.31 o	0.11 e
	150 m mol NaCl	3.220 n	0.300 f-j	3.110 k	0.420 k	1.250 mn	60.38 r	59.24 p	0.17 ab
C.L. Colocynthoides	Control	4.140 cd	0.420 a-d	3.570 c	0.610 c	2.200 d	191.2 e	91.25 b	0.02 k
	50 m mol NaCl	3.440 f	0.380 b-g	3.410 g	0.570 e	1.390 fg	105.4 g	84.54 f	0.05 hi
	100 m mol NaCl	3.430 fg	0.370 b-h	3.440 ef	0.540 g	1.360 i	104.6 h	81.21 j	0.09 f
	150 m mol NaCl	3.310 i	0.310 e-i	3.180 j	0.510 h	1.320 kl	98.68 k	68.19 m	0.16 bc
Luarens	Control	3.690 e	0.380 b-g	3.430 f	0.560 ef	2.150 e	147.1 f	80.86 k	0.04 ij
	50 m mol NaCl	3.410 h	0.320 d-i	3.110 k	0.480 i	1.380 gh	96.52 m	59.45 n	0.07 g
	100 m mol NaCl	3.270 l	0.270 h-j	3.210 i	0.420 k	1.310 l	75.71 q	57.36 s	0.15 c
	150 m mol NaCl	2.200 o	0.200 j	3.020 m	0.390 l	1.240 n	53.80 u	48.37 w	0.18 a

Different letters indicate significant differences between treatments (Duncan's multiple range test at $P < 0.05$)

Table 4. Effect of interaction of salinity and rootstocks on fruit quantity and quality of cucumber plants.

Root stock	Treatment	Total yield (g plant^{-1})	Fruit diameter (Mm)	Fruit dry matter (%)	Fruit dry weight (g)	Fruit fresh weight (g)	Fruit length (cm)	TSS (Brix^{O})
Super green	Control	828.0 ab	2.767 ab	5.973 bc	2.333 a-c	51.00 a	12.33 a	5.467 f-j
	50 m mol NaCl	406.7 c-h	2.500 a-c	5.533 c-e	1.970 b-f	33.74 b-d	9.800 c-e	6.033 b-f
	100 m mol NaCl	275.8 e-h	2.400 a-d	5.093 c-e	1.650 c-h	29.33 b-e	9.333 d-f	6.267 b-d
	150 m mol NaCl	206.7 gh	2.050 c-g	4.907 c-f	1.420 e-j	23.00 c-h	9.000 d-h	7.200 a
Just	Control	530.3 b-f	2.800 a	5.773 b-d	3.003 a	53.67 a	12.33 a	5.267 h-j
	50 m mol NaCl	470.8 c-g	2.500 a-c	5.560 b-e	1.780 c-g	32.67 b-d	11.10 a-c	5.733 d-i
	100 m mol NaCl	409.3 c-h	2.400 a-d	5.560 b-e	1.273 f-k	22.33 d-h	8.600 e-i	6.033 b-f
	150 m mol NaCl	330.0 e-h	1.900 e-g	5.547 c-e	1.213 g-k	21.67 d-h	7.833 g-k	6.333 bc
Guard 1	Control	1024 a	2.833 a	5.640 b-d	2.230 b-d	41.33 ab	11.40 ab	4.300 lm
	50 m mol NaCl	420.0 c-h	2.800 a	5.627 b-d	1.170 g-k	19.67 e-h	10.23 b-d	5.163 i-k
	100 m mol NaCl	311.3 e-h	2.767 ab	5.507 c-e	0.913 h-k	16.67 f-h	7.543 i-k	6.133 b-e
	150 m mol NaCl	200.0 gh	2.000 d-g	5.200 c-e	0.713 jk	12.00 gh	7.000 jk	6.367 b
Guard 2	Control	820.0 ab	2.867 a	7.493 a	2.323 a-c	41.67 ab	11.09 a-c	5.367 h-j
	50 m mol NaCl	669.2 b-d	2.767 ab	7.253 a	1.843 c-g	25.00 c-f	8.200 f-j	5.433 g-j
	100 m mol NaCl	587.8 b-e	2.300 b-e	6.667 ab	1.273 f-k	17.33 e-h	7.970 f-k	5.633 e-j
	150 m mol NaCl	240.0 f-h	1.733 fg	4.707 d-f	0.597 k	15.33 f-h	7.033 jk	5.767 c-h
C.L. Colocynthoides	Control	712.0 a-c	2.833 a	5.893 bc	2.673 ab	52.67 a	11.01 a-c	5.067 jk
	50 m mol NaCl	358.3 d-h	2.433 a-d	5.667 b-d	1.570 d-i	25.00 c-f	8.333 f-j	5.167 ij
	100 m mol NaCl	250.0 f-h	2.150 c-f	5.493 c-e	1.183 g-k	24.00 c-g	7.700 h-k	6.167 b-e
	150 m mol NaCl	240.0 f-h	2.067 c-g	4.493 ef	1.140 g-k	19.67 e-h	7.100 jk	6.467 b
Luarens	Control	401.7 c-h	2.733 ab	5.253 c-e	2.050 b-e	35.50 bc	9.167 d-g	3.900 m
	50 m mol NaCl	330.0 e-h	2.233 c-e	5.000 c-e	0.847 i-k	19.00 e-h	8.170 f-k	4.567 kl
	100 m mol NaCl	230.0 f-h	2.100 c-g	4.987 c-e	0.683 jk	13.83 f-h	7.133 jk	6.000 b-g
	150 m mol NaCl	115.0 h	1.633 g	3.867 f	0.543 k	11.00 h	6.833 k	6.067 b-e

Different letters indicate significant differences between treatments (Duncan's multiple range test at $P < 0.05$) and ± value indicates to stander

EL values were observed with the Just, Gourd 1, and watermelon rootstocks.

Regarding yield (Fig. 1C), grafted plants consistently outperformed non-grafted ones across different salinity levels. This indicated that yield generally decreased with escalating salinity levels. Gourd 1, Super Green, Gourd 2, and Watermelon rootstocks resulted in the highest total yields in the control treatment. The interaction between rootstocks and salinity treatments highlighted Gourd 2 rootstock as achieving the highest total yield at the 50 mmol salinity level.

Analyzing fruit quantity and quality (Table 4), grafted plants consistently excelled in yield and related factors, regardless of salinity levels. The data showed that yield and its components declined with increasing salinity, except for total soluble solids (TSS), which increased. The highest total yield among rootstocks occurred in the control treatment with Gourd 1, Super Green, Gourd 2, and Watermelon rootstocks.

Regarding fruit diameter, no significant differences were observed among rootstocks in the control treatment. Gourd 2 rootstock yielded the highest fruit dry matter across varying salinity levels. Regarding fruit dry weight, Just, Watermelon, Super Green, and Gourd 2 rootstocks demonstrated the highest values at the 0 mmol salinity level. For fruit fresh weight (FW), optimal results were achieved using Just, Watermelon, Super Green, Gourd 1, and Gourd 2 rootstocks in the control treatment.

Concerning fruit length, the Super Green, Just, Gourd 1, Gourd 2, and Watermelon rootstocks yielded the best outcomes at the 0 mmol salinity level. The interaction between rootstocks and salinity revealed that Gourd 2 rootstock at the 50 mmol salinity level achieved the highest yield and fruit dry matter. For fruit diameter, the Super Green, Just, and Gourd 1 rootstocks at 50 and 100 mmol salinity levels and Gourd 2 and Watermelon rootstocks at 50 mmol salinity levels resulted in the greatest fruit diameters. At the 50 mmol salinity level, the Super Green rootstock yielded the highest fruit dry weight.

Focusing on fruit FW characteristics, the Super Green and Just rootstocks yielded the best results. The Just rootstock performed well for fruit length at the 50 mmol salinity level. Conversely, total soluble solids (TSS) increased with rising salinity levels, with the Super Green rootstock recording the highest TSS values at the 150 mmol salinity level.

CAT and POD concentrations were lowest in the control treatment compared to various salinity treatments. Proline, CAT, and POD concentrations exhibited an upward trend with increasing salinity concentration. Different rootstocks led to increased enzyme concentrations compared to non-grafted plants (Fig. 2). The Super Green rootstock displayed the highest proline concentration in the control treatment (Fig. 2A), maintaining this trend at the 150 mmol salinity level. Similarly, CAT concentration was highest in the control treatment when the Super Green rootstock was employed (Fig. 2B). Regarding salinity treatments, the Gourd 2 rootstock yielded the highest CAT concentration at the 150 mmol salinity level. The highest POD concentration was noted in Gourd 1 in the control treatment (Fig. 2C). In salinity conditions, the Watermelon and Super Green rootstocks exhibited

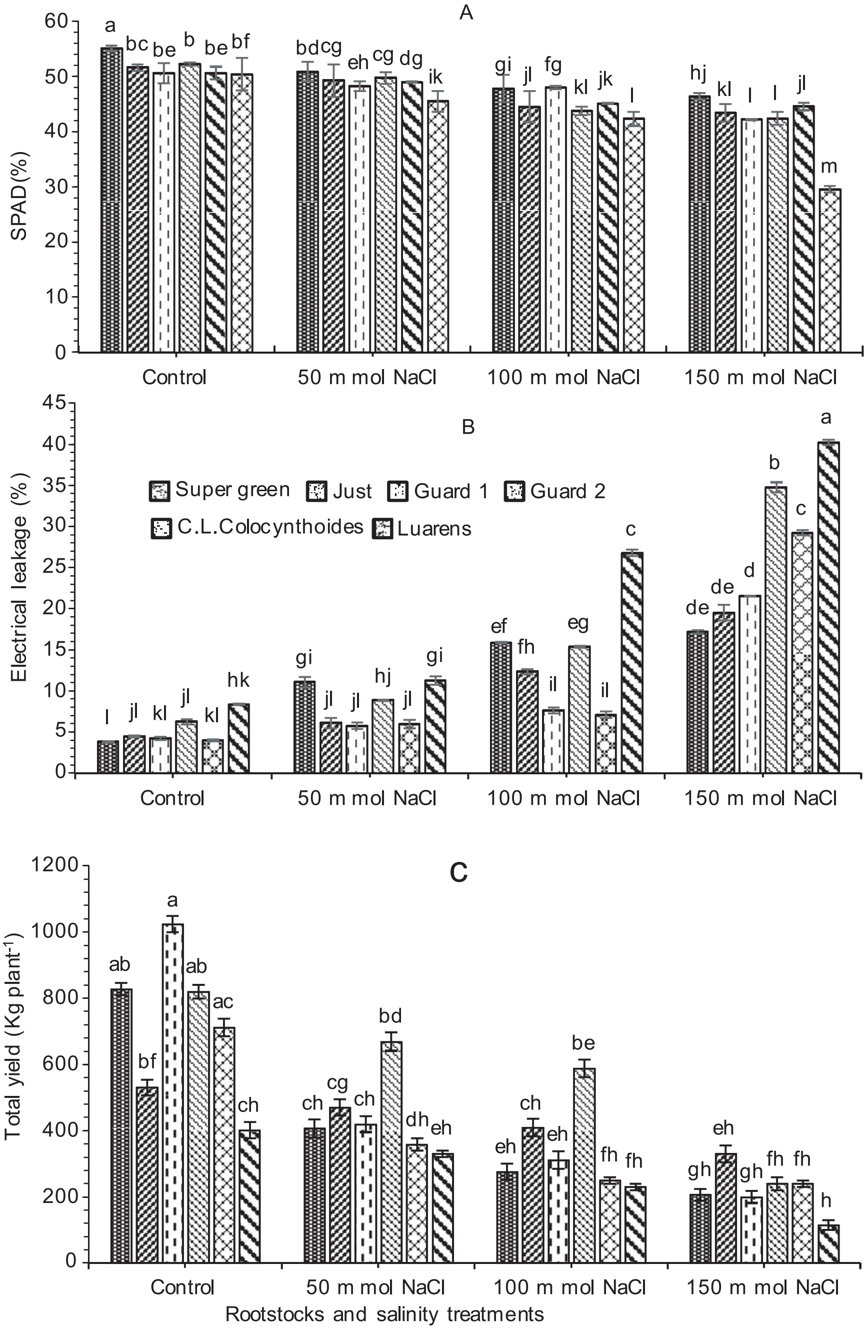

Fig. 1. Effect of interaction between salinity and rootstocks on (A) SPAD, (B) Electrical leakage, and (C) Total yield. Different letters indicate significant differences between treatments (Duncan's multiple range test $P < 0.05$).

the best POD concentration at the 150 mmol salinity level.

Discussion

Greenhouse vegetable production is experiencing rapid expansion in semiarid and arid regions worldwide, and Egypt is no exception, with greenhouses being established as part of the national sustainable development strategy for 2030 (Abdelaziz and Abdeldaym, 2018). However, cultivating greenhouse cucumbers in salt-affected soils, especially during winter, presents substantial challenges due to saline soil conditions and cold stress (Wei *et al.,* 2019; Fan *et al.,* 2020). Salinity, arising from soil or water, stands as a critical environmental stressor that detrimentally affects various physiological aspects of plants, including photosynthesis parameters, biochemical components, nutrient uptake, water absorption, vegetative growth features, and yield (Abdelaziz and Abdeldaym, 2019; Abdelaziz *et al.,* 2019; Mahmoud *et al.,* 2019; Mahmoud *et al.,* 2022).

To address these challenges, grafting is a commonly employed technique in cucumbers to confer resistance to abiotic and biotic stresses (Abdeldaym *et al.,* 2020; Farajimanesh and Haghighi, 2020; Bayoumi *et al.,* 2022; El-Mogy *et al.,* 2022; Shehata *et al.,* 2022). Pumpkin rootstocks, when used for grafting, have been shown to enhance cucumber's salinity tolerance by reducing sodium (Na) and chloride (Cl) accumulation in leaves (Chen *et al.,* 2020). The current study revealed that grafted cucumber plants outperformed their non-grafted counterparts regarding vegetative growth, even across various salinity levels (0, 50, 100, and 150 mmol). These findings align with previous research (El-Shraiy *et al.,* 2011; Bohm *et al.,* 2017; Chen *et al.,* 2020), which demonstrated that salinity-induced stress adversely affects plant growth by diminishing osmotic potential and triggering water stress and nutritional imbalances (Shannon and Grieve, 1998; Rouphael and Kyriacou, 2018).

The advantages of grafting were not limited to growth; grafted plants consistently exhibited higher yields and better quality than non-grafted plants under varying saline conditions. These results agree with prior studies (El-Shraiy *et al.,* 2011; Farajimanesh and Haghighi, 2020; El-Sayed *et al.,* 2021). Among the rootstocks studied, Gourd 1 demonstrated the most significant yield at 50 and 100 mmol salinity levels, while Gourd 2 excelled in fruit diameter and dry matter at the 50 mmol salinity level. Conversely, Super Green rootstock yielded the highest fruit dry weight at the same salinity level.

Grafted plants' superior performance could be attributed to factors such as enriched macro- and micro-elements (Abdeldaym *et al.,* 2020), increased respiration rate, reduced stomatal conductance, and enhanced net photosynthesis (Rouphael *et al.,* 2012; Amaro *et al.,* 2014). Moreover, total soluble solids (TSS) increased with escalating salinity, particularly in the Super Green rootstock. Notably, the type of rootstock influenced the concentration of mineral elements within plants. Leaves exhibited the highest concentrations of nitrogen (N), phosphorus (P), magnesium (Mg), calcium (Ca), and iron (Fe) when Watermelon rootstock was employed. Similar observations were reported in earlier studies, highlighting grafting's role in enhancing nutrient uptake compared to non-grafted plants (Abdeldaym *et al.,* 2018; Ceylan *et al.,* 2018; Abdeldaym, 2019; Sayed *et al.,* 2021).

Grafted plants also showcased differences in nutrient concentrations compared to non-grafted plants (El-Sayed *et al.,* 2021). Grafting's positive influence on nutrient concentrations could be attributed to robust root systems, facilitating efficient water and nutrient absorption from the

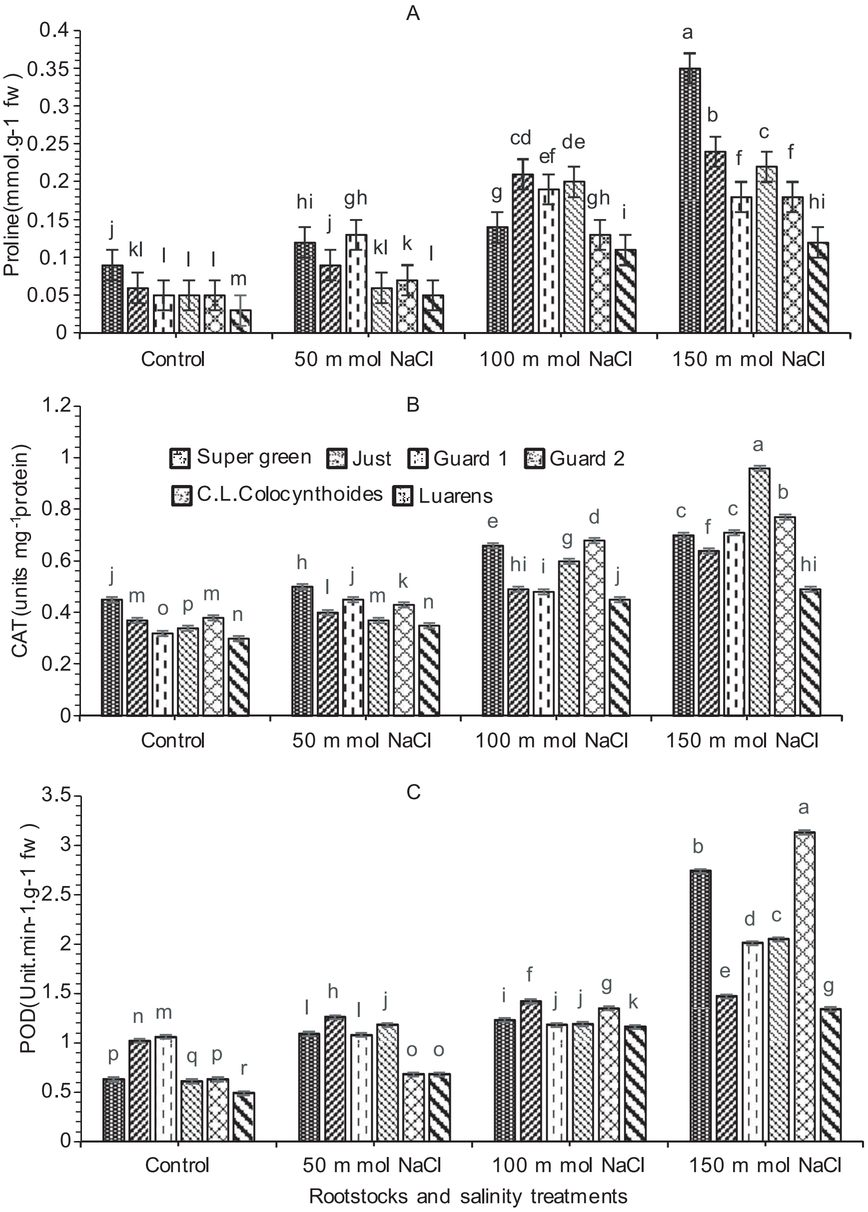

Fig. 2. Effect of interaction between salinity levels and rootstocks on (A) Proline, (B) Catalase enzyme activity (CAT), and (C) peroxidase enzyme activity (POD). Different letters indicate significant differences between treatments (Duncan's multiple range test

soil (Huang *et al.,* 2016) and sustaining leaf chlorophyll content (measured by SPAD). However, the positive effects of grafting might diminish as salinity concentrations increase (Abdallah *et al.,* 2021; Mahmoud *et al.,* 2022).

Under salinity-induced stress, electrolyte leakage (EL) increased, and antioxidant enzyme activities like superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) increased. Grafted plants exhibited superior antioxidant enzyme activities compared to non-grafted plants, indicating their enhanced ability to mitigate oxidative stress caused by environmental pressures. Reactive oxygen species (ROS) generated under environmental stress can damage plant cells, but antioxidant enzymes like POD, SOD, and CAT help counteract such damage.

In summary, grafting has emerged as a solution, enhancing cucumber plants' resilience to stressors, with grafted plants showing better growth, yield, and quality under varying salinity levels. This approach positively influences nutrient uptake, antioxidant enzyme activities, and overall plant performance, offering promise for sustainable cultivation in challenging environments.

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