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Original Article

The Effect of Foliar Spraying of Ascorbic Acid and Glycine Betaine on some Morpho-physiological and Biochemical Characteristics of Carola (*Momordica charantia L.*) under Salt Stress

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ABSTRACT

In arid and semi-arid locations like Iran, salinity is increasingly viewed as a severe hazard to agriculture. This study examined the effects of foliar spraying Carola plants with different doses of ascorbic acid and glycine betaine under varying watering circumstances. So, at the Research Farm of Velayat University of Iranshahr, an experiment was carried out in a split-plot factorial design with a randomized complete block structure with three replications. The experiment was run across two crop years (2020–2021 and 2021–2022). Applying ascorbic acid (2 mM) improved fruit length and diameter, fruit dry weight, fruit number, and yield, but salt stress decreased all of these characteristics. Photosynthetic pigments and relative water content (RWC) in leaves were both diminished by salt stress. Adding 2 mM ascorbic acid and 100 mM glycine betaine enhanced these characteristics. The phenolic compounds, momordicin, charantin, and proline were all upregulated by salt stress. Furthermore, by enhancing proline activity and RWC, the addition of ascorbic acid (2 mM) and glycine betaine (100 mM) to Carola plants made them more resistant to salt stress. In plants subjected to salt stress, the most abundant secondary metabolite momordicin was found in those treated with ascorbic acid and glycine betaine. These results showed that ascorbic acid and glycine betaine can be used to increase pigments and secondary metabolites in Carola plants under salt stress conditions.
Keywords: Ascorbic acid, Glycine betaine, Salinity stress, Secondary metabolites

INTRODUCTION

Carola from the Cucurbitaceae family is an annual plant with three irregular grooves [1]. The extent to which salinity affects agricultural output is a major environmental concern, particularly in dry and semi-arid parts of the globe. Salinity affects half of the world's rainfed fields and almost 20% of Faryab's agricultural lands [2]. Salinity stress is capable of reducing the photosynthetic capacity in plants by influencing various factors [3]. In salinity stress, plants need to maintain low internal soil water potential, maintain cell turgor, and absorb water for growth. These functions require increasing osmotic activity through absorption from the soil solution or synthesis of metabolic solutions [4]. The manufacture of growth-regulating chemicals is greatly aided by this combination, which is also crucial for enzymes involved in photosynthesis and the regulation of plant stomatal openings [5]. In addition, ascorbic acid is an important antioxidant compound to protect organs and cells from the destructive effects of oxygen-free radicals [6]. In most cases, the internal level of ascorbic acid is not sufficient to reduce the negative effects of various types of stress [7]. Glycine betaine promotes plant growth in its dissolved form by putting it into a dormant condition, and spraying it causes cell division to increase [8]. Under salt stress conditions (150 mmol sodium chloride), Malekzadeh [9] investigated how the antioxidant system and growth of soybean plants were affected by the external application of glycine-betaine. The scientists found that adding 50 mmol of glycine betaine to soybeans boosted their growth, reduced their proline level, and lowered their sodium ion content. Furthermore, in salt stress conditions, catalase activity was enhanced by glycine betaine treatments. When pea plants were subjected to salt stress, Kanwal et al. [10] studied the effects of spraying ascorbic acid onto their leaves. Researchers found that adding ascorbic acid to plants helped minimize the negative effects of salinity stress on growth, photosynthetic activity, and yield. In light of the foregoing, this article examines the role of ascorbic acid and glycine betaine in the salinity tolerance of the Carola plant.

MATERIALS AND METHODS

This randomized complete block design study aims to investigate the biochemical and morpho-physiological features of the Carola plant in a salt-and-fresh water management system as a result of foliar spraying with ascorbic acid and glycine-betaine. The design, which included 18 treatments with three replications over two crop years, 2020 and 2022, was implemented at the research farm of Velayat University of Iranshahr. Soil samples (in depth from 0 to 30 cm) were taken before planting, with the soil characteristics summarized in Table 1.

Table 1 Soil physical and chemical characteristics from 0 to 30 centimeters below the surface at the research location

Soil texture (%)	Sand (%)	Silt (%)	Clay (%)	Magnesium (meq/lit)	Ca (meq/lit)	K (ppm)	P (ppm)	N (meq/lit)	SAR	pH	EC (ms/cm)
Sandy loam	62.9	19.3	17.8	12.3	16.9	202.7	4.55	0.025	8.58	8.19	6.34

Plant Material, Method of Planting and Treatments

The planting distance in the row was 90 cm, the row distance in the plot was 1 m (3 x 4), and the distance between the repetitions was 3 m. One row of plants on the stack was cultivated with the plants of two adjacent stacks arranged in a zigzag pattern. Direct sowing was done in the field on 3 October 2020, and the seeds germinated one week after sowing. Carola seeds were purchased from Pakan Seed Company of Isfahan and soaked for 24 h to accelerate germination. The seeds were soaked in Carboxin Thiram before being planted 2 cm deep. For 10 days, each plot's field was irrigated daily based on the field's capacity. Treatments with salt, ascorbic acid, and glycine-betaine were reapplied with vinegar containing urea fertilizer in the 4-5 leaf stage following plant establishment. Irrigation is the primary variable in three different experimental treatments: those using only salt water, those using only fresh water, and a hybrid of the two. The amount of ascorbic acid and glycine betaine was also considered a secondary factor. The control group included normal water, ascorbic acid at two levels (0 and 2 mmol), and glycine betaine at three levels (0, 100, and 150 mmol). Irrigation was done up to the farm's capacity plus 30% of the water used for washing. Ethanol and hot water were used to prepare ascorbic acid and glycine betaine solutions. The plots without foliar spraying were also foliar-sprayed with pure water. Next, 10 days after the last foliar spraying, young mature leaves were randomly sampled in the early hours of the day and taken to the laboratory to measure the desired traits. About 70 days after planting, when the fruits reached a length of 15 to 20 cm, harvesting began.

Method of Fruit Study

Fruits were collected four times during the fruiting period, at 20-day intervals, from 8 randomly selected plants in each experimental unit. Next, we used a digital scale to determine how much each fruit weighed, and then we averaged the results per square meter. The average length and diameter of a single fruit were calculated using a ruler and caliper, respectively. After the samples were baked at 91℃ for 8 hours, their mass was measured in grams using a digital scale that had a precision of 0.001. Eight plants, chosen at random from the middle rows of each experimental unit, were counted for their fruit yield at harvest. Subsequently, the mean fruit yield per plant for each treatment was documented.

Method of Physiological Study

Before being sent to the lab, samples were quickly frozen in liquid nitrogen. In order to determine the chlorophyll concentration, a wooden mortar was used to grind 10 grams of Carola leaves with 16 milliliters of acetone and 4 milliliters of water. Subsequently, a South Korean-made Optizen 3220UV spectrophotometer was used to evaluate the light absorption of carotenoids, chlorophylls a and b, and 663 nm, 646 nm, and 470 nm, respectively [11]. The total phenolic content of the fruit was determined using the Folin-Ciocalteu reagent. After 2 hours, the combination was tested for absorbance at 760 nm using a spectrophotometer, with a blank serving as a reference [12]. Utilizing an aluminum chloride reagent, the quantity of total flavonoids in recently collected leaf tissue was ascertained. The combination's absorbance was measured at 415 nm compared to a blank after 30 minutes of storage at room temperature [13]. Secondary metabolites, including momodicin and its derivatives from the fruit, were measured using HPLC. Micrograms per gram of dry weight were used to determine the actual levels of momordicin based on the calibration curve formula. Using liquid chromatography, momordicin was assessed. The detector was an array of 243 nm UV diodes [14]. After being prepared for 20 minutes at 20℃ using an ultrasonic instrument, the mobile phase for the Charantin test was degassed. It comprised a 98:2 ratio of HPLC-grade methanol to deionized water. Using a 220 nm wavelength and a flow rate of 1 mL/min, the Charantin assay was conducted. A calibration curve formula [15], which measures concentrations in micrograms per gram of dry weight, was used to determine the quantities of Charantin in each sample. The concentration of free proline was determined by the authors of this work using a spectrophotometer at a wavelength of 520 nm with a pure toluene control [16]. Using scissors, we collected a sample from each experimental treatment's last fully matured leaf for measuring of RWC. The following formula was used to generate RWC from the data acquired from a 1/10,000 precision scale:

$$RWC = Fw - Dw / Sw - Dw \times 100$$

The three variables here are the leaf's fresh weight (Fw), its dry weight (Dw) after being baked, and its saturated weight (Sw) after being immersed in distilled water.

Statistical Analysis

The data was analyzed with the help of SAS version 9.1 software. Duncan's multiple range test was used to compare means at the 5% level. The Bartlett test was also run.

RESULTS

Effects of Ascorbic Acid and Glycine Betaine on the Fruit Characteristics

The findings of the combined ANOVA of the data in the Carola plant demonstrated that, at the 1% level of significance, the effects of irrigation treatment, foliar spraying, and the interaction of the two on fruit characteristics were all present (Table 2). The highest fruit length (15.30 cm) was obtained in the combined treatments of irrigation with fresh water and foliar spraying with 2 mM ascorbic acid solution. Also, the lowest fruit length (8.40 cm) was related to the combined treatments of irrigation with salt water and foliar spraying with ordinary water (Table 3). The highest fruit diameter (6.30 cm) was obtained in the combined treatments of irrigation with fresh water and foliar application with 2 mM ascorbic acid solution (Table 3). The highest fruit dry weight (8.50 g) and fruit yield (3.55 kg/m2) were obtained in the combined treatments of unsaline irrigation and spraying with 2 mM ascorbic acid solution. Also, the lowest fruit dry weight (4.20 g) and the lowest fruit yield (1.35 kg/m2) were obtained in the treatments of saline irrigation and spraying with normal water (Table 3). The highest number of fruits (13.75) was obtained in the combined treatments of irrigation with fresh water and foliar spraying with a 2 mM ascorbic acid solution and the lowest number of fruits (7.41) was obtained in the combined treatments of irrigation with saline water and foliar spraying with ordinary water (Table 3).

Table 2 Variance analysis of the impacts of carola plant morphological features and fruit yield on irrigation, year, and foliar ascorbic acid and glycine

		Mean square				
Sources of variation	df	Fruit length	Fruit diameter	Fruit dry weight	Fruit yield	Number of fruits
Year	1	0.0018 ^{ns}	0.1648 ^{ns}	0.0889 ^{ns}	0.2811 ^{ns}	0.6378 ^{ns}
Block (year)	4	7.8518 ^{ns}	4.6300 ^{ns}	1.2945 ^{ns}	0.0483 ^{ns}	4.8875 ^{ns}
Irrigation	2	178.13 **	16.59029 **	48.0223 **	5.4547 **	172.6325 **
Year×Irrigation	2	0.00048 ^{ns}	0.20478 ^{ns}	0.000648 ^{ns}	0.0047 ^{ns}	0.000148 ^{ns}
Block×Year×Irrigation	8	0.08152	0.0898	0.00425	0.03872	0.02231
Spraying	5	10.10398 **	4.32500 **	4.8465 **	3.04207 **	9.03320 **
Irrigation×Spraying	10	0.76843 **	0.24477 **	0.2366 **	0.1382 **	0.2223 **
Year×Spraying	5	0.00054 ^{ns}	0.07824 ^{ns}	0.00053 ^{ns}	0.01203 ^{ns}	0.0074 ^{ns}
Year×Irrigation×Spraying	10	0.00181 ^{ns}	0.01308 ^{ns}	0.00054 ^{ns}	0.0124 ^{ns}	0.7700 ^{ns}
Error	60	0.71	0.11	0.09	10.34	0.02
CV (%)	---	1.60	4.27	2.58	3.19	0.68

betaine applications

*: significant at p≤0.05, **: significant at p≤0.01 and ns: non-significant

Irrigation	Spraying (ppm)	Fruit length (cm)	Fruit diameter (cm)	Fruit dry matter (g)	Fruit yield (kg/m2)	Number of fruits
	Control	13.40 d	4.22 f	6.58 e	2.08 g	11.44 j
	1 ASA	14.53 b	4.99 cd	7.20 cd	2.42 d	13.00 e
Unsaline water	2 ASA	15.30 a	6.30 a	8.50 a	3.55 a	13.75 a
	50 GB	13.53 d	4.78 d	7.10 d	2.31 e	13.11 d
	100 GB	14.30 c	5.58 b	7.48 b	2.77 b	13.40 b
	150 GB	13.54 d	5.17 c	7.35 bc	2.57 c	13.21 c
	Control	8.40 l	3.24 k	4.20 l	1.35 k	7.41 p
	1 ASA	9.86 j	3.71 ij	5.10 j	1.68 i	8.51 o
Saline water	2 ASA	10.93 i	4.28 f	5.83 h	2.28 e	9.41 l
	50 GB	9.38 k	3.58 j	4.70 k	1.58 j	8.75 n
	100 GB	10.00 j	4.18 fg	5.46 i	2.18 f	9.33 l
	150 GB	9.33 k	3.91 hi	5.23 j	2.03 g	9.11 m
	Control	10.73 i	3.96 gh	5.25 j	1.58j	10.81 k
	1 ASA	11.30 h	4.36 ef	5.78 h	1.88h	11.81 i
Unsaline/Saline water	2 ASA	12.86 e	4.98 cd	6.33 f	2.57c	12.45 f
	50 GB	11.70 g	4.16 fg	5.80 h	1.90h	11.41 j
	100 GB	12.71 e	4.79 d	6.20 fg	2.47d	12.35 g
	150 GB	11.93 f	4.54 e	6.10 g	2.34e	12.23 h

Table 3 Irrigation, ascorbic acid, glycine betaine, and their combined effects on Carola plant morphology and fruit production

*The same letters in each treatment and combination indicate no significant difference at the 5% probability level based on Duncan's multiple range test.

Effects of Ascorbic Acid and Glycine Betaine on Plant Physiology

At the 1% level of significance, the combined ANOVA of the data in the Carola plant revealed that the physiological characteristics were affected by the irrigation treatment, foliar spraying, and the interaction of the two (Table 4). The combination of fresh water irrigation and spraying with a solution of 100 mM glycine betaine and 2 mM ascorbic acid resulted in the highest concentration of chlorophyll a, measuring 16.59 mg/g fresh leaf. Chlorophyll a levels were 31% higher after treating with glycine betaine and ascorbic acid than after treating with saline water alone. Combining salty irrigation with normal water spraying yielded the lowest chlorophyll a levels (8.60 mg/g fresh leaf) and 1 mM ascorbic acid (9.60 mg/g fresh leaf), as shown in Table 5. The combination of unsaline irrigation and spraying with 100 mM glycine betaine solution and 2 mM ascorbic acid resulted in the maximum chlorophyll b content (9.60 mg/g fresh leaf), followed by 2 mM ascorbic acid. When comparing the salinity treatment with and without 2 mM ascorbic acid, the chlorophyll b level in the latter group increased by 50.58%. The combination of saline irrigation and regular water spraying resulted in the lowest chlorophyll b level of 25.4 mg/g fresh leaf, as shown in Table 5. The highest amount of carotenoids was obtained in the combined treatments of irrigation with fresh water and spraying with a solution of 2 mM ascorbic acid (6.06 mg/g of fresh leaf) and 100 mM glycine betaine (6.00 mg/g of fresh leaf). The treatment of salinity and 2 mM ascorbic acid showed a 30% increase in the amount of carotenoids compared to the treatment of salinity without the use of ascorbic acid (Table 5). The highest amount of phenol was obtained in the combined treatments of irrigation with saline water and spraying with a 2 mM ascorbic acid solution (33.63 mg/g fresh leaf) (Table 5). The flavonoid content in the combined treatments of saline irrigation and spraying with 2 mM ascorbic acid solution (0.47 mg quercetin/g/g fresh leaf) reached its highest level in this experiment, with a 17.5% increase compared to the saline treatment without the use of ascorbic acid (Table 5). Also, the lowest flavonoid content was obtained in the combined treatments of unsaline irrigation and spraying with normal water (0.30 mg quercetin/g/g fresh leaf) (Table 5). The highest amount of momordicin was observed in the combined treatments of irrigation with saline water and spraying with 2 mM ascorbic acid solution (2.05 µg/g dry matter) (Table 5). Also, the lowest amount of momordicin was obtained in the combined treatments of irrigation with unsaline water and spraying with ordinary water (0.83 µg/g dry matter) (Table 5). According to Table 5, the combination of saline water irrigation and spraying with a 2 mM ascorbic acid solution resulted in the highest concentration of Charantin, measuring 13.5 µg/g dry matter. The combination of unsaline irrigation and regular water spraying resulted in the lowest level of Charantin (2.23 µg/g dry matter), as shown in Table 5. Results showed that the combination of salty water irrigation and foliar spraying with a solution of 2 mM ascorbic acid (6.96 µmol per

gram of fresh weight) and 100 mM glycine betaine (6.86 µmol per gram of fresh weight) produced the highest proline concentration. In this experiment, the leaf proline concentration increased by 77.09% when treated with salt plus 2 mM ascorbic acid, compared to when treated with salinity alone. Additionally, according to Table 7, the combination of unsaline irrigation and regular water spraying resulted in the lowest proline level at 3.20 µmol/g wet weight. The average relative leaf water content was found to be highest at a concentration of 2 mmol ascorbic acid (83.58%) and 100 mmol glycine betaine (83%), according to the data shown in Table 5. Spraying ascorbic acid or glycine betaine onto leaves under salt stress circumstances resulted in a 35.52% and 35.67% increase in relative leaf water content, respectively, as compared to spraying ordinary water. Table 5 shows that the combination of saline irrigation and normal water spraying resulted in the lowest relative leaf water content at 40.43 percent.

Table 4 The amount of photosynthetic pigments and phenolic compounds in Carola plants by analyzing the variance of the effects of year, irrigation, and the foliar application of ascorbic acid and glycine betaine

		Mean square								
Variation Sources	df	Chlorophyll a	Chlorophyll b	Carotenoid	Phenol	Flavonoid	Momordicin	Charantin	Prolin	RWC
Year	1	4.26 ^{ns}	1.23 ^{ns}	2.10 ^{ns}	49.22 ^{ns}	0.01 ^{ns}	0.108 ^{ns}	0.094 ^{ns}	1.60 ^{ns}	4.04 ^{ns}
Block (year)	4	5.07 ^{ns}	1.95 ^{ns}	2.54 ^{ns}	51.43 ^{ns}	0.03 ^{ns}	0.009 ^{ns}	0.824 **	1.89 ^{ns}	1.01 ^{ns}
Irrigation	2	172.86 **	58.30 **	23.81 **	2790.75 **	0.07 **	1.997 **	16.621 **	29.46 **	3297.45 **
Year×Irrigation	2	4.16 ^{ns}	0.01 ^{ns}	0.03 ^{ns}	14.86 ^{ns}	0.01 ^{ns}	0.049 ^{ns}	0.428 ^{ns}	0.02 ^{ns}	8.109 ^{ns}
Block×Year×Irrigation	8	3.54	0.18	0.27	22.09	0.01	0.018	0.111	0.37	5.66
Spraying	5	38.22 **	25.55 **	7.30 **	481.83 **	0.03 **	0.715 **	4.215 **	12.73 **	2218.95 **
Irrigation×Spraying	10	30.74 **	1.31 **	0.73 **	92.99 **	0.03 **	0.040 **	0.223 **	0.96 **	12.19 **
Year×Spraying	5	1.10 ^{ns}	0.14 ^{ns}	0.16 ^{ns}	4.73 ^{ns}	0.01 ^{ns}	0.010 ^{ns}	0.011 ^{ns}	0.21 ^{ns}	1.04 ^{ns}
Year×Irrigation×Spraying	10	0.56 ^{ns}	0.02 ^{ns}	0.14 ^{ns}	1.75 ^{ns}	0.01 ^{ns}	0.010 ^{ns}	0.038 ^{ns}	0.03 ^{ns}	2.79 ^{ns}
Error	60	0.71	0.11	0.09	10.34	0.02	0.14	0.12	0.13	36.08
CV (%)	---	6.93	5.21	7.39	8.59	2.01	10.07	3.38	7.65	1.76

*: significant at p≤0.05, **: significant at p≤0.01 and ns: non-significant

Table 5 The amounts of photosynthetic pigments and phenolic compounds in Carola plants affected by irrigation, the administration of solutions of ascorbic acid and glycine betaine, and the interaction between the two

Irrigation	Spraying (ppm)	Chlorophyll a (mgg-1 fw)	Chlorophyll b (mgg-1 fw)	Carotenoid (mgg-1 fw)	Phenol (mgg-1 fw)	Flavonoid (mg quercetin. g-1 fw)	Momordicin (µgg-1 dw)	Charantin (µgg-1 dw)	Prolin (µmol/g Fw)	RWC (%)
Unsaline water	Control	10.78 ef*	5.75 g	3.74 c	28.33 e	0.28 k	0.83 j	2.23 n	3.20 g	61.18 h
	1 ASA	12.93 c	6.78 cd	4.44 b	31.33 de	0.33 g	1.08 gh	3.51 jk	3.65 fg	72.78 c
	2 ASA	16.29 a	9.56 a	6.06 a	34.66 d	0.35 e	1.33 de	3.76 hi	4.45 cd	83.58 a
	50 GB	14.79 b	6.83 c	4.43 b	28.65 e	0.32 h	1.00 hi	3.38 kl	3.68 f	73.33 c
	100 GB	16.59 a	9.90 a	6.00 a	31.05 de	0.28 k	1.18 fg	3.66 ij	4.48 cd	83.00 a
	150 GB	15.50 ab	8.90 ab	5.50 ab	29.16 e	0.29 j	0.94 i	2.80 m	4.36 cde	76.21 b
Saline water	Control	8.60 h	4.25 i	2.96 e	41.44 c	0.40 c	1.34de	4.13 f	3.93 ef	40.43 l
	1 ASA	9.60 gh	4.80 h	3.23 de	54.66 b	0.42 b	1.40cd	4.53 d	4.75 bc	54.11 j
	2 ASA	11.27 e	6.08 efg	3.86 c	63.33 a	0.47 a	2.05a	5.13 a	6.96 a	63.01 g
	50 GB	9.92 fg	4.91 h	3.31 de	42.95 c	0.40 c	1.47c	4.53 e	5.20 b	55.53 i
	100 GB	11.25 e	6.40 de	3.83 c	40.35 c	0.35 e	1.59b	4.93 b	6.86 a	62.66 g
	150 GB	11.19 e	6.26 ef	3.80 c	41.35 c	0.36 d	1.32de	4.41 de	5.80 b	59.90 h
Unsaline/Saline water	Control	9.96 fg	5.00 h	3.73 d	31.33 de	0.32 gh	1.01hi	3.30 l	3.56 f	50.70 k
	1 ASA	11.18 e	5.85 fg	3.89 c	35.06 d	0.35 e	1.27ef	4.03 fg	4.03 def	66.63 f
	2 ASA	12.50 cd	7.08 bc	4.59 b	41.66 c	0.37 d	1.49bc	4.76 c	5.16 b	72.81 c
	50 GB	11.49 de	6.10 efg	3.95 c	35.16 d	0.34 f	1.18fg	3.90 gh	4.20 de	68.18 e
	100 GB	12.46 cd	7.42 b	4.62 b	31.00 de	0.31 i	1.30de	4.36 e	5.23 b	72.51 c
	150 GB	12.50 cd	7.30 b	4.53 b	32.15 de	0.32 gh	1.13g	3.56 j	5.06 b	70.46 d

*The same letters in each treatment and combination indicate no significant difference at the 5% probability level based on Duncan's multiple range test.

DISCUSSION

In response to salt stress, fruit length decreases because of osmotic and ionic stress, which is brought on by several factors, including decreased water and nutrient consumption, reduced photosynthetic activity, buildup of sodium ions, cell membrane breakdown, and lower turgor pressure [17]. Research by Soomro et al. [18] showed that fruits grown in environments without saltwater have longer fruit durations than those grown in saltwater. In a study reported by Caetano et al. [19] in salt stress circumstances, it was found that applying 0.8 mmol of ascorbic acid enhanced the stem diameter and meat volume of passion fruit. Fruits irrigated with unsaline water weighed more than those irrigated with saline water, according to these findings, which are in agreement with those of Soomro et al. [18]. By boosting cell division and proliferation, as well as enhancing the activity of the Rubisco enzyme and bolstering membrane integrity, ascorbic acid raises the dry weight of fruits [19]. Water salinity not only causes severe yield reduction, but it also causes plant toxicity, soil salinization, and soil degradation over time [20]. The genes that control the production of cell wall polysaccharides, suberin, and lignin could potentially undergo alterations under these circumstances [21]. One possible explanation for the molecular breakdown of chlorophylls in stressed plants is the release of oxygen-free radicals or the increased activity of the chlorophyllase enzyme, which causes the phytol chain to separate from the porphyrin ring [22]. The reduction in the amount of photosynthetic pigments can be associated with damage to the thylakoid membrane due to excessive salinity [23], which leads to a decrease in carbon dioxide uptake, rapid stomatal closure, and inactivation of enzymes necessary for the dark reaction [24]. The application of exogenous ascorbic acid can cause stomatal opening, reduce transpiration, and protect photosynthetic pigments under stress conditions. Additionally, it acts as a substrate for the enzyme ascorbate peroxidase, neutralizing free oxygen produced in the thylakoid membrane, and improves stomatal conductance [25]. The effects of salinity on photosynthesis depend on the plant species [26]. Osmoprotection, detoxification of reactive oxygen species, and protection of biological membranes by ascorbic acid have been shown to prevent damage to photosynthetic pigments in canola plants affected by salt stress [20]. One way to stop free radicals from destroying chlorophyll and instead boost its production is to use exogenous ascorbic acid, a detoxifier and neutralizer of singlet oxygen species and superoxide radicals [27]. The increase in photosynthetic pigments with glycine betaine application may be due to endogenous water conservation. Moreover, glycine betaine can protect photosynthetic cells by stabilizing the activity of proteins under salt stress conditions [28]. Higher leaf relative water and better stomatal conductance by glycine betaine application were responsible for better photosynthetic rate and reduced harmful effects of salt stress [29]. Emami Bistgani et al. [30] found that thyme's total phenolic content rose in response to increasing salt stress. The application of ascorbic acid also increases membrane stability, which in turn causes plant defense by changing the activity of enzymes and phenolic compounds, eliminating free electrons, and protecting cells and the defense system [31]. These results are consistent with reports by Taibi et al. [32], who reported an increase in leaf flavonoid content with increasing salinity stress in green bean and pea, respectively. Beneficial secondary metabolites momordicin and charantin shield plants from abiotic stressors, inhibit stressed plants' free oxygen generation and, potentially, lipid peroxidation, and boost plant development [33]. Furthermore, the positive results of foliar application of glycine betaine in increasing proline content can be attributed to its involvement in the internal increase of the proline precursor (glutamate) [34]. Exogenous application of ascorbic acid increased relative leaf water and improved potassium uptake in sorghum and bean plants under salt stress [35].

CONCLUSION

The combination of fresh water irrigation and spraying with a 2 mM ascorbic acid solution produced the best results in terms of fruit length, diameter, dry weight, yield, and quantity of fruits. The combined treatments of fresh water irrigation and spraying with 2 mM ascorbic acid and 100 mM glycine betaine solutions produced the highest levels of photosynthetic pigments (chlorophylls a and b and carotenoids) as well as relative leaf water content. Finally, the highest levels of total phenols, flavonoids, momordicin, and proline in Carola leaves were obtained in the combined treatments of irrigation with saline water and spraying with 2 mM ascorbic acid solution.

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Authorship Contribution Statement

Dr. Mohammad Fahramand: Conceptualization, Investigation, Data curation, Data analysis, Writing – review & editing. Dr. Isa Khammari and Dr. Alireza Sirousmehr: Conceptualization, Supervision, Dr. Mehdi Dahmardeh: Formal analysis, Dr. Mahboubeh Zamanipour: Investigation, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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