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LIQUEFIED WATER AND ANTIOXIDANTS INFLUENCE ON VEGETATIVE GROWTH AND PHYSIOLOGICAL TRAITS OF SWEET ORANGE

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SUMMARY

Citrus production faces many problems; a major one consists of irrigating citrus seedlings grown at Agricultural Research Stations and various nurseries with saline well water, which determines the growth, reproduction, and spread of citrus in Iraq. The said problem needs addressing to reduce its effects on the growth and reproduction of citrus fruits. Therefore, the latest research aimed to determine the effects of regular liquefied water and saline well water, in addition to a foliar spray of aspartic and ascorbic acids, on the growth and physiological properties of the grafted orange seedlings. The local citrus seedlings of the same age and size were selected and used as the original and grafted with local orange buds. They were shifted and tested for the above three factors at the Horticulture and Forestry Division, Najaf Agriculture Directorate, Iraq. The findings showed that irrigation with regular liquefied water had improved the growth traits, i.e., plant height, leaves plant^{-1}, leaf area plant^{-1}, and dry biomass weight of sweet orange seedlings, compared with saline well water. Results further revealed that foliar application of aspartic acid (100 mg L^{-1}) positively affected the leaves plant^{-1} and dry biomass weight compared with the control. The ascorbic acid (4000 mg L^{-1}) application gave highly superior and well-responsive reactions for most of the traits, i.e., leaves plant^{-1} (69.3 leaves plant^{-1}), leaf area plant^{-1} (3888 cm^2), dry biomass weight (0.3629 g), and total chlorophyll content (7.03 mg 100 g^{-1} fresh weight) in orange seedlings.

Keywords: Sweet orange transplants, aspartic, ascorbic acids, water quality

Key findings: Salinity impacts agriculture and crop plants must be induced for salt tolerance and sustained economic output. Antioxidants, such as, aspartic acid and ascorbic acid have auxinic action and also have a synergistic effect on plant growth and salinity tolerance.

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INTRODUCTION

Salinity is an environmental issue that impacts agriculture significantly. The salt-affected land is estimated at 953 million ha, around 7% of the total land and 20% of the planet's irrigated area (Abdelfattah *et al*., 2009). Hyperosmolarity, ion disequilibrium, nutritional imbalance, and the reactive oxygen species (ROS) generation in soil result in plant growth retardation (Nawaz *et al*., 2010). Plants need genetic engineering to tolerate salts to sustain their economic output. Genetic changes and pharmacologic therapies can help accomplish

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this (Hamdia and Shaddad, 2010). Plant breeding and genetic engineering efforts to develop salt tolerance proved long-term and complex endeavors with limited results (Ashraf *et al*., 2008).

Exogenous application of plant growth-regulating chemicals showed an effective and technically simpler way to deal with the negative effects of salinity on plants (Ashraf *et al*., 2010). The depletion of the endogenous levels of growth regulators resulted from stressful environments, which exogenous treatments can remedy. Plant growth regulators, fertilizers, and nonenzymatic antioxidants have all been employed successfully to reduce the negative effects of salinity on plant growth and output (Kaya *et al*., 2010). Antioxidants, such as, ascorbic and citric acids have auxinic action and a synergistic effect on fruit tree flowering and fruiting. Recently, employing antioxidants instead of auxins and other chemicals improved the growth and fruiting of various fruit trees (Ragab, 2002; Fatonah *et al*., 2018). Furthermore, antioxidants proved to have a good effect in trapping free radicals, potentially increasing the shelf life of plant cells and encouraging growth (Rao *et al*., 2000).

Ascorbic acid served as one of the most efficient growth regulators against abiotic stressors (Conklin, 2001). Not only does ascorbic acid work as an antioxidant, but at the cellular level, it links to activate sophisticated biological defense mechanisms (Conklin and Barth, 2004). In several crop plants, ascorbic acid has alleviated the negative effects of salt stress and hypothesized the functions in plant metabolism (Khan *et al*., 2010).

Aspartate is a precursor for the biosynthesis of multiple biomolecules required for plant growth and defense, including nicotinamide adenine dinucleotide (NAD), amino acids, organic acids, nucleotides, and their metabolites; it also constitutes proteins and serves as an active residue in many enzymes (Han *et al*., 2021). Four of eight essential amino acids, i.e., methionine, threonine, lysine, and isoleucine are generated from aspartic acid (Li *et al*., 2017). Aspartic and derived intermediates' exchanges and competition substantially affect plant metabolism (Han *et al*., 2021). Several studies have found a link between variations in aspartic acid concentration and plant stress.

The level of aspartic (Asp) increased 11-fold in the root and around 6.2-fold in the shoots of mangrove grass (*Aeluropus lagopoides*) in response to 250 mM NaCl salt stress (Shahzad *et al*., 2017). In the shoot, methionine, threonine, lysine, isoleucine, glycine, and proline increased by 1.46 to 9.98 times under the same circumstances, whereas glycine (Gly), proline (Pro), and ethanolamine increased by 2.5 to 15.6 fold in the roots compared with the control. Similarly, plants treated with *Bacillus amyloliquefaciens* RWL-1 showed a considerable increase (2.7-fold) in Asp when exposed to saline stress. Under salty circumstances, the large accumulation of Asp and other amino acids, such as proline, has played an important function in plants by maintaining intracellular osmotic potential and stabilizing the proteins (Hayat *et al*., 2012). Furthermore, a study observed that Asp content changes interlinked with changes in protein metabolism in salt-stressed plants (El-Shintinawy and El-Shourbagy, 2001).

Ascorbic acid is a natural chemical and an organic antioxidant (Hafez *et al*., 2010), as well as, a vital compound for plant tissues because of its ability to operate as a co-enzyme and plant growth regulator (Gomez and Lajolo, 2008). With its effects on cell division and differentiation, ascorbic acid is involved in a wide range of vital processes, including antioxidant defense, UV protection, photosynthesis regulation, and growth regulation (Al-janabi *et al*., 2021a).

Furthermore, aspartate serves as a precursor to several amino acids in plants, including threonine, methionine, and isoleucine (Rawia *et al*., 2011). Three amino acids—glutamic acid, aspartic acid, and alanine—are employed in the creation of bio-stimulant compounds (Colla *et al*., 2015). These can be added as is or as part of the raw ingredients from hydrolyzed proteins. In plants, a transamination event between glutamate and oxaloacetate produces aspartic acid. It is then metabolized to create the earlier-mentioned amino acids via the aspartic acid metabolic pathway. Plants that receive these three amino acids have much ability to withstand severe climatic circumstances, such as, drought, salinity, and toxicity of heavy metals (Rai, 2002). Therefore, the latest study aimed to determine the impact of quality irrigation water and spraying with antioxidants (ascorbic and aspartic acids) on the vegetative growth of budded sweet orange transplants.

MATERIALS AND METHODS

The problem of irrigating citrus seedlings grown in various research stations and nurseries with saline well water negatively affects the growth, reproduction, and spread of

citrus in Iraq. The selected local citrus sweet orange saplings of homogeneous age and size served as the original and were grafted in autumn with local orange buds as a graft on 10 December 2020. Shield grafting in the certified citrus production nursery belonged to the General Directorate of Horticulture and Forests, Holy Karbala Governorate, Iraq. The grafted orange saplings were transported and distributed in the canopy of the Horticulture and Forestry Division, Najaf Agriculture Directorate, to test the effect of three factors. In the randomized complete block design with a factorial arrangement, the irrigation water of two types (tap water and saline well water) was kept in the main plots. The second and third factors consist of foliar spraying with aspartic acid at a concentration (0, 100, and 150 mg L^{-1}) and ascorbic acid at a concentration (0, 4000 mg L^{-1}) were considered as sub-Sub-Plots. The saplings received irrigation with the quality factor of irrigation water starting from 1 May 2020, while the foliar spraying with the aspartic and ascorbic acids comprised of seven dates: 1April, 1 May, 1 June, 1 July, 1 August, 1 September and 1 November 2020. In 2021, some vegetative and chemical traits were studied on the orange grafts grown on the original, and the results were analyzed statistically according to ANOVA using the statistical program (Genstat Var 2012).

Growth characteristics

The measurement of vegetative growth indicators began a month after the last date for adding chemical fertilizers, on 10 November 2021 CE, and the measurements continued until the end of May.

Plant height

The plant height measurement started from the surface of the soil to the highest peak in the plant, for the saplings of the experimental unit. Then the average length of one plant in the experimental unit was divided by the total lengths of six, as shown in the following equation, with three replications for each treatment.

Average height per plant = total of the tallest six saplings divided by six

Leaves plant^{-1}

The number of leaves for all saplings in the experimental unit was counted, then calculating the average number of leaves is shown in the following equation, with three replications for each treatment.

Average number of leaves per plant = number of total sapling leaves divided by six

Leaf area plant^{-1}

After removing the vegetative parts of the plant, the leaves were washed with liquid soap, followed by distilled water to remove dust and plankton on the plant. After drying them, the leaf area of the leaves was measured using a computer scanner using Image J program. Then the washed and relatively dry leaves were placed on the scanner and then the leaf area was calculated, with the process being repeated several times until all the leaves of one plant have been scanned. The results were recorded each time using Microsoft Excel 2010. For ease of calculation, the leaf area of one plant in each experimental unit was calculated, as follows:

Average total leaf area per plant = experimental units for three total area divided by three

Dry biomass plant^{-1}

The vegetative parts of the plant were removed along with roots (the crown area), then quickly washed with water and liquid soap (brightening) to remove dirt and dust, rinsed with distilled water, then entered into the laboratory to dry. Then these were placed in perforated leaf bags and inserted in an electric oven at a temperature of 65°C until the mass was established, where the mass measurement used a sensitive scale.

Total chlorophyll content

Pigment in leaves got estimated in the laboratory for graduate studies at the College of Agriculture, University of Kufa, Iraq, by taking six samples of full-width leaves in the experimental unit saplings. The sample weight of 0.5 g was placed in the ceramic jar, then added with 10 ml of 80% acetone and mashed. The mixture was then filtered to separate the dye solution from the leaf tissue using a Whatman No.1 filter leaf. The process employed a repetition to extract the remaining dyes by adding 5 ml of acetone until the tissue bleached. The resulting filtrate was collected from the two filtration processes, then completing the volume to 15 ml using acetone.

With a spectrophotometer the readings consisted of two wavelengths of 645 and 663 nanometers (Goodwin, 1976), then the total chlorophyll amount calculation in units of mg (100 g fresh weight^{-1}) applied the following equation:

$$\text{Total chlorophyll} = 20.2 \times D\,(645) + 8.02 \times D\,(663)\,(V.W\ -1 \times 1000) \times 100$$

Where
D (645): the optical absorption reading at a wavelength of 645 nm.
D (663): the optical absorption reading at a wavelength of 663 nm,
V: the volume of the total extract,
W: the weight of the leaf tissue (g),

After finding the total chlorophyll for each plant, its average was determined per experimental unit, repeating the process three times.

Carbohydrate content in leaves

The Joslyn method (1970) estimated the number of total carbohydrates in the leaves, taking 0.2 g of dry sample powder for each experimental unit, then, adding perchloric acid solution, putting the sample in a 60-mo bathroom for 60 minutes, and repeating it three times. The brilliant solution was collected in a sizes duct, completed to 100 ml by adding distilled water, took 1 ml of diluted solution, added 1 ml of 5% phenol solution and 5 ml of concentrated sulfuric acid, then took the absorption reading of the solutions with Spectrophotometer along the 490 nm wavelength and calculated the percentage of carbohydrates as follows:

$$\text{Carbohydrates (\%)} = (\text{Concentration} \times \text{capacitors}) / (10 \times 1\text{ml} \times \text{sample weight})$$

RESULTS

Plant height

The irrigation with liquefied water had a significant impact on the vegetative growth of sweet oranges, and the said treatment provided the highest plant height (57.4 cm) compared with the saline well water (40.5 cm) (Table 1). The aspartic acid with different concentrations had a significant positive impact on the plant height and other growth traits. The aspartic acid spray with a concentration of 150 mg L^{-1} increased the plant height (55.8 cm) compared with the control treatment (45.0 cm). The ascorbic acid spray had no significant effect on the plant height. Also, the binary interactions of the regular liquefied water with aspartic and ascorbic acids and the interaction between aspartic and ascorbic acids showed no significance for the said trait. Moreover, the triple interaction among the liquefied water, aspartic, and ascorbic acids had no significant effect on the plant height.

Leaves plant^{-1}

The liquefied water irrigation revealed a significant effect on orange seedling's growth, recording the highest number of leaves per plant (73.1 leaves plant^{-1}) compared with the lowest values for the said trait obtained in saline well water (40.8 leaves plant^{-1}) in sweet orange grafts (Table 2). The aspartic acid concentrations did not have a significant effect. However, the influence of ascorbic acid (4000 mg L^{-1}) spray displayed a significant impact, recording an increased number of leaves (69.3 leaves plant^{-1}) compared with the control (44.5 leaves plant^{-1}). The interaction outcomes showed no effects for the leaves per plant.

Leaf area plant^{-1}

The results revealed that irrigation with normal liquefied water has a relevant positive impact on the average leaf area of sweet orange grafts, with a recorded highest average leaf area per plant (4206 cm^2) compared with the saline well water (1584 cm^2) (Table 3). The aspartic acid spray on the orange seedlings revealed a significant positive effect on the leaf area and other growth traits. Aspartic acid with a concentration of 150 mg L^{-1} provided the highest leaf area plant^{-1} (3851 cm^2) compared with control with no spray (2119 cm^2). The ascorbic acid also had a significant effect on the average leaf area of the orange seedlings. Ascorbic acid spray at a concentration of 4000 mg L^{-1} gave the highest leaf area plant^{-1} (3888 cm^2) compared with the control (1902 cm^2). However, the interaction of the above treatments had no significant impact on the average leaf area of the sweet orange seedlings.

Table 1. Effect of regular irrigation water and Salt well irrigation water and aspartic and ascorbic acids on average number of leaves sapling $^{-1}$.

Irrigation Water Quality	Aspartic acid concentration	Ascorbic acid concentration		Average of Irrigation Water Quality
		0 mg.L^{-1}	mg.L^{-1}4000	
Regular irrigation water	0 mg.L^{-1}	45.3	90.5	73.1
	100 mg.L^{-1}	52.6	89.3	
	150 mg.L^{-1}	68.4	92.3	
Salt Salt well irrigation water	0 mg.L^{-1}	28.5	58.2	40.8
	100 mg.L^{-1}	34.1	36.0	
	150 mg.L^{-1}	38.2	49.7	
Average of Ascorbic acid		44.5	69.3	28.65 = $LSD_{0.05}$ Irrigation Water Quality
$LSD_{0.05}$ aspartic*ascorbic* Irrigation Water Quality =N.S.				

Irrigation Water Quality	Aspartic acid concentration			Average of Irrigation Water Quality
	0 mg.L^{-1}	100 mg.L^{-1}	150 mg.L^{-1}	
Regular irrigation water	67.9	70.9	80.3	73.1
Salt Salt well irrigation water	43.4	35.0	43.9	40.8
Average of Aspartic acid	55.6	53.0	62.1	
$LSD_{0.05}$ Irrigation Water Quality* aspartic =				

Irrigation Water Quality	Ascorbic acid concentration		Average of Irrigation Water Quality
	0 mg.L^{-1}	mg.L^{-1}4000	
Regular irrigation water	55.4	90.7	73.1
Salt Salt well irrigation water	33.6	48.0	40.8
Average of Ascorbic acid	44.5	69.3	
$LSD_{0.05}$ Irrigation Water Quality * ascorbic= N.S.			

Aspartic acid concentration	Ascorbic acid concentration		Average of Aspartic acid
	0 mg.L^{-1}	mg.L^{-1}4000	
0 mg.L^{-1}	36.9	74.4	55.6
100 mg.L^{-1}	43.3	62.7	53.0
150 mg.L^{-1}	53.3	71.0	62.1
Average of Ascorbic acid	44.5	69.3	$LSD_{0.05}$ aspartic =N.S.
			$LSD_{0.05}$ ascorbic = 16.15
N.S.=aspartic*ascorbic $LSD_{0.05}$			

Table 2. Effect of regular irrigation water and Salt well irrigation water and ascorbic acids on average plant height (cm. plant^{-1}).

Irrigation Water Quality	Aspartic acid concentration	Ascorbic acid concentration		Average of Irrigation Water Quality
		0 mg.L^{-1}	mg.L^{-1}4000	
Regular irrigation water	0 mg.L^{-1}	53.1	53.9	57.4
	100 mg.L^{-1}	55.6	55.5	
	150 mg.L^{-1}	62.2	63.8	
Salt well irrigation water	0 mg.L^{-1}	31.7	41.2	40.5
	100 mg.L^{-1}	37.5	35.7	
	150 mg.L^{-1}	40.5	56.7	
Average of Ascorbic acid		46.8	51.1	9.75 =Irrigation Water Quality $LSD_{0.05}$
$LSD_{0.05}$ aspartic*ascorbic* Irrigation Water Quality =N.S.				

Irrigation Water Quality	Aspartic acid concentration			Average of Irrigation Water Quality
	0 mg.L^{-1}	100 mg.L^{-1}	150 mg.L^{-1}	
Regular irrigation water	53.5	55.6	63.0	57.4
Salt well irrigation water	36.4	36.6	48.6	40.5
Average of Aspartic acid	45.0	46.1	55.8	
$LSD_{0.05}$ Irrigation Water Quality* aspartic = N.S.				

Irrigation Water Quality	Ascorbic acid concentration		Average of Irrigation Water Quality
	0 mg.L^{-1}	mg.L^{-1}4000	
Regular irrigation water	57.0	57.8	57.4
Salt well irrigation water	36.5	44.5	40.5
Average of Ascorbic acid	46.8	51.1	
$LSD_{0.05}$ Irrigation Water Quality * ascorbic= N.S.			

Aspartic acid concentration	Ascorbic acid concentration		Average of Aspartic acid
	0 mg.L^{-1}	mg.L^{-1}4000	
0 mg.L^{-1}	42.4	47.6	45.0
100 mg.L^{-1}	46.6	45.6	46.1
150 mg.L^{-1}	51.3	60.3	55.8
Average of Ascorbic acid	46.8	51.1	$LSD_{0.05}$ Aspartic=7.84
			$LSD_{0.05}$ Ascorbic = N.S.
N.S.=aspartic*ascorbic $LSD_{0.05}$			

Table 3. Effect of regular irrigation water and Salt well irrigation water and aspartic and ascorbic acids on average total leaf area of the plant (cm^2. plant $^{-1}$).

Irrigation Water Quality	Aspartic acid concentration	Ascorbic acid concentration		Average of Irrigation Water Quality
		0 mg.L^{-1}	mg.L^{-1}4000	
Regular irrigation water	0 mg.L^{-1}	1081	4166	4206
	100 mg.L^{-1}	2937	6106	
	150 mg.L^{-1}	4552	6394	
Salt well irrigation water	0 mg.L^{-1}	603	2624	1584
	100 mg.L^{-1}	827	992	
	150 mg.L^{-1}	1413	3045	
Average of Ascorbic acid		1902	3888	2158.5 =Irrigation Water Quality $LSD_{0.05}$
$LSD_{0.05}$ aspartic*ascorbic* Irrigation Water Quality =N.S				

Irrigation Water Quality	Aspartic acid concentration			
	0 mg.L^{-1}	100 mg.L^{-1}	150 mg.L^{-1}	
Regular irrigation water	2624	4521	5473	4206
Salt well irrigation water	1613	910	2229	1584
Average of Aspartic acid	2119	2716	3851	
$LSD_{0.05}$ Irrigation Water Quality* aspartic = N.S				

Irrigation Water Quality	Ascorbic acid concentration		Average of Irrigation Water Quality
	0 mg.L^{-1}	mg.L^{-1}4000	
Regular irrigation water	2857	5555	4206
Salt well irrigation water	948	2220	1584
Average of Ascorbic acid	1902	3888	
$LSD_{0.05}$ Irrigation Water Quality * ascorbic= N.S			

Aspartic acid concentration	Ascorbic acid concentration		Average of Aspartic acid
	0 mg.L^{-1}	mg.L^{-1}4000	
0 mg.L^{-1}	842	3395	2119
100 mg.L^{-1}	1882	3549	2716
150 mg.L^{-1}	2982	4719	3851
Average of Ascorbic acid	1902	3888	$LSD_{0.05}$ Aspartic = 1110.1
			$LSD_{0.05}$ Ascorbic =1205.8
N.S= aspartic*ascorbic $LSD_{0.05}$			

Dry biomass plant^{-1}

The irrigation of regular liquefied water and saline well water revealed a significant effect on the average dry mass of the grafted orange seedlings (Table 4). Observations on the regular liquefied water demonstrated the highest rate of dry vegetative mass (0.4138 g), compared with the least value obtained in the treatment with saline well water irrigation (0.2391 g). The aspartic acid spray (150 mg. L-1) also showed significant effects on the dry weight mass of the vegetative parts, recording an increased in the dry weight of the leaves (0.4215 g) compared with the no spray treatment (0.2606 g). It also gave similar results from the aspartic application at a concentration of 100 mg L^{-1} (0.2973). The ascorbic acid treatment displayed a significant effect on the dry vegetative mass of the sweet orange seedlings. The ascorbic acid at a concentration of 4000 mg L^{-1} increased the dry weight of the leaves (0.3629 g) compared with the lowest value obtained from the control (0.2900 g). However, the bilateral interaction between the regular liquefied water and aspartic/ascorbic acid revealed no significant effect on the average dry vegetative mass of the sweet orange seedlings.

The interaction between the treatments of aspartic acid (150 mg L^{-1}) and ascorbic acid (4000 mg L^{-1}) provided the highest significant impact on the average dry weight of orange seedling leaves, amounting to 0.4918 g, compared with no spray of both acids. The latter gave the lowest value for the said trait (0.2495 g) (Table 4). The triple interaction between the three experimental factors, i.e., normal liquefied water, aspartic acid (150 mg L^{-1}), and ascorbic acid (4000 mg L^{-1}), reached 0.5740 g compared with the saline well water and no spray of aspartic and ascorbic acids, which gave the lowest value (0.1577 g) in the sweet orange seedlings.

Total chlorophyll content

The results revealed that both irrigation regimes and aspartic acid had no significant impact on the total chlorophyll content in the leaves of sweet orange grafts (Table 5). However, numerically the liquefied water irrigation helped reach the highest chlorophyll content in the leaves compared with the saline

Table 4. Effect of regular irrigation water and Salt well irrigation water and aspartic and ascorbic acids on average dry weight of the vegetative growth (g. plant $^{-1}$).

Irrigation Water Quality	Aspartic acid concentration	Ascorbic acid concentration		Average of Irrigation Water Quality
		0 mg.L^{-1}	mg.L^{-1}4000	
Regular irrigation water	0 mg.L^{-1}	0.3413	0.2847	0.4138
	100 mg.L^{-1}	0.3513	0.4770	
	150 mg.L^{-1}	0.4547	0.5740	
Salt well irrigation water	0 mg.L^{-1}	0.1577	0.2587	0.2391
	100 mg.L^{-1}	0.1873	0.1737	
	150 mg.L^{-1}	0.2477	0.4097	
Average of Ascorbic acid		0.290	0.363	$LSD_{0.05}$ Irrigation Water Quality = 0.06365
$LSD_{0.05}$ aspartic*ascorbic* Irrigation Water Quality=0.10630				
Irrigation Water Quality	Aspartic acid concentration			
	0 mg.L^{-1}	100 mg.L^{-1}	150 mg.L^{-1}	
Regular irrigation water	0.3130	0.4142	0.5143	0.4138
Salt well irrigation water	0.2082	0.1805	0.3287	0.2391
Average of Aspartic acid	0.2606	0.2973	0.4215	
$LSD_{0.05}$ Irrigation Water Quality* aspartic = N.S				
Irrigation Water Quality		Ascorbic acid concentration		Average of Irrigation Water Quality
		0 mg.L^{-1}	mg.L^{-1}4000	
Regular irrigation water		0.3824	0.4452	0.4138
Salt well irrigation water		0.1976	0.2807	0.2391
Average of Ascorbic acid		0.290	0.363	
$LSD_{0.05}$ Irrigation Water Quality * ascorbic= N.S				
Aspartic acid concentration	Ascorbic acid concentration		Average of Aspartic acid	
	0 mg.L^{-1}	mg.L^{-1}4000		
0 mg.L^{-1}	0.2495	0.2717	0.2606	
100 mg.L^{-1}	0.2693	0.3253	0.2973	
150 mg.L^{-1}	0.3512	0.4918	0.4215	
Average of Ascorbic acid	0.2900	0.3629	$LSD_{0.05}$ Aspartic =0.07711	
			$LSD_{0.05}$ Ascorbic = 0.03674	
0.08434= aspartic*ascorbic $LSD_{0.05}$				

well water irrigation. Ascorbic acid displayed positive effects on the total chlorophyll content in the leaves of sweet orange seedlings. Ascorbic acid (4000 mg L^{-1}) increased the total chlorophyll content (7.03 mg 100 g^{-1} fresh weight) compared with the control (5.63 mg 100 g^{-1} fresh weight).

The binary interaction between the liquefied water and aspartic acid and the interaction between aspartic and ascorbic acids exhibited nonsignificant differences in the total chlorophyll content in the leaves of sweet orange seedlings (Table 5). The interaction between regular liquefied water and ascorbic acid was significant. However, saline well water with ascorbic (4000 mg. L^{-1}) produced the highest total chlorophyll content in the leaves (7.25 mg 100 g^{-1} fresh weight) compared with the saline well water with no ascorbic acid spray having the lowest value for the said trait (4.95 mg 100 g^{-1} fresh weight). No significant effect of the triple interaction of liquefied water, aspartic and ascorbic acids on the total chlorophyll content in the leaves of sweet oranges occurred.

Carbohydrate content

Both water irrigation regimes positively affected the carbohydrate content in the sweet orange leaves (Table 6). However, the saline well water treatment showed the highest carbohydrate content in the leaves (46.20 mg g^{-1} dry weight) compared with the regular liquefied water, which recorded the lowest rate of carbohydrate content (37.04 mg g^{-1} dry weight). Results further enunciated that aspartic acid alone and in combination with liquefied water and ascorbic acid has no significant impact on the carbohydrate content in the leaves of sweet oranges.

In orange seedlings, the ascorbic acid effects showed significance for carbohydrate content in the leaves, and ascorbic acid (4000 mg L^{-1}) increased the total carbohydrate content (44.59 mg g^{-1} dry weight) compared with the control (38.64 mg g^{-1} dry weight) (Table 6). The bilateral interaction between saline well water and ascorbic acid (4000 mg. L^{-1}) exhibited excellent results with the highest carbohydrate content of leaves (54.04 mg g^{-1} dry weight) compared with the interaction of regular liquefied water and ascorbic acid (4000

Table 5. Effect of regular irrigation water and Salt well irrigation water and aspartic and ascorbic acids on total chlorophyll (mg. 100 g^{-1} fresh weight).

Irrigation Water Quality	Aspartic acid concentration	Ascorbic acid concentration		Average of Irrigation Water Quality
		0 $mg.L^{-1}$	$mg.L^{-1}$4000	
Regular irrigation water	0 $mg.L^{-1}$	6.49	6.75	6.57
	100 $mg.L^{-1}$	6.62	6.61	
	150 $mg.L^{-1}$	5.85	7.11	
Salt well irrigation water	0 $mg.L^{-1}$	3.02	7.09	6.10
	100 $mg.L^{-1}$	6.08	6.89	
	150 $mg.L^{-1}$	5.74	7.75	
Average of Ascorbic acid		5.63	7.03	$LSD_{0.05}$ Irrigation Water Quality= N.S
$LSD_{0.05}$ aspartic*ascorbic* Irrigation Water Quality =N.S.				
Irrigation Water Quality	Aspartic acid concentration			Average of Irrigation Water Quality
	0 $mg.L^{-1}$	100 $mg.L^{-1}$	150 $mg.L^{-1}$	
Regular irrigation water	6.62	6.62	6.48	6.57
Salt well irrigation water	5.06	6.48	6.74	6.10
Average of Aspartic acid	5.84	6.55	6.61	
$LSD_{0.05}$ Irrigation Water Quality* aspartic = N.S.				
Irrigation Water Quality		Ascorbic acid concentration		Average of Irrigation Water Quality
		0 $mg.L^{-1}$	$mg.L^{-1}$4000	
Regular irrigation water		6.32	6.82	6.57
Salt well irrigation water		4.95	7.25	6.10
Average of Ascorbic acid		5.63	7.03	
$LSD_{0.05}$ Irrigation Water Quality * ascorbic= 0.674				
Aspartic acid concentration	Ascorbic acid concentration		Average of Aspartic acid	
	0 $mg.L^{-1}$	$mg.L^{-1}$4000		
0 $mg.L^{-1}$	4.76	6.92	5.84	
100 $mg.L^{-1}$	6.35	6.75	6.55	
150 $mg.L^{-1}$	5.79	7.43	6.61	
Average of Ascorbic acid	5.63	7.03	$LSD_{0.05}$ Aspartic =N.S.	
			$LSD_{0.05}$ Ascorbic = 0.639	
N.S.=aspartic*ascorbic $LSD_{0.05}$				

Table 6. Effect of regular irrigation water and Salt well irrigation water and aspartic and ascorbic acids on carbohydrate content in leaves.

Irrigation Water Quality	Aspartic acid concentration	Ascorbic acid concentration		Average of Irrigation Water Quality
		0 $mg.L^{-1}$	$mg.L^{-1}$4000	
Regular irrigation water	0 $mg.L^{-1}$	42.17	37.98	37.04
	100 $mg.L^{-1}$	37.72	36.02	
	150 $mg.L^{-1}$	36.89	31.43	
Salt well irrigation water	0 $mg.L^{-1}$	32.93	54.56	46.20
	100 $mg.L^{-1}$	45.12	48.88	
	150 $mg.L^{-1}$	37.01	58.69	
Average of Ascorbic acid		38.64	44.59	8.225 $LSD_{0.05}$ Irrigation Water Quality=
$LSD_{0.05}$ aspartic*ascorbic* Irrigation Water Quality=9.640				
Irrigation Water Quality	Aspartic acid concentration			
	0 $mg.L^{-1}$	100 $mg.L^{-1}$	150 $mg.L^{-1}$	
Regular irrigation water	40.08	36.87	34.16	37.04
Salt well irrigation water	43.75	47.00	47.85	46.20
Average of Aspartic acid	41.91	41.94	41.00	
$LSD_{0.05}$ Irrigation Water Quality* aspartic = N.S.				
Irrigation Water Quality		Ascorbic acid concentration		Average of Irrigation Water Quality
		0 $mg.L^{-1}$	$mg.L^{-1}$4000	
Regular irrigation water		38.93	35.14	37.04
Salt well irrigation water		38.35	54.04	46.20
Average of Ascorbic acid		38.64	44.59	
$LSD_{0.05}$ Irrigation Water Quality * ascorbic= 6.298				
Aspartic acid concentration	Ascorbic acid concentration		Average of Aspartic acid	
	0 $mg.L^{-1}$	$mg.L^{-1}$4000		
0 $mg.L^{-1}$	37.55	46.27	41.91	
100 $mg.L^{-1}$	41.42	42.45	41.94	
150 $mg.L^{-1}$	36.95	45.06	41.00	
Average of Ascorbic acid	38.64	44.59	$LSD_{0.05}$ Aspartic = 3.047	
			$LSD_{0.05}$ Ascorbic = N.S.	
N.S.=aspartic*ascorbic $LSD_{0.05}$				

mg. L^{-1}) that recorded the lowest carbohydrate content (35.14 mg g^{-1} dry weight). In the triple interaction, the treatments of liquefied water with aspartic and ascorbic acids showed the highest and leading carbohydrate content in the leaves (58.69 mg. g^{-1} dry weight) of sweet orange seedlings.

DISCUSSION

The study findings authenticated ascorbic acid has a positive effect on the growth traits of grafted local orange seedlings and revealed a significant increase in the number of leaves and dry weight mass of the leaves, as well as, enhanced chlorophyll content in the leaves. The total chlorophyll content directly affects the process of photosynthesis because chlorophyll is the main component of this physiological process (Rawia *et al*., 2011). Chlorophyll also provides the basic units for building new cells and tissues, which ascorbic acid catalyzed through its function as a coenzyme for several enzymes responsible for the metabolism of carbohydrates and proteins and regulation of cell division and expansion processes in plants (Blokhina *et al*., 2003; Abd-El-Aziz *et al*., 2006). Ascorbic acid also affects the ratio between photosynthesis and respiration, which increases the carbohydrate content of leaves and improves the vegetative growth in crop plants (Dhopte and Lall, 1987). Likewise, ascorbic acid plays an essential role in increased chlorophyll content of leaves, leading to an enhancement in the rate of photosynthesis and providing the energy needed for growth and building processes (Kramer and Kozlowski, 1979).

Ascorbic acid not only enhances the total chlorophyll content of leaves but also plays a crucial role in protecting the chlorophyll from oxidation due to salinity as an antioxidant (Oertli, 1987). Given that ascorbic acid may increase the absorption of some basic elements and improve plant health. The soil nutrients, such as, iron and magnesium, and their concentration in the leaves are necessary elements for building chlorophyll (Fayed, 2010). The foliar spray of ascorbic acid increases the ratio of Fe++ to total iron, which is the active formula of iron in the electron transport chain in the cell processes, including chlorophyll synthesis (Crane *et al*., 2007).

The increased percentage of dry matter in the leaves, as well as, the increased carbohydrate content, results from the vital role of ascorbic acid in increasing the leaf area and total chlorophyll content. This may improve the efficiency of the leaves to carry out the process of photosynthesis, positively affecting the ratio between photosynthesis and respiration, consequently increasing the carbohydrate content and the percentage of dry matter in the leaves (Al-Janabi *et al*., 2021a, b). As the seedlings respond morphologically to the ascorbic acid, the responses stimulate the formation of roots and increase their live mass and dry weight (AL-Hadrawi and Al-Janabi, 2020), thus, increasing the ability of sweet orange seedlings to absorb larger quantities of mineral elements from the soil.

CONCLUSIONS

In comparing both water regimes in the irrigation of orange seedlings, the saline well water severely affected the vegetative and root growth characteristics and the chemical characteristics of the local citrus. The aspartic and ascorbic acids reduced the negative effects of salts caused by saline water irrigation and also improved the vegetative and root growth indicators and chemical properties. The application of foliar application of ascorbic acid individually, and in interaction with other factors, proved superior and had a significant positive effect on the growth characteristics of orange seedlings, protecting and enhancing the resistance of seedlings to the harmful effects of salinity from saline water irrigation.

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