J. Agric. Sci. Technol. (2024) Vol. 26 (2): 371-385

Effects of NaCl and Alkaline pH Stress on Some Morphophysiological and Biochemical Parameters of Two Citrus Rootstocks

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ABSTRACT

Citrus is one of the most important fruits whose growth performance and production is significantly affected by environmental stresses. Abiotic stresses, such as salinity and alkaline pH, strikingly limit citrus growth and development. The aim of the present study was to investigate the effect of four NaCl concentrations (0, 30, 60, 90 mM) and two pH levels (6.5 and 8.2) on some of morphological, physiological, and biochemical parameters of two citrus rootstocks (Sour orange and Bakraei rootstocks). The experiment was conducted as factorial based on a completely randomized design with four replications, at the Faculty of Agriculture, Shahid Chamran University of Ahvaz, Iran. The results showed that the value of shoot dry weight, fresh and dry weight of roots, and transpiration were significantly decreased in both Bakraei and Sour orange rootstocks when receiving irrigation with 90 mM supplement of salinity at pH= 8.2. Proline and carbohydrates of citrus rootstocks were considerably increased by increasing the levels of salinity (90 mM NaCl) and alkaline stress (pH= 8.2) in each rootstock, at which condition the photosynthesis rate of Sour orange and Bakraei also declined by 34.77 and 50.80%, respectively. The activity of antioxidant enzymes such as peroxidase, catalase, and superoxide dismutase were increased by 57.42, 42.10, and 45.86% in Sour orange rootstock and 42.04, 26.78, and 37.92% in Bakraei rootstock, respectively. Overall, it can be concluded that the growth performance of Sour orange rootstock is more suitable than Bakraei to tolerate salt-alkali conditions.

Keywords: Abiotic stress, Antioxidant enzymes, *Citrus aurantium*, Salinity stress, Sour orange.

INTRODUCTION

Citrus, as one of the important fruit crops, has been widely cultivated in arid and semi-arid regions where these trees are exposed to chlorine toxicity and iron deficiency by salt-alkali stress (Maldonado-Trres *et al*., 2013; Cimen and Yesiloglu, 2016). Several studies reported the citrus trees as salt and alkali-sensitive plants (Maas, 1993; Bañuls and Primo-Millo, 1992; Boman, 1993; Kelley and Thomas, 1920). Fang *et al*. (2021) noted that simultaneous salinity and alkaline stresses severely reduced microelements and macronutrients absorption, which could be considered one of the most important restrictions affecting plant growth and development (Boukari *et al*., 2019). Gentile *et al*. (2020) reported that exposure of citrus trees to abiotic stress, including salinity, increased the ratio of Na^{+}/Ca^{+2} and Na^{+}/K^{+}, resulting in disruption of water absorption from the soil by the plant roots and reduced growth. According to Snoussi *et al*. (2022), several physiological processes in plants can be affected by salinity stress, such as oxidative mechanism, and ion toxicity. Furthermore, this abiotic stress can cause membrane disruption, nutritional

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[DOI: 10.22034/JAST.26.2.371]

disorders, metabolic process changes, and decreased cell division and expansion. The imbalance of ions and water potential in citrus plant cells results in stunted plant growth under saline conditions (Khalid *et al*., 2022).

Cimen and Yesiloglu (2016) stated that citrus production was affected by abiotic stresses, such as drought, salinity, temperature, and alkalinity. These stresses can extremely influence growth and development of both rootstocks and/or scions of citrus trees. Ibrahim *et al*. (2018) reported that abiotic stresses have negative effects on citrus root growth rate, chlorophyll content and catalase enzyme activity. Numerous reports have indicated that combination of salinity and alkaline stresses in the soil lead to decline osmotic regulatory capacity and inhibition of the antioxidant system in which consequently enforced to cease the plant growth severely (Fang *et al*., 2021; Wang *et al*., 2017). Several research studies have demonstrated that response of citrus trees to salt-alkaline stress could be dependent on type of rootstock and interaction between the combinations of rootstock and scion (Syvertsen and Yelenosky, 1988; Zekri and Parsons, 1992). One way to increase citrus resistance under stress is to use rootstocks with different tolerances over the harsh conditions (Khoshbakht *et al.,* 2014). Cimen and Yesiloglu (2016) argued that rootstocks play a key role in the rapid development of citrus and the production of new tolerant cultivars against soil limiting factors such as abiotic stresses. Appropriate rootstocks can control tree vigor and size, to let high-density planting, and reduce the negative effects of stress on citrus plants to provide tolerance to disease and abiotic stresses (Snoussi *et al*., 2022). Some citrus rootstocks are tolerant to various stresses such as water shortage, chilling, nutrient deficiency and salinity. Accordingly, these seedlings can accumulate low amounts of toxic ions under salinity (Khalid *et al*., 2022). Citrus rootstocks have been reported to increase stress tolerance by excluding chlorine (Khalid *et al*., 2022). Similarly, Maldonado-Torres *et al*. (2013) state that the use of compatible rootstocks can increase the tolerance of citrus trees against abiotic stress, iron deficiency, and control chlorosis. Sing *et al.* (2014) also have demonstrated that selection of stress-resistant rootstock is an effective strategy to reduce the adverse impacts of harsh environments on commercial cultivars. Physiological performance of rootstocks against abiotic stresses was associated with changes in root growth, gas exchange, carbohydrate, and plant water potential (Pedroso *et al.,* 2014).

Considering the importance of citrus rootstocks for stress tolerance and the limited information on these rootstocks under simultaneous stress, the aim of this study was to evaluate the morphological, physiological, and biochemical changes of two citrus rootstocks (Sour orange and Bakraei) under salinity and alkaline stress conditions.

MATERIALS AND METHODS

Plant Culture and Experimental Site

Sour orange (*Citrus aurantium*) and Bakraei *(Citrus reticulata* x *Citrus limonia Osbeck*) seeds were provided from Citrus Research Center and planted in small plastic pots (height 15, diameter 10, and bottom 8 cm) containing sand bed, on November 2, 2019. The sands were sieved with sieve number 2, and 4 seeds were planted in each pot. When seedlings reached the four-leaf stage, they were transferred into 5-liter pots containing sand; then, irrigated the seedlings with Hoagland nutrient solution. Plants grew in the greenhouse (Latitude= 31.30592968, Longitude= 48.65828650 and Altitude= 18 m) of the Faculty of Agriculture, Shahid Chamran University, Ahvaz, Iran during the 2019-2020. The mean day/night temperature was 27-35/19-25°C, relative humidity of 50-70%, and light intensity of 7500 lux.

Treatments

After acclimatization of the Sour orange and Bakraei to new conditions, salinity and alkaline treatments were applied to 7-month-

[DOI: 10.22034/JAST.26.2.371]

old seedlings. The treatment of salinity stress consisted of concentrations of 0, 30, 60, and 90 mM sodium chloride, which were used gradually at three stages in order to prevent osmotic shock to plants. In this way, first, all plants were exposed to 30 mM sodium chloride, then 60 mM NaCl and the end 90 mM was applied. Alkaline stress was also applied by increasing the pH of the nutrient solution using sodium carbonate from 6.5 to 8.2 in irrigation water. Plants were exposed to both treatments at various levels for 70 days. The pots were irrigated three times per week using 500 mL of Hoagland solution in order to prevent salt accumulation around the plant roots, and the bed was washed-out with distilled water every 10 days. At the end of the experiment, the fully and healthy expanded leaves were collected and immediately frozen in liquid nitrogen to measure biochemical properties.

Morphological Parameters

The shoot was separated from the root at the end of the experiment. Using a digital scale (model EK-600, Japan), we measured the fresh weight of shoots and roots. (After washing the roots and removing excess water, the fresh weight of the roots was measured). To measure dry weight, the plant samples were placed in the oven at 70°C for 48 hours (Haddoudi *et al*., 2021).

Gas Exchange Parameters

Net photosynthetic rate (A) and transpiration (E) were measured with a portable gas exchange device (Lci Console model, UK). Measurements were performed in full light conditions (morning at 8 to 10:30 AM) on leaves of 3-6 nodes.

Chlorophylls and Carotenoids

Lichtenthaler (1987) method was used to measure total chlorophyll and carotenoids after extraction with 10 mL acetone 80% and centrifuged for 10 minutes at 3,000 rpm. Then, samples absorbance was record at 663.6, 646.6, and 470 nm by visible ultraviolet spectrophotometer (7315 model, Jenway, UK). Calculation of chlorophyll concentration in mg g^{-1} FW was used according to the Porra (2002) relation.

Free Proline and Total Soluble Carbohydrate

Proline content was determined using 0.5 g of fresh leaves homogenized in 15 mL of the 95% ethanol. Then, equal volumes (2 mL) of glacial acetic acid and ninhydrin were added to 2 mL of homogenate mixed and was placed in a hot water bath for 1 hour. After placing in cold water, toluene was added to the mixture and samples were vortex for 30 seconds. After 15 minutes, the absorbance of samples was record at 520 nm by visible ultraviolet spectrophotometer (7315 model, Jenway, UK) according to Bates *et al*. (1973).

Total soluble carbohydrate was measured according to the Irigoyen *et al*. (1992). Briefly, 3 mL of freshly Antron reagent was added to 100 µL of the alcoholic extract. The mixture was placed in a boiling water bath (Grant JB2 model, UK) for 10 minutes. After cooling, the absorbance of samples was read at 625 nm with a visible ultra violet spectrophotometer (7315 model, Jenway, UK).

Hydrogen Peroxide (H_2O_2) and Malondialdehyde (MDA).

The amount of H_2O_2 was determined as described by Loreto and Velikova (2001). Briefly, 0.5 g of the fresh leaf was homogenized in 5 mL of 1% TCA. After centrifuged 1 mL 1M KI was added to equal value (0.5 mL) of supernatant and 10 mM potassium phosphate buffer (pH 7.0). Then, sample absorbance was read by visible ultra violet spectrophotometer (7315 model, Jenway, UK) at 390 nm.

[DOI: 10.22034/JAST.26.2.371]

MDA was measured according to the method of Heath and Parker (1968): 0.1 g of the leaf was homogenized in 5 mL of 0.1% TCA solution and centrifuged for 15 minutes at 15,000 rpm. One mL of the solution containing 20% trichloroacetic acid (TCA) and 0.5% thiobarbituric acid TBA was added to 1 mL homogenate mixed. After placing in boiling water bath and ice water, the absorbance of samples was read at 532 and 600 nm.

Antioxidant Enzymes Activity

Citrus fresh leaves (0.5 g) were homogenized on ice with 100 mg polyvinylpyrrolidone and 3 mL of extraction buffer (potassium phosphate buffer, and sodium metabisulfite). The obtained solution was centrifuged at 4 °C, at 15000 rpm for 25 min. Subsequently, the supernatant was used as an extract to assay antioxidant enzymes such as Peroxidase (POD), Catalase (CAT), Ascorbate Peroxidase (APX), and Superoxidase Dismutase (SOD).

Peroxidase (POD) Enzyme Activity

POD activity was calculated by Hemeda and Klein (1990) method. The reaction mixture (1 mL) for measuring POD contained 810 µL of 50 mM potassium phosphate buffer (pH= 6.6), 90 µL 1% guaiacol, 90 µL 0.3% hydrogen peroxide, and 10 µL enzyme extract. The enzyme activity was measured by a visible ultraviolet spectrophotometer (7315 model, Jenway, UK) at 470 nm ($\underline{\varepsilon}$ = 26.6 $mmol^{-1}$ cm^{-1}).

Catalase (CAT) Enzyme Activity

CAT activity was assayed according to the disappearance of H_2O_2 at 240 nm for 2 min (ε= 39. 4 $mmol^{-1}$ cm^{-1}) as described by Beers and Sizer (1952). Accordingly, 10 µL Leaf enzyme extract was added to a reaction mixture containing 870 µL of sterile distilled water, 100 µL 1 mM potassium phosphate buffer (pH= 7.8), and 20 µL 1M hydrogen peroxide. The reaction mixture with no enzyme extract was used as blank.

Ascorbate Peroxidase (APX) Enzyme Activity

APX activity of the leaves was measured according to Nakano and Asada (1978). First, the enzyme extract (10 µL) obtained from the leaves was added to the reaction mixture containing 100 µL 1 mM potassium phosphate buffer (pH 7.8), 50 µL 10 mM ascorbate, 50 µL 10 mM H_2O_2, and 790 µL sterile distilled water. APX enzyme activity was recorded by spectrophotometer at 290 nm (ε= 2.8 $mmol^{-1}$ cm^{-1}) for 2 min.

Superoxidase Dismutase (SOD) Enzyme Activity

The activity of Superoxidase Dismutase (SOD) enzyme was determined by monitoring its ability to prevent the photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm according to the Dhindsa *et al.* (1981). The reaction mixture contained 50 mM potassium phosphate buffer (pH= 7.8), 13 mM methionine, 75 µM NBT, 0.1 mM EDTA, and enzyme extract. Subsequently, a mixture of riboflavin (100 µL) with a concentration of 12 mM was added to each reaction and vortexed. The reaction mixtures were then placed under a fluorescent lamp for 15 minutes. Eventually, the absorbance was recorded by a visible ultraviolet spectrophotometer (7315 model, Jenway, UK) at 560 nm.

Experimental Design and Statistical Analysis

Treatments were set up as factorial in a completely randomized design, with four

[DOI: 10.22034/JAST.26.2.371]

replications (two plants per replicate). Data were analyzed using SAS (v. 9.1) software and Duncan's multi-range test at the 5% ($P< 0.05$) level.

RESULTS

Growth Parameters

Fresh and dry weight of shoot

As shown in Table 1, fresh and dry weight of the shoot decreased by increasing salinity and alkalinity pH. Shoot fresh weight significantly decreased in Sour orange rootstock treated with pH= 6.5 at 90 mM NaCl solely, while pH increasing to 8.2 this parameter significantly decreased at all salinity concentrations. In Bakraei rootstock, the shoot fresh weight also decreased significantly at all treatments. In the pH= 8.2, 90 mM NaCl treatment, shoot fresh weight of both rootstocks (Sour orange and Bakraei), decreased by 34.6 and 70.5%, respectively. The highest dry weights of shoot were obtained in Sour orange treated with pH= 6.5 and 0 mM NaCl, and the lowest for Bakraei at pH= 8.2 and 90 mM NaCl, respectively.

Fresh and dry weight of root

The results revealed that salinity and alkalinity stress significantly decreased root fresh and dry weight in both root stocks, which caused both rootstocks to have the lowest fresh and dry weight at the most severe salinity concentration. Root fresh weight decreased up to 57.79% due to application of pH= 8.2, 90 mM NaCl in Sour orange and 76.28% (pH=8.2, 90 mM NaCl) in Bakraei as compared to the control, at pH= 6.5 and 0 mM NaCl. Furthermore, the highest (12.19 g) and lowest (2.23 g) root dry weight was observed in the Sour orange rootstock treated with pH= 6.5 and 0 mM

Table 1. Effect of salinity and alkalinity stress on the fresh and dry weight of shoot and fresh and dry weight of root in two citrus rootstocks. [a]

Rootstocks	Alkalinity (pH)	Salinity (mM NaCl)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Sour orange	6.5	0	51.25a	23.76a	38.5a	12.19a
		30	48.5ab	23.41a	34ab	9.51b
		60	47.25abc	20.48bc	22.5d	6.08d
		90	36.5ef	16.95e	21.75d	6.11d
	8.2	0	45.25bc	19.39cd	31bc	7.97bc
		30	43cd	16.66e	33.25b	8.26b
		60	37.5ef	13.06f	27.75c	6.42cd
		90	33.5fg	11.35fg	16.25e	3.98ef
Bakraei	6.5	0	51.75a	21.98ab	39a	11.98a
		30	46.25bc	19.40cd	15.5ef	4.19e
		60	38.75de	18.38cde	13.25efg	3.57ef
		90	30g	17.88de	11.75efg	3.42ef
	8.2	0	39.5de	18.88cde	21.5d	6.25cd
		30	20.5h	9.61gh	11.5efg	3.04ef
		60	17hi	8.12hi	10.25fg	2.78ef
		90	15.25i	6.99i	9.25g	2.23f

[a] (a-i) Means with at least one same letter (in the same column) are not significantly different at the 5% level ($P< 0.05$) using Duncan's multiple range test.

[DOI: 10.22034/JAST.26.2.371]

NaCl and Bakraei treated with pH= 8.2 and 90 mM NaCl (Table 1).

Gas Exchange Parameters

The result showed that gas exchange including photosynthesis rate (A) and transpiration rate (E), significantly declined in both rootstocks under salt-alkali stress (Table 2). In Sour orange seedling treated with pH= 8.2, the rate of photosynthesis decreased 34.77% by increasing salinity concentration as compared to the control. In Bakraei, the photosynthesis rate decreased 50.80% under both pH levels. Similarly, the rate of transpiration decreased dramatically with increasing salinity concentration in both cultivars with pH 8.2 and 6.5. The highest (1.14 mmol m^{-2} s^{-1}) and lowest (0.85 mmol m^{-2} s^{-1}) rate of transpiration was observed in Sour orange (pH= 6.5, 0 mM NaCl) and Bakraei (pH= 8.2, 90 mM NaCl) (Table 2).

Chlorophyll (Chl) and Carotenoid (CAR)

The results illustrated a negative relationship between salinity-alkalinity treatments and chlorophyll content (Table 2). With increasing NaCl concentration and pH level, chlorophyll content significantly decreased to 41.17% and 64.88% in Sour orange and Bakraei, respectively, under pH= 8.2 and 90 mM NaCl compared to the control. The highest (1.7 mg g^{-1} FW) and lowest (0.59 mg g^{-1} FW) Chl content was obtained in Sour orange (pH= 6.5, 0mM Nacl) and Bakraei (pH= 8.2, 90 mM NaCl). The CAR content also decreased under salt-alkali stress. Although this reduction was not significant in sour oranges, but it decreased significantly under all treatments in Bakraei. The lowest (0.02 mg g^{-1} FW) and highest (0.24 mg g^{-1} FW) CAR content was observed in Bakraei with (pH= 6.5, 0 mM NaCl) and (pH= 8.2, 0 mM NaCl), respectively.

Table 2. Effect of salinity and alkalinity stress on the photosynthesis rate (A), transpiration rate (E) chlorophyll and carotenoid in two citrus rootstocks. [a]

Rootstocks	Alkalinity (pH)	Salinity (mM NaCl)	Photosynthesis (µmol $m^{-2}s^{-1}$)	Transpiration (mmol $m^{-2}s^{-1}$)	Chlrophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
Sour orange	6.5	0	12.28a	1.14a	1.70a	0.15bc
		30	11.73ab	1.09c	1.49b	0.17b
		60	11.43b	1.05d	1.43bc	0.15bc
		90	9.95cd	0.97g	1.33cd	0.15bc
	8.2	0	11.32b	1.04e	1.27de	0.15bc
		30	10.11c	0.94h	1.21e	0.13bcd
		60	9.14e	0.91i	1.09f	0.15bc
		90	8.01f	0.89j	1.00fg	0.14bcd
Bakraei	6.5	0	11.85ab	1.12b	1.68a	0.24a
		30	10.25c	1.03e	1.48b	0.17b
		60	9.23e	1.00f	1.35cd	0.11cd
		90	8.22f	0.96g	0.99fg	0.05e
	8.2	0	9.47de	0.99f	0.85h	0.10cd
		30	8.20f	0.91i	0.90gh	0.02e
		60	7.13g	0.87k	0.79h	0.05e
		90	5.83h	0.85l	0.59i	0.04e

[a] (a-l) Means with at least one same letter (in the same column) are not significantly different at the 5% level ($P< 0.05$) using Duncan's multiple range test.

[DOI: 10.22034/JAST.26.2.371]

Proline Content

Salt-alkaline stress significantly increased the amount of proline in both rootstock leaves. This enhancement was greater in Sour orange than Bakraei. The highest proline content up to 26.35 mmol g^{-1} FW was obtained in the Sour orange rootstock when treated with pH= 8.2 and 90 mM NaCl, while the proline content up to 7.02 mmol g^{-1} FW was observed in sour orange rootstock receiving pH= 6.5 and 0 mM NaCl (Table 3).

Total Soluble Carbohydrate

As shown in Table 3, total soluble carbohydrate significantly increased at high NaCl concentration and alkalinity levels. However, the amount of total soluble carbohydrate significantly increased in both rootstocks treated with pH 8.2 under all salinity treatments as compared to the control. But, this index was significantly higher in Bakraii when treated with pH= 6.5 and 90 mM NaCl. The highest (10.40 mg g^{-1} FW) and lowest (3.36 mg g^{-1} FW) total soluble carbohydrate content was obtained in Sour orange under (pH= 8.2 and 90 mM NaCl) and (pH= 6.5 and 0 mM NaCl), respectively.

Malondialdehyde (MDA) and Hydrogen Peroxide (H2O2)

Our result demonstrated that salt-alkali treatments increased accumulation of MDA in both rootstocks. According to the results, the increase rate of MDA content was higher in Bakraei than Sour orang. The greatest amount of MDA (16.43 mM g^{-1} FW) was obtained in the Bakraei treated with pH= 6.5 and 90 mM NaCl (Table 3). The present study indicated that H_2O_2 increased under salinity and alkalinity stress in both rootstocks and strongly suggested that such enhancement was directly due to stress intensity. The highest amount (129.97 mmol L^{-1}) of H_2O_2 was record in Bakraei with pH= 8.2 and 90 mM NaCl. This index tended to increase to 42.83 and 32.38% in, respectively, Bakraei and Sour orang

Table 3. Effect of salt-alkali stress on proline, total soluble carbohydrate, Malondi Aldehyde (MDA) and the content of hydrogen peroxide. [a]

Rootstocks	Alkalinity (pH)	Salinity (mM NaCl)	Proline (mmol g^{-1} FW)	Total soluble carbohydrate (mg g^{-1} FW)	MDA (mmol g^{-1} FW)	Hydrogen peroxide (mmol L^{-1})
Sour orange	6.5	0	7.02h	3.36d	4.65f	93.07g
		30	13.42efg	7.75b	4.65f	107.10f
		60	22.41abc	9.71a	8.53e	116.46de
		90	23.71ab	10.14a	9.69bcde	120.61cd
	8.2	0	21.05bcd	8.00b	10.46bcde	108.66f
		30	23.57ab	9.54a	11.63b	111.26ef
		60	24.18ab	10.31a	11.24bc	122.69bc
		90	26.36a	10.40a	10.85bcd	123.21bc
Bakraei	6.5	0	9.95gh	3.73d	4.65f	90.99g
		30	11.31fgh	3.90d	10.85bcd	108.66f
		60	13.42efg	4.50d	9.69bcde	114.90e
		90	18.26cde	5.95c	8.53e	123.73bc
	8.2	0	13.97efg	4.41d	9.30cde	112.30ef
		30	15.54ef	5.95c	8.91de	122.17bc
		60	17.24de	6.04c	8.91de	127.89ab
		90	22.00abcd	9.90a	16.43a	129.97a

[a] (a-h) Means with at least one same letter (in the same column) are not significantly different at the 5% level (P< 0.05) using Duncan's multiple range test.

[DOI: 10.22034/JAST.26.2.371]

rootstocks when treated with pH= 8.2 and 90 mM NaCl, (Table 3).

POD, CAT, APX, and SOD Activities

Peroxidase (POD) Activity

Antioxidant enzyme activities are enhanced with an increase in salt-alkali stress (Table 4). All the treatments significantly increased the activity of peroxidase. POD activity in the leaves of Sour orange and Bakraei significantly increased at various levels of NaCl and pH up to 57.42 and 42.04% in the Sour orange and in the Bakraei rootstock, respectively. Moreover, the highest (32.76 U mg^{-1} protein) POD activity were detected at pH= 8.2 and 90 mM NaCl in both cultivars.

Catalase (CAT) activity

As shown in Table 4, increasing salinity concentration and pH levels significantly enhanced the activity of CAT in the leaves of Sour orange and Bakraei rootstocks. Increased CAT activity was significant in all treatments for both rootstocks with the amount of 42.10% in the Sour orange and 26.78% in the Bakraei rootstock. On the other hand, the highest (0.81 U mg^{-1} protein) and lowest (0.56 U mg^{-1} protein) activity of CAT enzyme was obtained in Sour orange and Bakraei, respectively (Table 4).

Ascorbate Peroxidase (APX) Activity

The present study revealed a positive relation between the activity of APX and the intensity of stress. APX activity enhanced in both Sour orange and Bakraei rootstocks under salt-alkali stress as compared to the control. Salinity stress induced APX activity in both pH levels. However, there was no significant difference between 0 mM NaCl and 30 mM NaCl, while the highest (22.84 U mg^{-1} protein) and lowest (5.79 U mg^{-1} protein) APX activity was observed in Sour orange and Bakraei, respectively (Table 4).

Table 4. Effect of salt-alkali stress on the activity of Peroxidase (POD), Catalase (CAT), Ascorbate Peroxidase (APX), and Superoxidase Dismutase (SOD) in leaves of two citrus rootstocks.

Rootstocks	Alkalinity (pH)	Salinity (mM NaCl)	Peroxidase (U mg^{-1} protein)	Catalase (U mg^{-1} protein)	APX (U mg^{-1} protein)	SOD (U mg^{-1} protein)
Sour orange	6.5	0	20.81i	0.57e	5.88g	72.80i
		30	24.15fgh	0.61d	8.37efg	80.51ghi
		60	27.07d	0.63d	11.05de	86.76efgh
		90	28.70c	0.69c	13.36cd	88.22efgh
	8.2	0	23.57gh	0.62d	8.34efg	77.58hi
		30	26.55de	0.71bc	13.95cd	94.85cdef
		60	28.72c	0.74b	16.23bc	98.67bcde
		90	32.76a	0.81a	22.84a	106.19abc
Bakraei	6.5	0	21.36i	0.56e	5.79g	84.85fghi
		30	22.97h	0.56e	7.74fg	83.19fghi
		60	24.95fg	0.56e	11.20de	89.08defgh
		90	26.87d	0.62d	9.57ef	93.68cdefg
	8.2	0	24.54fg	0.57e	9.97ef	106.47abc
		30	25.18ef	0.64d	11.52de	101.94bcd
		60	27.70cd	0.72bc	14.81bc	108.91ab
		90	30.34b	0.71bc	17.64b	117.03a

[a] (a-i) Means with at least one same letter (in the same column) are not significantly different at the 5% level (P< 0.05) using Duncan's multiple range test.

[DOI: 10.22034/JAST.26.2.371]

Super Oxidase Dismutase (SOD) Activity

SOD activity revealed an increasing trend at more severe salinity and pH levels (Table 4). This enhancement was not significant between 0 mM NaCl and 30 mM NaCl in both cultivars. It elucidated that the increase in SOD enzyme activity were 45.86 and 37.92% in Sour orange and in Bakraei, respectively. Salinity stress induced SOD activity in both pH levels, in both cultivars.

DISCUSSION

Findings of this study indicated that morphological factors could decrease under salinity and alkalinity stresses. In addition, the results showed that the morphological indices were affected by the rootstocks, while the Sour orange had less decrease than Bakraei. These results are in agreement with the report documented by Safdar *et al*. (2019) who asserted that environmental stresses reduced photosynthesis, and the event depended on stress intensity and plant species; and the end result ultimately inhibited plant growth performance and development. Also, Feng *et al*. (2021) stated that salt-alkali stress decreased fresh and dry weight of shoot by inducing high osmotic pressure, ion toxicity, inhibited the net Photosynthetic rate (Pn), reduced chlorophyll and carotenoid, and had negative effect on the absorption of light energy. Several studies demonstrate that salinity stress reduces nutrient uptake and plant water availability, soil-plant osmotic imbalance, oxidative stress and, ultimately, reduces shoot and root dry weight (Zarei and Paymaneh, 2014; Safdar *et al*., 2019). Lin *et al*. (2017) also reported that salt-alkali stress reduced the fresh and dry weight of *Leymus chinensis* by reducing water uptake by the roots. Similarly, Hassan and Ali. (2014) reported the decrease in plant height, number of branches, number of leaves, fresh and dry weight of jojoba plant under salinity stress was due to a decrease in cell division, cell elongation, and ionic toxicity. It was stated by Snoussi *et al*. (2022) that stress conditions affect all aspects of plant development and decrease growth, development, and survival in citrus trees. It is possible to grow citrus under different environmental conditions by using convenient rootstocks, which can improve tolerance to such restrictions. According to the present study, rootstocks affected physiological parameters such as photosynthesis and transpiration rate under salt-alkali stress. The rate of photosynthesis and transpiration in Sour orange was higher than Bakraei. Rootstocks can increase tolerance to stress conditions by excluding sodium and chlorine, increasing the absorption of essential elements such as nitrogen and potassium, (Khalid *et al*., 2020 a), and enhancing photosynthetic pigments and gas exchanges (Roussos *et al*., 2013). One of the primary responses of plants to stress is stomatal closure, which limits gas exchange between the atmosphere and leaves (Mafakheri *et al*., 2010; Golbashy *et al*., 2010). Shahid *et al*. (2019) found that salinity stress reduced photosynthetic rate and stomatal conductance in sensitive citrus rootstocks more than in tolerant rootstocks. The reduction of gas exchanges is a major factor in reducing plant growth under stress conditions. This phenomenon could be explained by decreased cell swelling and accumulation of sodium chloride in leaves, which alters enzyme activity (Tabatabaei, 2006). Additionally, another explanation could be the decrease in the content of chlorophyll and carotenoids, which reduces the enzyme activity of Rubisco. Generally, photosynthesis is an important indicator of physiologic susceptibility to abiotic stress, which is significantly reduced in salt-alkali environments. (Lin *et al*., 2017). These results are consistent with previous researches (Mafakheri *et al*., 2010; Golbashy *et al*., 2010). The rate of chlorophyll content decreased under salt-alkali stress conditions leading to decreased photosynthesis (Taïbi *et al*., 2016; Manzari *et al*., 2016; Ye *et al*., 2019). Several factors affect chlorophyll and CAR content, such as

[DOI: 10.22034/JAST.26.2.371]

nutrition imbalance, the increasing amount of Na, decreasing absorption of K, Mg, and other elements involved in chlorophyll synthesis under salinity stress. In addition, an increase in the activity of antioxidant enzymes, a decrease in the activity of enzymes involved in the synthesis of photosynthetic pigments, an increase in the rate of active oxygen species, formation of proteolytic enzymes such as chlorophyllase have been reported as the other effects of salt stress that could decrease the amount of chlorophyll and carotenoids (Hassan and Ali, 2014; Zarei and Paymaneh, 2014). The present study demonstrated that salt-alkaline stress significantly enhanced the proline content in both rootstocks. The proline content enhancement in Sour orange was higher than Bakraei. These results are consistent with those of Zarei and Paymaneh (2014), who reported that proline is one of the most common changes in stress that, as an osmolyte, can protect cytosolic enzymes and cellular organelles and maintain normal osmotic conditions. Ye *et al.* (2019) reported that plants can increase their tolerance to salinity-alkaline stress through a variety of biochemical pathways, such as the production of proline, dynamic osmotic metabolites, and certain proteins that manage ion and water flow. Proline, an osmotic regulator in plants, can stabilize subcellular structures and eliminate free radicals and protect membranes and enzymes from oxidative stress. It has been widely reported that proline may play a role in adapting to intracellular stress. Increased proline levels due to stress are well known (Hassan and Ali, 2014). Under stress conditions, the amount of soluble sugar increases to regulate the osmotic potential, maintain metabolism, increase energy, and promote recovery after stress. Moreover, salinity stress could increase the activity of sucrose phosphate synthetase, as a key enzyme in the sucrose pathway synthesis, and finally, soluble sugars could be increased (Hassan and Ali, 2014). These findings are similar to a previous study (Stoop and Pharr, 1994; Amirjani, 2011). When the plant faces a reduction in photosynthesis, there is an accumulation of ROS in the cell organelles, which affects cell metabolism in the chloroplasts and mitochondria and leads to cell death. To determine the damage to cell membranes, we determined lipid peroxidation by measuring MDA (Khalid *et al*., 2022). Salt-alkali treatments increased MDA accumulation in both rootstocks, but this increase was greater in Bakraei than in Sour Orange. These results are consistent with previous studies (Ye *et al*., 2019) who reported MDA is the breakdown product of unsaturated fatty acids in cell membranes. In other words, under stress conditions, the cell membrane is damaged and increased MDA levels are generated because of changes in reactive oxygen species (Soltabayeva *et al*., 2021). Therefore, it could be a valuable indicator to assess the severity of oxidative damage. To protect cellular systems from reactive oxygen species, plants under stress conditions increase the concentration of active non-enzymatic compounds and the activity of antioxidant enzymes (Khalid *et al*., 2020b). Our findings indicated that antioxidant enzyme activities enhanced under abiotic stress, and the increase in sour orange was higher than in Bakraei rootstock.

Similar results were reported by Apel and Hirt (2004) and M'barek *et al*., (2007), who found that stress conditions increased POD enzyme activity to prevent the toxic effects of Reactive Oxygen Species (ROS) and lipid and protein oxidation. Antioxidant enzymes play an important role in the plant's defense mechanism against oxidative damage caused by stress. The changes in the activity of antioxidant enzymes is the defense system against abiotic stresses (Ghasemi *et al*., 2014). In response to alkaline stress, plants stimulate antioxidant defense systems, including POD, CAT, APX, and SOD to remove excess ROS from their cells. These results are consistent with the findings of Naliwajski and Skłodowska (2021), who reported that there was a positive correlation between the activity of CAT and the intensity of salt stress, suggesting that the

[DOI: 10.22034/JAST.26.2.371]

increase in enzyme activity is proportional to the concentration of NaCl. Nevertheless, long-term ROS production under stress may exceed the antioxidant defense capacity and result in oxidative damage to seedlings (Zhang *et al*., 2017). Likewise, increased activity of antioxidant enzymes such as APX under salinity stress was reported by (Zhang *et al*., 2013). The increased activity of antioxidant enzymes under saline and alkaline stress conditions reduces reactive oxygen species and provides balance in the plant (Ye *et al*., 2019). Similarly, Hassan and Ali (2014) demonstrated production of ROS, including singlet Oxygen ($^1O2$), superoxide anion (O^{2-}), Hydrogen peroxide (H_2O_2), and Hydroxyl radicals (HO), causes oxidative stress. Following the production of ROS, plant cells must activate an antioxidant defense system, including the enzymatic antioxidant, to inhibit reactive oxygen species. Therefore, mechanisms that reduce ROS and increase the antioxidant enzyme system in plants play an important role in plant tolerance to environmental stress. Sarker *et al.* (2020) found that SOD activity increases under stress conditions due to its role in salt tolerance, which increases the conversion of superoxide radicals into H_2O_2. Stress causes an imbalance of reactive oxygen species due to a disruption of the electron transfer chain during light cessation or a reduction in water potential. ROS increase dramatically with salt stress, and their first reaction is an oxidative explosion. Higher levels of ROS are toxic to cells, leading to cell damage and death. Furthermore, ROS act as a signaling molecule that may alter transcription. It is well documented that the amount of ROS is regulated by increasing the activity of enzymes such as peroxidase, catalase, and ascorbate peroxidase (Soltabayeva *et al*., 2021). Furthermore, abiotic stresses can cause unfavorable physiological, morphological, and biochemical effects on various organs of the citrus tree (Snoussi *et al*., 2022). The use of rootstocks that exclude sodium and chlorine ions is one way citrus trees tolerate stress conditions (Khalid *et al*., 2020 a). Rootstocks have different effects on growth, development, and response to stress due to their particular root structure, ion uptake, xylem anatomy, and hormonal and biochemical processes (Snoussi *et al*., 2022).

CONCLUSIONS

The present study indicated that salinity and alkaline stress reduced morpho-physiological characteristics, and increased osmotic regulators and antioxidant enzyme activity in both Sour orange and Bakraei rootstocks. Overall, it can be concluded that Sour orange rootstock can be more suitable than Bakraei under salt-alkali conditions.

ACKNOWLEGEMENTS

This study was supported by Shahid Chamran University of Ahvaz, Iran. We thank the anonymous reviewer for his/her most helpful comments.

REFERENCES

1. Amirjani, M. R. 2011. Effect of Salinity Stress on Growth, Sugar Content, Pigments and Enzyme Activity of Rice. *Int. J. Bot*. **7**: 73-81.
2. Apel, K. and Hirt, H. 2004. Reactive Oxygen Species: Metabolism, Oxidative Stress, and Signal Transduction. *Annu. Rev. Plant Biol.,* **55:** 373-399.
3. Bañuls, J. and Primo-Millo, E. 1992. Effect of Chloride and Sodium on Gas Exchange Parameters and Water of Citrus Plants. *Physiol. Plant,* **86:** 115 123.
4. Bates, L. S., Waldaren, R. P. and Teare, I. D. 1973. Rapid Determination of Free Proline for Water Stress Studies. *Plant Soil,* **39:** 205-208.
5. Beers, R. F. and Sizer, I. W. 1952. A Spectrophotometric Method for Measuring the Breakdown of Hydrogen Peroxide by Catalase. *J. Biol. Chem.,* **195:** 133-140.
6. Boman, B.J. 1993. First-Year Response of 'Ruby Red' Grapefruit on Four Rootstocks

[DOI: 10.22034/JAST.26.2.371]

to Fertilization and Salinity. *Proc. Annu. Meet. Fla. State Hort. Soc.,* **106:** 12-18.
7. Boukari, N., Jelali, N., Renaud, J. B., Youssef, R. B., Abdelly, C. and Hannoufa, A. 2019. Salicylic Acid Seed Priming Improves Tolerance to Salinity, Iron Deficiency and Their Combined Effect in Two Ecotypes of Alfalfa. *Environ. Exp. Bot. ,* **167:** 1-12.
8. Cimen, B. and Yesiloglu, T. 2016. Rootstock Breeding for Abiotic Stress Tolerance in Citrus. In: "*Abiotic and Biotic Stress in Plants-Recent Advances and Future Perspectives*", (Eds.): Shanker, A. K. and Shanker, C. IntechOpen. https://www.intechopen.com/chapters/49717
9. Dhindsa, R. S., Plumb-Dhindsa, P. A. M. E. L. A. and Thorpe, T. A. 1981. Leaf Senescence: Correlated with Increased Levels of Membrane Permeability and Lipid Peroxidation, and Decreased Levels of Superoxide Dismutase and Catalase. *J. Exp. Bot.,* **32:** 93-101.
10. Fang, S., Hou, X. and Liang, X. 2021. Response Mechanisms of Plants under Saline-Alkali Stress. *Front. Recent Dev. Plant Sci.,* **12**: 1-20.
11. Feng, N.,Yu, M., Li, Y., Jin, D. and Zheng, D. 2021. Prohexadione-Calcium Alleviates Saline-Alkali Stress in Soybean by Improving the Photosynthesis and Up-Regulating Antioxidant Defense. *Ecotoxicol. Environ. Saf.,* **220:** 1-13..
12. Gentile, A., LaMalfa, S. and Deng, Z. 2020. *The Citrus Genome.* Springer Nature.
13. Ghasemi, S., Khoshgoftarmanesh, A. H., Afyuni, M. and Hadadzadeh, H. 2014. Iron (II)–Amino Acid Chelates Alleviate Salt-Stress Induced Oxidative Damages on Tomato Grown in Nutrient Solution Culture. *Sci. Hortic.,* **165:** 91-98.
14. Golbashy, M., Ebrahimi, M., Khorasani, S. K. and Choukan, R. 2010. Evaluation of Drought Tolerance of Some Corn (*Zea mays* L.) Hybrids in Iran. *Afr. J. Agric. Res.,* **5:** 2714-2719.
15. Haddoudi, L., Hdira, S., Hanana, M., Romero, I., Haddoudi, I., Mahjoub, A. and Badri, M. 2021. Evaluation of the Morpho-Physiological, Biochemical and Molecular Responses of Contrasting *Medicago truncatula* Lines under Water Deficit Stress. Plants, **10:** 2-21.
16. Hassan, F. and Ali, E. 2014. Effects of Salt Stress on Growth, Antioxidant Enzyme Activity and Some Other Physiological Parameters in Jojoba [*'Simmondsia chinensis'* (link) schneider] Plant. *Aust. J. Crop Sci.,* **8:** 1615-1624.
17. Heath R. L. and Parker, L. 1968. Photoperoxidation in Isolated Chloroplasts. I. Kinetics and Stoichiometry of Fatty Acid Peroxidation. *Arch. Biochem.,* **125:** 189–198.
18. Hemeda, H. M. and Klein, B. P. 1990. Effects of Naturally Occurring Antioxidants on Peroxidase Activity of Vegetable Extracts. *J. Food Sci.,* **55:** 184-185.
19. Ibrahim, D. S., Eissa, A. M., Attala, A. Z. M., Sabbah, S. M. and Khalil, H. A. 2018. Alleviation of Salinity Stress by Exogenous Plant Growth Regulators in Three Citrus Rootstocks. *Middle East J.,* **7:** 437-455.
20. Irigoyen, J. J. and Einerich, D. W. and Sánchez Díaz, M. 1992. Water Stress-Induced Changes in Concentrations of Proline and Total Soluble Sugars in Nodulated Alfalfa (*Medicago sativa*) Plants. *Physiol. Plant,* **84:** 55-60.
21. Kelley, W. P. and Thomas, E. E. 1920. *The Effects of Alkali on Citrus Trees.* Bull. No. 318, Berkeley University, California. USA.
22. Khalid, M. F., Hussain, S., Anjum, M. A., Ali, M. A., Ahmad, S., Ejaz, S. and Morillon, R. 2020a. Efficient Compartmentalization and Translocation of Toxic Minerals Lead Tolerance in Volkamer Lemon Tetraploids More than Diploids under Moderate and High Salt Stress. *Fruits,* **75:** 204-215.
23. Khalid, M. F., Hussain, S., Anjum, M. A., Ahmad, S., Ali, M. A., Ejaz, S. and Morillon, R. 2020b. Better Salinity Tolerance in Tetraploid vs Diploid Volkamer Lemon Seedlings Is Associated with Robust Antioxidant and Osmotic Adjustment Mechanisms. *J. Plant Physiol.,* **244:** 1-37.
24. Khalid, M. F., Morillon, R., Anjum, M. A., Ejaz, S., Rao, M. J., Ahmad, S. and Hussain, S. 2022. Volkamer Lemon Tetraploid Rootstock Transmits the Salt Tolerance When Grafted with Diploid Kinnow Mandarin by Strong Antioxidant Defense Mechanism and Efficient Osmotic Adjustment. *J. Plant Growth Regul.,* **41**: 1125-1137.

[DOI: 10.22034/JAST.26.2.371]

25. Khoshbakht, D., Ramin, A. A. and Baninasab, B. 2014. Citrus Rootstocks Response to Salinity: Physio-Biochemical Parameters Changes. *Res. J. Environ. Sci.,* **8:** 29-38.
26. Lichtenthaler, H. K. 1987. ChloropHylls and Carotenoids: Pigments of Photosynthetic Biomembranes. *Meth. Enzymol.,* **148:** 350-382.
27. Lin, J., Wang, Y., Sun, S., Mu, C. and Yan, X. 2017. Effects of Arbuscular Mycorrhizal Fungi on the Growth, Photosynthesis and Photosynthetic Pigments of *Leymus chinensis* seedlings under Salt-Alkali Stress and Nitrogen Deposition. *Sci. Total Environ.,* **576:** 234-241.
28. Loreto, F. and Velikova, V. 2001. Isoprene Produced by Leaves Protects the Photosynthetic Apparatus against Ozone Damage, Quenches Ozone Products, and Reduces Lipid Peroxidation of Cellular Membranes. *Plant Physiol.,* **127:** 1781-1787.
29. Maas, E. V. 1993. Salinity and Citriculture. *Tree Physiol.,* **12(2):** 195-216. Mafakheri, A., Siosemardeh, A. F., Bahramnejad, B., Struik, P. C. and Sohrabi, Y. 2010. Effect of Drought Stress on Yield, Proline and Chlorophyll Contents in Three Chickpea Cultivars. *Aust. J. Crop Sci.,* **4:** 580-585.
30. Maldonado-Torres, R., Alvarez-Sánchez, M. E. and Etchevers, J. D. 2013. Adaptation Strategies of Mexican Lemon Rootstock in Response to Iron Deficiency. *Terra Latinoamericana,* **31:** 23-34.
31. Manzari, T. M., Roosta, H. R. and Hamidpour, M. 2016. Effects of Alkali Stress and Growing Media on Growth and Physiological Characteristics of Gerbera Plants. *J. Agric. Sci. Technol.,* **18:** 453-466.
32. M'barek, B. N., Raoudha, A. and Leila, B. K. 2007. Relationship between Peroxidase Activity and Salt Tolerance during Barley Seed Germination. *J. Agron.,* **6:** 433-438.
33. Nakano, Y. and Asada, K. 1981. Hydrogen Peroxide Is Scavenged by Ascorbate-Specific Peroxidase in Spinach Chloroplasts. *Plant Cell Physiol.,* **22:** 867-880.
34. Naliwajski, M. and Skłodowska, M. 2021. The Relationship between the Antioxidant System and Proline Metabolism in the Leaves of Cucumber Plants Acclimated to Salt Stress. *Cells,* **10:** 1-15.
35. Pedroso, F. K., Prudente, D. A., Bueno, A. C. R., Machado, E. C. and Ribeiro, R. V. 2014. Drought Tolerance in Citrus Trees Is Enhanced by Rootstock-Dependent Changes in Root Growth and Carbohydrate Availability. *Environ. Exp. Bot.,* **101:** 26-35.
36. Porra, R. J. 2002. The Chequered History of the Development and Use of Simultaneous Equations for the Accurate Determination of Chlorophylls a and b. *Trends Photosynth. Res.,* **73:** 149-156.
37. Roussos, P. A., Gasparatos, D., Kyriakou, C., Tsichli, K., Tsantili, E. and Haidouti, C. 2013. Growth, Nutrient Status, and Biochemical Changes of Sour Orange Plants Subjected to Sodium Chloride Stress. *Commun. Soil Sci. Plant Anal.,* **44:** 805-816.
38. Safdar, H., Amin, A., Shafiq, Y., Ali, A., Yasin, R., Shoukat, A. and Sarwar, M. I. 2019. A Review: Impact of Salinity on Plant Growth. *Nat. Sci.,* **17:** 34-40.
39. Sarker, U. and Oba, S. 2020. The Response of Salinity Stress-Induced *A. tricolor* to Growth, Snatomy, Physiology, Non-Enzymatic and Enzymatic Antioxidants. *Front. Plant Sci.,* **11:** 1-14.
40. Shafiei, N., Khaleghi, E. and Moallemi, N. 2019. Effect of Salicylic Acid on Some Morphological and Physiological Characteristics of Olive cv. 'Konservalia' under Water Deficit Condition. *Iran. J. Hortic. Sci.,* **49(4):** 881-890.
41. Shafieizargar, A., Awang, Y., Ajamgard, F., Juraimi, A. S., Othman, R. and Ahmadi, A. K. 2015. Assessing Five Citrus Rootstocks for NaCl Salinity Tolerance Using Mineral Concentrations, Proline and Relative Water Contents as Indicators. *Asian J. Plant Sci.,* **14:** 20-26.
42. Shahid, M. A., Balal, R. M., Khan, N., Simón-Grao, S., Alfosea-Simón, M., Cámara-Zapata, J. M. and Garcia-Sanchez, F. 2019. Rootstocks Influence the Salt Tolerance of Kinnow Mandarin Trees by Altering the Antioxidant Defense System, Osmolyte Concentration, and Toxic Ion Accumulation. *Sci. Hortic.,* **250**: 1-11.
43. Singh, A., Prakash, J., Srivastav, M., Singh, S. K., Awasthi, O. P., Singh, A. K. and Sharma, D. K. 2014. Physiological and Biochemical Responses of Citrus Rootstocks under Salinity Stress. *Indian J. Hortic.,* **71:** 162-167.

[DOI: 10.22034/JAST.26.2.371]

44. Soltabayeva, A., Ongaltay, A., Omondi, J. O. and Srivastava, S. 2021. Morphological, Physiological and Molecular Markers for Salt-Stressed Plants. *Plants,* **10**: 1-18.
45. Snoussi, H., Askri, H., Nacouzi, D., Ouerghui, I., Ananga, A., Najar, A., El Kayal, W., 2022. Comparative Transcriptome Profiling of Salinity-Induced Genes in Citrus Rootstocks with Contrasted Salt Tolerance. *Agriculture,* **12:** 350-361.
46. Stoop, J. M. and Pharr, D. M. 1994. Growth Substrate and Nutrient Salt Environment Alter Mannitol-to-Hexose Partitioning in Celery Petioles. *J. Am. Soc. Hortic. Sci.,* **119:** 237-242.
47. Syvertsen, J. P. and Yelenosky, G. 1988. Salinity Can Enhance Freeze Tolerance of Citrus Rootstock Seedlings by Modifying Growth, Water Relations, and Mineral Nutrition. *J. Am. Soc. Hortic. Sci.,* **113(6):** 889-893.
48. Tabatabaei, S. J. 2006. Effects of Salinity and N on the Growth, Photosynthesis and N Status of Olive (*Olea europaea* L.) Trees. *Sci. Hortic.,* **108:** 432-438.
49. Taïbi, K., Taïbi, F., Abderrahim, L. A., Ennajah, A., Belkhodja, M. and Mulet, J. M. 2016. Effect of Salt Stress on Growth, Chlorophyll Content, Lipid Peroxidation and Antioxidant Defense Systems in *Phaseolus vulgaris* L. *S. Afr. J. Bot.* **105:** 306-312.
50. Wang, X. -S., Ren, H. -L., Wei, Z. -W., Wang, Y. -W. and Ren, W. B. 2017. Effects of Neutral Salt and Alkali on Ion Distributions in the Roots, Shoots, and Leaves of Two Alfalfa Cultivars with Differing Degrees of Salt Tolerance. *J. Integr. Agric.,* **16:** 1800–1807.
51. Ye, L., Zhao, X., Bao, E., Cao, K. and Zou, Z. 2019. Effects of Arbuscular Mycorrhizal Fungi on Watermelon Growth, Elemental Uptake, Antioxidant, and Photosystem II Activities and Stress-Response Gene Expressions under Salinity-Alkalinity Stresses. *Front. Plant Sci.,* **10:** 1-12.
52. Zarei, M. and Paymaneh, Z. 2014. Effect of Salinity and Arbuscular Mycorrhizal Fungi on Growth and Some Physiological Parameters of *Citrus jambheri*. *Arch. Agron. Soil Sci.,* **60:** 993-
53. Zekri, M. and Parsons, L. R. 1992. Salinity Tolerance of Citrus Rootstocks: Effect of Salt on Root and Leaf Mineral Concentrations. *Plant Soil,* **147(2):** 171-181
54. Zhang, H., Liu, X. L., Zhang, R. X., Yuan, H. Y., Wang, M. M., Yang, H. Y. and Liang, Z. W. 2017. Root Damage under Alkaline Stress Is Associated with Reactive Oxygen Species Accumulation in Rice (*Oryza sativa* L.). *Front. Recent Dev. Plant Sci.,* **8:** 1-12.
55. Zhang, M., Fang, Y., Ji, Y., Jiang, Z. and Wang, L. 2013. Effects of Salt Stress on Ion Content, Antioxidant Enzymes and Protein Profile in Different Tissues of *Broussonetia papyrifera. S. Afr. J. Bot.*, **85:** 1-9.

تأثیر تنش شوری و قلیایی بر برخی از پارامترهای مورفوفیزیولوژیکی و بیوشیمیایی دو پایه مرکبات

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چکیده

مرکبات یکی از مهم‌ترین محصولات هستند که رشد آنها تحت تأثیر تنش قرار می‌گیرد. تنش‌های غیر زنده مانند شوری و قلیایی رشد و نمو مرکبات را محدود می‌کنند. در مطالعه حاضر اثر چهار غلظت سدیم کلرید در آب آبیاری (۰، ۳۰، ۶۰ و ۹۰ میلی‌مولار) و دو سطح تنش قلیایی در آب (pH=۶/۵ و pH=۸/۲) بر برخی از

[DOI: 10.22034/JAST.26.2.371]

پارامترهای مورفولوژیکی، فیزیولوژیکی و بیوشیمیایی دو پایه مرکبات (نارنج و بکرایی) بررسی شد. این آزمایش به صورت فاکتوریل در قالب طرح بلوک کاملاً تصادفی در چهار تکرار در دانشکده کشاورزی دانشگاه شهید چمران اهواز اجرا شد.نتایج نشان داد که در pH=۸/۲ با افزایش غلظت شوری به ۹۰ میلی‌مولار وزن خشک اندام هوایی، وزن تر و خشک ریشه و تعرق در هر دو پایه نارنج و بکرائی به طور معنی‌داری کاهش یافت. همچنین با افزایش شوری (۹۰ میلی مولار) و تنش قلیایی (pH=۸/۲) پرولین و کربوهیدرات در هر پایه به طور قابل توجهی افزایش یافت. درpH=۸/۲ وشوری ۹۰ میلی مولار NaCl سرعت فتوسنتز در نارنج به میزان ۳٤/۷۷ درصد و در بکرائی ۵۰/۸۰ درصد کاهش یافت. فعالیت آنزیم های آنتی اکسیدانی پراکسیداز، کاتالاز و سوپراکسید دیسموتاز در پایه نارنج به ترتیب ۵۷/٤۲، ٤۲/۱۰ و ٤۵/۸٦ درصد و در پایه بکرایی ٤۲/۰٤، ۲٦/۷۸ و ۳۷/۹۲ درصد افزایش یافت. در مجموع می توان نتیجه گرفت که پایه نارنج برای تحمل شرایط شوری- قلیایی نسبت به بکرایی مناسب تر باشد.

[DOI: 10.22034/JAST.26.2.371]