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EFFECT OF SALINE IRRIGATION WATER ON SOME SOUR ORANGE RACES.

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ABSTRACT

Seedlings of Brazilian, Spanish and Balady sour orange rootstocks were studied for their tolerance to five salinity levels : tap water (control), 1000, 2000, 3000 and 4000 ppm of NaCl, $CaCl_2$ and $MgCl_2$ .. All growth parameters of Balady, Brazilian and Spanish seedlings measured were significantly affected by salinity levels. Brazilian sour orange seedlings gave the highest values of all growth parameters such as stem length and diameter, leaf number per plant, leaf area and dry weight of top and roots followed by Balady sour orange and Spanish seedlings, respectively. Chlorophyll a, b and its total value decreased as salinity increased in irrigation water. In contrary, free proline amino acid, soluble and non soluble sugars were increased with increasing saline water. Moreover, Brazilian rootstock had higher amount of chlorophyll, proline and soluble and non soluble sugars than those recorded for Balady and Spanish rootstocks. On the other hand, leaf tissue analysis showed that increasing the salinity level in irrigation water caused a significant increase in N, Ca, Mg, Na, Cl and Fe values, but decreased content of P, K, Mn, Zn and Cu . Moreover, root Ca, Mg, Na, Cl, and Fe, contents were increased with increasing salinity level, while, N, P, K, K, Mn, Zn, and Cu values were decreased . Brazilian seedling tended to contain the lowest Na, and Cl concentrations when compared with those of both Spanish and Balady seedlings . This may be explain why Brazilian is the most tolerant rootstock to salinity .there for, The three rootstocks can be arranged in the following descending order due to their salt tolerance: Brazilian> Balady> Spanish.

INTRODUCTION

Salinity is one of the most problems for introduce and cultivate much more citrus varieties in the new reclaimed areas in Egypt, most of these new areas were irrigated with underground waters, which have poor quality, due to its high salt content.

workers such as Anjum *et al*. (2000), Anjum *et al*. (2001), Moya *et al*. (2002) The effect of salinity on growth parameters have been studed by many and Garcia-Sanchez *et al*. (2002) they concluded that, vegetative growth parameters were depressed with further increase in water salinity. Also, Murkute *et al*. (2006) reported that irrigation of Karna khatta and Troyer citrange with salinized water significantly reduced plant height, stem diameter and number of leaves. Similar findings were obtained by Balal *et al*. (2011) on ten citrus rootstock genotypes. Dry matter accumulation in leaves, shoots and roots of citrus rootstocks were affected by salinity as shown by Ruiz *et al*. (1999), Garcia-Sanchez and Syvertsen (2006) and Abadi *et al*. (2010), they concluded that, dry weight of citrus rootstocks decreased under high salinity stress.

Biochemical indicators such as proline, plant pigments and sugars showed variable response to salinity. El-Hammady *et al*. (1996) studied Sour orange, Cleopatra mandarin, Carrizo citrange and Volkamer lemon seedlings for proline and found accumulated proline in their leaves under salt stress. Similar results were observed by Perez-Perez *et al*. (2007) and Ferreira-Silva *et al*. (2008). The plant pigment contents decrease in response to salt stress in several citrus rootstocks (Garcia-Sanchez *et al*., 2006b; Murkute *et al*., 2006 and Balal *et al*., 2011). Garcia-Sanchez *et al*. (2002) reported that, the loss of chlorophyll is due to Na^+ and Cl^- accumulation. Salinity treatments tended to increase soluble sugars (Ferreira-Silva *et al*., 2008 and Balal *et al*., 2011), due to osmotic adjustment to lower down the osmotic potential of plant cells (Ezz and Nawar 1994).

Sour orange (*Citrus aurantium,*L.) has been and still ranks as one of the world's greatest and widely used citrus rootstock. Its popularity mainly due to adequate yields, good fruit quality, tolerance to foot rot, cold and compatibility with the most important citrus species and varieties. Sour orange races showing different morphological and physiological traits, for selecting the most resistance to abiotic stress such as salinity. On the other hand, salinity decreases citrus tree growth and yield du to (1) salinity level (Murkute *et al*., 2006) (2) water deficit (Moya *et al*., 2002), (3) effects on plant metabolism (Al-Yassin,2005) and ion toxicity and nutritional imbalance as accumulation of Na^+,or Cl^-, or both. Large differences among citrus species in their ability to root uptake and transport of Na+ and Cl^- from roots to shoots and leaves have been reported (Raveh and Levy 2005), it was showed that there is little effect of scion on Cl transport in citrus plant, however the effect of root stock is a major importance, (Camara – Zapata *et al*., 2003 and 2004) . Such differences may be of great importance in determining the resistance of a species to saline stress. Therefore, the present study was carried out to evaluate the effect of different salinity levels on vegetative growth, leaf proline , and chlorophyll , soluble and non soluble sugars of Balady, Brazilian and Spanish sour orange rootstock seedlings .

MATERIALS AND METHODS

The present study was carried out during 2010/2011 and 2011/2012 growing seasons in the greenhouse of a private nursery at Samanoud city, Al-Gharbiya governorate in order to study the effect of different salinity levels on vegetative growth and biochemical parameters of sour orange (*Citrus aurantium,* L.) races namely: Balady, Brazilian and Spanish.

In the first week of March, one year old seedlings were growing in 40 cm wide plastic pots containing clay soil taken from the top 30 cm of the nursery soil. Mechanical and chemical analysis of this soil was done as shown in Table 1.

Table 1. Mechanical and chemical analysis of experimental soil.

Mechanical			Chemical			Available					
Sand (%)	Silt (%)	Clay (%)	pH	Ec Mmhos\cm	o.m (%)	N (%)	P (%)	K (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)
17.4	35.2	46.9	7.8	1.45	1.9	0.51	0.06	220	19.8	3.8	6.2

In both seasons, all seedlings were irrigated twice weekly with tap water from the first of March until late of May. During this period the seedlings produced new growths. Then , they subjected to different salinity treatments. Saline solutions were prepared by mixing NaCl, $CaCl_2$ and $MgCl_2$ salts according to Ibrahem and El-Kobbia (1986) as follow: {3NaCl : 1(3$CaCl_2$+ 1 $MgCl_2$)}, at different concentrations of 1000, 2000, 3000 and 4000 ppm, whereas, tap water was used as control treatment. Salinity treatments started at the first week of July in both seasons on the same seedling, and continued for 12 months in the first season, and 24 months in the second season. Each seedling was irrigated twice weekly with one litter of salt solution. The volume of saline water was increased in the third irrigation time by tap water about 25% as leaching requirements to avoid salt accumulation in soil pots. In addition, 1500 ml of complete Hoagland solution (Hoagland and Arnon, 1950) was add at biweekly intervals until the end of the experiment as a source of the essential nutrients. Thus, the factorial experiment consisted of 5 treatments x 3 rootstocks x 3 replicates (each replicate contained 3 plants, one plant/pot). Total seedlings were 45 for each rootstock. The seedlings were arranged in a randomized complete block design and the least significant differences (LSD at 5% level) was used to compare the main values according to Snedecor and Cochran(1967) .

The following data were recorded:

1. Vegetative growth:

1.1. Stem length (cm), stem diameter (cm).

1.2. Leaf number per seedling and leaf area (cm^2)

Number of leaves per seedling was accounted at the end of October.of each eason (2010/2011) and (2011/2012)

Leaf area was measured by planimeter (licore 3110 area meterb) according to Singh and Snyder (1984)

1.3. Shoots and roots dry weight:

At the end of both seasons in November, seedlings were removed and divided to top (leaves, stem and branches) and roots for dry weight; it was oven dried to constant weight at 60-65C° then, dry weight (g) was weighted.

2.Biochemical parameters:

2.1. Leaf proline content as μ mole/gm fresh weight: leaf proline content was determined in 0.5 gm fresh weight of fully mature leaves according to the method described by Bates ***et al***. (1973).

2.2. Leaf chlorophyll content as μ gm/cm^2: fresh leaf sample was taken from each replicate to determine chlorophyll a, b and its total according to the methods described by Moran and Porath (1980).

2.3. Soluble and non soluble sugars: soluble and non soluble sugars were determined according to the methods described by (AOAC, 1980). Fresh leaves (1 g) were ground in 10 ml of 80% ethanol (v/v). Centrifuged and filtered the extract and supernatant was used for the estimation of sugars. Plant extract (1ml) was taken in 25ml test tubes and 6 ml enthrone reagent was added to each tube. The mixture was heated in boiling water bath for 10 minutes. The test tubes were ice-cooled for 10minutes, and incubated for 20 minutes at room temperature (25^{O}C). Optical density was recorded at 625nm on a spectrophotometer. The concentration of soluble sugars was calculated from the standard curve developed with different concentrations of glucose using the above method.

3. Macro – and micro nutrients

At the end of both seasons(November), seedlings were removed from their pots: roots were washed free of soil with tap water, and separated from the shoots. Fully mature leaves were also separated from shoots. The roots and mature leaves were oven dried to constant weight at 60-65^{O}C and weighted. The dried leaves and roots samples of each replicate were grounded and digested with H2SO4 and H2O2 according method described by Evenhuis and Dewaard (1980). In digested solution samples N, P, K, Ca, Na, Ng, Fe, Mn, Zn, and Cu were determined as follows: nitrogen was determined by micro-Kjeldahl method(AOAC,1980).K by flame photometer, P by spectrophotometer, Ca, Na, Mg, Mn, Fe, Zn, and Cu were assayed with atomic absorption spectrophotometer (Unican SP 1900) according to method described by Chapman and Pratt (1961) . Cl was determined in leaves and roots dry matter by silver nitrate method due to method described by Brown and Jackso,1955.

RESULTS AND DISCUSSION

1. Vegetative growth:

1.1. Stem characters:

Data in Table 2 showed that irrigating Balady, Brazilian and Spanish seedlings with saline water at different concentrations reduced length and diameter of the stem in both seasons. This reduction was significantly among all treatments in both seasons. These results were

Table 2: Effect of different salinity levels on stem length and diameter of Balady, Brazilian and Spanish sour orange rootstocks.

Season	Salinity Levels ppm	Stem length (cm)				Stem diameter (cm)			
		Balady	Brazilian	Spanish	mean	Balady	Brazilian	Spanish	mean
2010/ 2011	Control	83.7	93.8	74.1	83.8	1.20	1.34	1.07	1.20
	1000	77.9	88.3	66.5	77.57	1.12	1.26	0.95	1.11
	2000	72.8	83.3	65.7	73.9	1.04	1.19	0.94	1.05
	3000	71.6	79.4	61.2	70.7	1.02	1.13	0.87	1.00
	4000	64.0	71.3	60.4	65.2	0.91	1.02	0.86	0.93
	Mean	74.0	83.2	65.6	----	1.05	1.18	0.93	-----
	L.S.D. 5%	Salinity	Rootstock	interaction		Salinity	Rootstock	interaction	
		0.74	0.67	1.40		0.02	0.02	0.05	
2011/ 2012	Control	103.4	114.9	85.6	101.3	1.49	1.66	1.24	1.46
	1000	96.9	107.8	75.6	93.4	1.40	1.56	1.09	1.35
	2000	88.0	99.6	71.8	86.4	1.27	1.44	1.04	1.25
	3000	82.3	92.4	63.2	79.5	1.19	1.33	0.91	1.14
	4000	66.5	74.3	61.0	67.2	0.96	1.07	0.88	0.97
	Mean	87.4	97.8	71.4	----	1.26	1.41	1.03	-----
	L.S.D. 5%	Salinity	Rootstock	interaction		Salinity	Rootstock	interaction	
		0.74	0.66	1.48		0.02	0.02	0.04	

Similar to those obtained by Anjum *et al*. (2001) on six citrus rootstocks and Balal *et al*. (2011) on ten citrus rootstocks.

Brazilian seedlings recorded the highest stem length and diameter values in both seasons of irrigation with salinized water followed in a decreasing order by Balady and Spanish seedlings in both seasons. Differences in stem length and diameter among the three citrus rootstock seedlings were significant in both seasons. These results are in line with the findings of Murkute *et al*. (2006) and Boman (1993).

Data also indicated a significant interaction between rootstock and salinity levels, where Brazilian seedlings irrigated with tap water showed the greatest stem length and diameter, whereas Spanish seedlings treated with 4000 ppm level had the lowest stem length and diameter (Table 2). These results are in harmony with those of Anjum *et al* (2000) who reported that, stem length was depressed by increasing the salt concentration in irrigation water. Such reduction in stem length and diameter means depression in plant growth. This depression could be attributed to inhibited cell division and cell elongation under condition of increasing salinity at high salt concentration (4000ppm). This explanation is in agreement with that reported by Moya *et al* (2002) and Garcia-Sanchez *et al* (2006a).

1.2. Leaf characters:

Data in Table 3 revealed that applying saline water for irrigating Balady, Brazilian and Spanish seedlings significantly decreased leaf number per plant and leaf area (cm^2) in both seasons. The obtained results dealing with leaf characters are in line with those reported by Ruiz *et al*. (1999), Murkute *et al*. (2006) and Balal *et al*. (2011) they revealed that, salinity had a great deleterious effect on leaves number per plant.

Also, data presented in Table 3 showed that leaf number per plant and leaf area of Brazilian seedlings had higher value than those of Balady and Spanish seedlings. The differences were only significant between Brazilian and other tow rootstocks in both seasons. Similar observation recorded by Anjum *et al*. (2001) and Garcia-Sanchez *et al*. (2002) stated that, applying saline water for irrigation inhibited vegetative growth of ten citrus rootstocks such as number of leaves and leaf area.

Table 3: Effect of different salinity levels on leaf number per plant and leaf area (cm^2) of Balady, Brazilian and Spanish sour orange rootstocks.

Season	Salinity Levels ppm	Leaf number per plant				Leaf area (cm2)			
		Balady	Brazilian	Spanish	mean	Balady	Brazilian	Spanish	Mean
2010/ 2011	Control	76.41	78.58	70.19	75.39	15.40	14.70	14.50	14.86
	1000	72.96	70.19	61.90	68.35	14.90	14.50	13.70	14.36
	2000	57.06	62.59	53.61	57.75	12.60	13.40	12.10	12.70
	3000	38.41	52.23	43.93	44.85	10.00	11.90	10.70	10.86
	4000	39.10	44.63	40.48	41.40	9.90	10.80	10.10	10.26
	Mean	56.78	60.34	54.02	----	12.56	13.06	12.14	----
	L.S.D. 5%	**Salinity**	**Rootstock**	**interaction**		**Salinity**	**Rootstock**	**Interaction**	
		1.79	**1.60**	**3.58**		**0.61**	**0.55**	**1.23**	
2011/ 2012	Control	92.27	97.80	88.12	92.73	14.80	15.60	14.20	14.86
	1000	82.59	90.19	83.28	85.35	13.40	14.50	13.50	13.80
	2000	72.23	86.05	69.46	75.91	11.90	13.90	11.50	12.43
	3000	69.46	68.77	68.08	68.77	11.50	13.40	11.30	12.06
	4000	66.70	61.90	66.01	64.87	11.10	11.30	11.00	11.13
	Mean	76.65	80.94	74.99	----	12.54	13.74	12.30	----
	L.S.D. 5%	Salinity	Rootstock	interaction		Salinity	Rootstock	interaction	
		2.70	2.41	5.40		0.62	0.55	1.24	

Regarding, the interaction between salinity levels and rootstocks, leaf number per plant and leaf area were significantly decreased in both seasons. Moreover, the negative effect of salinity was more pronounced at high salt concentration of 4000 ppm, followed by 3000, 2000 and 1000 ppm, respectively. This result was true in the three citrus rootstocks in both seasons. The obtained results are in line with the findings of Anjum *et al*. (2000) and Moya *et al*. (2002),they recorded that salinized of irrigation water caused a conspicuous reduction in leaf number and leaf area of citrus rootstocks. In this respect, Paranychianakis and Chartzoulakis (2005) found that, the reduction in leaf area was due to a reduction in cell size. This reduction in leaf growth could be resulted from increasing osmotic pressure of medium (Lea-Cox and Syvertsen 1993), depressing water absorption (Chatzissavvidis *et al.,* 2008), or excess of certain ions, which seem to have specific impact, especially Na and Cl (Ben-Hayyim *et al.*,1985; Zekri *et al*., 1992).

1.3. Plant dry weight (g):

It is clear from Tables 4 that, dry weight of top and roots was gradually reduced with increasing salinity concentration. The highest values of dry weight were recorded in the control seedlings, while the least values of dry weight were resulted from high salinity treatment of 4000 ppm in both seasons. The differences were significant between low concentrations (control, 1000 and 2000 ppm) and high concentrations (3000 and 4000 ppm) in both seasons. These results are in agreement with those published by Zekri (1993), who stated that dry weight for shoots and roots of eight citrus rootstock seedlings decreased with the increase of salt concentration in irrigation water.

In addition, Brazilian seedlings produced more dry weight of top and roots followed by Balady and Spanish seedlings. The differences were significant among them only in the first season, in the second season the difference were found between Spanish and other rootstocks. These results are similar to those obtained by Fernandez-Ballester *et al*. (2003),they revealed that dry weight of sour orange and *Citrus Macrophylla* rootstocks irrigated with saline water was decreased as the salinity level in the irrigation water increased.

As for interaction between salinity and rootstock, it is clear that dry weight of three rootstock seedlings were significantly decreased by all salinity treatments in both seasons. Moreover, top and roots dry weight were always lower in all salinity treatments than the control one, during the two seasons. Similar results were obtained by Ezz and Nawar (1993), they showed that, irrigated sour orange seedlings with saline water tended to decrease top and roots dry weight by increasing salinity level in irrigation water. Such reduction in plant dry weight could be attributed to the reduction of photosynthetic products, which in turn were influenced by low available water needed for tissue development. This explanation finds support in the findings of Downton (1977) and Behboudian *et al*. (1986).

From the result presented in Tables 2, 3 and 4, it is obvious that all growth parameters of Balady, Brazilian and Spanish seedlings measured were significantly affected by salinity treatments. In this respect, the higher levels of salinity (3000 and 4000) were more effective than the lower levels (1000 and 2000). Also, Spanish sour orange seedlings were the most affected rootstock by salinity treatments. On the other hand, Brazilian sour orange seedlings gave the highest values of all growth parameters such as stem length and diameter, leaf number per plant and leaf area followed by Balady sour orange seedlings in both seasons.

Finally, it could be concluded from the above discussed results that, Brazilian sour orange seedlings were more tolerant to salinity followed by Balady sour orange and Spanish seedlings.

Table 4: Effect of different salinity levels on top (leaves, shoots and stem) and roots dry weight (g) of Balady, Brazilian and Spanish sour orange rootstocks.

season	Salinity Levels ppm	Top dry weight (g)				Root dry weight (g)			
		Balady	Brazilian	Spanish	mean	Balady	Brazilian	Spanish	Mean
2010/ 2011	Control	35.6	36.5	22.7	31.6	21.7	22.6	13.8	19.3
	1000	28.5	33.8	20.8	27.7	17.3	18.2	12.7	16.0
	2000	21.7	22.3	17.9	20.6	13.1	13.8	10.9	12.6
	3000	16.6	15.1	13.7	15.1	10.0	13.2	8.3	10.5
	4000	12.5	15.1	14.7	14.1	7.5	12.6	8.9	9.6
	Mean	22.9	24.5	17.9	----	13.9	16.0	10.9	---
	L.S.D. 5%	Salinity	Rootstock	interaction		Salinity	Rootstock	Interaction	
		2.10	1.90	4.30		0.89	0.80	1.70	
2011/ 2012	Control	36.1	42.8	30.8	36.5	31.4	27.4	22.6	27.1
	1000	36.5	35.7	24.9	32.3	26.2	26.7	18.2	23.7
	2000	26.6	32.7	18.9	26.0	23.9	19.2	13.8	18.9
	3000	21.5	18.9	18.7	19.7	13.8	18.8	13.2	15.2
	4000	17.7	15.4	17.2	16.7	11.2	13.0	12.6	12.2
	Mean	27.6	29.1	22.1	----	21.3	20.4	16.0	----
	L.S.D. 5%	Salinity	Rootstock	interaction		Salinity	Rootstock	interaction	
		2.50	2.20	5.10		1.70	1.50	3.50	

2. Biochemical parameters:

2.1. Leaf chlorophyll content:

Data in Tables 5 and 6 showed that, amount of chlorophyll a, b and its total value per µg/cm^2 leaf area was decreased with increasing salinity in both seasons. The amount of chlorophyll a, b and its total value were significantly decreased as salt concentration increased especially at high level (3000 and 4000 ppm) when compared with control and low level in both seasons. Similar results were reported by Morinaga and Sykes (2001), who reported that leaf chlorophyll content was negatively affect with increasing salinity of irrigation water.

As for sour orange rootstocks, it is clear from Tables 5 and 6 that leaves of Balady seedlings recorded the highest values of chlorophyll a, b and its total content in the first season, in the second season Brazilian came the first in this respect. However, leaves from Spanish seedlings had the lowest values of chlorophyll a, b and its total content as compared with other tested rootstocks. Differences were significant among all tested rootstocks except chlorophyll b in both seasons. Similar results were obtained by El-Hammady *et al*.(1996), Murkute *et al*. (2006) and Balal *et al*. (2011), they reported that, increasing salt concentration in irrigation water decreased leaf chlorophyll content of several citrus rootstocks.

Regarding the interaction between rootstock and salinity, data in Tables 5 and 6 indicated that leaves of control seedlings recorded the highest values of chlorophyll a, b and its total value as compared with all salinity treatments with significant differences between control and other treatments in both seasons. On the other hand, the treatment of 4000 ppm recorded the least values of chlorophyll. Similar results are reported by Ennab (2003) indicated that chlorophyll a, b and its total value decreased with increasing salinity levels in irrigation water. The deficiency in chlorophyll content could be explained by a suppression of nutrient absorption (N, K, Ca, Fe and Mn) due to uptake Na^+ and Cl^- in competition with nutrient ions (Garcia-Sanchez *et al*., 2002). Also, plastid breakdown could be responsible for chlorophyll deficiency under salinity conditions (Puritch and Barker, 1967 and Garcia-Sanchez *et al*, 2006b).

Table 5: Effect of different salinity levels on leaf chlorophyll a and b ($\mu g/cm^2$) of Balady, Brazilian and spanish sour orange rootstocks.

Season	Salinity Levels ppm	Leaf chlorophyll a $\mu g/cm^2$				Leaf chlorophyll b µg/cm2			
		Balady	Brazilian	Spanish	mean	Balady	Brazilian	Spanish	Mean
2010/ 2011	Control	120.5	111.0	121.7	117.7	41.1	37.8	41.5	40.1
	1000	105.0	98.9	105.5	103.1	35.8	33.7	35.9	35.1
	2000	94.0	86.5	94.4	91.6	32.1	29.5	32.2	31.2
	3000	86.8	79.9	84.6	83.7	29.6	27.3	28.9	28.6
	4000	86.0	78.6	82.6	82.4	29.3	26.8	28.2	28.1
	Mean	98.4	90.9	97.7	---	33.5	31.0	33.3	----
	L.S.D. 5%	**Salinity**	**Rootstock**	**Interaction**		**Salinity**	**Rootstock**	**interaction**	
		5.30	**4.70**	**10.60**		**3.40**	**NS**	**6.80**	
2011/ 2012	Control	107.3	104.1	106.4	105.9	59.6	57.8	59.1	58.8
	1000	93.4	95.7	93.3	94.1	51.8	53.1	51.8	52.2
	2000	85.7	86.9	83.4	85.3	47.5	48.2	46.3	47.3
	3000	79.6	81.3	75.3	78.7	44.2	45.1	41.8	43.7
	4000	77.0	79.9	73.8	76.9	42.7	44.4	41.0	42.7
	Mean	88.6	89.5	86.4	----	49.1	49.7	48.0	----
	L.S.D. 5%	**Salinity**	**Rootstock**	**interaction**		**Salinity**	**Rootstock**	**interaction**	
		5.80	**5.20**	**11.60**		**3.70**	**NS**	**7.40**	

NS= non significant

2.2. Leaf proline content:

Data presented in Table 6 indicated that citrus rootstock seedlings irrigated with tap water had lower leaf proline content than those irrigated with different concentrations saline water. Moreover, leaf proline content was significantly increased with increasing salinity level in irrigation water in both seasons. These results are in line with those obtained by Ferreira-Silva *et al*.(2008), who concluded that leaf proline content tended to increase as salinity levels increased.

Table 6: Effect of different salinity levels on leaf total chlorophyll ($\mu g/cm^2$) and proline content (µmole/gm fresh weight) of Balady, Brazilian and Spanish sour orange rootstocks.

Season	Salinity Levels ppm	Leaf total chlorophyll µg/cm2				Leaf proline µmole/gm fresh weight			
		Balady	Brazilian	Spanish	mean	Balady	Brazilian	Spanish	mean
2010/ 2011	Control	161.6	148.8	163.2	157.8	0.43	0.46	0.40	0.43
	1000	140.8	132.6	141.4	138.2	0.59	0.62	0.63	0.61
	2000	126.1	116.0	126.6	122.9	0.69	0.73	0.71	0.71
	3000	116.4	107. 2	113.5	112.3	0.81	0.91	0.82	0.84
	4000	115.3	105.4	110.8	110.5	0.85	0.93	0.83	0.87
	Mean	132.0	122.0	131.1	---	0.67	0.73	0.67	----
	L.S.D. 5%	Salinity	Rootstock	Interaction		Salinity	Rootstock	Interaction	
		5.90	5.30	11.80		0.03	0.02	0.06	
2011/ 2012	Control	166.9	161.9	165.5	164.7	0.36	0.43	0.35	0.38
	1000	145.2	148.8	145.1	146.3	0.53	0.61	0.51	0.56
	2000	133.2	135.1	129.7	132.6	0.68	0.73	0.67	0.69
	3000	123.8	126.4	117.1	122.4	0.75	0.89	0.68	0.77
	4000	119.7	124.3	114.8	119.6	0.77	0.92	0.83	0.84
	Mean	137.7	139.3	134.4	----	0.62	0.71	0.62	----
	L.S.D. 5%	Salinity	Rootstock	Interaction		Salinity	Rootstock	Interaction	
		3.60	3.30	7.30		0.04	0.04	0.09	

Also data in Table 6 showed that Brazilian seedlings had high values of leaf proline content, whereas Balady and Spanish sour orange had similar value of leaf proline content. These results are in agreement with Abadi *et al*. (2010) showed that proline amino acid concentration in the leaves of different citrus rootstocks tended to increase drastically with increasing salinity. Concerning the interaction of rootstock and salinity, it is clear in both season that leaf proline content was significantly increased as salt concentration increased especially at 3000 and 4000 ppm. Similar results were reported by Perez-Perez *et al*. (2007) and Balal *et al*. (2011). The increase in proline may be due to the increase in hydrolysis of proteins under high salinity conditions. Murkute *et al*. (2005) found that the adverse effects of salinity caused an accumulation of free amino acid especially proline.

2.3. Soluble and non soluble sugars:

It is clear from Table 7 that, the value of soluble and non soluble sugars of the three sour orange rootstock seedlings were significantly increased by increasing salinity levels in irrigation water in both seasons. Similar results are obtained by Perez-Perez *et al*. (2007). Such increase in soluble and non soluble sugars could be attributed to the ability to make osmotic adjustment to maintain favorable water balance in sour orange seedlings (Ezz and Nawar 1993). The comparison among the citrus rootstocks for soluble and non soluble sugars, Brazilian seedling showed high sugar accumulation under salt stress, while Balady and Spanish seedlings indicated similar values under this conditions. Similar results were reported by Balal *et al*. (2011) showed that salt stress caused an increase in total soluble sugars in ten citrus rootstocks. Increased concentration of soluble and non soluble sugars in response to salinity could be attributed as osmotic adjustment to down the osmotic potential of plant cells. These findings are in accordance with those reported by Ferreira-Silva *et al*. (2008), they reported under salinity that the tolerant rootstock showed greater ability to accumulate compatible organic solutes such soluble sugars.

Table7: Effect of different salinity levels on leaf soluble and insoluble carbohydrate (mg/g fresh weight) of Balady, Brazilian and Spanish sour orange rootstocks.

season	Salinity Levels ppm	Soluble mg/g fresh weight				Insoluble mg/g fresh weight			
		Balady	Brazilian	Spanish	mean	Balady	Brazilian	Spanish	mean
2010/ 2011	Control	21	25	24	23	64	67	60	63
	1000	26	28	31	28	74	79	72	75
	2000	36	38	33	35	85	89	85	86
	3000	42	45	42	43	92	95	91	92
	4000	53	58	50	53	99	101	94	98
	Mean	35	38	36	----	82	86	80	----
	L.S.D. 5%	Salinity	Rootstock	Interaction		Salinity	Rootstock	Interaction	
		3.13	2.80	3.61		1.48	1.32	1.71	
2011/ 2012	Control	20	28	19	22	62	66	62	63
	1000	33	35	30	32	77	77	75	76
	2000	40	40	35	38	86	90	85	87
	3000	44	53	41	46	93	97	92	94
	4000	52	58	52	54	98	99	97	98
	Mean	37	42	35	-----	83	85	82	----
	L.S.D. 5%	Salinity	Rootstock	Interaction		Salinity	Rootstock	Interaction	
		3.92	3.50	4.52		1.78	1.59	2.05	

From results presented in Tables 5, 6 and 7, it is clear that chlorophyll a, b and its total value were decreased as salinity increased in irrigation water. In contrary free proline amino acid, soluble and non soluble sugars were increased with increasing saline water. Moreover, these biochemical parameters were also differed according to different rootstocks. Brazilian rootstock had higher amount of chlorophyll, proline and soluble and non soluble sugars than those recorded on Balady and Spanish rootstocks. These results confirm the pervious findings dealing with different vegetative growth and dry matter of citrus rootstocks under this study, which in general indicated that Brazilian was more tolerant to salt stress when compared with Balady and Spanish seedlings.

3. Leaf macro – and micro nutrients:

Data in table 8 – 9 showed that leaf N- Ca – Mg – Na- Cl – contents were significantly increased with the increase of salt concentration in irrigation water .On the other hand , K- P–Fe–Zn– Mn–Cu content were significantly decreased with increasing salinity levels in irrigation water in both season .Also, data showed that Brazilian Sour orange seedling had lower Na and Cl than that on Balady and Spanish sour orange seedlings. These results are in line with those reported by (Garcia – Sanchez *et al*. (2002, 2003, 2006b) and Chatzissavvidis *et al*. (2008) and Lea-Cox and Syvertsen (1992).

Table 8: Effect of different salinity levels on leaf mineral content of Balady, Brazilian and Spanish sour orange rootstocks during 2010/2011.

rootstock	salinity levels, ppm	N %	P%	K %	Ca %	Mg %	Na %	Cl %	Fe ppm	Mn ppm	Zn ppm	Cu ppm
Balady	Control	2.30	0.19	1.49	1.68	0.33	0.39	0.49	141	51	49	21
	1000	2.38	0.16	1.19	1.88	0.35	0.43	0.70	147	48	47	19
	2000	2.54	0.17	0.95	2.32	0.40	0.70	0.75	151	33	45	16
	3000	2.60	0.14	0.91	2.88	0.43	0.81	0.79	155	32	44	13
	4000	2.65	0.13	0.88	2.90	0.45	0.85	0.88	153	30	41	10
Brazilian	Control	2.29	0.28	1.70	2.02	0.35	0.41	0.61	125	43	50	22
	1000	2.39	0.28	1.54	2.32	0.37	0.50	0.64	127	42	47	19
	2000	2.53	0.25	1.46	2.52	0.42	0.63	0.75	136	41	46	15
	3000	2.60	0.22	1.38	2.67	0.46	0.67	0.79	141	40	43	13
	4000	2.69	0.22	1.32	2.82	0.47	0.71	0.85	185	38	42	11
Spanish	Control	2.27	0.29	1.57	1.62	0.32	0.44	0.53	119	45	60	21
	1000	2.37	0.28	1.49	1.98	0.35	0.53	0.71	128	43	60	17
	2000	2.48	0.27	1.40	2.28	0.39	0.68	0.79	139	42	56	14
	3000	2.49	0.27	1.31	2.51	0.39	0.74	0.89	149	41	51	12
	4000	2.52	0.27	1.22	2.62	0.41	0.75	0.99	153	40	49	10
L.S.D. at 5%		NS	0.08	0.09	0.72	0.08	0.10	0.13	10.8	7.1	6.2	4.5
Average salinity	Control	2.28	0.25	1.58	1.77	0.33	0.41	0.54	128	46	46	21
	1000	2.38	0.24	1.40	2.06	0.35	0.48	0.68	134	44	44	18
	2000	2.51	0.23	1.27	2.37	0.40	0.67	0.76	142	38	38	15
	3000	2.56	0.21	1.20	2.68	0.42	0.74	0.82	148	37	37	12
	4000	2.62	0.20	1.14	2.78	0.44	0.77	0.90	167	36	36	10
L.S.D. at 5%		NS	0.04	0.04	0.36	0.04	0.05	0.06	5.4	3.5	3.1	2.2
Average rootstocks	Balady	2.49	0.15	1.08	2.33	0.39	0.63	0.72	151	39	45	15
	Brazilian	2.50	0.25	1.48	2.47	0.41	0.58	0.72	135	41	46	16
	Spanish	2.42	0.27	1.39	2.20	0.37	0.62	0.78	138	42	55	14
L.S.D. at 5%		NS	0.03	0.04	0.32	0.03	0.04	0.05	4.8	3.2	2.7	NS

NS = non significant

Table 9: Effect of different salinity levels on leaf mineral content of Balady, Brazilian and Spanish sour orange rootstocks during 2011/2012.

Rootstock	salinity levels ppm	N %	P %	K %	Ca %	Mg %	Na %	Cl %	Fe ppm	Mn ppm	Zn ppm	Cu ppm
Balady	Control	2.32	0.19	1.42	1.63	0.43	0.54	0.63	111	52	56	21
	1000	2.40	0.20	1.39	1.75	0.45	0.56	0.76	111	35	50	20
	2000	2.60	0.20	1.29	2.67	0.45	0.64	1.16	121	34	50	18
	3000	2.69	0.14	1.14	2.82	0.46	0.81	1.43	137	33	47	13
	4000	2.70	0.14	1.02	2.93	0.48	0.90	1.55	141	33	45	11
Brazilian	Control	2.33	0.20	1.66	2.42	0.40	0.33	0.67	112	45	67	22
	1000	2.45	0.20	1.53	2.48	0.44	0.41	0.76	116	44	64	20
	2000	2.67	0.18	1.31	2.57	0.44	0.54	0.95	121	41	62	16
	3000	2.73	0.15	1.18	2.63	0.47	0.53	1.22	133	40	61	14
	4000	2.78	0.14	1.09	2.63	0.54	0.59	1.32	137	39	59	11
Spanish	Control	3.31	0.22	1.46	1.54	0.46	0.54	0.71	96	42	70	20
	1000	2.44	0.20	1.40	2.52	0.48	0.63	0.90	98	42	69	19
	2000	2.59	0.18	1.28	2.55	0.52	0.67	1.18	100	39	69	17
	3000	2.61	0.17	1.22	2.67	0.53	0.79	1.61	102	38	67	15
	4000	2.68	0.15	1.15	2.71	0.53	0.92	1.68	112	35	65	10
L.S.D. at 5%		0.16	NS	0.14	0.16	0.08	0.13	0.14	7.3	6.7	8.8	4.2
Average salinity	Control	2.32	0.20	1.51	1.86	0.43	0.47	0.67	106	46	64	21
	1000	2.43	0.20	1.44	2.25	0.46	0.53	0.80	108	40	61	19
	2000	2.62	0.18	1.29	2.59	0.47	0.61	1.09	114	38	60	17
	3000	2.67	0.15	1.18	2.70	0.48	0.71	1.42	124	37	58	14
	4000	2.72	0.14	1.08	2.75	0.51	0.80	1.51	130	35	56	10
L.S.D. at 5%		0.8	NS	0.07	0.08	0.04	0.06	0.07	3.6	3.3	4.4	2.4
Average rootstocks	Balady	2.54	0.17	1.25	2.36	0.45	0.69	1.10	124	37	50	16
	Brazilian	2.59	0.17	1.35	2.54	0.45	0.48	0.98	124	42	63	16
	Spanish	2.52	0.18	1.30	2.39	0.50	0.71	1.21	102	39	68	16
L.S.D. at 5%		0.07	NS	0.06	0.07	0.03	0.05	0.06	3.2	3.0	3.9	NS

NS = non significant

3. 1 – Root macro – and micro - nutrients:

Data in table 10 and 11 revealed that increasing salinity level in the irrigation water caused significantly decrease in root N – P – K – Mn – Zn and Cu contents of the three studied Sour orange root stocks as compared with control in both seasons, these results are in line with those found by Garcia-sanchez *et al*. (2002) . On the other hand , root Ca–Mg–Na–Cl–Fe– contents were significantly increased with increasing salinity level in both seasons , these results agree with those obtained by Banuls *et al*. (1991),and Zekri and Parsons (1992) , and Garcia – Sanchez *et al*. (2006b) and Ferreire-Silva *et al*.(2008).

The results showed that all nutrients in roots are affected by salt stress. Brazilian seedlings tended to contain the lowest Na and Cl concentrations as compared with those of both Spanish and Balady seedlings . However, the accumulation of Na and Cl in roots may explain why Brazilian is the most tolerant rootstock to salinity.

Finally, it could be concluded that the three sour orange races used in this study were arranged according to their relative tolerance to irrigation water containing mixture of NaCl ,$CaCl_2$ and $MgCl_2$ up to 4000 ppm in the following descending order; Barzilian > Balady> Spanish .

Table 10: Effect of different salinity levels on root mineral content of Balady, Brazilian and Spanish sour orange rootstocks during 2010/2011.

rootstock	salinity levels (ppm)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)	Cl (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
Balady	Control	1.31	0.17	0.57	0.97	0.25	0.34	0.47	183	49	52	13
	1000	1.10	0.14	0.50	0.94	0.26	0.48	0.51	195	47	50	13
	2000	0.99	0.14	0.42	0.99	0.26	0.55	0.54	204	41	48	13
	3000	0.91	0.13	0.34	1.06	0.35	0.63	0.63	217	40	45	12
	4000	0.89	0.11	0.31	1.16	0.35	0.65	0.69	222	38	42	11
Brazilian	Control	1.33	0.24	0.66	0.97	0.26	0.30	0.41	213	52	63	15
	1000	1.14	0.22	0.62	0.94	0.28	0.45	0.48	228	50	59	13
	2000	1.05	0.21	0.55	0.99	0.32	0.53	0.50	240	48	56	12
	3000	0.97	0.20	0.50	1.06	0.37	0.63	0.59	260	46	53	12
	4000	0.93	0.18	0.43	1.16	0.37	0.65	0.63	265	43	50	12
Spanish	Control	1.23	0.18	0.54	0.85	0.21	0.42	0.50	194	55	50	13
	1000	1.02	0.17	0.51	0.86	0.23	0.52	0.52	198	52	48	12
	2000	0.95	0.16	0.43	1.01	0.26	0.61	0.57	212	50	45	11
	3000	0.92	0.16	0.36	1.07	0.31	0.65	0.62	219	49	43	11
	4000	0.90	0.15	0.35	1.17	0.31	0.66	0.70	235	41	40	10
L.S.D. at 5%		0.08	0.05	0.14	0.14	NS	0.10	0.08	9.3	9.5	5.6	Ns
Average salinity	Control	1.25	0.19	0.59	0.97	0.24	0.35	0.46	196	52	55	13
	1000	1.08	0.17	0.54	1.06	0.25	0.48	0.50	207	49	52	12
	2000	0.99	0.17	0.46	1.27	0.28	0.56	0.53	218	46	49	12
	3000	0.93	0.16	0.40	1.41	0.34	0.63	0.61	232	45	47	11
	4000	0.90	0.14	0.36	1.52	0.34	0.65	0.67	240	40	44	11
L.S.D. at 5%		0.04	0.02	0.07	0.07	NS	0.05	0.04	4.6	4.7	2.8	Ns
Average rootstocks	Balady	1.02	0.13	0.42	1.02	0.29	0.53	0.56	204	43	56	12
	Brazilian	1.08	0.21	0.55	1.02	0.32	0.51	0.52	241	48	56	12
	Spanish	1.00	0.16	0.43	0.99	0.26	0.57	0.58	212	49	45	11
L.S.D. at 5%		0.04	0.02	0.06	0.06	NS	0.04	0.03	4.1	4.2	2.5	Ns

NS = non significant

Table11: Effect of different salinity levels on root mineral content of Balady, Brazilian and Spanish sour orange rootstocks during 2011/2012.

rootstock	salinity levels (ppm)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)	Cl (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
Balady	Control	1.51	0.18	0.68	0.84	0.29	0.45	0.48	231	43	56	14
	1000	1.44	0.17	0.65	0.87	0.30	0.49	0.77	235	41	53	14
	2000	1.41	0.15	0.51	0.89	0.31	0.53	0.82	241	40	49	13
	3000	1.30	0.13	0.36	0.97	0.31	0.62	0.87	255	39	43	12
	4000	1.22	0.12	0.35	1.11	0.32	0.62	0.99	263	34	40	11
Brazilian	Control	1.66	0.19	0.85	1.55	0.30	0.41	0.45	232	35	61	13
	1000	1.59	0.16	0.70	1.85	0.30	0.44	0.49	241	52	59	12
	2000	1.56	0.16	0.68	2.07	0.31	0.53	0.66	248	51	55	12
	3000	1.53	0.15	0.52	2.22	0.31	0.58	0.74	265	44	52	11
	4000	1.42	0.13	0.44	2.33	0.31	0.60	0.89	269	40	50	10
Spanish	Control	1.42	0.19	0.75	1.24	0.24	0.47	0.51	197	45	54	15
	1000	1.39	0.17	0.71	1.70	0.28	0.54	0.73	211	44	52	14
	2000	1.36	0.17	0.62	2.05	0.28	0.59	0.90	232	41	48	13
	3000	1.28	0.14	0.50	2.51	0.31	0.65	1.03	246	40	45	11
	4000	1.15	0.14	0.45	2.59	0.31	0.70	1.15	253	39	42	11
L.S.D. at 5%		0.12	0.06	0.07	0.12	NS	0.15	0.12	11.8	6.4	8.4	NS
Average salinity	Control	1.53	0.18	0.76	1.21	0.27	0.44	0.48	220	41	57	14
	1000	1.47	0.16	0.68	1.47	0.29	0.49	0.66	229	45	54	13
	2000	1.44	0.16	0.60	1.82	0.30	0.55	0.79	240	44	50	12
	3000	1.37	0.14	0.46	1.90	0.31	0.61	0.88	255	41	46	11
	4000	1.26	0.13	0.41	2.01	0.31	0.64	1.01	261	37	44	10
L.S.D. at 5%		0.06	0.03	0.03	0.06	NS	0.07	0.06	5.9	3.2	4.2	NS
Average rootstocks	Balady	1.37	0.15	0.50	0.93	0.30	0.54	0.78	245	40	48	12
	Brazilian	1.55	0.15	0.63	2.00	0.30	0.51	0.64	251	48	55	11
	Spanish	1.32	0.16	0.60	2.01	0.28	0.59	0.86	228	42	48	12
L.S.D. at 5%		0.05	NS	0.03	0.05	NS	0.06	0.05	5.2	2.8	3.7	NS

NS = non significant

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تأثير ملوحة ماء الري على بعض سلالات النارنج .

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قسم الموالح - محطة بحوث البساتين بسخا – كفر الشيخ

أجريت هذه الدراسة على شتلات ثلاث سلالات بذرية من أصل النارنج وهى البلدي و الاسباني و البرازيلي و ذلك لمعرفة تحملها للري بخمسة تركيزات من مخلوط أملاح كلوريد الصوديوم و كلوريد الكالسيوم و كلوريد الماغنسيوم : والماء العادي (مقارنة) و تركيزات المحاليل الملحيه هى 1000 و 2000 و 3000 و 4000 جزء في المليون و قد أثرت الملوحة بكل مستوياتها و بشكل معنوي على كل قياسات النمو الخضري.وقد أعطت شتلات النارنج البرازيلي أفضل نمو خضري و المتمثل في طول و سمك الساق ومساحة الورقة الواحدة وعدد الأوراق على النبات وكذلك الوزن الجاف لكل من القمة و الجذور يليه النارنج البلدي ثم الاسباني.بينما انخفضت قيم كل من كلوروفيل a وb و الكلوروفيل الكلى و ذلك عند زيادة الملوحة في ماء الري فى حين زاد كل من الحمض الامينى البرولين و السكريات الذائبة و الغير ذائبة عند زيادة الملوحة في ماء الري ، وقد اظهر التحليل الكيماوي لأوراق شتلات النارنج البرازيلي أن محتواها من كلوروفيل a وb و الكلوروفيل الكلى و الحمض الامينى البرولين و السكريات الذائبة و الغير ذائبة أعلى من تلك المقدرة في أوراق النارنج البلدي و الاسباني. من ناحية أخرى اختلف تركيز العناصر المعدنية في ألورقه وذلك باختلاف تركيز الملوحة في ماء الري ،فقد وجد أن تركيز النتروجين والكالسيوم و الماغنيسيوم و الصوديوم و الكلور و الحديد زاد بينما انخفض تركيز الفوسفور و البوتاسيوم و المنجنيز و الزنك و النحاس . عند تقدير نفس العناصر في الجذور فقد وجد أن تركيز الكالسيوم و الماغنيسيوم و والصوديوم و الكلور و الحديد قد زاد بينما انخفض تركيز كل من النتروجين و الفوسفور و البوتاسيوم و المنجنيز و الزنك و النحاس بزيادة مستويات الملوحة في ماء الري .أيضا أظهرت شتلات النارنج البرازيلي اتجاه إلى خفض محتوى ألأوراق و الجذور من الصوديوم والكلور وذلك عند ألمقارنه بالنارنج البلدي والاسباني . مما سبق يمكن ترتيب الثلاث سلالات البذرية من أصل النارنج تنازليا حسب نحملهم للملوحة كما يلي النارنج البرازيلي – البلدي – الاسباني.

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