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Citrus Rootstocks Response to Salinity: Physio-biochemical Parameters Changes

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ABSTRACT
Salinity is amongst the most significant environmental factors responsible for substantial losses in agricultural production worldwide. This is a critical problem especially in citrus since they are one of the most globally important horticultural crops considered salt sensitive. The effects of salinity (0, 25, 50, or 75 mmol NaCl) investigated on visible symptoms of leaf damage, electrolyte leakage, relative chlorophyll contents (Chl) (spot values), relative water content (RWC), proline and chlorophyll fluorescence yields (Fv/Fm) in nine citrus rootstocks including: Sour orange, Bakraii, Cleopatra mandarin, Rangpur lime, Rough lemon, Macrophylla, Swingle citrumelo, Citrange and Trifoliate orange. The lowest visible symptoms of leaf damage were found in Cleopatra mandarin. The highest and the lowest electrolyte leakage levels were observed in the Trifoliate orange and Sour orange, respectively. By increasing the levels of salt Chl, RWC and Fv/Fm decreased. The lowest rate of reduction in Chl observed in Cleopatra mandarin and Sour orange. RWC level was higher in the leaves of Sour orange, Cleopatra mandarin and Bakraii, respectively. The lowest rate of reduction in Fv/Fm observed in Cleopatra mandarin, Sour orange and Rangpur lime. Increasing the salt levels led to significant increase in proline content of leaves in all rootstocks. Based on the results it was found that Sour orange, Cleopatra mandarin were the most tolerant rootstocks to salinity of all nine studied. In addition Trifoliate orange, Citrange and Swingle citrumelo were the most sensitive citrus rootstocks to salt stress followed by the Rough lemon and Macrophylla that showed a low-to-moderate tolerance to salt stress, and Rangpur lime and Bakraii, with a moderate-to-high tolerance to high salinity.

Key words: Citrus, salinity, chlorophyll content, chlorophyll fluorescence

INTRODUCTION
Citrus plants are one of the most important horticultural crops and is relatively salt sensitive (Al-Yassin, 2005) because of the specific toxicity of Cl^- and/or Na^+ and to the osmotic effect caused by the high concentration of salts (Garcia-Sanchez *et al*., 2000). To graft scions onto salt resistance rootstocks is one of the ways of improving the salt tolerance of citrus. Rootstocks are different in their salinity tolerance as judged by the ability to inhibit the accumulation of Cl^- and/or Na^+ in leaves of the scion (Storey and Walker, 1998). Nearly 25 million ha of the Iran's cultivated area are affected by salinity stress (Khayyat *et al*., 2007). Water salinity is a serious problem in arid and semi-arid regions because of the excessive use of water resources (Al-Yassin, 2004). An extra amount of salty water at root zone may often be found in Mediterranean areas during the long

summer season, due to high temperatures and both reduced water availability and the quality of water used for irrigation (Tattini *et al*., 2002). Plant responses to excessive NaCl concentration are complex and include several changes in their morphology, physiology and metabolism (Hilal *et al*., 1998). Current evidence indicates that salt toxicity is the principal cause of leaf senescence (Lutts *et al*., 1995). There is a correspondence between leaf senescence with the reduction of chlorophyll content (Chen *et al*., 1991) and increase of membrane permeability at high NaCl concentrations (Dhindsa *et al*., 1981). Salinity was reported to induce in chlorophyll content and photosystem II (PSII) activities in many plants (Nishihara *et al*., 2003). The use of chlorophyll fluorescence provided ways of analyzing potential photosynthetic performance in field or laboratory conditions (Maxwell and Johnson, 2000). The principles of these techniques depend on the basis that light energy absorbed by chlorophyll molecules at the reaction centers: (1) It can be used to drive photosynthesis through the photosynthetic electron chain, (2) The energy can be dissipated as heat or, (3) The energy can be re-emitted as light which is chlorophyll fluorescence. Chlorophyll fluorescence measurement provides a good way to evaluate the photosynthetic performance in stressed plants (Maxwell and Johnson, 2000; Epitalawage *et al.,* 2003). The reduction in nutrient absorption caused by Na^+ and Cl^- is a well-known competitive process in plant roots. Salinity can influence the nutrient availability to plant by influencing the ion selectivity of membranes (Behboudian *et al*., 1986). In halophytes, there is a positive correlation between proline content and the amount of Cl^- and Na^+ in cell sap (Taylor, 1996). Plants respond to salinity stress by accumulation non-toxic and compatible osmolytes such as proline. Proline can increase in protection of cellular structures against oxidative damage by scavenging free radicals (Ashraf and Foolad, 2007). Salt tolerance in citrus is associated with various mechanisms such as accumulation of Cl^- and /or Na^+ in cell vacuoles (Yeo, 1998), the osmotic adjustment by accumulation of proline and inorganic ions in the cytoplasm (Heuer and Feigin, 1993), an ability to decrease the uptake (Walker and Douglas, 1983) and/or transport of toxic salt ions from roots to shoots (Zekri, 1991). The objective of this experiment was to study the relationship between physio-biochemical parameters changes and salt tolerance of nine citrus rootstocks under saline conditions.

MATERIALS AND METHODS

Plant material and growth conditions: Seeds of nine citrus rootstocks included: Sour orange (*Citrus aurantium*), Bakraii (*Citrus reticulata*×*Citrus limetta*), Cleopatra mandarin (*Citrus reshni*), Rangpur lime (*Citrus limonia*), Rough lemon (*Citrus jambhiri*), Macrophylla (*Citrus macrophylla*), Swingle citrumelo (*Poncirus trifoliata*×*Citrus paradisi*), Citrange (*Poncirus trifoliata*×*Citrus sinensis*) and Trifoliate orange (*Poncirus trifoliata*) were obtained from Citrus Research Station, Ramsar, Iran. Plants were grown in a greenhouse located at the Department of Horticulture, College of Agriculture, Isfahan University of Technology. Seeds were sown in earthen pots containing sterile sand and perlite. When seedlings were two-month old, uniform sized seedlings of nine citrus rootstocks cultivars were selected and transplanted in to 30 cm wide plastic pots containing of fine sand. Prior to the onset of treatment, plants were allowed to acclimation in the greenhouse 3 months at 25/17°C day/night temperature and 60% relative humidity. Plants were watered three times per week with 500 mL (This volume was sufficient to leach from the bottom of all pots to avoid any build up of salts) and fertilized three times per week with a commercial water soluble fertilizer containing macro-and-micro-nutrients (Floral Mixed fertilizer B+Cu+Fe+Mn+Mo+Zn + 20-20-20 N-P-K, Italian product and company CIFO).

Treatments: After the period of acclimation (five-month-old seedlings) four levels sodium chloride (NaCl) including 0, 25, 50 and 75 mM NaCl were used. In order to avoid osmotic shock, sodium chloride was applied in increments of 25 mM each day to give final NaCl treatment concentration of 0, 25, 50 or 75 mM. Plants were watered three times per week. Treatments was maintained for 60 days.

Visible symptoms of leaf damage: Presence of yellowish spots at the leaf tip that lead to progressively severe burning injuries was considered to be a good visible estimate of chloride induced damage to leaves (Lopez-Climent *et al.*, 2008). The NaCl treatment produced irregular necrotic spots in the interveinal regions of the leaf lamina and necrosis along the margins and tips in the leaves (Ruiz *et al.*, 1999). The number of damaged leaves in each treatment was recorded during the experimental period and percentages of leaves showing toxic symptoms in salt stressed plants were measured. At the end of the experiment results were expressed as a mean of all three salt levels in each rootstock.

Relative chlorophyll contents (Chl): At the end of the experiment (after 60 days), RCC (spot values) were recorded using SPAD Chlorophyll Meter (Hansatech Instrument Ltd., King's Lynn, UK). Chlorophyll meter readings were used as relative values for chlorophyll content.

Chlorophyll fluorescence yields (Fv/Fm): After 60 days of applying treatments, Fv/Fm was measured between 8.30 and 14.45 h, using a Plant Efficiency Analyzer (Hansatech Instrument Ltd, King's Lynn, Norfolk, UK). When using a PEA, the undamaged mature leaves are darkened with a lightweight plastic leaf clipped for 30 min before the measurement. During measurement, the PEA sensor unit is held over the clip and the shutter is opened. A simple button-press activates the high intensity of the LED array of the sensor head which provides a maximum light intensity. Maximal PSII photochemical efficiency (Fv/Fm) was calculated automatically. The Fv/Fm ratio was calculated as: Fv/Fm = (Fm-Fo)/Fm. Where Fm and Fo are the maximum and basal fluorescence yields, respectively, of dark-adapted leaves.

Ion leakage: This parameter was included in order to have more information on the membrane stability and thereby on the relative ion content in the apoplastic space. Electrolyte leakage was assessed as described by Lutts *et al.* (1995) using young leaf discs of nine plants for each treatment (three per replicate).

Relative water content (RWC): After 60 days of applying treatments, RWC was measured in fully expanded leaves of two plants per replicate (Ghoulam *et al.*, 2002).

Proline: After 60 days of applying treatments the proline content Proline was extracted from the leaf tissues according to the method described by Bates *et al.* (1973).

Statistical analysis: A factorial experiment through Complete Randomized Design (CRD) with three replications and four levels of salt including 0, 25, 50 and 75 mM NaCl were used. Data were analyzed statistically using Statistical Analysis System (SAS), version 9.1. Differences between means of the treatments were compared with the Least Significant Difference (LSD) range at $p \leq 0.05$ and interaction effects were compared with MSTAT-C programmer.

RESULTS AND DISCUSSION

Leaf symptoms (Mean from all 3 salt levels): Study of means of damaged leaves in three salinity levels (25, 50 and 75) in rootstocks during two months of treatments showed significant differences between rootstocks. Cleopatra plants that were exposed to salinity showed the lowest leaf damage (11.3%). In this case the most sensitive rootstock was Trifoliate orange because it indicated the highest percentage of leaf damage (56%) (Table 1). In this study, seedlings of nine citrus rootstocks, commonly used as rootstocks, were used to study their ability to cope with salt stress. Leaf toxic symptoms are important character in determining the salt tolerance ability of different citrus rootstocks. Data presented herein indicates that Cleopatra mandarin was the most tolerant rootstock to salinity of all nine studied. Studies indicate that salinity produced leaf toxicity symptoms. This is due to accumulation toxicity level of sodium and chloride in leaves (Storey and Walker, 1998). The chloride accumulation in the leaves produced yellowish spots at the leaves tips of citrus (Lopez-Climent *et al.*, 2008). The NaCl treatment produced irregular necrotic spots in the interveinal regions of the leaf lamina and necrosis along the margins and tips in the leaves (Ruiz *et al.*, 1999).

Relative leaf chlorophyll content (Chl): Chl was significantly affected by both salinity and rootstocks. Chl values were significantly decreased with the increasing salinity stress (Table 2). In

Table 1: Percentage of leaves showing toxic symptoms in salt stressed plants (Mean from all 3 salt levels)

Rootstock	Day 60
Sour orange	14.4^{*g**}
Cleopatra	11.3^{h}
Rangpur lime	19.0^{f}
Bakraii	25.7^{e}
Rough lemon	30.5^{d}
Macrophylla	28.9^{d}
Citrumelo	36.0^{c}
Citrange	43.0^{b}
Trifoliate orange	56.0^{a}

*: Mean of 9 plants (3 replication from all 3 salt levels), **: Means followed by the same letters are not significantly different at $p \leq 0.05$ (LSD test)

Table 2: Effects of rootstock and NaCl (0, 25, 50 and 75 mM) on relative chlorophyll content (RCC, spad values) of nine citrus rootstocks after 60 days of applying salinity treatments

	NaCl levels (mmol)				
Rootstock	0	25	50	75	Means
Sour orange	57.7^{d*}	$53^{ef\,(8)**}$	$46^{hi\,(20)}$	$38^{km\,(34)}$	48.7^{a}
Cleopatra	$60.7^{b\text{-}d}$	$57^{de\,(6)}$	$48^{g\text{-}i\,(20.9)}$	$35^{l\text{-}n\,(42)}$	50.2^{a}
Rangpur lime	57.7^{d}	$52^{fg\,(9.9)}$	$45^{i\,(22)}$	$30^{op\,(48)}$	46.2^{b}
Bakraii	59.3^{d}	$50^{f\text{-}h\,(15.7)}$	$38^{km\,(35.9)}$	$27^{pq\,(54.5)}$	43.6^{c}
Rough lemon	65.7^{ab}	$40^{jk\,(39.2)}$	$30^{op\,(54.4)}$	$22^{r\text{-}t\,(66.5)}$	39.4^{d}
Macrophylla	60.0^{cd}	$44^{ij\,(26.7)}$	$34^{mo\,(43)}$	$24^{qs\,(60)}$	40.5^{d}
Citrumelo	64.0^{bc}	$39^{kl(39)}$	$26^{pr\,(59)}$	$18^{tv\,(71.8)}$	36.7^{e}
Citrange	64.3^{bc}	$32^{no\,(50)}$	$24^{qs\,(62.7)}$	$17^{uv\,(73.6)}$	34.3^{f}
Trifoliate orange	69.6^{a}	$30^{op\,(56.9)}$	$20^{su\,(71)}$	$14^{v\,(79.8)}$	33.4^{f}

*: Within each column, means followed by the same letters are not significantly different at $p \leq 0.05$ (LSD test), **: Reduction in comparison to the control

Table 3: Effects of rootstock and NaCl (0, 25, 50 and 75 mM) on chlorophyll fluorescence yields (Fv/Fm) of nine citrus rootstocks after 60 days of applying salinity treatments

	NaCl levels (mmol)				
Rootstock	0	25	50	75	Means
Sour orange	0.79^{a}*	$0.75^{ac\,(4.4)}$**	$0.67^{bf\,(15.6)}$	$0.56^{fh\,(28.8)}$	0.69^{a}
Cleopatra	0.80^{a}	$0.79^{a\,(1.5)}$	$0.73^{ad\,(8.6)}$	$0.61^{eh\,(24.4)}$	0.73^{a}
Rangpur lime	0.78^{ab}	$0.76^{ac\,(2.7)}$	$0.72^{ae\,(7.8)}$	$0.51^{hi\,(34.8)}$	0.69^{a}
Bakraii	0.75^{abc}	$0.74^{ac\,(1.3)}$	$0.65^{cg\,(13.9)}$	$0.38^{jk\,(49.3)}$	0.63^{b}
Rough lemon	0.78^{ab}	$0.73^{ad\,(6.4)}$	$0.59^{fh\,(24.7)}$	$0.35^{jk\,(55.1)}$	0.60^{b}
Macrophylla	0.77^{ab}	$0.74^{ac\,(4)}$	$0.61^{dh\,(20.5)}$	$0.29^{k\,(62.4)}$	0.60^{b}
Citrumelo	0.75^{abc}	$0.74^{ac\,(1.3)}$	$0.53^{gi\,(29)}$	$0.33^{jk\,(55.5)}$	0.58^{bc}
Citrange	0.81^{a}	$0.67^{bf\,(18.1)}$	$0.35^{jk\,(56.8)}$	$0.30^{k\,(63.4)}$	0.53^{c}
Trifoliate orange	0.84^{a}	$0.43^{ij\,(48.6)}$	$0.30^{k\,(64)}$	$0.27^{k\,(67.4)}$	0.46^{d}

*: Within each column, means followed by the same letters are not significantly different at $p \le 0.05$ (LSD test), **: Reduction in comparison to the control

25 mM NaCl, Chl showed a significant decrease compared to the control in all rootstocks except the Cleopatra. In this salinity level, Sour orange (8% reduction) and Cleopatra (6% reduction) showed highest and Citrange (50% reduction) and Trifoliate orange (56.9% reduction) showed the lowest Chl. In 50 mM NaCl, Sour orange (20% reduction), Cleopatra (20.9% reduction) and Rangpur lime (22% reduction) retained higher Chl and Citrange (62.7% reduction) and Trifoliate orange (71% reduction) showed the lowest Chl. In 75 mM NaCl, the highest Chl value was obtained from Sour orange (34% reduction) and Cleopatra (42% reduction) and lowest Chl was obtained from Citrumelo (71.8% reduction), Citrange (73.6% reduction) and Trifoliate orange (79.8% reduction). This result is in agreement with the results obtained by the Garcia-Sanchez *et al.* (2002). Anjum (2008) found that under salt stress, chlorophyll contents, have decreased in Troyer citrange and Cleopatra mandarin and the seedlings of Troyer citrange were more affected than those of Cleopatra mandarin. Mg^{2+} deficiency may be one of the reasons for the chlorosis observed in NaCl treated rootstocks leaves (Tozlu *et al.*, 2000). Sabater and Rodrguez (1978) suggested that, decreases in chlorophyll concentration are a consequence of proteolytic enzymes formation such as chlorophyllase.

Chlorophyll fluorescence yields (Fv/Fm): The Fv/Fm significantly decreased with the increasing salinity stress. Fv/Fm reduction by salinity level 50 mM NaCl, was lowest in Rangpur lime (7.8% reduction), Cleopatra (8.6% reduction), Bakraii (13.9% reduction), Sour orange (15.6% reduction) but it was greater reduced in Citrange (56.8% reduction) and Trifoliate orange (64% reduction). When NaCl concentration increased to 75 mM, the lowest reduction in Fv/Fm was observed in Cleopatra (24.4% reduction), Sour orange (28. 8% reduction) and Rangpur lime (34.8% reduction) (Table 3). In this experiment Fv/Fm were reduced by the salt treatment, which indicates that salinity is effect on efficiency of PSII. Fv/Fm of a good way to evaluate the photosynthetic performance in stressed plants. Fv/Fm is correlated with the efficiency of leaf photosynthesis. The reduction in Fv/Fm suggesting that salt stress induced photo-inhibitory damage caused by the incident photon flux density (Bjorkman and Demmig, 1987). It has been reported that Fv/Fm variation in Cleopatra mandarin and Citrange were markedly reduced by the salt treatment. Rate of this reduction was higher in Citrange than Cleopatra mandarin (Anjum, 2008; Lopez-Climent *et al.*, 2008).

Ion leakage: In 75 mM of NaCl the largest increase in ion leakage was seen in Trifoliate orange (66.2%) and the lowest values of ion leakage occurring in the seedlings of Sour orange (35.9%) and Cleopatra mandarin (37%) (Table 4). In this experiment, salinity leads to increasing ion leakage in leaf that shows damage to membrane in salinity condition. It is clear that under salt stress substituting potassium with sodium in the cell membrane, leads to changes in membrane stability (Cheeseman, 1988). High concentrations of NaCl caused severe membrane damage (Hasegawa *et al.*, 2000). Mansour *et al.* (2002) reported that salinity decreased molar percentage of sterols and phospholipids. Electrolyte leakage measurements provide a good way to evaluate the membrane injury in stressed plants. Maintaining integrity of the cellular membranes under salt stress is successful strategies that enhanced salinity resistance (Stevens *et al.*, 2006).

Relative water content (RWC): RWC was significantly affected by salt stress and rootstocks. Salinity significantly decreased RWC. In 25 and 50 mM of NaCl, Bakraii with 84 and 82.4% respectively and in 75 mM of NaCl, Sour orange with 79% showed the highest of RWC. The lowest RWC was recorded in Trifoliate orange (in 25 mM of NaCl, with 70%, in 50 mM with 61% and in 75 mM with 54.5%). This difference between the rootstocks indicates differences in the figures of the amount of salt tolerance (Table 5). RWC in leaves is known as an alternative measure of plant

Table 4: Effects of rootstock and NaCl (0, 25, 50, 75 mM) on ion leakage (%) in leaves of of nine citrus rootstocks after 60 days of applying salinity treatments

	NaCl levels (mmol)				
Rootstock	0	25	50	75	Means
Sour orange	10.4^{uv}*	12.0^{su}	17.0^{pq}	35.9^{hi}	18.8^{g}
Cleopatra	11.9^{sv}	13.3^{rs}	20.5^{n}	37.0^{h}	20.7^{f}
Rangpur lime	10.3^{v}	16.0^{q}	30.0^{k}	48.0^{e}	26.1^{e}
Bakraii	11.9^{sv}	17.9^{op}	36.4^{hi}	40.7^{g}	26.7^{e}
Rough lemon	10.5^{uv}	19.2^{no}	35.0^{i}	48.5^{e}	28.3^{d}
Macrophylla	12.4^{rt}	17.0^{pq}	32.0^{j}	54.0^{d}	28.9^{d}
Citrumelo	11.0^{tv}	20.1^{n}	39.1^{g}	62.8^{b}	33.2^{c}
Citrange	14.0^{r}	22.3^{m}	42.7^{f}	60.2^{c}	34.8^{b}
Trifoliate orange	11.1^{tv}	24.0^{l}	53.0^{d}	66.2^{a}	38.6^{a}

*: Within each column, means followed by the same letters are not significantly different at $p \leq 0.05$ (LSD test)

Table 5: Effects of rootstock and NaCl (0, 25, 50 and 75 mM) on relative water content (%) (RWC) of nine citrus rootstocks after 60 days of applying salinity treatments

	NaCl levels (mmol)				
Rootstock	0	25	50	75	Means
Sour orange	86.0^{c}*	82.0^{ef}	79.7^{hi}	79.0^{i}	81.7^{a}
Cleopatra	89.4^{a}	81.0^{fh}	79.0^{i}	75.8^{jk}	81.3^{a}
Rangpur lime	83.0^{de}	77.0^{j}	75.0^{kl}	72.8^{mn}	77.0^{c}
Bakraii	86.5^{bc}	84.0^{d}	82.4^{ef}	73.2^{mn}	81.5^{a}
Rough lemon	88.0^{ab}	80.0^{gi}	74.0^{lm}	72.0^{no}	78.5^{b}
Macrophylla	77.0^{j}	71.0^{op}	67.0^{q}	65.0^{r}	70.0^{e}
Citrumelo	88.0^{ab}	75.0^{kl}	64.7^{r}	61.0^{s}	72.2^{d}
Citrange	83.0^{de}	75.6^{jk}	64.2^{r}	58.0^{t}	70.2^{e}
Trifoliate orange	81.4^{fg}	70.0^{p}	61.0^{s}	54.5^{u}	66.7^{f}

*: Within each column, means followed by the same letters are not significantly different at $p \leq 0.05$ LSD test

Table 6: Effects of rootstock and NaCl (0, 25, 50 and 75 mM) on proline (μmol proline g^{-1} Fw) of nine citrus rootstocks after 60 days of applying salinity treatments

	NaCl levels (mmol)				
Rootstock	0	25	50	75	Means
Sour orange	27^{op}*	44^{l}	72^{g}	98^{b}	60.2^{b}
Cleopatra	25^{pq}	55.5^{i}	69^{g}	96^{b}	61.4^{b}
Rangpur lime	32^{mo}	53^{ij}	87^{de}	110^{a}	70.5^{a}
Bakraii	27^{op}	46^{kl}	63^{h}	79^{f}	53.7^{c}
Rough lemon	34^{m}	46^{kl}	94^{bc}	99^{b}	68.2^{a}
Macrophylla	30^{mp}	34^{m}	48^{jl}	90^{cd}	50.5^{d}
Citrumelo	28.3^{np}	43^{l}	50^{ik}	84^{ef}	51.3^{cd}
Citrange	21^{q}	33^{mn}	48^{jl}	86^{de}	47.0^{e}
Trifoliate orange	33^{mn}	43^{l}	50^{ik}	80^{f}	51.5^{cd}

*: Within each column, means followed by the same letters are not significantly different at $p \leq 0.05$ (LSD test)

water status, reflecting the metabolic activity in tissues (Gonzalez and Gonzalez-Vilar, 2001). Katerji *et al.* (1997) reported that RWC decrease duo to exposure to stress indicated a loss of turgor that resulted in limited water availability for the cell extension process. Cause of this issue can be replaced Ca^{2+} with Na^{+} in cell wall in salinity conditions. Similar reports have been made for many plant species under salinity stress conditions (Stepien and Klbus, 2006; Zekri, 1991) about the citrus. This decrease in RWC could be because of lower water availability under stress conditions (Shalhevet, 1993), or root systems which are not able to compensate for water lost by transpiration through a reduction of the absorbing surface (Gadallah, 2000).

Proline: Proline was significantly increased with the increasing salinity stress in all rootstocks. This indicates a possible role of proline in osmotic adjustment of Citrus leaves. Maximum proline accumulation was recorded at higher salinity level i.e., 75 mM followed by 50 and 25 mM, respectively. The amount of changes was different depending on rootstocks and salinity levels. In 25 mM NaCl, Cleopatra and Rangpur lime showed the highest (55.5 and 53 μmol proline g^{-1} Fw, respectively) and Citrange and Macrophylla showed the lowest proline (33 and 34 μmol proline g^{-1} Fw, respectively). In 50 mM NaCl, than 25 mM NaCl, proline increased significantly. In this level, Rough lemon showed the highest proline (94 μmol proline g^{-1} Fw). In 75 mM NaCl, most proline was obtained from Rangpur lime (110 μmol proline g^{-1} Fw) and the lowest proline was obtained from Bakraii, Citrumelo and Trifoliate orange (79, 84 and 80 μmol proline g^{-1} Fw, respectively) (Table 6). It has been shown that there is a positive correlation between salt tolerance and concentration of proline (Hokmabadi *et al.*, 2005). In a previous study, NaCl treatment increased the concentration of proline in the leaves of sweet orange seedlings (Banuls and Primo-Millo, 1992). In this study for all the nine species, proline content of the leaves increased in response to salt stress. These results indicate that proline accumulation is a result of the increase in leaf chloride and sodium levels. Proline is organic compounds that might be involved in facilitate osmotic adjustment under salinity stress and sink of energy for growth after stress. Proline is considered to act as an enzymatic regulator during stress conditions (Rontein *et al.*, 2002).

CONCLUSION

It is concluded that exists difference in salinity resistance among seedling of nine rootstocks. The application of non-destructive based techniques [(Chlorophyll fluorescence (Fv/Fm), relative chlorophyll contents (spot values) and visible symptoms of leaf damage] combine with leaf Relative Water Content (RWC), proline content and electrolyte leakage provides a good way to evaluate the salt tolerance of citrus rootstocks and obtain a full picture of the response of citrus rootstocks to salinity. High concentration of salts in the root zone causes substantial reduction in chlorophyll, chlorophyll fluorescence yields and relative water content. The greater growth reduction was observed in rootstocks which have higher ion leakage and have low Chl, Fv/Fm and RWC. It is concluded that Sour orange, Cleopatra mandarin, Rangpur lime and Bakraii rootstocks were more resistant to changes in the level of salt. But, Trifoliate orange, Citrange and Swingle citrumelo were the most sensitive ones to salt.

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