Research Journal of

Environmental Sciences

ISSN 1819-3412

Academic Journals Inc.

www.academicjournals.com

Research Journal of Environmental Sciences 4 (2): 158-165, 2010
ISSN 1819-3412

Biomass Accumulation and Proline Content of Six Citrus Rootstocks as Influenced by Long-Term Salinity

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Abstract: Citrus species are sensitive to salinity and such conditions greatly reduce their growth and yield. This study was conducted to evaluate the biomass and proline level changes in six citrus rootstocks, namely alemow, citromelo, rough lemon, volkamerlemon, sour orange and Mexican lime under salt stress. The study was performed in a greenhouse with NaCl and $CaCl_2$ induced salinity treatments as 0.57 (control) and 2.5 and 5 (dS m^{-1}) for twelve weeks. Biomass accumulation and proline content of leaves and roots measured at the end of the experiment. Biomass accumulation decreased with increasing salinity level and the lowest rate of reduction in biomass accumulation observed in sour orange. Increasing the salt levels led to significant increase in proline content of leaves and roots regardless to the salt types. The salt type did not significantly affect the proline level and or biomass accumulation. Proline level was higher in the leaves of all rootstocks compared to their roots. The highest and the lowest proline levels in the leaves were observed in the citromelo and rough lemon, respectively; and the highest and the lowest levels of root proline were found in alemow and citromelo, respectively. Since, the species displayed similar trends in proline increments in responding to the salinity levels and there found no illustrative correlation between sensitivity to salinity and proline accumulation in leaves or roots. It is concluded that proline accumulation is a better index of salinity levels exerted on the plant than the salinity tolerance index.

Key words: Calcium chloride, citrus, growth, proline, salinity stress, sodium chloride

INTRODUCTION

Citrus trees are one of the most important commercial trees that grown in semi arid areas in the world. In such circumstances, many soils and waters contain amounts of salts that lead to more salt accumulation in soil. Citrus species are glycophyte and the differences in tolerance do exist among species (Maas, 1993) and they need to be tested individually. Soil salinity is a major constraint limiting agricultural productivity on nearly 20% of the cultivated area and half of the irrigated area worldwide (Zhu, 2001). Increasing salinization of arable lands is problem of paramount importance to crop production in many parts of the world

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and especially in irrigated fields of arid and semi-arid regions (Grattan and Grieve, 1992), where soil salt content is high and precipitation is insufficient for their leaching. Citrus are grown preferentially in semiarid areas where irrigation is required to produce maximum yield. Citrus species are classified as salt-sensitive (Maas, 1990, 1993). Growth and fruit yield of citrus are impaired at a soil salinity of about 2 (dS m^{-1}) without any concomitant expression of leaf symptoms (Cerdá *et al*., 1977). There is great variation in the ability of citrus trees to tolerate salinity depending on rootstock (Cerdá *et al*., 1977; Walker and Douglas, 1983; Zekri and Parsons, 1992) and scion (Lloyd *et al*., 1990; Nieves *et al*., 1990).

Saline soils contain sufficient soluble salts to suppress plant growth through a series ofinteracting factors such as osmotic potential effect, ion toxicity and antagonism, which induce nutrient imbalances (Sepaskhah and Maftoun, 1988; Grattan and Grieve, 1992; Neuman, 1997). Salt stress has been reported to cause an inhibition of growth and development and reduction in photosynthesis in sensitive species (Boyer, 1982). Salinity and its effects on biomass production have been considered by numerous authors (Khan *et al*., 2000a, b; McKell, 1994; Mehari *et al*., 2005). Adaptation of plants to salinity is associated with osmoregulation adjustment. Accumulation of metabolites that act as compatible solutes is one of the probable universal responses of plants to changes in the external osmotic potential (Abou-El-Khashab *et al*., 1997). These small molecules are important physiological indicators for evaluating osmotic adjustment ability (Zhu, 2001). Proline accumulation in the plant tissue due to salinity stress reported in wide range of plants, e.g., in alfalfa (Irrigoyen *et al*., 1992), sweet orange (Banuls and Primo-Millo, 1992), pistachio (Hokmabadi *et al*., 2005) and in citrus species (Anjum, 2008). In halophytes, there is a positive correlation between proline content and the amount of Cl^- and Na^+ in cell sap (Taylor, 1996). Proline has been known to serve as a compatible osmolyte, protectant of macromolecules and also as a scavenger of reactive oxygen species under stressful conditions (Hellman *et al*., 2000; Girija *et al*., 2002; Ashraf and Foolad, 2007).

It is necessary to search and find rootstocks that have notable tolerance to salinity. Therefore, this study was carried out to assess and compare six citrus rootstocks for their salt tolerance, organic composition and biomass production under saline conditions.

MATERIALS AND METHODS

Growth Condition and Plant Material

The experiment was conducted during the spring and summer seasons of 2008 at the research greenhouse of the Jahrom Pars Narang Co., Jahrom, Iran.

Seeds of six citrus rootstocks alemow (*Citrus macrophylla*), rough lemon (*C. jambhiri*), volkamerlemon (*C. volkameriana*), citromelo (*Poncirus trifoliate* ×*C. paradise*), Mexican lime (*C. aurantifolia*) and sour orange (*C. aurantium*) were obtained from Darab Agricultural Research Station, Darab, Fars, Iran and were sown in a greenhouse at Jahrom Pars Narang Co.. The physicochemical properties of the soil (Table 1) and water (Table 2) were determined by Soil and Water Laboratory of Fars Regional Water Organization.

Table 1: Physicochemical properties of soil used

Soil texture	Value	Sandy clay loam	Value
Basic saturation (%)	56.00	Nitrogen (%)	0.18
Electrical conductance (dS m^{-1})	01.47	Organic carbon (%)	1.11
pH of Saturated soil solution	08.60	Phosphorus (%)	0.22
Neutralizing agents (%)	42.50	Potassium (%)	3.40
Clay (%)	22.00	Iron (mg kg^{-1} of soil)	4.30
Silt (%)	34.00	Zinc (mg kg^{-1} of soil)	2.40
Sand (%)	44.00	Magnesium (mg kg^{-1} of soil)	2.70
		Copper (mg kg^{-1} of soil)	0.66

Table 2: Physicochemical properties of irrigation water used

Properties	Value	Properties	Value
Electrical conductance (dS m^{-1})	0.56	Sodium (mg L^{-1})	26.91
pH	7.45	Calcium (mg L^{-1})	70.00
Carbonates (mg L^{-1})	0.00	Potassium (mg L^{-1})	1.17
Bicarbonates (mg L^{-1})	244.00	Magnesium (mg L^{-1})	14.40
Sulphates (mg L^{-1})	58.08	Chlorides (mg L^{-1})	23.08

Uniform eight months old seedlings were selected and transplanted in eight liter pots containing typical soil of southern Iran. Seedlings were grown in greenhouse at day/night temperatures of(30/25±4°C), relative humidity of 55/75% and a 16 h photoperiod. Seedlings were irrigated using irrigation water with the electrical conductivity of 0.56 (dS m^{-1}). After 120 days, salinity treatments were applied to the pots with irrigation water containing NaCl and $CaCl_2$ at 5 day intervals (irrigation to FC level). Salts were added to the irrigation water gradually such that after four courses of irrigation the desired treatment concentrations were achieved, to prevent salinity shock. Then, rootstocks were treated with saline water for 12-week experimental period. Irrigation water was employed as the control treatment. Salinity treatments were made by adding sodium chloride (SIGMA™) and calcium chloride (SIGMA™) up to 2.5 and 5 (dS m^{-1}) to the irrigation water, based on the formula of (Sparks, 2002):

$$\text{Salt (mg L}^{-1}\text{)} = 640 \times \text{EC (dS m}^{-1}\text{)}$$

Data Recorded

At the end of the experimental period, seedlings were harvested and the biomass accumulation and proline content of leaves and roots were assessed.

Dry Weight

Citrus seedlings were harvested by washing roots from the soil and plants divided into root and shoot. Plant material was washed thoroughly with tap water and then rinsed twice with distilled water, before being oven-dried at 75°C to a constant weight to estimate dry weight.

Proline Analysis

Proline was extracted from the leaf and root tissues according to the method described by Bates *et al.* (1973). Five hundred milligrams of fresh leaf material was homogenized in 10 mL of 3% aqueous sulfosalicylic acid and filtered through Whatman's No. 1 filter paper. Two milliliter of the filtrate was mixed with 2 mL of acid-ninhydrin and 2 mL of glacial acetic acid in a test tube. The reaction mixture was extracted with 4 mL toluene and the chromophore containing toluene was aspirated, cooled to room temperature and the absorbance was measured at 520 nm with a spectrophotometer (CEIL CE 2301). L-proline (SIGMA™) was used as standard.

Statistical Design and Data Analyses

Treatments were set in a factorial experiment based on a Complete Randomized Design (CRD) with 4 replications per treatment. Data were analyzed using SPSS 16.0 software for windows. Means were separated using Tukey's HSD test-at $p \leq 0.05$.

RESULTS

Dry weight decreased with increasing salt concentration for all rootstocks. The highest value of total dry weight observed in the control treatment. The maximum amount of biomass found in volkamerlime under control condition (Table 3). The minimum value of total dry weight found in NaCl induced salinity at 5 (dS m^{-1}). Both NaCl and $CaCl_2$ induced salinities lead to significant decrease in biomass accumulation significantly; however there was no significant difference between them. Biomass of rootstocks differed significantly by salinity treatments. The minimum quantity of biomass observed in sour orange rootstock under high salinity stress induced by NaCl. However, there were no significant differences between sour orange and Mexican lime, alemow and rough lemon.

The proline accumulation in leaves and roots of the citrus rootstocks was increased in response to increasing salt concentrations in irrigation water. Leaves accumulate more proline than roots in response to salinity. The maximum amount of leaves proline was observed at the highest salinity concentrations (5 dS m^{-1}) for all species. However, there were no significant differences between proline content and different concentrations of sodium chloride and calcium chloride salinity (Table 4). The lowest levels of leaves and roots proline were observed at the control treatments. Proline accumulation in leaves of different Citrus species was not analogous. Citromelo accumulate much proline than the other rootstocks and the minimum proline content of leaves found in rough lemon, Mexican lime and sour orange (Table 4).

Generally, the root proline content of all rootstocks increased as the increasing salinity (Table 5). The highest root proline level was observed at the salinity level of 5 (dS m^{-1}) of

Table 3: Effect of salinity on biomass accumulation of different citrus rootstocks

Salinity (dS m^{-1})	Alemow	Rough lemon	Citromelo	Volkamer-lemon	Mexican lime	Sour orange	Average
Control	26.59b†	22.01b-e	17.39b-e	37.94a	10.8f-j	9.38g-j	21.18a
NaCl							
2.5	17.65c-g	17.64c-g	14.00e-j	23.61b-d	9.78g-j	9.31g-j	15.3b
5	14.78e-i	15.54d-h	11.23f-j	22.89b-d	7.92j	6.74ij	12.85b
$CaCl_2$							
2.5	16.74c-g	18.86b-f	12.69f-j	23.39b-d	8.31h-j	9.76g-j	14.96b
5	13.77f-j	16.72c-g	11.43f-j	24.06bc	9.92 g-j	6.24j	13.69b

†Values not associated with the same letter are significantly different (Tukey's HSD test, at $P \leq 0.05$)

Table 4: Effect of sodium chloride and calcium chloride salt level on leaf proline content of citrus rootstocks (µmol g^{-1} of fresh weight)

		Sodium chloride		Calcium chloride	
Rootstock	Control	2.5 (dS m^{-1})	5 (dS m^{-1})	2.5 (dS m^{-1})	5 (dS m^{-1})
Alemow	46.67lm†	92.45e-k	128.3b-f	83.87g-l	150.2bc
Rough lemon	32.49m	45.60lm	108.0d-i	59.53klm	94.19e-k
Citromelo	60.00klm	133.1b-e	141.1bc	118.6b-g	170.8a
Volkamerlemon	33.66m	72.93h-m	113.1c-h	60.23klm	127.0b-f
Mexican lime	38.37m	60.00klm	106.6d-i	102.0d-j	65.18j-m
Sour orange	32.49m	90.57fk	106.7d-i	71.52i-m	87.04f-k

†Values not associated with the same letter are significantly different (Tukey's HSD test at $p \leq 0.05$)

Table 5: Effect of sodium chloride and calcium chloride salt level on root proline content of citrus rootstocks (µmol g^{-1} of fresh weight)

		Sodium chloride		Calcium chloride	
Rootstock	Control	2.5 (dS m^{-1})	5 (dS m^{-1})	2.5 (dS m^{-1})	5 (dS m^{-1})
Alemow	51.94d-i†	80.69cd	130.8a	74.35cde	65.35c-i
Rough lemon	38.37i	57.42d-i	80.52cd	53.36d-i	67.80c-h
Citromelo	39.95hi	50.71efghi	73.99c-f	46.65e-i	55.30d-i
Volkamerlemon	44.71f-i	59.00d-i	71.88c-g	43.30ghi	71.70c-g
Mexican lime	52.30d-i	48.95e-i	90.92bc	62.31d-i	47.89e-i
Sour orange	48.60e-i	60.59d-i	88.2bc	71.52c-g	68.88c-h

†In each column, the cells with the same letters are not significantly different based on Tukey's HSD test, at $p \leq 0.05$

treatment. NaCl treatments also caused no significant difference at 2.5 (dS m^{-1}). Thereupon, the only significant increase of proline content of roots, observed in NaCl treatments at 5 (dS m^{-1}) salinity induced (except in volkamerlemon roots).

DISCUSSION

In the present study, biomass and proline accumulation in leaves and roots of different Citrus species under salinity stress were investigated.

Dry weight of Citrus rootstocks decreased with increasing water salinity for all rootstocks; however we found no significant difference between the two salt types in the case of reduction in biomass of rootstocks. The minimum and maximum quantity of biomass observed in sour orange and volkamerlemon rootstocks under high salinity stress of both salts, respectively (Table 3), but it should be noted that the rate of reduction in biomass by increasing salinity also varied among rootstocks. Figure 1 shows the percentage of growth of different rootstocks to their respective control treatments in respond to salinity stress. Sour orange rootstock proved less sensitivity in biomass reduction (28.1% of respective control). In fact volkamerlemon rootstock shows much sensitivity to biomass reduction than the other rootstocks in respond to increasing salinity (Fig. 1). The sensitivity to salinity based on biomass reduction was in the following descending order: alemow (32.6%), volkamerlemon (30.4%), citromelo (23.2%), rough lemon (17.5%), Mexican lime (14.3%) and sour orange (11.6%). These results are also in agreement with Ream and Furr (1976) and Storey and Walker (1987).

Compatible solutes are known to accumulate under conditions of salt stress to play a role in the process of osmotic adjustment in many crops. Proline is a dominant organic molecule that acts as a mediator of osmotic adjustment under salinity stress, a stabilizer of sub-cellular structures, a sink for energy and even a stress-related signal. It is also involved in cell osmoregulation, protection of proteins during dehydration and can act as an enzymatic regulator during stress conditions (Rontain *et al*., 2002). Somehow, some studies demonstrated that in conditions where growth of citrus is affected by an imposed salinity, the elevation in the proline level is less than two-fold (Nieves *et al*., 1990; Walker *et al*., 1993) or there is no proline response (Syvertsen and Yelenosky, 1988; Lloyd *et al*., 1989).

Based on the results of the present study, as the salinity level increased, the leaf and root proline level increased in all the rootstocks. Furthermore, the leaf proline level was

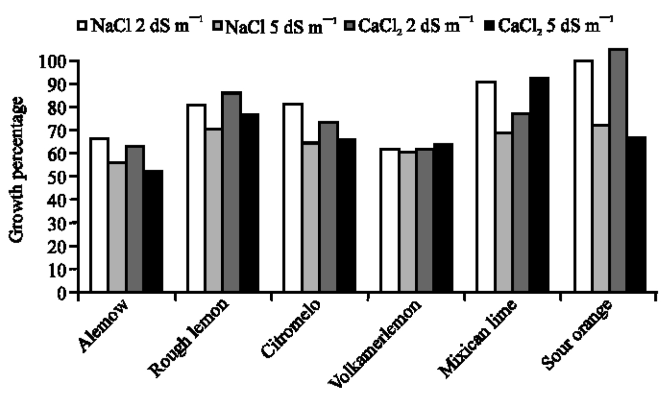

Fig. 1: Percentages of biomass accumulation of citrus rootstocks in respond to salinity stress

higher than the root proline level. A significant difference was observed among the proline content of leaves at all three salt levels and the highest of which was observed at the salinity level of 5 (dS m^{-1}). This finding is in accordance with the results of other studies conducted by Banuls and Primo-Millo (1992), Ashraf and Harris (2004), Ashraf and Orooj (2006) and Anjum (2008). Proline concentration in the leaves of citromelo at all salinity levels was higher than other rootstock types (Table 4). The leaf proline level of other rootstock types was in the following descending order: alemow, sour orange, volkamerlemon and Mexican lime.

The proline contents of the leaves obtained from the treatments of the two salts did not vary significantly. But the salt types made significant differences in the case of root proline contents. Root proline contents of citrus rootstocks did not increased significantly, except in the NaCl induced salinity at 5 (dS m^{-1}) level. Such a difference may be due to the difference between the mobility and toxicity of the ions of the salts. Taylor (1996) stated that accumulation of Cl^- and Na^+ may responsible of proline accumulation in plants under salt stress. Since, the Cl^- is more mobile and toxic than the Na^+ ions, it accumulates in leaves and induces proline accumulation (Zekri and Parsons, 1992); thereupon as the results show, there should not be any significant differences between NaCl and $CaCl_2$ (Table 4). But Na^+ ions are less mobile and more toxic than the Ca^{++} ions and they often accumulate in roots (Zekri and Parsons, 1992), so as the result, in NaCl treated plants more proline will be produced and accumulate in the roots than the $CaCl_2$ treated plants (Table 5). The lower proline content of $CaCl_2$ treated plants also may due to ameliorative effects of Ca^{++} ions on plants against high concentrations of Cl^- ions (Al-Yassin, 2004). These results are in agreement with Zekri and Parsons (1990) and Zekri (1993a, b).

It has been shown that there is a positive correlation between salt tolerance and concentration of proline (Hokmabadi *et al*., 2005; Karimi *et al*., 2009); however, in this study for all the six species, proline content of the leaves increased in response to salt stress and there was no good correlation between sensitivity to salinity and proline accumulation. Since, the species displayed similar trends in proline increments in responding to the salinity levels, these results would also support the theory of Bokhari and Trent (1985) regarding plant responses to the drought stress. Therefore, in this regard, these results suggest that proline accumulation in leaves is a better index of salinity levels exerted on the plant, than the salinity tolerance index regardless to the salt types. We also concluded that proline contents of roots may not be as useful as proline content of leaves in determination of level of salt stress which plants deal with.

It is also concluded that NaCl and $CaCl_2$ may not have different significant effects on growth of citrus rootstocks and response of citrus is may dependant to the composition of salts.

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