American-Eurasian J. Agric. & Environ. Sci., 3 (2): 264-268, 2008
ISSN 1818-6769

Effect of Salinity on Tolerance of Two Bitter Almond Rootstocks

Sh. Najafian, M. Rahemi and V. Tavallali

Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz, Iran

Abstract: Salinity is a major problem particularly in arid and semi-arid areas and more than 30% of cultivated land is under the influence of salinity, consequently the growth of plants has declined considerably. Selection of tolerant genotypes is an alternative against salinity stress. The objective of this research was to evaluate tolerance of two bitter almond rootstocks (number 31 and 41) to salinity stress. This experiment was conducted in the framework of completely randomized design with three replicates for each rootstock and 6 levels of NaCl were applied including 0, 15, 30, 45, 60 and 75 mmol L^{-1}. In this study, the effect of salinity on the accumulation of Cl^-, Na^+ and K^+ in the root and shoot and also its effect on plant growth traits including plant height, leaf area, upper and lower stem diameter, internodes number, chlorophyll content, proline, root and shoot fresh and dry weights and K^+:Na^+ ratio of root and shoot were assessed. Based on the results of this study, the increase of salinity in irrigation water increased the accumulation of Na^+ and Cl-in the shoot and root of the two rootstocks 31 and 41. In rootstock number 41 in all treatments K^+:Na^+ ratio of whole plant was significantly decreased while in rootstock number 31 only in the case of 75 mmol L^{-1} a significant decreases was noticed. Since the extent of reduction in this item (K^+:Na^+) has reverse correlation with tolerance degree, it is concluded that rootstock number 31 is more tolerant than 41. Furthermore, salinity reduced stem length, number of internodes, fresh and dry weights of root and shoot in the two wild rootstocks but this reduction was not significant. Chlorophyll content was not affected by salinity but leaf area was reduced and just in rootstock 41 at higher levels of salinity was significant. Proline (mg g^{-1} DW) content of the seedling rootstock was increased as salinity level increased in comparison to control but was not significant at 5% of probability.

Key words: Almond · NaCl · rootstock · salinity · tolerance

INTRODUCTION

Salinity is a widespread problem especially in arid and semiarid regions. Some studies indicate that 20-50% of all irrigated croplands are affected by high salt concentration, resulting in considerable economic losses [1]. There are two main negative effects of high salt concentration that influence plant growth and development: water deficit and ion toxicity associated with excessive Cl and Na, leading to a Ca and K deficiency and to other nutrient imbalances [2].

Temperate fruit trees are generally rated as sensitive to soluble salts [3, 4] and particularly sensitive to chloride [5] and irrigation with saline water may significantly reduce yield. woody plants usually are relatively salt tolerant during seed germination, much more sensitive during the emergence and young seedling stages and become progressively more tolerant with increasing age through the reproductive stage (with the exception of anthesis) [6].

Most fruit trees including *Prunus armeniaca*, *Prunus domestica* and *Prunus persica* are sensitive to salinity [7]. Control mechanisms of salt load at whole plant level highly integrate growth rates and plant morphology [8-11] as well as leaf water relations and osmotic adjustment [12, 13]. From several previous decades making use of interspecies hybrids in prunus as a rootstock for number of stone fruits such as almond and peach is strongly recommended, therefore the selection of salt tolerant genotypes to hybridization is more important.

In frequent rains only partially leach accumulated salts out of the soil. As a consequence salinity declines plant growth. The salt effect differs among genotypes and selection of salt tolerant genotypes is an efficient means to cope with salinity [14].

Corresponding Author: Dr. Vahid Tavallali, Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz, Iran

Table1: Some prior physico-chemical properties of the soil

			Soil texture			Water content (%, dry wt basis)	
$EC\times10^3$(dS m^{-1})	pH Paste	Organic matter (%)	Sand (%)	Silt (%)	Clay (%)	Permanent wilting point	Field capacity
0.6	7.5	1.5	50.84	34	15.16	13.86	20.05

Selection of tolerant genotypes is an alternative against salinity stress. The objective of this research was to evaluate tolerance of two bitter almond genotypes (Number 31and 41), when exposed to NaCl concentrations in the range of 0-75mM in terms of (i) plant growth and morphology (ii) leaves characteristics (iii) Na^+, Cl^- and K^+ accumulation in shoots and roots.

MATERIALS AND METHODS

The experimental site was located at Shiraz University Glasshouse, Shiraz, Iran (29°36'N, 52°32'E). The experiment was conducted in randomized complete design with three replications in each six salinity levels.

A dry, loamy, calcareous soil was collected from the top 20-cm layer of Ramjerdi series (Fine, Mixed, Mesic, Fluventic haploxerepts) of a Bajgah soil at the Agricultural Experimental Station of Shiraz University, 16km north of Shiraz. Some of the physico-chemical properties of this soil are shown in Table 1.

Seedling of the bitter almond genotypes were planted in 7 kilograms plastic pots containing a 1:1:1 soil, sand and mold mixture. Pot were maintained under controlled condition at temperature of 24/17°C day/night, light intensity from 800 to 1600 μmol m^{-2} s^{-1} and relative humidity of 65% for 15 weeks.

In order to vegetative growth of seedlings, Nitrogen and phosphorous were applied uniformly to all pots at the rate of 50 ppm each as NH_4NO_3 and KH_2PO_4, respectively.

Each pot irrigated with distilled water to near field capacity by weighting the pots, no water was lost by drainage. After 21 days the salinity treatments started. Salt treatments were 0 (distilled water), 15, 30, 45, 60, 75 mM, obtained by adding NaCl to the distilled water. NaCl levels were added in two equal parts on a 7-day interval. The experiment was continued for 15 weeks after planting. Shedded leaves from each plant were collected and at the end were weighted with dry weight of shoot and leaves.

At 105 days after planting, the seedlings were cut at soil surface and the roots also washed free of soil. The numbers of leaves per shoot, total leaf area, stem and leaf fresh and dry weights were recorded. Leaf areas were measured with a portable area meter model LI-3000 (ΔT Device, England). Stem height and diameter at top and bottom were also measured. Shoots and roots were dried at 70°C for 48 h, dry weights were recorded and the tissues (leaves, shoots and roots) were ground in a Wiley mill to pass 40-mesh screen. Chloride was measured by the method outlined by Chapman and Pratt [15]. Representative samples were dry-ashed and analyzed for Na by Corning 405 flame photometry. Electrical conductivity (total soluble salts) was measured in the soil at the end of the experiment by metrohm 644 conductometer (Switzerland).

The methods described by Lichtenthaler [16] were employed for the extraction of chlorophyll (mg/g FW) from leaves. The amount of chlorophyll exists in leaf extract was determined by chlorophyllmeter (model: Spectronic 20, USA).

Collected data were analyzed using MSTATC statistical software. Treatment means were compared using Duncan's test (P = 0.05).

RESULTS AND DISCUSSION

Growth characteristics: The effects of salt treatments on growth characteristic of two bitter almond genotypes (number 31 and 41) were evaluated (Table 2). In genotype 31, stem length was higher than genotype 41 at high salinity levels, but the number of internodes was higher in genotype 31 at all treatments. In the higher NaCl treatment, both stem length and number of internodes were the lowest. In genotype 41, leaf area was significantly higher than genotype 31 at all salinity treatments. Leaf area was reduced by increasing the salinity level.It was significantly lower at the highest salinity level in genotype 41. In spite of expectance, the chlorophyll content of genotype 41 was the highest by increasing salinity level up to 75 mM L^{-1} of NaCl, but genotype 31 had lower chlorophyll content than genotype 41 at 75 mM NaCl. Similar results were reported by Gale and Polijakoff-Mayber [9] for the leaf area of *Atriplex halimus*. They showed that leaf area per plant was increased about 85% at the NaCl-induced osmotic potential of about-2.5 atm compared with the check. Then

Table2: Effects of salinity treatments on stem length (cm), number of internodes, leaf area (cm^2), chlorophyll content (mg/g) and proline content (mg/g) of two bitter almond genotypes

Treatment	Stem length (cm)	Number of internodes	Leaf area (cm^2)	Chlorophyll content (mg/g FW)	Proline content (mg/g DW)
Genotype 31					
Control	87.67a†	58.00a	0.06d	6.75ab	1459b
*S1	78.67ab	52.00abc	0.07d	3.66b	5167a
S2	68.83bcd	48.67bcd	0.09d	7.20ab	1327b
S3	74.75abc	56.67ab	0.08d	6.85ab	2543ab
S4	69.00bcd	49.33abcd	0.07d	7.74a	1877ab
S5	70.83ab	50.67abcd	0.09d	5.18ab	1562b
Genotype 41					
Control	70.25bcd	43.33cd	12.73a	4.52ab	1930ab
S1	73.17abcd	47.33cd	10.73b	7.30ab	2134ab
S2	72.27ab	43.67cd	13.36a	7.21ab	1146b
S3	86.17ab	50.00abcd	13.72a	6.62ab	2706ab
S4	57.00d	42.00d	8.85bc	6.56ab	3003ab
S5	60.00cd	42.00d	8.45c	7.76a	2663ab

*S1:15mM NaCl, S2:30 mM NaCl, S3:45 mM NaCl, S4:60 mM NaCl, S5:75 mM NaCl, †Means followed by the same letter in each column are not significantly different at P=0.05

Table 3: Effects of salinity levels on Na, K and Cl Concentrations in roots of two bitter almond genotypes

Treatment	Na (μg g^{-1})	K (μg g^{-1})	Cl (mgg^{-1})
Genotype 31			
Control	325.0abc†	2283a	4.26bc
*S1	300.0abc	1567ab	2.13c
S2	291.7abc	1350ab	7.81ab
S3	383.3ab	2017ab	4.85abc
S4	333.3abc	1758ab	3.31c
S5	408.3ab	1125ab	3.78c
Genotype 41			
Control	150.0c	1275ab	2.13c
S1	225.0bc	1708ab	4.14bc
S2	283.3abc	900b	2.84c
S3	325.0abc	1450ab	4.26bc
S4	450.0a	1842ab	8.40a
S5	300.0abc	1200ab	6.15abc

*S1:15mM NaCl, S2:30 mM NaCl, S3:45 mM NaCl, S4:60 mM NaCl, S5:75 mM NaCl, †Means followed by the same letter in each column are not significantly different at P=0.05

Table 4: Effects of water salinity levels on Na, k and Cl Concentrations in shoots of two bitter almond genotypes

Treatment	Na (μg g^{-1})	K (μg g^{-1})	Cl (mgg^{-1})
Genotype 31			
Control	408.3abcd†	4208ab	0.75bc
*S1	233.3cd	3017bc	2.18a
S2	383.3abcd	3225abc	1.30abc
S3	458.3abc	3892abc	1.12abc
S4	358.3bcd	4400a	1.83ab
S5	500.0ab	3433abc	2.17a
Genotype 41			
Control	0.29c	2850bc	166.7d
S1	0.47c	2750c	250.0bcd
S2	0.47c	3250abc	391.7abcd
S3	1.29abc	3250abc	383.3abcd
S4	1.24abc	2833c	466.7abc
S5	1.83ab	3058abc	625.0a

*S1:15mM NaCl, S2:30 mM NaCl, S3:45 mM NaCl, S4:60 mM NaCl, S5:75 mM NaCl, †Means followed by the same letter in each column are not significantly different at P=0.05

the leaf area followed a decreasing trend at the lower values of NaCl-induced osmotic potentials. Decrease in chlorophyll contents induced by salinity in different Pistacia species have been reported earlier by Sepaskhah and Maftoun [17]. Decrease in chlorophyll content under salinity stress may be the result of chlorophyll degradation and /or reduced rate of synthesis, together with a decrease in thylakoid membrane stability [18]. Rao *et al.* [19] suggested that the reduction in chlorophyll concentration of salt treated plants could be attributed to the increase activity of chlorophyll degrading enzyme, chlorophyllase.

Proline content was increased under high salinity level especially in genotype 41 compared with the control

Table 5: Effects of salinity treatment on the total of K/Na ratio of two bitter almond genotypes

	K/Na ratio	
Treatment	Genotype 31	Genotype 41
Control	8.99b†	13.41a
*S1	9.07b	9.27b
S2	7.11bc	6.35bc
S3	7.23bc	6.93bc
S4	9.14b	5.23c
S5	4.97c	4.65c

*S1:15mM NaCl, S2:30 mM NaCl, S3:45 mM NaCl, S4:60 mM NaCl, S5:75 mM NaCl, †Means followed by the same letter in each column are not significantly different at P=0.05

treatment, but in both genotypes there is no clear trend. Data in Table 2 indicated that the highest proline content was recorded when genotype 31 received the lowest level of salinity (15mM NaCl). However, at genotype 41 the highest proline content was obtained when plants received 60 mM NaCl.

Chemical composition: Susceptibility of a plant to salinity is controlled by several factors other than Na and Cl [11]. The impact of the salinity stress on Na itself within plants is not unexpected, with very large elevations in Na concentrations in both the roots and, especially, the leaves. Under salt stress the Na content in both root and shoot of two genotypes 41 and 31 was increased by increasing salinity levels, but it had no clear trend. These data are in agreement by Satti and Al-Yahyai [20] on tomato, Asch *et al.* [21] on rice. From Table 3 and 4, it is clear that salt treatments greatly reduced shoot and root K concentrations especially in genotype 31, but not in genotype 41, compared with unstressed plants. In saline soils, Na competes with K for uptake across the plasma membrane of plant cells. This can result in low K^+:Na^+ ratios that reduce plant growth and eventually become toxic [22]. The present results show that the salt-sensitive rootstock, genotype 41, had much lower K^+:Na^+ ratios than genotype 31 under salt treatments (Table 5). Increasing of salinity levels reduced K^+:Na^+ ratios in both genotypes (Table 5). This reduction was higher in genotype 41 than genotype 31. Sodium chloride caused a decrease in the total K content; this reduction in uptake undoubtedly reflects competition between Na and K [23]. Potassium, unlike Na, was accumulated to a relatively higher level in the shoot than in the root. In this study the K^+:Na^+ ratio was considerably decreased in both plant parts especially in the shoot due to salinity.

It seems that the rate of decrease in K^+:Na^+ ratio had inverse relationship with the rate of rootstocks resistance. A wide K^+:Na^+ ratio is recommended as a sensible criterion of salt tolerance in higher plant species [19]. Grieve and Walker [24] described that competitive character of K and Na uptake is the most important factor of the reduction of K^+:Na^+ ratio at high salinity levels. The selection of appropriate genotype improves the K^+:Na^+ selectivity by shifting the uptake ratio in favor of K at the expense of Na.

In both genotypes root and shoot Cl were significantly increased compared with the control treatment by increasing salinity levels (Table 3 and 4). The root Cl concentrations of genotype 41 was higher than genotype 31 (Table 3), but there was not the same result for shoot Cl (Table 4).

As mentioned before, the shoot Na concentrations were excessively less than Cl concentrations at high salinity level. This is in agreement with the findings of Maftoun *et al.* [25] on soybean (Glycin max L. merr) and Sepaskhah and Maftoun [17] on pistachio. Lessani and Marschner [26] reported that the Cl concentration varied in various Salt-stressed crops, excepting sugar beet where Cl concentrations were always higher than Na. Bernal *et al.* [27] suggested that the higher Cl than Na uptake in salt-stressed crops could be responsible for growth suppression by reducing the uptake of No_3-N.

CONCLUSION

According to the above data, the increase of salinity in irrigation water increased the accumulation of Na and Cl in the shoot and root of the two genotypes. Under all salinity treatments, rootstock number 41, K^+:Na^+ ratio of whole plant was significantly decreased, while in rootstock number 31 that ratio was significantly decreased only at 75 mM L^{-1} NaCl. Since the extent of reduction in this item (K^+:Na^+) has reverse correlation with tolerance degree, it is concluded that rootstock number 31 is more tolerant than 41.

REFERENCES

1. Flowers, T.J., 1999. Salinisation and horticultural production. Sci. Hortic., 78 : 1-4.
2. Marschner, H., 1995. Mineral Nutrition of Higher Plants. Academic press, London, pp: 379-396.
3. Mass, E.V. and G.J. Hoffman, 1997. Crop salt tolerance-salt tolerance-current assessment. J. Irrig. Drain. Div. ASCE, 103: 115.

4. Mass, E.V., 1986. Salt tolerance of plants. Appl. Agric. Res., 1: 12-26.
5. Bernstein, L., 1980. Salt tolerance of fruit Crops. USDA Agric. Inf. Bull., 292: 1-8.
6. Shannon, M.C., C.M. Grieve and L.E. Francois, 1994. Whole palnt response to salinity. In plant-Environment Interactions. Wilkinson, R.E. (Ed.). Marcel Dekker, New York, pp: 199-244.
7. Gucci, R. and M. Tattini, 1997. Salinity tolerance in olive. Hortic. Rev., 21: 177-214.
8. Cheeseman, J.M., 1988. Mechanisms of salinity tolerance in plants. Plant Physiol., 87: 547-550.
9. Gale, J. and A. Poljakoff-Mayber, 1970. Interrelations between growth and photosynthesis of saltbush (*Atriplex halimus* L.) growth in saline media. Aust. J. Biol. Sci., 23: 937-954.
10. Moya, J.L.E., Primo-Millo and M. Talon, 1999. Morphological factor determining salt tolerance in citrus seedlings: The shoot to root ratio modulates passive root uptake of chloride ions and their accumulation in leaves. Plant Cell Environ., 22: 1425-1433.
11. Munns, R., 1993. Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant Cell Environ., 16: 15-24.
12. Donovan, L.A., J.H. Richards and M.W. Muller, 1996. Water relations and leaf chemistry of *chrysothamnus nauseosus* spp. *consimilis*__(Asteraceae) and *Sarcobatus vermiculitis* (chenopodiaceae). Am. J. Bot., 83: 1637-1646.
13. Jacoby, B., 1994. Mechanisms Involved in Salt Tolerance by Plants. In: Hand book of plant and crop stress. Pessarakli, M. (Ed.). Marcel Dekker, New York, pp: 97-123.
14. Noitsakis, B., K. Dimassi and I. Therios, 1997. Effects of Nacl induced salinity on growth, chemical composition and water relation of two almond (*Prunus amygdalus* L.) cultivars and the hybrid GF_{677} (*Prunus amygdalus* X *P. persica*). Acta. Hort., 449: 641-648.
15. Chapman, H.D. and D.F. Pratt, 1961. Methods of Analysis for Soil, Plant and Water. Univ. Calif., Div. Agric. Sci., pp: 60-62.
16. Lichtenthaler, H.K., 1987. Chlorophylls and carotenoides: Pigments of photosynthetic bio-membrances. In: Methods in Enzymology. Colowick, S.P. and N.O. Kaplan, Academic Press. New York, 48: 350-382.
17. Sepaskhah, A.R. and M. Maftaun, 1988. Relative salt tolerance of pistachio cultivars. J. Hortic. Sci., 63: 157-162.
18. Vieira, S.C.L., A. Campos, H. Azevedo and G. Calderia, 2001. *In situ* and *in vitro* senescence induced by KCl stress: Nutritional imbalance, lipid peroxidation and antioxidant metabolism. J. Exp. Bot., 52: 351-360.
19. Rao, K.B., S. Panchaksharaiah, K.V. Janardhan and J.S.P. Yardav, 1981. Saline water irrigation and plant growth in vertisol. Proceedings of the Hungoro-Indian Seminar on Salt Affected Soil. Szabolc, J. (Ed.). Budapest, pp: 129-138.
20. Satti, S.M.E. and R.A. Al-Yahyai, 1995. Salinity tolerance in tomato: Implications of potassium, calcium and phosphorus. Commu. Soil Sci. Plant Anal., 26 (17 & 18): 2749-2760.
21. Asch, F., M. Dingkuhn, C. Wittstock and K. Doerffling, 1999. Sodium and potassium uptake of rice panicles as affected by salinity and season in relation to yield and yield components. Plant Soil, 207: 133-145.
22. Schachtman, D. and W. Lio, 1999. Molecular pieces to the puzzle of the interaction between potassium and sodium uptake in plants. Tren. Plant Sci., 4: 281-287.
23. Laties, G.G., 1969. Dual mechanisms of salt uptake in relation to compartmentation and long distance transport. Ann. Rev. Plant Physiol., 20: 89-116.
24. Grieve, A.M. and R.R. Walker, 1983. Uptake and distribution of chloride, sodium and potassium ions in salt-treated citrus plants. Aust. J. Agric. Res., 34: 133-143.
25. Maftoun, M., A. Bassiri, A.M. Sameni and J. Yasrebi, 1982. Growth and chemical composition of soybeans as affected by trifluralin and soil salinity. Weed Res., 22: 89-94.
26. Lessani, H. and H. Marschner, 1978. Relation between salt tolerance and long distance transport of sodium and chloride in various crop species. Aust. J. Plant Physiol., 5: 27-37.
27. Bernal, C.T., F.T. Bingham and J. Orehi, 1974. Salt tolerance of Mexican wheat. ll. Relation of variable sodium chloride and length of growing season. Proc. Soil Sci. Soc. Amer., 38 : 777-780.