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Growth and Chemical Composition of Hybrid GF_{677} (*Prunus amygdalus*×*Prunus persica*) Influenced by Salinity Levels of Irrigation Water

Sh. Najafian, M. Rahemi and V. Tavallali
Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz, Iran

Abstract: The objective of this research was to evaluate response of GF_{677} hybrid (*Prunus amygdalus*× *Prunus persica*) to salinity stress. This experiment was conducted in the framework of completely randomized design with three replicates 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, root fresh and dry weights and K^+:Na^+ ratio of root and shoot were assessed. In GF_{677} rootstock, salinity led to the accumulation of Na^+ and Cl^- in the shoot. K^+/Na^+ ratio of whole plant only at 75 mmol L^{-1} NaCl was decreased significantly. GF_{677} rootstock was tolerant to the salinity up to 60 mmol L^{-1} of NaCl. The result of this study indicated that this rootstock has lower sensitivity to higher salinity levels. Chlorophyll content and root dry and fresh weights were reduced only under 75 mmol L^{-1} NaCl treatment and the number of internodes, stem length and fresh and dry weights of shoot were reduced but not significantly at 5% of probability.

Key words: Almond, hybrid GF_{677}, rootstock, salt stress

INTRODUCTION

Salinity is a widespread problem especially in arid and semiarid regions. Some studies indicate that 20-50% of all irrigated croplands are damaged by high salt concentration, resulting in considerable economic losses (Flowers, 1999). 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 (Marschner, 1995).

Temperate fruit trees are generally rated as sensitive to soluble salts (Mass and Hoffman, 1997; Mass, 1986) and particularly sensitive to chloride (Bernstein, 1980) 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) (Shannon *et al*., 1994).

Most fruit trees including *Prunus armeniaca*, *Prunus domestica* and *Prunus persica* are sensitive to salinity (Gucci and Tattini, 1997). Control mechanisms of salt load at whole plant level highly integrate growth rates and plant morphology (Cheeseman, 1998; Flowers and Yeo, 1989; Moya *et al*., 1999; Munns, 1993) as well as leaf water relations and osmotic adjustment (Donovan *et al*., 1996; Jacoby, 1994). From several previous decades, using interspecies hybrids in prunus as a rootstock for number of stone fruits such as almond and peach is strongly recommended.

This necessity is due to the homogeneity of these hybrids with different cultivars of these hybrids and adaptation to environment, resistance to parasites, growth provocation according to scion and using possibility of them in many unfavorable soils. GF_{667} (*Prunus persica*× *Prunus amygdalus*) has been planted extensively on calcareous soils (Loreti, 1994). We evaluated the responses of this rootstock (the almond-peach hybrid GF_{667}) when exposed to NaCl concentrations in the range 0-75 mM in terms of (i) plant growth and morphology (ii) leaves characteristics and (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 2003 and 2004 in randomized complete design with three replications in each six salinity levels.

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

Table 1: Some prior physico-chemical properties of the soil

$EC \times 10^3$ ($dS\ m^{-1}$)	pH paste	Organic matter (%)	Soil texture: Sand (%)	Silt (%)	Clay (%)	Water content (%, dry wt. basis): Permanent wilting point	Field capacity
0.6	7.5	1.5	50.84	34	15.16	13.86	20.05

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, 16 km north of Shiraz. Some of the physico-chemical properties of this soil are shown in Table 1.

Prepared cuttings were established under mist system with a temperature of 25/18°C day/night, relative humidity of 75% and soil moisture at Saturation level. One week after cuttings planting, callus tissue was formed at the bottom of cuttings. In order to root facility, cuttings were sprayed 3 times a day at 10, 12 am and 2 pm with mist system until soil moisture reached field capacity.

At early March, uniform rooted cuttings were planted in 7 kg plastic pots containing a 1:1:1 soil, sand and mold mixture. Before transferring of cuttings to pots, roots of cuttings were pretreated with tap water and 0.2% benomil solution.

After cuttings planting, pots were maintained under controlled condition at temperature of 24/17°C day/night, light intensity from 800 to 1600 μmol m^{-2} sec^{-1} and relative humidity of 65% for 15 weeks.

In order to vegetative growth of GF_{667} cuttings, 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 weighing 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 and 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.

One hundred and five days after planting, the seedlings were cut at soil surface and the roots also washed free of soil. The numbers of internodes per shoot, total leaf area, stem and leaf dry and fresh weights were recorded. Leaf area was measured with a portable leaf 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 (leaf, shoot and root) were ground in a Wiley mill to pass 40 mesh screen. Chlorine was measured by the method outlined by Chapman and Pratt (1961). 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 method described by Lichtenthaler (1987) was employed for the extraction of chlorophyll 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. Means were compared using Duncan's test ($p = 0.05$).

RESULTS AND DISCUSSION

The effects of salt treatments on leaf characteristic of GF_{677} were evaluated. Leaf chlorophyll content did not show a clear trend. The leaf area was reduced by increasing the salinity level, but it was not significant (Table 2). Similar results were reported by Gale and Poljakoff-Mayber (1970) 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 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 (Behboudian *et al*., 1988; Ranjbar *et al*., 2000). 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 (Vieira *et al*., 2001). Rao and Rao (1981) suggested that reduction in chlorophyll concentration of salt treated plants could be attributed to the increase activity of chlorophyll degrading enzyme, chlorophyllase.

The salinity levels of irrigation water did not significantly influence the shoot dry and fresh weight (Table 3). However, lower shoot dry and fresh weights were obtained at higher salinity levels. The effect of salinity levels on the internodes number did not show a clear trend. The salinity levels of irrigation water had no effect on stem length and upper and bottom stem diameter. Longer stems were obtained at salinity levels of 0, 30 and 45 mM. At the highest salinity the minimum

Table 2: Leaf characteristics of GF_{677} rootstock at various water salinity levels

Leaf characteristics	Salinity levels ($mmol\ L^{-1}$) 0	15	30	45	60	75
Chlorophyll ($mg\ g^{-1}$)	8.23a*	9.57a	5.56a	7.33a	9.21a	6.05a
Leaf area (cm^{-2})	12.99a	11.69a	11.72a	10.13a	9.59a	8.36a

*Means followed by the same letter(s) in each row are not significantly different at $p = 0.05$

Table 3: Vegetative growth responses of GF_{677} rootstock at various water salinity levels

Growth responses	Salinity levels ($mmol\ L^{-1}$) 0	15	30	45	60	75
Shoot fresh weight (g)	7.33a*	5.96a	6.70a	5.13a	5.300a	3.16a
Shoot dry weight (g)	2.66a	2.23a	2.70a	2.16a	2.03a	1.20a
Number of internodes	24.33a	20.00a	25.33a	20.33a	21.50a	18.00a
Length of stem (cm)	39.00a	24.33a	31.67a	29.00a	23.00a	21.67a
Bottom diameter of stem (cm)	0.27a	0.28a	0.28a	0.25a	0.23a	0.22a
Top diameter of stem (cm)	0.16b	0.22a	0.19ab	0.18ab	0.16b	0.16b
The ratio of top diameter to bottom diameter of stem (cm)	0.60a	0.78a	0.70a	0.73a	0.72a	0.72a

*Means followed by the same letter(s) in each row are not significantly different at $p = 0.05$

Table 4: Effects of water salinity levels on Cl^-, Na^+ and K^+ concentrations in shoot of GF_{677} rootstock

Concentrations	Salinity levels ($mmol\ L^{-1}$) 0	15	30	45	60	75
Cl^- ($mg\ g^{-1}$)	2.25b*	1.42b	2.25b	3.67b	11.13a	14.05a
K^+ ($\mu g\ g^{-1}$)	11750a	10830a	10670a	8167a	9667a	9354a
Na^+ ($\mu g\ g^{-1}$)	441.7b	466.7b	458.3b	475b	600b	2325a
$K^+:Na^+$	31.05a	24.79ab	24.64ab	19.02ab	19.72ab	4.39b

*Means followed by the same letter(s) in each row are not significantly different at $p = 0.05$

length of stem was recorded. The ratios of upper diameter to the lower diameter of stem are shown in Table 3. The salinity levels of the water had no effect on this ratio.

The Na^+ concentration of the shoots of GF_{677} rootstock increased significantly only at the highest salinity level. Mean shoot K^+ concentration decreased by increasing the salinity levels, but it was not significant. Mean shoot Cl^- concentration increased significantly with NaCl application (Table 4). The shoot Cl^- concentration was higher than that of Na^+ at each salinity level. The shoot Na^+ concentrations were excessively less than Cl^- concentration at the highest salinity.

Roots of GF_{677} under various salinity levels in comparison with control had lower Na^+ content. The Na^+ content of the roots was considerably lower than the Cl^- content. The root Cl^- concentration decreased

Table 5: Effects of water salinity levels on Cl^-, Na^+ and K^+ Concentrations in root of GF_{677} rootstock

Concentrations	Salinity levels ($mmol\ L^{-1}$) 0	15	30	45	60	75
Cl^- ($mg\ g^{-1}$)	20.41a*	9.47b	8.87b	8.88b	11.24b	13.61ab
K^+ ($\mu g\ g^{-1}$)	1983a	1483a	1317a	1067a	2600a	1017a
Na^+ ($\mu g\ g^{-1}$)	1150a	783.3ab	966.7ab	633.3b	900ab	683.3ab
$K^+:Na^+$	1.68a	1.95a	1.38a	1.63a	2.68a	1.43a

*Means followed by the same letter(s) in each row are not significantly different at $p = 0.05$

Table 6: Effects of water salinity levels on total concentration of Cl^-, Na^+ and K^+ in shoot and root of GF_{677} rootstock

Concentrations	Salinity levels ($mmol\ L^{-1}$) 0	15	30	45	60	75
Cl^- ($mg\ g^{-1}$)	22.66a*	10.30b	11.13b	14.91a	24.73a	23.52a
K^+ ($\mu g\ g^{-1}$)	13730a	12320a	11980a	9233a	12270a	10370a
Na^+ ($\mu g\ g^{-1}$)	1592b	1250a	1425a	1108b	1500b	3008a
$K^+:Na^+$	8.61a	9.83a	8.65a	7.93a	8.83a	3.55b

*Means followed by the same letter(s) in each row are not significantly different at $p = 0.05$

significantly by increasing the salinity levels (except at 60 mM NaCl) (Table 5). The effect of salinity levels on the root. K^+ content was not significant, but with increasing the salinity levels, K^+ concentrations of roots were reduced. (Except at 60 mM NaCl) (Table 5).

The total concentration of Cl^-, K^+ and Na^+ of shoots and roots and the $K^+:Na^+$ ratios is shown in Table 6. The total $K^+:Na^+$ ratio shown significant reduction only at the highest salinity level (Table 6).

Sodium chloride caused a decrease in the total K^+ content; this reduction in uptake undoubtedly reflects competition between Na^+ and K^+ (Laties, 1969). However there was no significant change in the percentage of K^+ in the stem and root as a result of the salinity treatment (Table 4, 5). Potassium, unlike Na^+, was accumulated to a relatively lighter 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. Reduction of the $K^+:Na^+$ ratio at higher salinity levels is the reason for the higher accumulation of Na^+ in sensitive root stock. A wide $K^+:Na^+$ ratio is recommended as a sensible criterion of salt tolerance in higher plant species (Rao *et al*., 1981). Grieve and Walker (1983) 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.

As mentioned earlier, the shoot Na^+ concentrations were excessively less than Cl^- concentration at the highest salinity. This is in agreement with the findings of Maftoun *et al*. (1982) in soybean (*Glycin max* L. Merr) and Sepaskhah and Maftoun (1988) in pistachio. Lessani and Marschner (1978) 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*. (1974) 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. Naitsakis *et al.* (1997) suggested that GF_{677} is less tolerant to NaCl than almond cultivars like Truoito and equally tolerant to Ferragness. The possible explanation of this behavior is the higher uptake and/or transport of Cl^- and partially of sodium to tops. He also indicated that sodium concentration of GF_{677} was considerably lower in almond tops than Cl^-. Massai *et al*. (2004) showed that peach leaves grafted on GF_{677} were able to store most of the incoming salt in the basal (old) leaves, thus protecting the actively growing (apical) leaves from a massive accumulation of unwanted ions. Salt loading in to basal leaves, that can be eventually lost during an intense salinity treatment, may also contribute to increasing salt tolerance. Although the assimilation of carbon at whole plant level may decrease (Massai *et al*., 2004).

CONCLUSION

According to the above data, GF_{677} rootstock could tolerance NaCl stress to the extent of 60 mM and it is sensitive at higher concentration, because the significant reduction of K^+:Na^+ ratio was only obtained at 75 mM NaCl treatment.

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