Evaluation of NaCl Salinity Tolerance of Four Fig Genotypes Based on Vegetative Growth and Ion Content in Leaves, Shoots, and Roots

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Abstract. The effects of NaCl stress on some growth parameters and ion accumulation in roots, shoots, and leaves of four fig genotypes ($S \times P$, $S \times K$, $S \times Sh$, and $S \times D$) were investigated. Eight-month-old fig plants growing in a mixture of sand, leaf mold, and clay (1:1:1) were irrigated with solutions containing NaCl at various levels: 0.6 (S0), 4 (S1), 6 (S2), and 8 (S3) dS·m⁻¹. Salinity stress decreased growth parameters to a different extent in each genotype. Leaf water potential, stomatal conductance (g_s), leaf number, shoot height, and root fresh weight were significantly decreased by salinity; and among the four fig genotypes studied, $S \times P$ and $S \times K$ were the most sensitive and the most tolerant genotypes, respectively. Furthermore, the highest reduction in shoot diameter and shoot fresh and dry weight were observed in $S \times Sh$ and the lowest reduction in $S \times K$. Root dry weight decreased by increasing salinity, mainly in $S \times D$. At S1 salinity treatment in both $S \times Sh$ and $S \times D$ genotypes, Na⁺ ion concentration was higher in leaves than in roots, but this pattern was not evident in $S \times P$ and $S \times K$ genotypes in NaCl treatments below S2 and S3, respectively. Chloride concentrations in all organs increased and were higher in roots than in both leaves and shoots, except in $S \times D$ genotype that accumulated more Cl⁻ ion in leaves than in roots at S2 and S3 levels. These results indicate that the ability to sequester Na⁺ and Cl⁻ ions in roots differs among the genotypes used in this study. Overall, results indicated that salinity tolerance in fig tree is strongly associated with Na⁺ and Cl⁻ ions exclusion mechanism from shoots. Moreover, to our surprise, salinity stress considerably increased K⁺ ion concentration in leaves and shoots of salt-sensitive genotypes. Our proposed explanation is that the inability of salt-sensitive fig genotypes to prevent delivery of hazardous ions to shoot is compensated by tissue tolerance mechanism. Keeping high cytosolic K⁺ ion may lead to better sequestration of Na⁺ ion in vacuoles and, therefore, enable the genotypes with poor Na⁺ exclusion mechanism to handle large amounts of Na⁺ ion in leaves. Finally, $S \times K$ is the most salt-tolerant genotype due to efficient exclusion of Na⁺ and Cl⁻ ions and lower reduction in growth factors.

Recently, archaeobotanical evidences have demonstrated that the fig tree is the first domesticated plant (Kislev et al., 2006). Although, figs may now be found in most warm and temperate climates, the tree has originated in Persia, Asia Minor, and Syria and has been spread throughout the Mediterranean region (Ferguson et al., 1990). Iran is ranked as the fifth fig producer in

the world and the third largest exporter of dried figs (FAO, 2014). Ninety percent of Iranian dried fig is produced in Fars Province. ‘Sabz’ is the major cultivar in Fars and about 98% of cultivated trees are of this type. ‘Sabz’ belongs to the smyrna figs, requiring caprification for fruit development (Rahemi and Jafari, 2008). Traditionally, fig trees are propagated by hardwood cuttings and have a fibrous root system, which is very shallow (Flaishman et al., 2008). After several successive drought years in Iran, salinity has appeared as a problem to local fig cultivation, especially in some regions of Fars. Salinity problems are most common in arid and semiarid areas where annual precipitation is less

than annual evapotranspiration (Ferguson and Grattan, 2005). Salinity severely restricts the growth and production of most fruit trees (Gucci and Tattini, 1997) such as fig. Fig trees are moderately salt tolerant (Golombek and Lüdders, 1990), but commonly encountered salinity levels reduce the number and length of new shoots on fig trees (Qrunfleh et al., 2013). In woody species, different cultivars and rootstocks within a specific species may considerably vary in salt tolerance. Mechanisms of salt tolerance that might account for these differences include the ability to selectively avoid uptake of Cl⁻ and Na⁺ by roots (so-called root exclusion), preventing Na⁺ from being loaded into xylem, removing it from upper organs, Na⁺ sequestration, potassium retention in the cytosol, osmotic adjustment, and enzymatic and nonenzymatic detoxification of reactive oxygen species (ROS) (Shabala and Munns, 2012). Therefore, for crops cultivated in areas at risk of salinization, selection of amplified salt tolerance genotypes is required. This involves finding new genetic sources of salt tolerance and identifying salt-tolerant germplasms.

Obligatory outcrossing in smyrna figs can be considered as a source of profiting from genetic variability in the caprifig genotypes, consuming for caprification, especially to screen salinity-resistant rootstocks. Indeed, given the fact that seedlings have a deep tap root system, (in comparison with plants grown from cuttings), and that cultivation of deep-rooted perennial plants can be a solution to limit salinization (Munns et al., 2002), seedling rootstocks of figs might be particularly advantageous. However, little literature has provided information on the salinity tolerance of fig trees. To the best of our knowledge, the first short-term fig salinity tolerance research was conducted on two varieties, Bardajik and Faro, using cuttings grown in nutrient solution (Golombek and Lüdders, 1990). There was a negative correlation between leaf Na⁺ and Cl⁻ contents and net photosynthesis; moreover, transpiration rate reduced as a result of diminished g_s . Metwali et al. (2014) investigated the effects of different levels of NaCl salinity stress on in vitro cultivation of three fig cultivars, viz Black Mission, Brown Turkey, and Brunswick. They determined Na⁺, K⁺, Fe²⁺, and Zn²⁺ concentrations in leaves and concluded that salinity decreased K⁺, Fe²⁺, and Zn²⁺ ion contents and unsurprisingly increased Na⁺. Further, they reported that salinity decreased shoot length, number of shoots per explants, fresh weight, dry weight, and chlorophyll content while proline increased. Vegetative growth of two cultivars of fig seedlings, Royal and Brown Turkey, produced from tissue culture, grown in a mixture of sand and clay and subjected to NaCl salinity stress was investigated (Alswalmeh et al., 2015). The results indicated that salinity decreased plant diameter, leaf area, root length, root fresh and dry weight, chlorophyll a and b, and total chlorophyll; meanwhile, seedling height, leaf number, and free proline content did not change. AbdoliNejad and Shekafandeh (2014) evaluated salt tolerance of seedlings of

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two open-pollinated fig cultivars, namely Anjir Sabz and Shah Anjir, raised in Murashige and Skoog medium. They observed that salinity increased Na^+ and Cl^- concentrations and depressed K^+ in both roots and shoots. Their data showed that concentration of Cl^- in leaves was higher than in roots while in terms of Na^+ the two cultivars showed different trends. Thus with the exception of AbdoliNejad and Shekafandeh (2014), none of the aforementioned researches have tried to address overall salinity tolerance mechanisms or tissue tolerance in their experiments. None of the previous researchers have attempted to screen fig seedlings (grown in the soil as their natural habitat) from known parents for salinity tolerance, with a view to finding promising rootstocks with specified parents. Therefore, we designed this study with the 2-fold aim of filling gaps in the published knowledge about physiological mechanisms of salinity tolerance in fig, while simultaneously screening offspring of variety Sabz and four caprifig varieties for salinity tolerance.

Materials and Methods

Plant material and salinity treatments.

Four new genotypes (Table 1) were produced by crossing the female cultivar Sabz (*Ficus carica* L.) at Estahban Fig Research Station, with four caprifigs from Fars Province, namely 'Khormaei', 'Pouz Donbali', 'Dane Sepid', and 'Shahanjiri'. The offspring of these crosses will be referred to as S × K, S × P, S × D, and S × Sh, respectively. The seeds were sown in a sand medium in a greenhouse where average day/night temperature was $30 \pm 2/21 \pm 2$ °C with relative humidity of 50%. When seedlings reached the three to four leaf stage, they were transplanted into 10-L plastic pots containing 8 kg (air-dried weight) of a sterilized potting mix composed of sand, leaf mold, and clay (1:1:1). In addition, the pots had a layer of 0.5 kg gravel at the bottom, for drainage. Some of the physicochemical properties of the media are shown in Table 2. The seedlings were irrigated with tap water until commencement of salt treatment and pruned to a single shoot.

Table 1. Abbreviation of new genotypes produced by crossing.

Genotypes		Abbreviations
♀	♂	
<i>Ficus carica</i> cv. Sabz ×		S × P
<i>F. carica</i> cv. Pouz Donbali		
<i>F. carica</i> cv. Sabz ×		S × K
<i>F. carica</i> cv. Khormaei		
<i>F. carica</i> cv. Sabz ×		S × Sh
<i>F. carica</i> cv. Shahanjiri		
<i>F. carica</i> cv. Sabz ×		S × D
<i>F. carica</i> cv. Daneh sepid		

Table 2. Some of physicochemical characteristics of potting soil.

Sand (%)	Silt (%)	Clay (%)	pH of saturation paste	Electrical conductivity ($\text{dS}\cdot\text{m}^{-1}$)	Organic carbon (%)	Na ($\text{meq}\cdot\text{L}^{-1}$)	Ca ($\text{meq}\cdot\text{L}^{-1}$)	Mg ($\text{meq}\cdot\text{L}^{-1}$)	Fe ($\text{meq}\cdot\text{L}^{-1}$)	Zn ($\text{meq}\cdot\text{L}^{-1}$)	N (%)
65.4	22	12.6	6.97	1.45	2.13	0.69	8.5	9	7.5	3.5	0.18

Salinity treatment was started when the seedlings were 8 months old and were conducted in the Shiraz University greenhouse (Bajgah, Shiraz, Iran, $29^{\circ}36'\text{N}$, $52^{\circ}32'\text{E}$). Sodium chloride treatment solutions (designated S0, S1, S2, and S3) containing 0.6 (tap water only), 4, 6, and 8 $\text{dS}\cdot\text{m}^{-1}$, respectively, were applied for irrigation from 3 Apr. to 13 May, 2015. Before commencing salt treatments, pots were irrigated with tap water to field capacity. Sodium chloride (Merck, Darmstadt, Germany) for the treatments was dissolved in tap water to achieve the desired conductivity. To avoid osmotic shock to the plants, treatments were applied incrementally, increasing by 1 $\text{dS}\cdot\text{m}^{-1}$ every 2 d, over a 2-week period, to achieve the highest level of salinity. Irrigation treatments were at 2-d intervals. For each irrigation, the soil moisture content was increased to field capacity level by weighing the pots. To prevent the salt accumulation in the pots, 20% more saline water was added to the irrigation water as a leaching requirement (Yarimi and Sepaskhah, 2015). The experiment was conducted on a 4×4 factorial basis in a completely randomized design with 10 replications, and one seedling in each replicate.

Growth parameters. At the beginning and end of the experiment period, the number of leaves, height of the stem (cm), and stem diameter (mm) were measured, results were reported as the difference in the last and first measurements. Stem diameter was measured at 4 cm above the soil surface using a Vernier caliper, and the stem height was measured also from 4 cm above the soil, to the highest point of the plants.

Fresh and dry weight. At the completion of the salinity treatment period, leaves were removed and plants were cut ≈ 1 cm above the soil surface and divided into stem and root. Roots and stems were rinsed in distilled water to be free of soil. Fresh weight of roots and stems was measured. Roots and stems were placed in separate envelopes and dried in an oven at 70 °C for 2 d and then the dry weight was measured on a digital scale with an accuracy of 0.01 g. In preparation for ion analysis, the harvested leaves were rinsed in distilled water to remove saline treatment solution that might have been splashed during irrigation and were dried as it was mentioned above.

Ion analysis. After weighing, the dried stems and roots were chopped into small pieces and ground to a fine powder. The dried leaves were also similarly ground. Sodium and potassium were determined in 1 g of powdered samples ashed at 550 °C for 8 h, digested in hot 2 M hydrochloric acid, filtered, and made up to 50 mL volume for analysis with a flame emission photometer (JENWAY, Ltd, UK) (Chapman and Pratt, 1982). Concentration of Cl^- was determined by colorimetry (ferricyanide method) (Munns et al., 2010).

Stomatal conductance. At 35 d after commencing treatments, g_s was measured on the first fully expanded leaves with a leaf porometer (SC-1; Decagon Devices Inc.). Measurements were taken between 10–12 and 14–15 h. Four replicates, for each treatment were measured.

Leaf water potential. The third fully expanded leaf on each plant was used to measure midday leaf water potential (ψ). After cutting a leaf with a sharp blade, it was put in the chamber of a pressure bomb (PMS Instrument Company, Corvallis, OR) and as soon as xylem sap came out data were taken. About 1 h before measurement, the selected leaf was enclosed in a plastic bag and covered with aluminum foil to equilibrate its xylem water potential with that in the stem. Measurements were taken on four randomly selected plants out of 10 plants for each treatment.

Statistical analysis. Data analysis was conducted using analysis of variance (ANOVA) procedure. The comparison of means and correlations were tested with least significant difference and Pearson's correlation, respectively. The SAS software version 9.00 was used for all of analysis. To calculate EC50 values, the electrical conductivity causing 50% decrease in the plant growth parameters (shoot height and diameter, shoot fresh and dry weight, and root fresh and dry weight) compared with control treatment, concentration–response curves were graphed using Excel. The EC50 of each curve was calculated by a noncomputational technique (Alexander et al., 1999).

Results

Leaf number. Salinity stress significantly ($P \leq 0.01$) reduced the number of leaves in all genotypes. The effect of salinity on this parameter showed a significant genotypic variation. Leaf number was progressively reduced by increasing salinity levels in S × P and S × D genotypes, whereas in S × K and S × Sh genotypes there were not any significant differences between leaf abscission at S1, S2, and S3 salinity levels and at S1 and S2, respectively (Table 3). In all genotypes, the highest decline in leaf number was observed under the highest level of salinity (S3). At this treatment, however, the genotypes S × P (20-fold reduction from 10.44 to -9.77), and S × K (2-fold reduction from 9.55 to 4.89) displayed the highest and lowest decline, respectively. Decreased leaf numbers per plant were negatively correlated with increased Na^+ and Cl^- concentrations in the leaves ($r = -0.71^{***}$ and $r = -0.63^{***}$, respectively) (Fig. 1A and B).

Shoot height. After 40-d salinity treatment, shoot height of control plants significantly increased and the growth acceleration was more pronounced in S × K and S × D

Table 3. Effect of NaCl salinity on leaf number; shoot height, diameter, fresh and dry weight; and root fresh and dry weight of four fig genotypes 40 d after salinity treatment.

NaCl (dS·m ⁻¹)	Leaf number	Shoot ht (cm)	Shoot diam (mm)	Shoot fresh wt (g)	Shoot dry wt (g)	Root fresh wt (g)	Root dry wt (g)
S × P							
0.6	10.44 ± 0.3 ^a	23.76 ± 0.58 ^{bc}	4.26 ± 0.29 ^b	37.21 ± 1.67 ^{bc}	16.27 ± 0.46 ^a	84.52 ± 4.16 ^a	37.66 ± 1.20 ^a
4	5.11 ± 0.63 ^b	12.89 ± 0.48 ^{ghi}	1.58 ± 0.11 ^{fg}	29.33 ± 1.14 ^d	12.90 ± 0.82 ^{bc}	53.89 ± 2.03 ^{cd}	31.56 ± 1.68 ^b
6	0.67 ± 0.42 ^{fg}	13.72 ± 0.96 ^{gh}	1.76 ± 0.09 ^{ef}	29.00 ± 1.39 ^d	12.67 ± 0.77 ^{bc}	52.36 ± 2.02 ^d	25.88 ± 1.09 ^c
8	-9.78 ± 0.89 ⁱ	12.44 ± 0.61 ^{ghi}	1.69 ± 0.09 ^{fg}	22.03 ± 0.92 ^{fg}	9.55 ± 0.39 ^{ef}	36.74 ± 1.71 ^{fg}	20.17 ± 0.91 ^{ef}
S × K							
0.6	9.55 ± 1.06 ^a	36.29 ± 3.10 ^a	5.20 ± 0.12 ^a	38.58 ± 2.03 ^{ab}	16.93 ± 0.96 ^a	58.43 ± 2.01 ^c	21.54 ± 0.98 ^{de}
4	6.55 ± 0.44 ^b	22.67 ± 1.33 ^{bc}	3.11 ± 0.17 ^c	29.85 ± 1.34 ^d	13.33 ± 0.79 ^{bc}	43.47 ± 1.58 ^e	17.32 ± 0.84 ^f
6	4.55 ± 0.53 ^{bcd}	21.33 ± 1.12 ^{cd}	2.12 ± 0.05 ^{de}	28.62 ± 1.79 ^d	13.38 ± 0.64 ^{bc}	35.24 ± 1.08 ^{fg}	13.47 ± 0.62 ^g
8	4.89 ± 1.20 ^{bcd}	18.05 ± 0.75 ^{ef}	1.73 ± 0.10 ^{fg}	28.16 ± 0.81 ^{de}	11.94 ± 0.73 ^{cd}	34.27 ± 1.57 ^{gh}	12.43 ± 0.52 ^g
S × Sh							
0.6	10.56 ± 0.4 ^a	25.22 ± 0.65 ^b	4.16 ± 0.15 ^b	35.13 ± 1.11 ^c	13.61 ± 0.19 ^b	66.34 ± 2.73 ^b	23.36 ± 1.42 ^{cd}
4	3.56 ± 1.38 ^{de}	13.33 ± 0.47 ^{gh}	3.18 ± 0.14 ^c	19.18 ± 0.56 ^{gh}	9.18 ± 0.41 ^{fg}	53.79 ± 1.79 ^{cd}	17.24 ± 0.65 ^f
6	2.56 ± 0.63 ^{def}	11.44 ± 0.55 ^{hi}	2.29 ± 0.17 ^d	15.13 ± 0.72 ⁱ	7.17 ± 0.32 ^h	38.69 ± 1.64 ^{efg}	12.82 ± 0.80 ^g
8	-1.11 ± 0.72 ^g	10.00 ± 0.32 ⁱ	1.37 ± 0.08 ^g	17.89 ± 0.70 ^{hi}	7.62 ± 0.40 ^{gh}	38.97 ± 1.25 ^{efg}	11.96 ± 0.39 ^g
S × D							
0.6	9.67 ± 0.55 ^a	34.28 ± 1.65 ^a	3.93 ± 0.15 ^b	41.17 ± 0.40 ^a	16.96 ± 0.43 ^a	59.32 ± 1.59 ^c	33.29 ± 1.31 ^b
4	5.33 ± 0.67 ^b	19.39 ± 0.90 ^{de}	1.92 ± 0.11 ^{def}	35.29 ± 1.83 ^{bc}	13.60 ± 0.25 ^b	40.24 ± 1.93 ^{ef}	24.04 ± 1.60 ^{cd}
6	1.56 ± 0.78 ^c	15.06 ± 0.92 ^{fg}	1.71 ± 0.11 ^{fg}	27.71 ± 0.77 ^{de}	13.93 ± 0.43 ^b	37.34 ± 2.00 ^{fg}	20.40 ± 1.03 ^e
8	-5.44 ± 1.46 ^h	14.11 ± 0.96 ^{gh}	1.93 ± 0.09 ^{def}	25.19 ± 0.78 ^{ef}	10.79 ± 0.26 ^{de}	29.42 ± 1.51 ^h	13.77 ± 0.69 ^g

Data (mean ± SE) in the same column followed by the same letter have no significant differences.

plants (Table 3). This growth spurt contrasted with a significant decrease ($P \leq 0.001$) of shoot height correlating with increasing salinity levels. Altogether, EC50 values showed that S × D and S × Sh presented more susceptibility to the salinity stress (Table 4), whereas the lowest shoot height was obtained in S × P and S × Sh ($P \leq 0.001$). The highest reduction of shoot height in comparison with control was observed in the S3 treatment in genotype S × Sh (64%). The correlation between shoot height and shoot sodium and chloride content was investigated. Reduction of shoot height was negatively correlated with shoot Na⁺ and Cl⁻ concentrations ($r = -0.59^{***}$ and $r = -0.72^{***}$, respectively) (Fig. 1C and D).

Shoot diameter. Salinity treatments significantly reduced shoot diameter in all genotypes. At both S1 and S2 salinity levels, shoot diameter reduction was significantly ($P \leq 0.001$) higher in S × P and S × D genotypes (Table 3). Furthermore, among the four fig genotypes, S × D and S × P had the lowest EC50 values (lower than 1.30 dS·m⁻¹) and showed the highest sensitivity to salinity stress (Table 4). At the highest salinity level (S3), shoot diameter was severely reduced and reached 72.8% of S0 in the S × Sh genotype (Table 3). S × K plants had the highest shoot diameter and were especially markedly vigorous under control condition.

Shoot fresh and dry weight. Under non-saline conditions, the highest shoot fresh weight was observed in S × D and S × K and the lowest in S × Sh genotypes. All salt treatments significantly decreased shoot fresh weight in all four genotypes ($P \leq 0.001$) (Table 3). At the highest salinity level, shoot growth of S × Sh plants decreased about to half of that in the control (from 35.13 to 17.89 g), although the exact EC50 values for all genotypes were over 8 dS·m⁻¹ (Table 4). The lowest decline in shoot fresh weight belonged to S × K genotype with 27% reduction in fresh growth as compared with S0 (from

38.58 to 28.16 g). There was a negative correlation between shoot fresh weight and shoot concentrations of Na⁺ ($r = -0.62^{***}$) and Cl⁻ ($r = -0.71^{***}$) (Fig. 1E and F). Salinity stress significantly reduced shoot dry weight in comparison with control in all genotypes (Table 3). A high positive correlation was observed between shoot dry weight and shoot fresh weight ($r = 0.79^{***}$). However, there were not any significant differences in shoot dry weight between S2 and S3 salinity treatments in any genotypes. Altogether, at S3 salinity treatment, the highest and the lowest reduction in shoot dry weights were found in S × Sh and S × K genotypes, with 44% (from 13.61 to 7.62 g) and 29% (from 16.93 to 11.94 g) decline, respectively. The calculated EC50 values for effect of salinity stress on dry weight of four fig genotypes were over 8 dS·m⁻¹ (Table 4).

Root fresh and dry weight. Root fresh weight was significantly reduced by salinity in the four studied genotypes. This reduction displayed a significant genotypic variation, although there were also significant differences among control plants of different genotypes (Table 3). At the highest level of salinity (S3), root fresh weight reduced and the lowest reduction reached by 41% for S × K and S × Sh and the highest by 56% for S × P with respect to control plants. However, under control condition, the highest root fresh weight belonged to S × P and S × D, and the lowest was in S × K genotypes. With regard to EC50, root fresh weight of S × D and S × P genotypes presented more sensitivity to salinity stress with the values of 6.71 and 6.06 dS·m⁻¹, whereas this was over 8 dS·m⁻¹ in S × K and S × Sh (Table 4). Root fresh weight was negatively correlated with sodium chloride concentration in the roots ($r = -0.77^{***}$) (Fig. 1G). Dry weight of roots was also affected by salinity level in all genotypes tested (Table 3). Salinity treatments had no severe effects on root dry weight in S × K and S × Sh plants. The lowest reduction in root

dry weight reached by 42% of control in the S × K genotype and the highest reduction (59%) was observed in the S × D genotype. Moreover, among the four investigated fig genotypes, only S × D genotype showed EC50 lower than 8 dS·m⁻¹. Root dry weight was negatively correlated with sodium chloride concentration ($r = -0.68^{***}$) and positively to the root fresh weight ($r = 0.74^{***}$) (Fig. 1H–J).

Leaf water potential. Results presented in Fig. 2 show that as salinity increased leaf water potential significantly decreased and it was more obvious under S3. Mean value of Ψ parameter decreased in S × P, S × D, S × Sh, and S × K genotypes from -0.76 to -1.46 MPa (92% decline), -0.93 to -1.76 MPa (89% decline), -0.96 to -1.80 MPa (87% decline), and -0.66 to -1.17 MPa (77% decline), respectively. Thus concerning the changes of leaf water potential, S × K and S × P genotypes showed the lowest and highest reductions and consequently were the most and the least salt-tolerant genotypes, respectively.

Stomatal conductance. G_s of all genotypes under S0 was higher than in other treatments. Salinity reduced g_s from 261.9 to 43.23; 289.4 to 97.87; 212 to 61.76; and 260.96 to 67.6 mmol (H₂O) m⁻²·S⁻¹ in S × P, S × K, S × Sh, and S × D genotypes, respectively. Altogether, S × K and S × P showed the lowest and highest alteration of g_s , respectively (Fig. 2).

Sodium (Na⁺). ANOVA revealed that Na⁺ and Cl⁻ concentrations in plant organs progressively increased with increasing salinity levels. Sodium concentration followed different trends in different plant parts. In the control treatment (S0), all genotypes had the same concentration of leaf Na⁺, with the exception of S × D that in S0 had the highest Na⁺ content in this study. Under S2 and S3 salinity treatments, S × K accumulated less Na⁺ in the leaves than any other genotypes did (Fig. 3A). Moreover, S × K plants had less

Table 4. EC50 values of plant growth factors.

Growth factor	EC50			
	S × P	S × K	S × Sh	S × D
Shoot height	>8	6.057	5.24	4.98
Shoot diameter	1.29	4.97	7.54	0.75
Shoot fresh weight	>8	>8	>8	>8
Shoot dry weight	>8	>8	>8	>8
Root fresh weight	6.71	>8	>8	6.06
Root dry weight	>8	>8	>8	6.87

All concentrations are in dS·m⁻¹.

(Fig. 4). Under S3 treatment, the accumulation of Na⁺ in leaves of S × K genotype was 8% higher than roots, whereas it was 25%, 12%, and 31% higher for S × P, S × Sh, and S × D, respectively (Fig. 4A). According to the variations between genotypes regarding accumulation and transport of Na⁺ in different organs, the studied genotypes can be divided into three groups. The first group contains only S × Sh, which was not able to inhibit accumulation of Na⁺ in leaves even at the lower level of salinity. The second group includes S × P and S × D, which at the low level of salinity treatment, accumulated more Na⁺ in the roots than in the leaves, but at middle level of salinity (S2) accumulated more Na⁺ in the leaves than roots. The third group contains S × K, which accumulated more Na⁺ in the roots and less transported to the leaves.

Potassium (K⁺). Like Na⁺ the leaf K⁺ concentration generally increased with the salinity treatment, but not as consistently as Na⁺, and not in the same proportion (Fig. 3A). Salinity at S2 and S3 levels significantly increased K⁺ concentration in the leaves of S × P, S × Sh, and S × D genotypes and at S3 level in S × K genotype (Fig. 3A). K⁺ concentration in leaves was significantly and positively correlated with Na⁺ concentration in the leaves ($r = 0.71^{***}$) (Fig. 1K). Moreover, salinity at the highest level (S3) significantly increased shoot K⁺ concentrations in S × P, S × K, and S × D genotypes ($P \leq 0.001$) (Fig. 3B). Salinity treatments resulted in decreased K⁺ content in the roots of all genotypes in comparison with control ($P \leq 0.001$) (Fig. 3C). Contrarily, increasing salinity levels from S1 to S3 led to the continuous increase in root K⁺ concentrations in all genotypes, except in S × P and S × K that did not display any significant differences between S1 and S2. Overall, there was a negative correlation between K⁺ and Na⁺ concentrations in the roots ($r = -0.58^{***}$) (Fig. 1L).

Dissecting the trend of K⁺ concentration changes in different organs of different fig genotypes revealed that as soon as salinity treatments started a pronounced increase of potassium concentration in the leaves was noticed in four genotypes (Fig. 4C), whereas exactly the opposite tendency, decrease of K⁺ concentration, occurred in the roots. Thus, at every level of salinity in which K⁺ concentration in leaves reached to its highest or lowest level, in contrast, K⁺ concentration in roots attained its lowest or highest level. The trend for potassium in shoots generally resembled the trend in leaves, with the most

Fig. 1. Relationship between leaf number and leaf Na⁺ (A) and leaf Cl⁻ (B) concentration, shoot height and shoot Na⁺ (C) and shoot Cl⁻ (D) concentration, shoot fresh weight and shoot Na⁺ (E) and shoot Cl⁻ (F) concentration, root fresh weight and root Na⁺ concentration (G), root dry weight and root Na⁺ (H) and root Cl⁻ (I) concentration, root dry and fresh weight (J), leaf K⁺ and Na⁺ concentration (K), root K⁺ and Na⁺ concentration (L), leaf Cl⁻ and Na⁺ concentration (M) and root Cl⁻ and Na⁺ concentration (N) of four fig genotypes. The correlation coefficient (r) values were obtained using Pearson's correlation.

Na⁺ concentration in both shoots and roots under S3 salinity treatment (Fig. 3B and C). Low level of salinity (S1) considerably increased Na⁺ concentration in roots contrasting with other plant parts in all genotypes,

except S × Sh that accumulated Na⁺ to the same extent in both shoots and leaves (Fig. 4). Only S × K plants did not show a trend of Na⁺ concentration increasing with treatment level in both leaves and roots

Fig. 2. Effect of salinity (NaCl) on (A) leaf water potential and (B) stomata conductance of four fig genotypes. Data are means $\pm$ SE of four replicates. Bars marked with the same letter were not significantly different at $P \leq 0.05$ level.

obvious exception being in the S $\times$ Sh genotypes (Fig. 4C).

Chloride (Cl^-). Salinity treatments increased Cl^- concentrations in fig tree organs. Under higher salinity treatments (S2 and S3) S $\times$ D had the highest concentration of Cl^- ion in the leaves (Fig. 3A), whereas in shoots, it had medium or low concentrations. The lowest Cl^- ion concentration for shoots was found in S $\times$ P or S $\times$ K, depending on the salinity level (Fig. 3B). The highest and the lowest concentrations of Cl^- in the shoots under S3 were observed in S $\times$ Sh and S $\times$ K plants, respectively. Chloride concentration in roots was significantly higher than in leaves and shoots of all four fig genotypes and increased progressively with increasing NaCl concentration in irrigation water (Fig. 4B). There was a significant difference between genotypes regarding accumulation of Cl^- ion in the roots and the highest concentration was found in the S $\times$ K genotype ($P \leq 0.001$) (Fig. 3C). Increases of Na^+ and Cl^- ions were significantly correlated. There were considerable positive correlations between Na^+ and Cl^- in leaves ($r = 0.95^{***}$) (Fig. 1M), shoots ($r = 0.92^{***}$), and in roots ($r = 0.93^{***}$) (Fig. 1N).

Discussion

Growth parameters. The effects of salinity treatments at all levels on the reduction of leaf numbers were notable and their magnitude became severe under S3 treatment, at which level S $\times$ K genotype had more leaves than other genotypes. Reduced leaf number was negatively correlated with Na^+ and Cl^- leaf concentrations. Decrease in leaf number under salinity is known from previous investigations (Ferguson et al., 2002; Incesu et al., 2014; Munns, 2002) and is due to increased leaf mortality before the senescence phase and precocious senescence (Yeo et al., 1991). Inordinate buildup of salts in cell walls and cytoplasm of transpiring leaves leads to cell shrinkage and dehydration, inhibition of enzymatic activities, leaf injury, premature senescence, and eventually leaf abscission (Munns, 2002).

For horticultural crops, the rate of biomass production can be used for the assessment of salinity tolerance (Munns, 2002). In our investigation under control condition, shoot and root fresh weight, shoot diameter, and height of the four fig genotypes were different. The observed variations among

genotypes may be associated with growth characteristics of new genotypes and different biomass partitioning among plant organs. Our results revealed that S $\times$ K genotype, with the lowest root dry weight and the highest shoot dry weight, portioned more biomass in shoots than roots. Fresh and dry weight of all four fig genotypes (shoots and roots) were reduced by increasing salinity, depending on salinity level and genotype, and the observed alterations were negatively correlated with excessive augmentation of sodium and chloride in both organs. In most cases, when salinity reached to 8 $\text{dS}\cdot\text{m}^{-1}$, the lowest shoot and root biomass reductions were noticed in S $\times$ K (29% and 42%, respectively) and the highest of those were found in S $\times$ Sh and S $\times$ D (44% and 58%, respectively). Results obtained in this study were in agreement with previous investigations on other fruit trees or other fig cultivars, which have shown that all growth parameters decreased as salinity increased (Anjum, 2008; Ferguson et al., 2002; Kchaou et al., 2010; Khalil et al., 2011). In fact, the occurrence of growth reduction due to lower cell division or/and elongation in all plants under salinity is universal. Growth reduction is regarded as an adaptive characteristic, which redirects cell resources and energy into stress tolerance (Zhu, 2001), although tolerance levels and the rates of growth suppression differ greatly among plants species (Hasegawa et al., 2000). The initial impact of salinity on photosynthesis is stomatal closure (Lloyd et al., 1990). As our results demonstrated, salinity decreased g_s , which resulted in reduced photosynthetic efficiency of mesophyll. Golombek and Lüdders (1990) reported that in two fig clones, NaCl treatment up to 100 mM resulted in reduced g_s and net photosynthesis rate. According to their results, net photosynthesis rate negatively changed as a function of Na^+ and Cl^- content in fig leaves. That reduction primarily was caused by the decrease of non- g_s of CO_2 and fairly of reduced g_s . Furthermore, the accumulation of toxic amounts of salts in the older leaves of plants and subsequently their premature senescence lead to the decline of photosynthetic leaf area to a level which disturbs growth (Munns, 2002).

Although leaf water potential must decrease to maintain the osmotic gradient during salinity stress, decreased water potential can be an indication of inability to maintain leaf turgor and therefore growth (Maas, 1986). Reduction of leaf water potential and g_s under salinity stress has been reported by many investigators (Ahmad and Prasad, 2012). Movement of water into and through plants is the result of a gradient of decreasing water potential (i.e., a lower potential in the leaf than the soil). Elevation of salts in soil water decreases water potential in the soil and will restrict absorption of water by plants, consequently reducing growth. To overcome this restriction, plants must reduce their leaf water potential during salinity stress. Decrease of leaf water potential is due to a rapid osmotic adjustment and an elevation of the concentration of osmotically active solutes

Fig. 3. Effect of salinity (NaCl) on Na^+ (left), K^+ (middle), and Cl^- (right) concentration of (A) leaves, (B) shoots, and (C) Roots of four fig genotypes 40 d after treatment. Data are means $\pm$ SE. Bars marked with the same letters were not significantly different at $P \leq 0.05$ level.

Fig. 4. Effect of salinity on (A) sodium, (B) potassium, and (C) chloride concentrations in different plant tissues (roots, shoots, and leaves). Data are means $\pm$ SE for each plant organ. Bars marked with the same letters were not significantly different at $P \leq 0.05$ level.

(Koyro, 2006). Our results revealed that leaf water potential (ψ) of control plants was higher than salinity-treated ones. Our results are in agreement with Gucci et al. (1997) in their study on two olive (*Olea europaea*)

cultivars indicating that exposure to 200 mM salt concentration reduced water potential.

Further, Askri et al. (2012) reported that exposure of two wild grapevine (*Vitis vinifera* L. ssp. *Sylvestris*) accessions to salt stress

negatively affected their water potential while in the salt-tolerant accession, the water potential was less reduced. We similarly found that leaf water potential was reduced most in the genotypes that were clearly less

tolerant of salinity, and was reduced less in the genotype S × K that was most tolerant.

Considering all growth parameters together as pointers for salinity tolerance, genotype S × K, with the least salinity-induced reduction of fresh and dry weight in root and shoot, the highest leaf retention and the largest shoot diameter, also the least change in leaf water potential and g_s can be regarded as the genotype most tolerant to salinity.

Effect of salinity on ions accumulation.

Glycophytes adapt to saline environment by limiting saline ions (Na^+ and Cl^-) net absorption by root cells and/or reducing their loading into the xylem and delivery to the shoot (Munns, 2002). Many other mechanisms also play a critical role. This includes efficient osmotic adjustment (Munns and Tester, 2008), vacuolar Na^+ sequestration (Undurraga et al., 2012), Na^+ retrieval from the shoot (Davenport et al., 2007), cytosolic K^+ retention (Shabala and Pottosin, 2014; Shabala et al., 2016), and ROS detoxification (Shabala and Pottosin, 2014). The most tolerant plant species known as halophytes also possess some specific anatomical features, such as salt glands or bladders that help them with dealing with huge salt loads (Shabala et al., 2014). Specifically in figs, Golombek and Lüdders (1990) found a negative linear relationship between Cl^- and Na^+ contents of leaves and photosynthesis, which did not discriminate between the effects of Cl^- and Na^+ on photosynthesis. Our results revealed an increasing gradient in the transfer of Na^+ and Cl^- ions from roots to leaves, although a significant difference was observed among genotypes concerning amount of their uptake and translocation. At S1 salinity, the sodium exclusion ability in all genotypes except in S × Sh was effective in reducing toxic ion transport to aerial organs, although the most effectiveness of this mechanism was observed only in S × K genotype at S2 level, which became apparent as the accumulation of Na^+ in roots and the restriction of its transport to leaves. Concerning Cl^- , our results indicate that an effective shoot chloride exclusion mechanism was operating in all genotypes at all levels of salinity treatment, except in S × D at S2 and S3. However, S × K and S × Sh genotypes displayed particularly strong shoot chloride exclusion mechanisms because despite having higher chloride concentrations in the roots, they accumulated less chloride in their leaves than the S × P and S × D genotypes did. Therefore, it can be speculated that this difference is due either to abscisic acid down regulating anion channels, responsible for passive Cl^- loading into the xylem, or elevated active restoration of that ion from xylem (Munns and Tester, 2008).

The variability in the concentration of sodium and chloride among roots, shoots, and leaves and in their sequestration among investigated fig genotypes are further supported by results found by Kchaou et al. (2010) where they investigated the effects of 0.5, 50, 100, and 200 mM NaCl on five

olive cultivars. Similarly, they found that Na^+ and Cl^- concentrations in most cultivars were lower in leaves and shoots than in roots, although the effectiveness of sodium and chloride exclusion mechanism in the roots was diverse among cultivars. From our results, it is apparent that in fig tree control over the rate of transmission of damaging ions from roots to shoots (excluding from shoot) is cultivar dependent and it is assumed that the mechanism is localized in roots, as has been reported in olive (Chartzoulakis, 2005). However, in most fruit trees species such as pomegranate (Karimi and Hasanpour, 2014; Khayyat et al., 2014), pistachio (Ferguson et al., 2002), olive (Chartzoulakis, 2005; Kchaou et al., 2010), and citrus (Ruiz et al., 1997), there is a negative correlation between the accumulation of Na^+ and Cl^- in aerial parts and salinity tolerance. As shown in Fig. 2, there is a negative correlation between the accumulation of Na^+ and Cl^- in fig aerial parts and vegetative growth parameters as salinity tolerance indicators. Further, results showed that more salinity tolerance in S × K genotype is strongly associated with the genotype ability to deliver lower Na^+ to the aerial tissues. It is plausible that reduced Na^+ xylem loading or increased Na^+ retrieval from xylem or both of them play a role to increase fig salinity tolerance and this may be related with *Nax* loci. James et al. (2006) identified *Nax1* and *Nax 2* loci and their respective candidate genes, *HKT1;4* and *HKT1;5* as the transporters which in roots retrieve Na^+ from xylem and, therefore, decrease the rate of transported Na^+ to shoots. A further study conducted by Zhu et al. (2016) revealed that *Nax* loci confer salinity tolerance by two mechanisms, the enhancement of Na^+ retrieval and regulating the activity and the expression of *SOS1*-like exchanger in the xylem tissue, reducing the rate of Na^+ loading to xylem. The mechanism is activated under salinity treatment but not in the absence of NaCl. To our great surprise, salinity increased K^+ concentration in leaf and shoot of salinity-treated figs and this was more pronounced in salt-sensitive ones especially at higher levels of salinity while salinity decreased K^+ concentration in roots (Fig. 4). The coincidence of Na^+ and K^+ accumulation in leaf reflects that salt tolerance in fig may profit from another salinity tolerance mechanism. Inefficient Na^+ exclusion mechanism is compensated by retaining high cytosolic K^+ content. However, high concentration of cytosolic K^+ is necessary to stimulate tonoplast H^+ -PPase activity (Serrano et al., 2007), which activates the Na^+/H^+ exchanger tonoplast (Blumwald, 2000; Fukuda et al., 2004). In roots and leaves of many cultivars, efficient sequestration of Na^+ in vacuoles confers plants the ability to handle a large amount of salts. The sequestration of damaging ions into the vacuoles and retaining high K^+ ratio in the cytosol not only play a vital role in ion detoxification, but also provides the necessary osmotic force for water absorption (Shabala, 2013). Our results are in agreement with Wu et al. (2015) used

detached leaves to show that the inability of sensitive wheat varieties to exclude Na^+ ion from shoot is compensated by better K^+ retention and vacuole Na^+ sequestration. It is also worth mentioning that Golombek and Lüdders (1990) concluded that the relatively higher salinity tolerance of fig compared with grapevine, citrus, and 'Valencia' orange was not only due to Na^+ and Cl^- exclusion mechanism. Alleviation of K^+ concentration in tissues by salinity increment was reported in some researches on fig, olive, and citrus (AbdoliNejad and Shekafandeh, 2014; Chartzoulakis, 2005; Kchaou et al., 2010; Khalil et al., 2011). Sepaskhah and Maftoun (1982) in their study on pistachio reported that salinity decreased root K^+ concentration, whereas markedly increased K^+ in the leaf. Khayyat et al. (2014) indicated that stressing two cultivar of pomegranate significantly increased the potassium concentration of leaves, whereas the concentration of shoots did not change. Also, Zrig et al. (2016) reported that salinity only at higher level (75 mM NaCl) increased K^+ concentration of sweet almond leaves on two different rootstocks. Salinity induced elevation of K^+ in shoots in contrast with its reduction in roots indicates shoot selective mechanism of potassium absorption.

Concluding Remarks

Our results revealed that S × K was the most salt-tolerant genotype, which can be used successfully as fig rootstock because of its ability to effectively exclude Na^+ and Cl^- from leaves up to 6 dS·m⁻¹ salinity. It showed the lowest growth reduction under salinity. Even at 6 dS·m⁻¹, despite accumulating more Na^+ and Cl^- in leaves than roots, growth parameters were not reduced considerably. On the other hand, although S × D was not able to exclude Na^+ and Cl^- effectively from leaves, its growth reduction was low and it seems that this genotype effectively profits from a second mechanism, namely effective compartmentalization of hazardous ions in leaf vacuoles. To our surprise, salinity increased K^+ content in leaves and shoots, whereas decreased it in roots. It seems that fig genotypes, especially sensitive ones, profit from a tissue tolerance composed of increased leaf cytosol K^+ content and compartmentalization of hazardous ions in leaf vacuoles.

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