

## Impact of NaCl on leaf abscission, ion content and photosynthetic indices of seven commercial Fig (*Ficus carica*.L) cultivars

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### Abstract

Figs (*Ficus carica*.L) are woody perennial crop, cultivated worldwide in subtropical regions. Since, salinity is the main concern of fig industry, therefore, studying the pattern of ion absorption and accumulation during stress in different plant organs can lead to better understanding of mechanism of tolerance/sensitivity. So, during 2016-2017, seven fig cultivars ('Sabz', 'Siyah', 'Shah Anjir', 'Atabaki', 'Kashki', 'Mati' and 'Bar') were subjected to saline water (0.5, 2, 4, 6, 8 and 10 dSm<sup>-1</sup>). Then, ion accumulation of different organs, leaf abscission, dry matter, relative water content, and photosynthetic indices were compared to introduce the most salt-tolerant cultivar. The results indicated salinity caused a decrease in leaf relative water content, photosynthesis rate, K content and an increase in leaf abscission, Cl and Na content. Principal component analysis of the data led to a reduction in the variables with major contributions from the content of K, Na and Na/K of leaf and root, shoot Na, leaf Cl, and RWC. The 'Siyah' and 'Sabz', as the most salt-tolerant cultivars, had relatively high leaf abscission, the least Na content and the maximum shoot dry matter under salt condition. The 'Shah Anjir', as the most salt-sensitive cultivar, could not restrict root Cl ex-flux into shoot and leaf.

**Kew words:** Dry matter, Intercellular CO<sub>2</sub> concentration, Ion contents, Leaf abscission, Photosynthetic rate, Relative water content

### Introduction

With respect to the global drought and consequently increment of water salinity, it is necessary to use salt-tolerant cultivars. Fig (*Ficus carica*.L), belongs to the Moraceae family and the most important species of Ficus genus, is originated from the southwest Asia and the east of the Mediterranean (Frodin, 2004).

Cultivation of fig (dry and table cultivars) has reached nearly 31,9494 ha (Duenas *et al.*, 2008), with more than one million tons annual worldwide production. Turkey, Egypt, Algeria, Iran, and Morocco are the main fig producers. Iran is the fourth world producer of table fig (70,178 tons), and the third one for dried fig (FAO, 2016).

Around 607 fig cultivars have already described (Condit, 1955; Garcia-Ruiz *et al.*, 2013; Toribio. and Montes, 1996). The most marketable cultivars of Iran, whether for local markets or export, are included The 'Sabz' (or Verde, means green): belonging to the Smyrna type (El-Gharably, AM *et al.*, 2009); The

'Siyah' (means black): a high quality cultivar which is the common table fig cultivar (Condit, 1955); The 'Shah Anjir' (means king fig): with bright fruits and high level of Quercetin-3-glucoside level (Pourghayoumi *et al.*, 2016); The 'Atabaki': which significantly possesses more Catechin content; The 'Kashki' and The 'Mati' which contains high glucoside and total acid; and The 'Bar' is the common Caprifig used (Pourghayoumi *et al.*, 2016).

Fig, a salt moderate-tolerance crop (Golombek and Ludders, 1990), withstands the stress by reduction of gas exchanges, lessening the photosynthesis rate, and loss of vegetative characteristics and fruit quality (Essam *et al.*, 2013). The reports on some fig cultivars under salt conditions have shown a variation in morphological characteristics, growth parameters as well as physiological behavior (Essam *et al.*, 2013; Metwali *et al.*, 2014; Alswalmeh *et al.*, 2015; Zarei *et al.*, 2016, 2017; Soliman and Abd Alhady, 2017).

The mechanisms of salt-tolerance, is varied among

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woody plants. Osmotic effective regulation (Munns and Tester, 2008); exclusion of vacuole sodium, sodium uptake from the shoot (Tester and Davenport, 2003), and scavenging of reactive oxygen species (Shabala and Pottosin, 2014) are the main mechanisms. In addition, salt-tolerant plants usually have lower concentrations of sodium chloride in leaves (Munns, 2002), fewer Na/K (Chen *et al.*, 2005) and lower leaf area index (Munns and Tester, 2008).

In fruit crop species, saline water has been found to regulate a number of growth, biochemical and physiological mechanisms (Munns, 2002). The defend mechanisms may be dissimilar, not only among plant species, but also between the cultivars.

Since, salinity is the main concern of fig industry, therefore, studying the pattern of ion absorption and accumulation contribute during the stress in different plant organs, can lead to better understanding of the mechanism of tolerance/sensitivity (Ashraf and Ahmad, 2000), and assistance cultivar screening. The present study was aimed to investigate growth parameters, photosynthetic capacity and ion uptake of seven commercial salt-exposed Fig cultivars to understand the salt tolerant (or sensitivity) mechanism and to identify the main characteristics which distinguish tolerant and sensitive cultivars. So, during 2016-2017, Fig cultivars (six edible cultivars and a Caprifig) were subjected to saline water (six concentrations). Then, ion accumulation of different organs, leaf abscission, dry matter, relative water content, and photosynthetic indices were compared to introduce the most salt-tolerant cultivar.

## Material and methods

The present study was carried out in the plant breeding department, Faculty of Agriculture, University of Shiraz (36° 29' N and 32° 52' E) during 2016-2017.

**Plant materials:** The plant materials (20-year-old rootstocks) were included six edible Fig cultivars ('Sabz', 'Siyah', 'Shah Anjir', 'Atabaki', 'Kashki', 'Mati') and one Caprifig ('Bar'), which were located at Estahban Fig Research Station (36° 29' N and 32° 52' E). Hard-wood cuttings (20 cm in length and 1 cm in diameter) were collected from one-year-old branches on March 25, 2016. The cuttings were treated with Benomyl (2000 ppm) and IBA (3000 ppm). Then the upper end of the cutting was covered by sealer to prevent the decay. The cuttings were placed in black plastic bags (25\*18 cm<sup>2</sup>, one cutting/bag), and the bags were filled by sand. The cuttings were placed in shade-house conditions (RH = 50%, temperature: 28 ± 2°C D/18± 2°C N, and 50% shade) and were irrigated twice a day.

In mid-June, 2016, rooted-cuttings were transferred to new pots (33\*36cm<sup>2</sup>, 25 L). About 500 gr gravel were poured to the bottom of each pot and then the pots were filled by media (20 kg). The media (Table 1), the mixture of soil, leaf compost and sand (1: 1: 1), was steam disinfected. A pressure plate extractor (Model

ADC, by Santa Barbara, United States) was used to determine the media water capacity. Then the pots were kept in shade-house condition (RH = 50%, temperature: 30 ± 1° C D/18± 0.5° C N, and 50% shade).

**Salt treatment:** Sodium chloride (Merck, Darmstadt, Germany), was treated thorough irrigation and was included low salinity (0.5 and 2 dSm<sup>-1</sup>), moderate salinity (4 and 6 dSm<sup>-1</sup>) and severe salinity (8 and 10 dSm<sup>-1</sup>) levels (Zarei *et al.*, 2016).

Salt treatment began by ¼ of the desire concentration and gradually rose to the final value, to avoid sudden stress. Plant irrigation regime was calculated based on the water requirement and media filed capacity (Essam *et al.*, 2013; Zarei *et al.*, 2016). Salt treatment took nine months (23/7/2016- 23/4/2017), then all the plant materials were irrigated by distilled water (three months) and the following parameters were measured.

**Leaf abscission:** The leaves of each plant were counted at the beginning of the experiment and were re-counted at the end. The difference was recorded as leaf abscission (Karnosky *et al.*, 1996).

**Relative water content (RWC):** Mature leaves were collected, nine months after salt application, at mid-day, and were transferred immediately to the lab. Afterward, five similar leaf discs which had no vein were separated from each sample and were weighted (W<sub>1</sub>). The disc samples were then placed in distilled water (4 hrs.) under laboratory conditions (24 ± 1° C). Subsequently, the samples were surface dried and re-weighted (W<sub>2</sub>). The discs were placed in an electric furnace (Model: Memmert, made by Karl Klob factory, Germany) (90° C, 60 mins.) and then were weighed again (W<sub>3</sub>). The relative water content was calculated using the following equation (Ritchie *et al.*, 1990).

$$RWC = (w_1 - w_3) / (w_2 - w_3) * 100 \quad (1)$$

**Dry matter (leaf, shoot and root):** Leaf samples (the fifth expanded leaf), shoot and root samples were collected and transferred to the laboratory, nine weeks after salt treatment. All samples were weighted (W<sub>1</sub>), then were dried in an oven at 75° C (48, 148 and 96 hrs. for leaf, shoot and root samples, respectively). The samples were re-weighted (W<sub>2</sub>) and the dry matter was calculated using the following equation (Zarei *et al.*, 2016).

$$\text{Dry matter} = 100 - ((w_1 - w_2) / w_1) * 100 \quad (2)$$

**Na and K content (leaf, shoot and root):** At the end of the experiment about one gram of dry sample (prepared in step 2.5), was grounded and put in an electric furnace (540° C for 6 hrs.). Then, 2N HCl (5 ml) was added to each sample, well mixed and was incubated at warm bath (80° C, 60 mins.). The yellow-turned extract was filtered (watman's filter paper No. 42) and boiling distilled water was added to each sample tube (up to 50 ml). Then, Na and K content were measured using a flame photometer (Model: JENWAY, PFP7, United Kingdom) (Chapman and Pratt, 1961).

**Table 1- Physico-chemical properties of the soil**

Soil texture	Sand (%)	Silt (%)	Clay (%)	EC (ds/m)	C.E.C (Me/100)	pH	Lime (%)
Sandy-clay-loam	58±1.01	26±1	16±0.9	1.45±0.21	10.84±0.81	7.7±0.17	35±1.33
Organic C (%)	N (%)	K (ppm)	P (ppm)	Cu (ppm)	Mn (ppm)	Fe (ppm)	Zn (ppm)
1.17±0.05	0.17±0.002	126±1.3	3.2±0.05	0.26±0.002	3.86±0.04	2.85±0.03	0.056±0.001

**Cl content (leaf, shoot and root):** About 0.5 g of dry sample (prepared in step 2.5) was mixed with 12 g of calcium oxide and distilled water and put in an electric furnace (540° C for 6 hrs.). Then boiling distilled water (15 ml) was added, each sample was filtered and boiling distilled water was added again (up to 50 ml).

The pH was adjusted to 7 (by adding 33 ml of acetic acid: 67 ml of distilled water). Then, a few drops of 5% potassium chromate (5 g of potassium chromate: 95 ml of distilled water) were added and titrated by 0.05N AgNO<sub>3</sub>, till the extract turned red. (One ml of potassium chromate with silver nitrate was used as blank). Cl content was calculated using the following equation (Chapman and Pratt, 1961).

$$\%Cl = \frac{(ml\ AgNO_3 - ml\ blank) \times N\ AgNO_3 \times 35.5 \times 100}{g\ sample \times 1000} \quad (3)$$

**Photosynthesis indices:** The photosynthesis indices were recorded using a compact-portable-photosynthesis-system (LCI, UK), nine weeks after salinity treatment. The device was put on attached leaf (1/3 of third expanded leaf) of each plant at mid-day and the photosynthesis indices (photosynthetic rate and intercellular CO<sub>2</sub> concentration) was recorded 2 mins. later (Evans and Caemmerer, 1996).

**Experimental design and data analysis:** This experiment was carried out in a Randomized Complete Block Design. The factors included fig cultivars (7 types) and sodium chloride (6 concentration), with 5 replications. Shapiro-Wilks test confirmed normality of data. Multivariate Analyses of variance, considering the cultivar and salinity levels as independent factor, were performed for the measured variables. Leven's test confirmed homogeneity of variance. The Tukey test was carried out for Mean comparisons (level of P < 0.01). Principal Component Analysis (PCA) was used to identify the main characteristics which distinguish tolerant and sensitive cultivars. KMO and Bartlett's test confirmed the suitability of data for PCA. These statistical analysis was carried out with the program MSTATC ("MSTATC"). SAS Version 9.1.3 ("SAS Institute Inc. Cary, NC, USA," 2002) was used for correlation analysis. Excel 2013 was applied to draw the figures.

## Results

All the traits were influenced by genotype and interaction of genotype and salinity. But salinity did not affect dry matter of the leaf and shoot, neither the RWC (Table 2).

**The influence of saline water on leaf abscission of fig cultivars:** The results revealed a decrease in leaf number by intensifying the salt stress. 'Bar' and 'Kashki' cultivars showed the most leaf abscission under high salt concentration (10 dSm<sup>-1</sup>). 'Shah Anjir' cultivar, lost the most leaves under high salt stress (6 dSm<sup>-1</sup> and more). Remarkably, 'Siyah' cultivar started to response to salinity by leaf drop under low salinity level (2 dSm<sup>-1</sup>) (Fig. 1 A).

**The influence of saline water on relative water content of Fig cultivars:** According to our findings, salinity had different effects on leaf relative water content in fig cultivars and the changes did not follow a certain trend. Under low salt concentration (2 dSm<sup>-1</sup>) an increase in RWC, and then a decrease under 4dSm<sup>-1</sup> were observed. An increase in RWC under 8 dSm<sup>-1</sup> NaCl was observed in 'Bar' and 'Mati' Among all cultivars, 'Bar' had the most RWC under 10 dSm<sup>-1</sup> NaCl (Fig.1 B).

**The influence of saline water on dry matter of fig cultivars (leaf, shoot and root):** The results indicated that salinity caused a slight decreasing trend of shoot dry matter in all cultivars (Fig. 2 A). 'Sabz' and 'Siyah' cultivars had the most leaf dry matter, while 'Bar' had the least value (Fig. 2 B). The effect of salinity on the root dry matter had a similar trend (Fig.2 C).

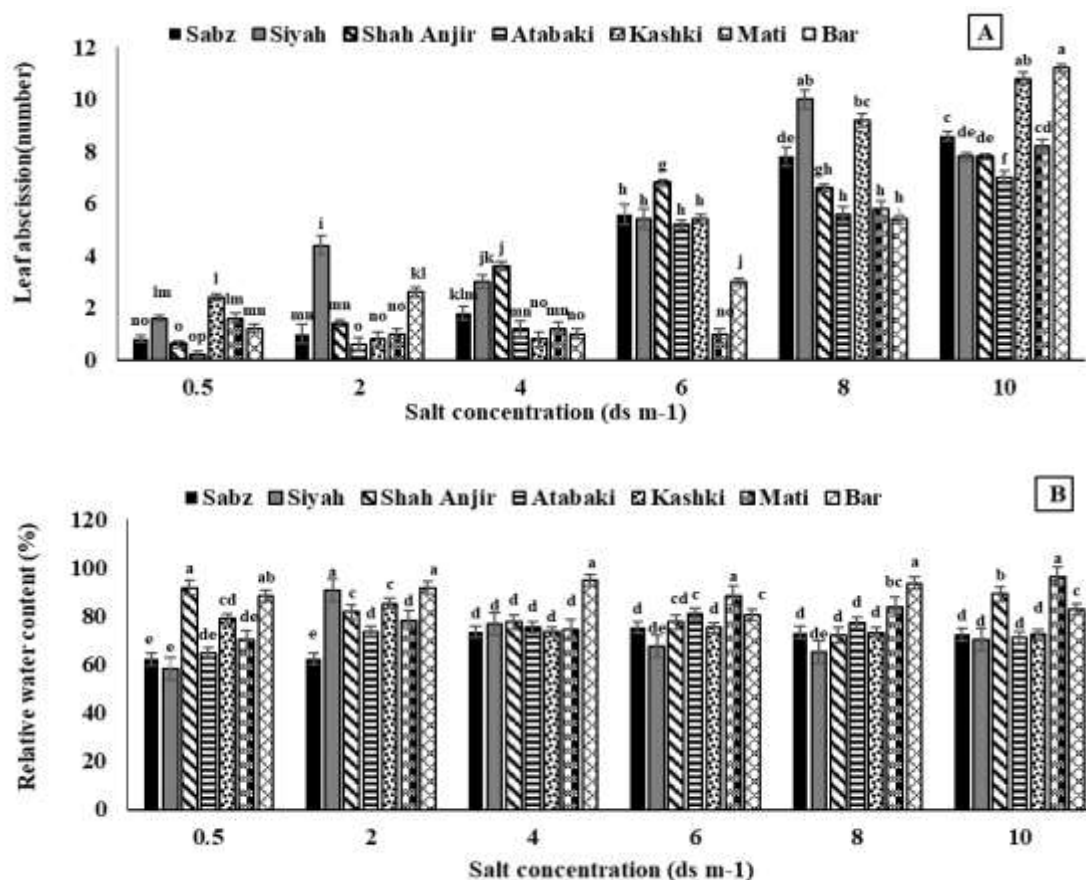
**3.4. The influence of saline water on Na content of fig cultivars (leaf, shoot and root):** A linear relationship was observed between Sodium content of leaf, root and shoot and salinity levels. The maximum and minimum sodium accumulation in shoot were observed in 'Shah Anjir' and 'Siyah', respectively (76.71 and 61.8 mg L<sup>-1</sup>, in 10 dSm<sup>-1</sup> salinity, respectively) (Fig. 3 A). The most leaf sodium content (80.43 mg L<sup>-1</sup> in 10 dSm<sup>-1</sup> salinity) was observed in 'Shah Anjir' cultivar and the least value belonged to 'Siyah' cultivar (65.34 mg L<sup>-1</sup> in 10 dS m<sup>-1</sup> salinity) (Fig. 3 B). In roots, the most and least values belonged to 'Shah Anjir' and 'Siyah' (82.48 and 71.31 mg L<sup>-1</sup>, in salinity 10 dS / m), respectively (Fig. 3 C).

**The influence of saline water on K content of fig cultivars (leaf, shoot and root):** By rising salt, a descending trend was observed in K content of different organs of all fig cultivars. The most impact of salt on shoot K content was observed in 'Bar' and 'Shah Anjir', it reached from 151.3 and 172.4 mg L<sup>-1</sup> in 2 dSm<sup>-1</sup> to 76.52 and 104.4 mg L<sup>-1</sup> in 10 dSm<sup>-1</sup>. In 'Sabz' cultivar, the shoot potassium was relatively high at low-moderate stress levels. By rising salt level, 'Siyah' cultivar had the most K content in its shoots (121.7 mg L<sup>-1</sup> in 10

**Table 2- The interaction of cultivar and salinity on physiological parameters of fig (the Mean Square value is given)**

Physiological parameters	Cultivar	Salinity	Cultivar * Salinity
Leaf abscission	107.80*	220.38*	11.25*
RWC	1540.57**	264.34 <sup>ns</sup>	289.57*
Shoot dry matter	226.45**	78.94 <sup>ns</sup>	20.57*
Leaf dry matter	771.58**	70.38 <sup>ns</sup>	97.50*
Root dry matter	89.92*	337.42*	37.05*
Shoot Na	254.51**	17053.22**	32.67*
Leaf Na	382.45**	14773.30**	27.87*
Root Na	274.91**	8424.03**	13.03*
Shoot K	2584.50**	15662.62**	184.63*
Leaf K	1332.34**	14731.03**	222.29*
Root K	1682.92**	17159.83**	174.03*
Shoot Na/K	3.81**	1.133*	0.177*
Leaf Na/K	0.28**	1.002**	0.103*
Root Na/K	0.097**	0.0079*	0.0074*
Shoot Cl	0.51**	3.50**	0.07*
Leaf Cl	0.36*	7.56**	0.09*
Root Cl	0.25**	2.75**	0.06*
Intercellular CO <sub>2</sub> concentration	23847.08**	23969.01**	35378.23**
Photosynthetic rate	434.96**	1699.89**	287.13**

ns, \* and \*\*: not significant, significant at 5 and 1% respectively (by Tukey mean comparison test)



**Fig 1. The influence of saline water on leaf abscission (A) and relative water content (B) of fig cultivars. Means  $\pm$  SE of five replicates are shown. The same letter denotes no differ significantly Tukey ( $P < 0.05$ ).**

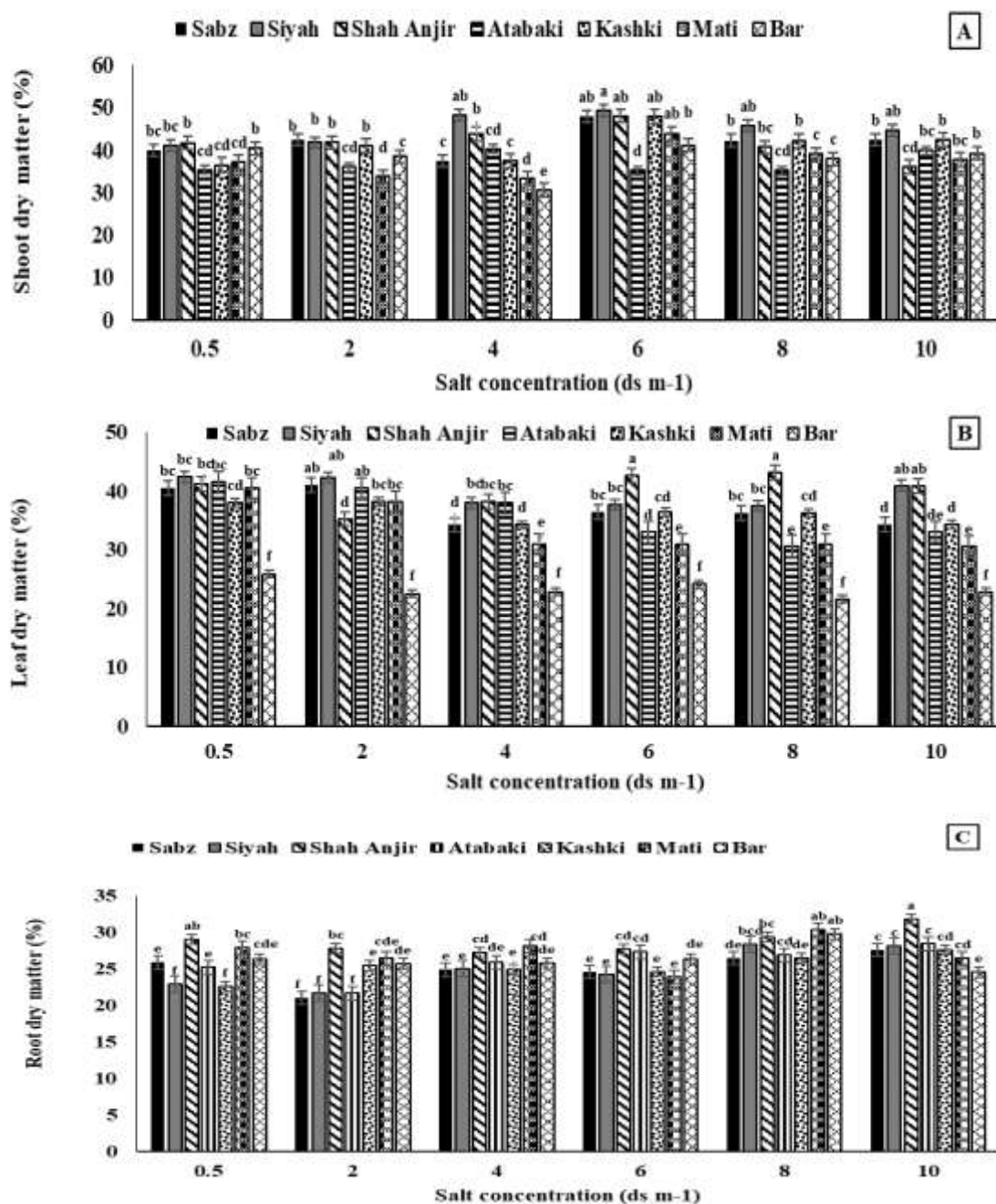


Fig 2. The influence of saline water on shoot (A), leaf (B) and root (C) dry matter of fig cultivars. Means  $\pm$  SE of five replicates are shown. The same letter denotes no differ significantly Tukey ( $P < 0.05$ ).

dSm<sup>-1</sup>) (Fig 4. A). The least leaf K content was observed in 'Shah Anjir' cultivar at 10 dSm<sup>-1</sup> salinity. The potassium amount in the leaf of 'Siyah' cultivar was higher than others at different levels of salinity stress, however, it decreased gradually by rising salt concentration, which reached to 68.14 mg L<sup>-1</sup> at 10 dSm<sup>-1</sup> NaCl (Fig 4. B). The most root potassium content was observed in 'Siyah' cultivar (77.1 mg L<sup>-1</sup>) and the least values belonged to 'Bar' and 'Shah Anjir' cultivars (67.36 and 69.31 mg L<sup>-1</sup>, respectively) in 10 dSm<sup>-1</sup>. The K content of 'Siyah' cultivar changed from 138.86 mg L<sup>-1</sup> in 2 dSm<sup>-1</sup> NaCl to 77.10 mg L<sup>-1</sup> in 10 dSm<sup>-1</sup>. The

'Shah Anjir' had the minimum potassium accumulation in its roots and reached from 106.96 mg L<sup>-1</sup> in 2 dSm<sup>-1</sup> salt to 69.31 mg L<sup>-1</sup> in 10 dSm<sup>-1</sup> salinity (Fig 4. C).

**The influence of saline water on Na/K of fig cultivars (leaf, shoot and root):** In all cultivars Na/K of shoot, leaf and root increased by salt rising (Fig. 5 A, B, C). The most values in shoot Na/K was observed at 10 dSm<sup>-1</sup> salt in 'Bar' cultivar (0.96) and then in 'Shah Anjir' cultivars (0.73). 'Siyah' and 'Sabz' cultivars had the least values in 10 dSm<sup>-1</sup> NaCl (Fig. 5 A). 'Shah Anjir' had the most leaf Na/K (Fig. 5 B), and root Na/k values (Fig. 5 C).

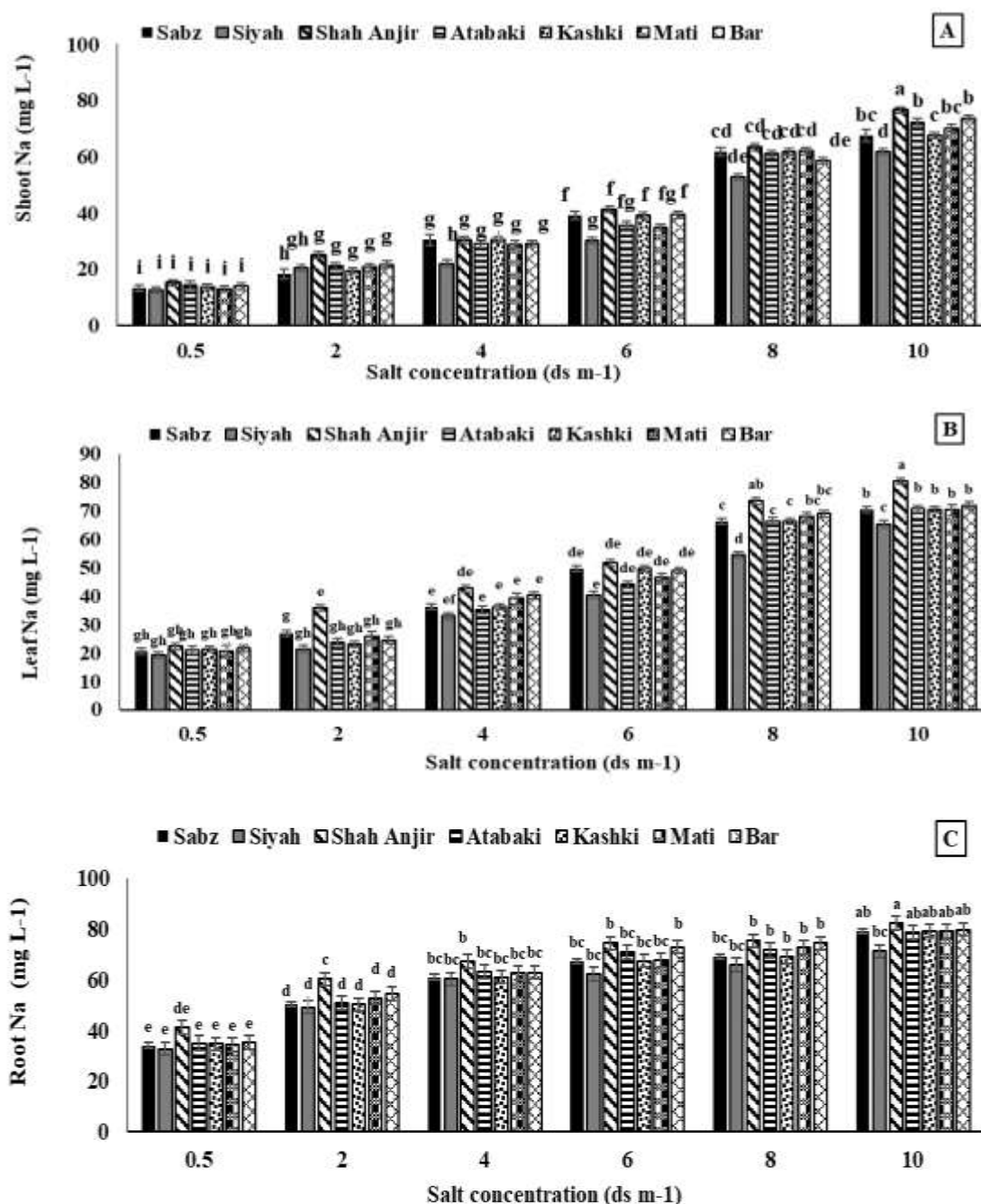


Fig 3. The influence of saline water on shoot (A), leaf (B) and root (C) Na content of fig cultivars. Means  $\pm$  SE of five replicates are shown. The same letter denotes no differ significantly Tukey ( $P < 0.05$ ).

**The influence of saline water on Cl content of fig cultivars (leaf, shoot and root):** Rising NaCl in irrigation water caused a slight ascending change in Cl content of three organs of fig cultivars. The minimum and maximum shoot Cl content under 10 dSm<sup>-1</sup> salt, belonged to 'Bar' (1.05 mg L<sup>-1</sup>) and 'Mati' (1.65 mg L<sup>-1</sup>) cultivars, respectively (Fig. 6 A). Under 10 dSm<sup>-1</sup> salt, the least leaves Cl was observed in 'Siyah' (1.29 mg L<sup>-1</sup>) and the most in 'Shah Anjir' (1.76 mg L<sup>-1</sup>) (Fig. 6 B). Regarding root Cl content, the least and the most content under this salt level, was accumulated in 'Shah Anjir' (1.22 mg L<sup>-1</sup>) and 'Mati' (1.79 mg L<sup>-1</sup>),

respectively (Fig. 6 C).

**The influence of saline water on photosynthetic indices of fig cultivars:** Saline water significantly influenced the photosynthetic indices. Salt rising decreased photosynthesis rate gradually in all cultivars, except 'Shah Anjir' (Fig. 7 A). 'Shah Anjir', exhibited a slight increase in intercellular CO<sub>2</sub> concentration under low salt (2 dSm<sup>-1</sup>). This rise had a sudden peak at 4 dSm<sup>-1</sup>, and then decreased and reached to its minimum value at 10 dSm<sup>-1</sup>. But 'Siyah' cultivar showed a decrease trend by salt rising, and reached to its minimum value at 10 dSm<sup>-1</sup> salt (Fig. 7 B).

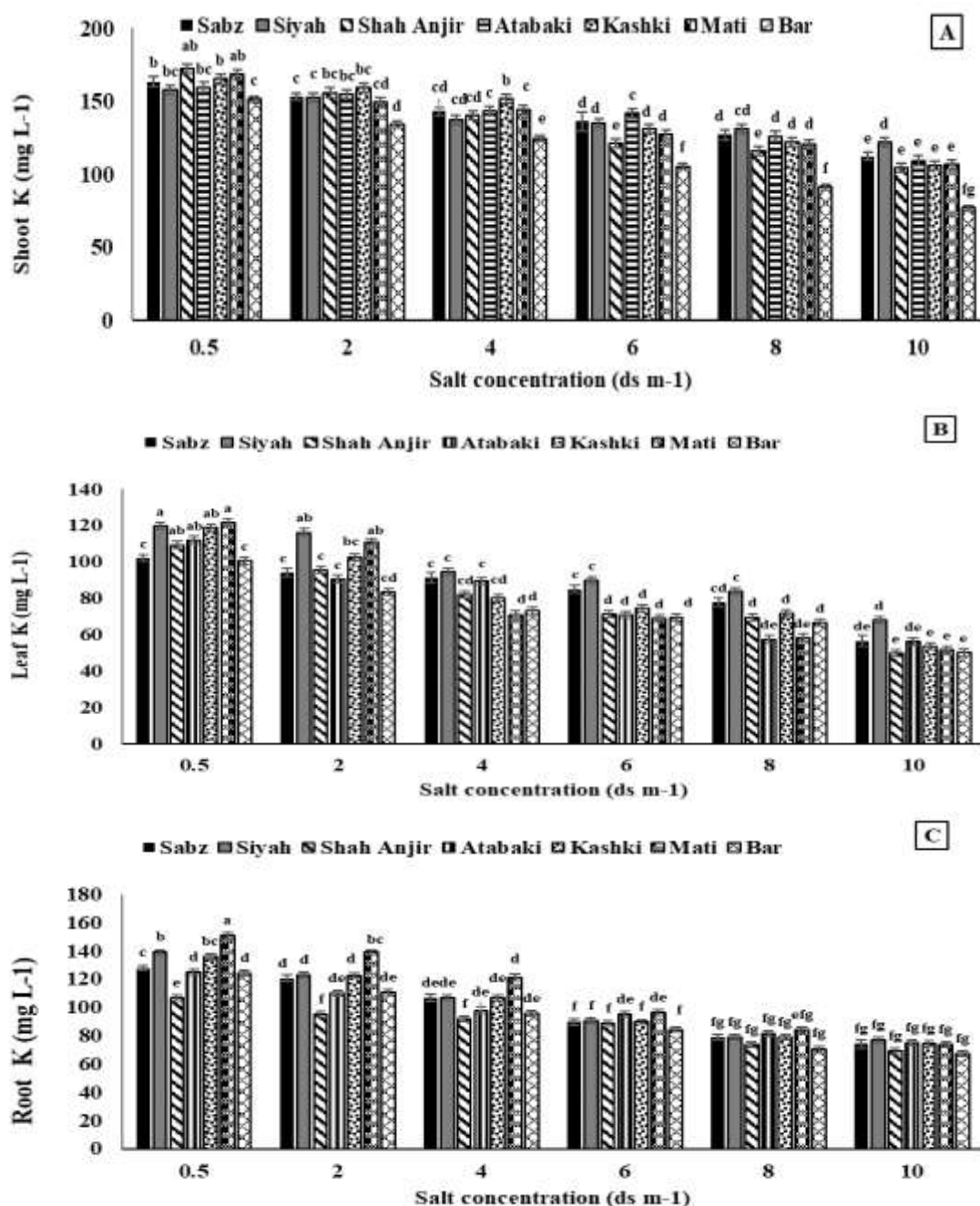


Fig 4. The influence of saline water on shoot (A), leaf (B) and root (C) K content of fig cultivars. Means  $\pm$  SE of five replicates are shown. The same letter denotes no differ significantly Tukey ( $P < 0.05$ ).

**Correlation analysis:** The bivariate Pearson correlations of the parameters are given in Table 3. The blue values indicate high correlated values (more than 0.7). Leaf abscission had high significant correlation with Na, Cl and Na/K in leave, root and shoot, however, were negatively correlated with K content. Correlation value varied between the different organs; the leaf abscission influenced by shoot Na more than leaf ones. However, root K showed higher correlation with leaf abscission than the K content of root or shoot.

The sodium content of the shoot strongly correlated with the sodium content of the root and leaf but,

negatively correlated with potassium contents of all three organs. In addition, it had a significant negative correlation with photosynthetic rate ( $-0.625^{**}$ ). Regarding Cl, the influence of leaf and root content on leaf abscission was greater than shoots. Photosynthetic rate had a strong relationship ( $0.608^{**}$ ) with intercellular  $\text{CO}_2$  concentration. Though, intercellular  $\text{CO}_2$  concentration and photosynthetic rate were negatively correlated with leaf abscission, but it was not statistically significant. Which is probably due to their indirect impact on leaf abscission.

**Principal Component Analysis:** Principal



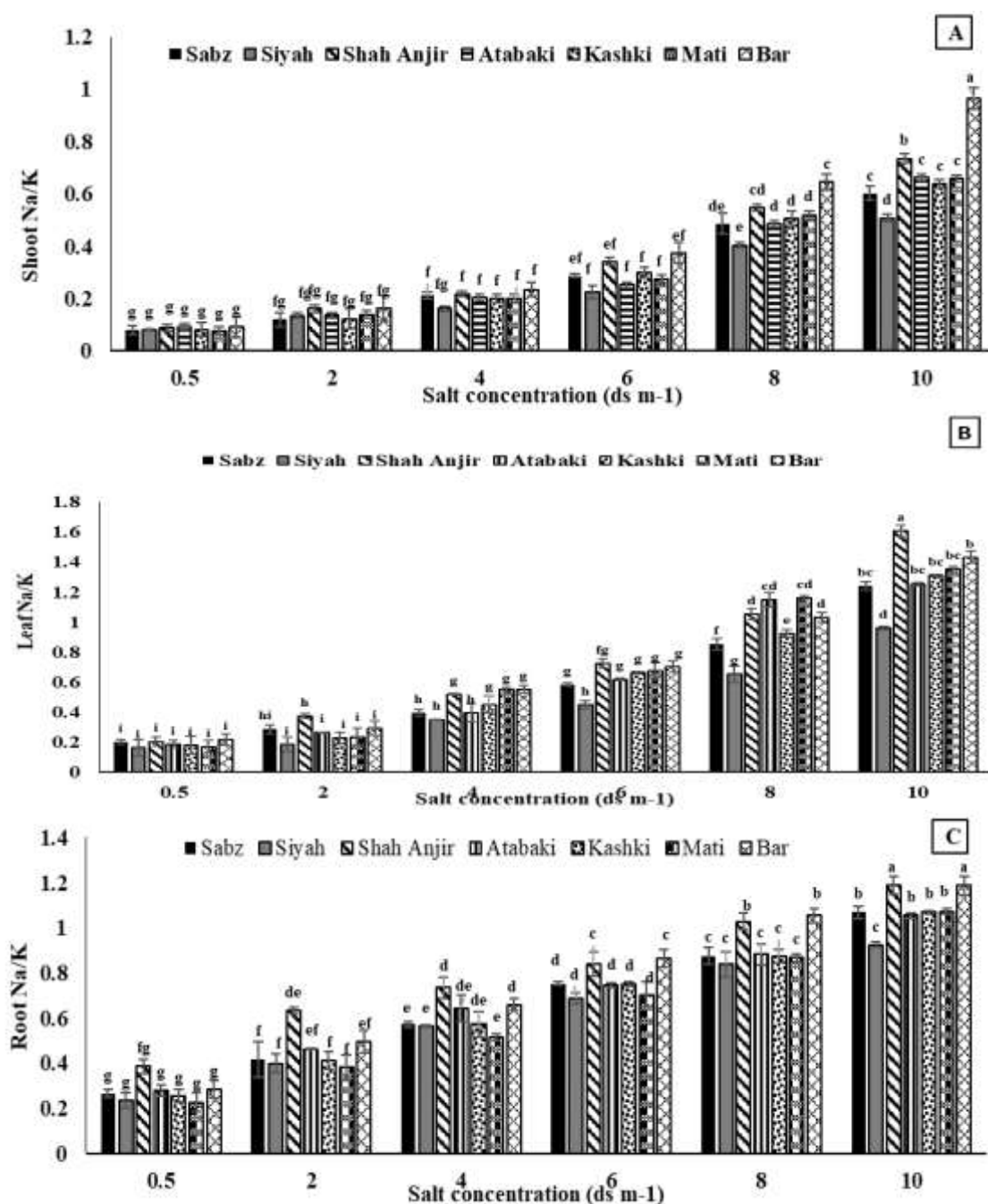


Fig 5. The influence of saline water on shoot (A), leaf (B) and root (C) Na/K of fig cultivars. Means  $\pm$  SE of five replicates are shown. The same letter denotes no differ significantly Tukey ( $P < 0.05$ ).

component analysis under high salt condition grouped the characteristics into four components (Table 4). PCA analysis indicated that the first PC has an eigenvalue of 8.21 and explains 43.22% of the total variation (Table 4). PC<sub>1</sub> represents the equivalent of nine variables included the content of K, Na and Na/K of leaf and root, shoot Na, leaf Cl and RWC (Table 5); therefore, it should be considered as main criteria which distinguished salt-tolerant (sensitive) Fig cultivars. PC<sub>2</sub> explains an additional 22.11% of the variation and has an eigenvalue of 4.20 (Table 4). This component was related to shoot K, shoot Na/K, leaf abscission and dry

matter of three organs (Table 5). PC<sub>3</sub>, which accounted for 17.90% of the variance (Table 4), included both the intercellular CO<sub>2</sub> and photosynthetic rate (Table 5), and was termed as photosynthetic indices factor. The forth PC, which accounted for 12.54% of the variance (Table 4), represented root and shoot Cl (Table 5).

### Discussion

High salt accumulation, in the cell wall and cytoplasm, causes dehydration and cellular leakage, prevents enzymatic activity, damages the leaves and ultimately leads to leaf abscission (Munns, 2002). The removal of



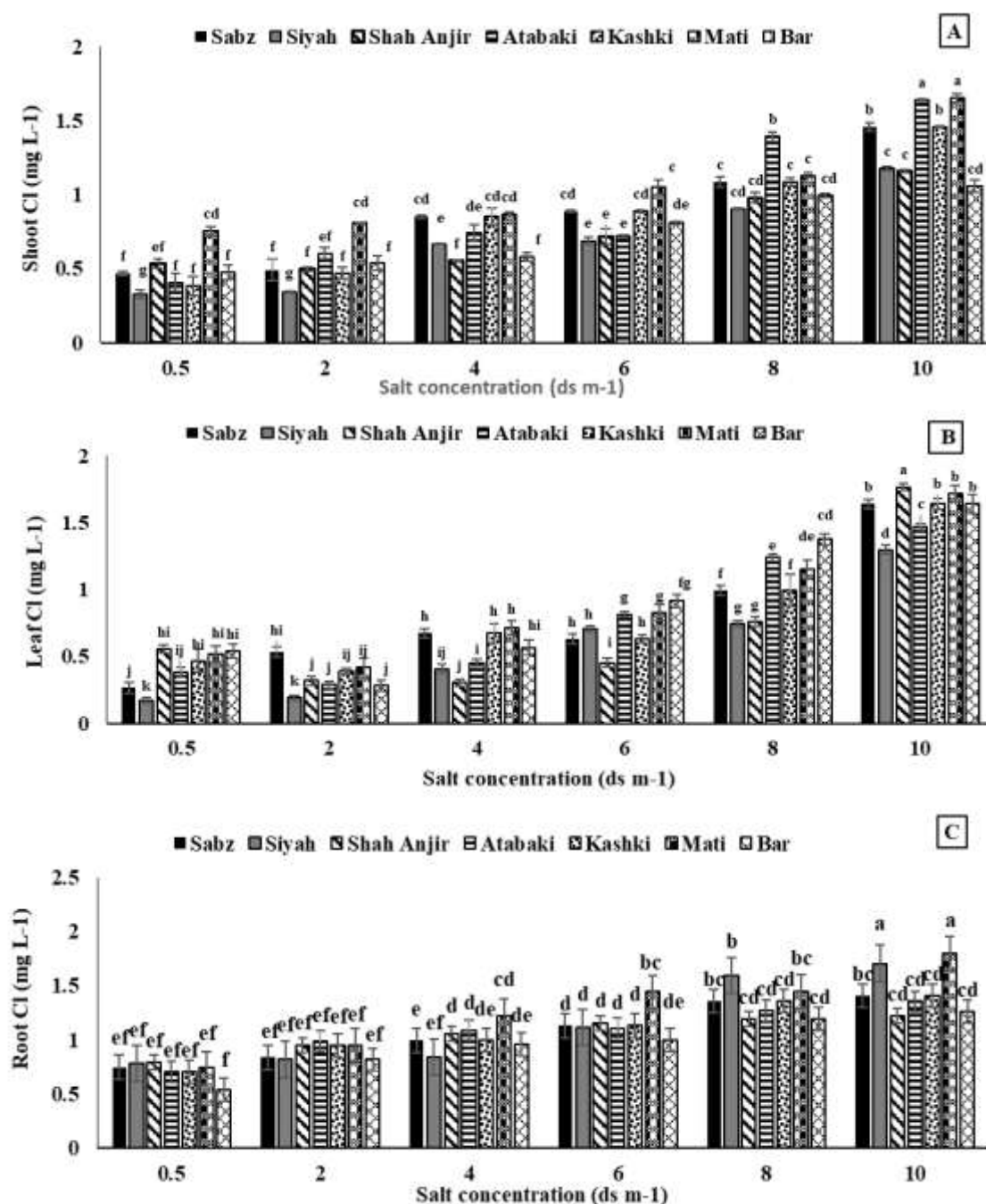


Fig 6. The influence of saline water on shoot (A), leaf (B) and root (C) Cl content of fig cultivars. Means  $\pm$  SE of five replicates are shown. The same letter denotes no differ significantly Tukey ( $P < 0.05$ ).

excess organs which have accumulated the injurious minerals is one of the different mechanisms of salt tolerance. Many plants, react with senescence intensification of some leaves and old leaves abscission when exposed to prolonged stress. While, in some others, under severe stress, only the youngest leaves will remain at the top of the shoots, and the rest will fall (Hopkins, 2008). In the present study, the 'Siyah' cultivar was the first cultivar who initiated leaf abscission under low salinity level. Leaf abscission as a response of fruit crops to salt stress was reported in previous experiments (Munns, 2002; Zarei *et al.*, 2016).

Under salt stress, cell wall will modify, leaf turgor and photosynthesis rates decrease, which leads to decrease in leaf area and leaf water content (Rodriguez-Dominguez *et al.*, 2016). According to our results, RWC changes under different salt concentrations were cultivar-dependent. Zarei *et al.* 2016 reported salinity had no significant effect on leaf area of fig cultivars.

It seems that prevention of the cellular development and growth initiated by a reduction of turgor pressure and water absorption causes a drop in plant dry matter and sensitive cultivars lose more dry matter under such condition (Syvertsen *et al.*, 2010). In our research,

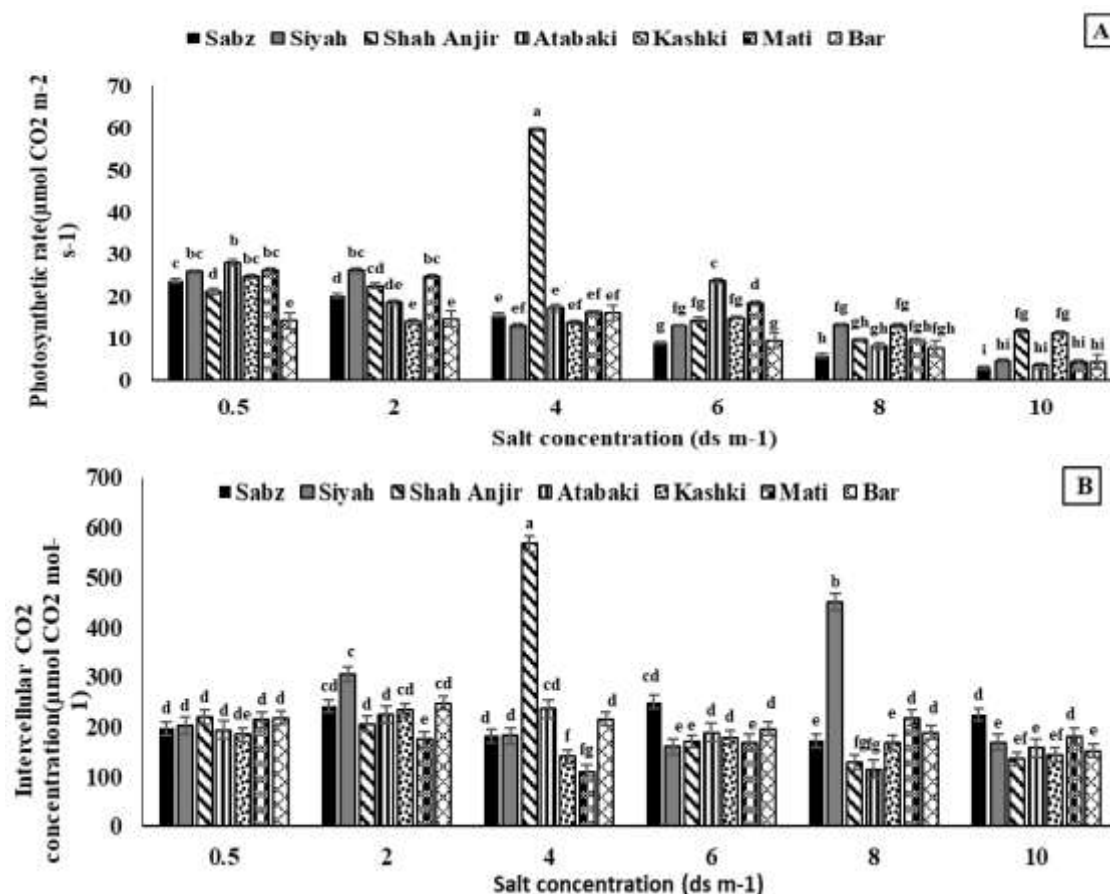


Fig 7. The influence of saline water on photosynthetic rate (A) and intercellular CO<sub>2</sub> concentration (B) of fig cultivar. Means  $\pm$  SE of five replicates are shown. The same letter denotes no differ significantly Tukey ( $P < 0.01$ ).

among all fig cultivars 'Siyah' cultivar loss less shoot dry matter than others. Moreover, salinity had less impact on the root system in tolerant-cultivars such as 'Siyah' and, while it had a greater effect on root dry matter in sensitive cultivars such as 'Shah Anjir'. The declining impact of salinity on root and shoot dry matter has reported already in both Fig (Zarei *et al.*, 2016) and Plum (Bolat *et al.*, 2016).

Salinity stress in the soil, by increasing the amount of Cl<sup>-</sup> and Na<sup>+</sup>, disrupts Ca<sup>2+</sup> absorption (Gaber, 2010), reduces K availability and increases the Na/K, which ultimately decreases plant yield and efficiency (Grattan and Grieve, 1999). The 'Siyah' cultivar, as a salt-tolerant, avoid excessive Na influx in younger leaves, by early leaf abscission. Hence, the Na content of 'Siyah' shoot was less than other cultivars. The 'Shah Anjir', as a sensitive cultivar, could not inhibit the sodium flux from root to shoot; therefore, it accumulated more Na in the shoot. The results also indicated that 'Shah Anjir' could not prevent the excessive sodium absorption by the root system. So, it had the most root Na content. According to a report, salinity stress has increased the sodium content in the root, shoot and leaf of 'Green', 'Pius' and 'King' fig cultivars (Zarei *et al.*, 2016).

Potassium decline in salt-exposed plants was probably due to the competition of sodium, on the

bonding sites to plasma membrane carriers, or potassium leakage as a result of the instability of the plasma membrane (Ferreira-Silva *et al.*, 2008; Gaber, 2010; Shabala and Pottosin, 2014). Electrophysiological evidence has indicated that potassium loss in salt-sensitive roots of wheat and barley is significantly higher than resistant cultivars. In fact, potassium flow from root to the leaf and its accumulation in the leaf is one of salinity resistance mechanism (Shabala and Pottosin, 2014). It is possible that in salt tolerant cultivars, 'Siyah' and "Sabz", higher potassium concentration is due to an increase in selective uptake of potassium, a rise in sodium removing from root, resulting in better activity of sodium/hydrogen carriers, the greater activity of H-ATPase to prevent prolonged depolarization of plasma membrane (Chakraborty *et al.* 2016).

Low Na/K has been considered as an important physiological criterion to screen salt-tolerant plant species (Szczerba *et al.*, 2009). Mostly, the root content less potassium than aerial organs, which results in a decrease in Na/K in the aerial compared to the underground parts of the plant (Szczerba *et al.*, 2008). 'Siyah' and "Sabz" cultivars had higher K concentration. While 'Shah Anjir', 'Mati' and 'Bar' had the least K value in their root, shoot, and leaf respectively. It has reported that in Fig (Zarei *et al.*, 2016)

**Table 3- The analysis of physiological parameters of fig cultivars**

		1	2	3	4	5	6	7	8	9
1	Leaf abscission	1.00								
2	RWC	-.29	1.00							
3	Leaf dry matter	-.06	-.13	1.00						
4	Shoot dry matter	.09	-.12	.24	1.00					
5	Root dry matter	.07	.06	.26	.534**	1.00				
6	Shoot Na	.874**	-.20	-.09	-.12	-.04	1.00			
7	Leaf Na	.845**	-.18	-.12	-.12	-.05	.985**	1.00		
8	Root Na	.729**	-.16	-.08	-.12	-.13	.876**	.903**	1.00	
9	Shoot k	-.765**	.19	.319*	.18	.15	-.875**	-.883**	-.866**	1.00
10	Leaf K	-.683**	.15	.18	.25	.18	-.881**	-.898**	-.920**	.873**
11	Root K	-.783**	.10	.06	-.01	.03	-.894**	-.916**	-.911**	.852**
12	Shoot Na/ k	.859**	-.16	-.16	-.13	-.08	.969**	.946**	.834**	-.924**
13	Leaf Na/ k	.817**	-.18	-.17	-.17	-.11	.974**	.968**	.867**	-.889**
14	Root Na/ K	.833**	-.15	-.12	-.08	-.09	.956**	.969**	.956**	-.922**
15	Shoot cl	.705**	-.23	-.12	-.24	.02	.886**	.864**	.782**	-.729**
16	Leaf cl	.747**	-.21	-.16	-.22	.00	.900**	.875**	.746**	-.811**
17	Root CL	.741**	-.345*	.09	-.02	.10	.842**	.834**	.809**	-.688**
18	Intercellular CO <sub>2</sub> concentration	-.04	.04	.03	.442**	.05	-.22	-.23	-.14	.21
19	Photosynthetic rate	-.505**	.19	.06	.28	-.17	-.625**	-.593**	-.514**	.596**

\* and \*\*: is significant at the 5 and 1% levels, respectively

**Continue Table 3-**

		10	11	12	13	14	15	16	17	18	19
1	Leaf abscission										
2	RWC										
3	Leaf dry matter										
4	Shoot dry matter										
5	Root dry matter										
6	Shoot Na										
7	Leaf Na										
8	Root Na										
9	Shoot k										
10	Leaf K	1.00									
11	Root K	.879**	1.00								
12	Shoot Na/ k	-.858**	-.858**	1.00							
13	Leaf Na/ k	-.916**	-.863**	.967**	1.00						
14	Root Na/ K	-.918**	-.960**	.940**	.946**	1.00					
15	Shoot cl	-.817**	-.726**	.828**	.878**	.814**	1.00				
16	Leaf cl	-.825**	-.752**	.913**	.933**	.846**	.889**	1.00			
17	Root CL	-.783**	-.779**	.758**	.789**	.810**	.837**	.736**	1.00		
18	Intercellular CO <sub>2</sub> concentration	.25	.07	-.24	-.28	-.16	-.328*	-.350*	-.08	1.00	
19	Photosynthetic rate	.570**	.546**	-.616**	-.589**	-.568**	-.638**	-.645**	-.515**	.608**	1.00

\* and \*\*: is significant at the 5 and 1% levels, respectively

**Table 4- Principal component analysis of 7 Fig cultivars under high salt concentration (10ds m<sup>-1</sup>)**

Principal components	Eigen Value	Absolute variation	Accumulated variation (%)
1	8.213	43.22	43.22
2	4.201	22.11	65.33
3	3.403	17.90	83.24
4	2.380	12.54	95.78

and Plum (Bolat *et al.*, 2016), that the leaf Na/K of salt-resistant cultivar, is less than the salt-sensitive cultivars. High levels of chlorine disrupt potassium, calcium, ammonium, and nitrate absorption. Also lessens enzymes activity, causes membrane destructive which finally, declines the plant's metabolism efficiency (Ashraf and Ahmad, 2000). Salt-resistant plants are distinguished by transferring less Cl into their leaves

(Munns, 2002). The 'Siyah' cultivar, did not transfer Cl to the aerial parts. While 'Mati' cultivar had the most chlorine content in both shoots and leaves. According to reports, sodium chloride causes an increase in root Cl, and consequently, it will accumulate in leaves and shoots (Duran-Zuazo *et al.*, 2003; Zarei *et al.*, 2016).

Salinity tolerance in citrus is related to low Na and Cl transfer into the shoot (Marschner, 1995), while in

**Table 5- PCA Analysis of the evaluated traits under high salt concentration (10ds m<sup>-1</sup>)**

	Component			
	1	2	3	4
Leaf dry matter	-.202	.971	.120	.053
Shoot dry matter	.110	-.970	.124	.083
Root dry matter	.248	.890	.301	.008
Leaf K	-.848	.394	-.328	-.106
Root K	-.701	.358	-.221	.572
Shoot K	-.446	.758	-.181	.433
Leaf Na	.846	.257	.398	-.230
Root Na	.850	-.203	.468	.120
Shoot Na	.875	-.150	.210	-.213
Leaf Cl	.875	-.140	.273	.168
Root Cl	-.322	.146	-.424	-.774
Shoot Cl	.021	-.083	-.133	.950
Leaf abscission	-.218	.868	.020	-.435
Relative water content	.853	-.006	-.447	-.014
Intercellular CO <sub>2</sub>	-.178	-.111	-.948	.238
Photosynthetic rate	.202	.378	.776	.145
Leaf Na/K	.922	-.103	.358	-.100
Root Na/K	.831	-.301	.359	-.298
Shoot Na/K	.547	-.649	.148	-.506

the grape rootstocks this issue is essentially related to low Cl transfer (Luachli and Wteneke, 1979).

Salinity encourages the excessive absorption of mineral nutrition and causes ionic imbalance, which restricts gas exchange in plants (Munns, 2002). Under such conditions, the maximum efficiency of photosystem II and CO<sub>2</sub> assimilation will decline (Joao- Correia *et al.*, 2006). This decrement is due to stomatal (such as reduction in stomatal conductance) or non-stomatal (such as chlorophyll degradation) factors (Qasim *et al.*, 2003). Actually, salt causes both reductions in plant growth (by transpiration decrease) and plant photosynthetic efficiency (related to changes in RWC, dry matter, leaf fall and stomata conductivity (Munns, 2002; Munns and Tester, 2008), by affecting the capacity of the plant for CO<sub>2</sub> assimilation (Walker *et al.* 1981, 1983), which lead to a reduction in stomatal and mesophyll conductivity and dwindling photosynthetic efficiency (Centritto *et al.*, 2003; Flexas *et al.*, 2004). In this study, salt stress especially high levels of salt NaCl (8 and 10 dsm<sup>-1</sup>), caused a decrease in intercellular CO<sub>2</sub> concentration and photosynthetic rate, indicating the possible impact of salt on photosynthetic rate due to stomatal factors. 'Shah Anjir' and 'Siyah' cultivars, had the least and the most intercellular CO<sub>2</sub> under sever salinity level (at 8 dSm<sup>-1</sup> salinity), respectively. Changes in photosynthesis rate in 'Shah Anjir' cultivar was different. This cultivar had the most intercellular CO<sub>2</sub> and photosynthetic rate under 4 dSm<sup>-1</sup> NaCl and the least value under 10 dSm<sup>-1</sup> salt. It seems that this cultivar consumes photosynthesis osmolyte to overcome moderate salinity stress while stomata closure occurred later for 'Siyah' and resulting in the effectiveness of tolerance mechanism. According to Golombek and Ludders, (1990) and Zarei *et al.* (2016), salinity reduced the stomatal conductivity of Fig cultivars and rootstocks and caused a reduction in photosynthetic efficiency of mesophilic cells.

A strong correlation between salt tolerance and salt ex-flux has been reported in many plant species (Ruiz-Sanchez *et al.*, 2000; Tester and Davenport, 2003; Zarei *et al.*, 2016).

PCA analysis reduces a large data set to few unrelated components. Variables which are strongly connected in the same component, may share some principal biological relation. These associations are often useful for understanding the behavior of complex traits (such as yield), or in classifying genotypes based on their behavior in different growth conditions (Iezzoni and Pritts, 1991). According to our results, principal component analysis of the data led to a reduction in the variables, with major contributions from the content of K, Na and Na/K of leaf and root, shoot Na, leaf Cl, and RWC. Moreover, PCA displayed significant differences between groupings of salt-sensitive cultivar ('Shah Anjir') and salt-tolerant cultivars ('Siyah' and 'Sabz'). According to Mathaba *et al.* (2013), PCA analysis has been used to separate the major contributions which distinguish chilling sensitive and non-chilling susceptible citrus fruits (Mathaba *et al.*, 2013).

## Conclusion

In conclusion, salinity significantly reduces growth parameters and photosynthetic indices, besides, it changes the ion uptake trends in salt tolerant and salt sensitive cultivars of Fig. The 'Siyah' and 'Sabz', as salt-tolerant cultivars, prevented damage to younger leaves and rise photosynthesis rate, by in-fluxing fewer Na and Cl, let more leaves to fall and exhibiting the quickest response to the negative impact of salinity. The 'Mati', as a moderate salt-tolerance cultivar, had the least leaf abscission under severe salinity levels and in-fluxed high Cl and Na into both aerial and foliar organs. The 'Shah Anjir', as the most salt-sensitive cultivar, could not restrict root Cl ex-flux into shoot and leaf. According to PCA, the content of K, Na and Na/K of

leaf and root, shoot Na, leaf Cl, and RWC were the main parameters which separated tolerant and sensitive cultivars. There was also a correlation between Leaf abscission and Na and Cl accumulation in salt exposed plants.

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### References

- Alswalmeh, H. A., Al-oheed, R. S., Khalil Omar, A. (2015) Effect of water salinity on seedlings growth of Brown Turkey and Royal fig cultivars. *The Journal of Agriculture and Natural Resources* 2:510-516.
- Ashraf, M., Ahmad, S. (2000) Influence of sodium chloride on ion accumulation, yield components and fibre characteristics in salt-tolerant and salt-sensitive lines of cotton (*Gossypium hirsutum* L.). *Field Crops Research* 66: 115-127.
- Bolat, I., Dikilitas, M., Ikinici, A., Ercisli, S., Tonkaz, T. (2016) Morphological, physiological, biochemical characteristics and bud success responses of myrobalan 29 c plum. *Canadian Journal of Plant Science* 96: 485-493.
- Centritto, M., Loreto, F. and Chartzoulakis, K. (2003) The use of low CO<sub>2</sub> to estimate diffusional and non-diffusional limitations of photosynthetic capacity of salt-stressed olive saplings. *Plant Cell Environment* 26: 585-594.
- Chakraborty, K., Bose, J., Shabala, L., Shabala, S. (2016) Difference in root K<sup>+</sup> retention ability and reduced sensitivity of K<sup>+</sup>-permeable channels to reactive oxygen species confer differential salt tolerance in three Brassica species. *Journal of Experimental Botany* 67: 4611-4625.
- Chapman, H. D. and Pratt, P. F. (1961) *Methods of analysis for soils, plants, and waters*. University of California, Berkeley, Calif.
- Chen, Z., Newman, I., Zhou, M., Mendham, N., Zhang, G. and Shabala, S. (2005) Screening plants for salt tolerance by measuring K<sup>+</sup> flux: a case study for barley. *Plant Cell Environment* 28: 1230-1246.
- Condit, I. (1955) *Fig varieties: A monograph*. Hilgardia 23.
- Duenas, M., Pérez-Alonso, J. J., Santos-Buelga, C. and Escribano-Bailón, T. (2008) Anthocyanin composition in fig (*Ficus carica* L.). *Journal of Food Composition and Analysis* 21: 107-115.
- Duran-Zuazo, V. H., Martínez-Raya, A. and Aguilar Ruiz, J. (2003) Salt tolerance of mango rootstock. *Spanish Journal of agricultural research* 1: 67-78.
- El-Gharably, A. M., Nagib, A. and Rizk, E. (2009) Evaluation of quality attributes for Egyptian apricot and fig cultivars produced by osmodying process. *Annals of Agricultural Sciences. Sci* 54: 359-369.
- Essam, M., Mohamad, M. and Zakaria I. (2013) Effect of different concentration of carbon source, salinity and gelling agent on in vitro growth fig (*Ficus carica* L.). *African Journal of Biotechnology* 12: 936-940.
- Evans, J. R., Caemmerer, S. V (1996) Carbon Dioxide diffusion inside leaves. *Plant Physiol* 110: 339-346.
- FAO (2016) [www.FAOstat.fao.org](http://www.FAOstat.fao.org).2016.
- Ferreira-Silva, S. L., Silveira, J., Voigt, E., Soares, L. and Viegas, R. (2008) Changes in physiological indicators associated with salt tolerance in two contrasting cashew rootstocks. *Brazilian Journal of Plant Physiology* 20: 51-59.
- Flexas, J. J., Bota, F., Loreto, G. and Cornic. Sharkey, T. D. (2004) Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. *Plant Biology* 6: 269-279.
- Frodin, D. G. (2004) History and concepts of big plant genera, Taxon. *International Association*. 53: 753-776.
- Gaber, M. A. (2010) Antioxidative defense under salt stress. *Plant Signal. Behav for Plant Taxonomy* 5: 369-374.
- García-Ruiz, M. T., Mendoza-Castillo, V. M., Valadez-Moctezuma, E. and Muratalla-Lua, A. (2013) Initial assessment of natural diversity in Mexican fig landraces. *Genetics and Molecular Research* 12: 3931-3943.
- Golombek, S. D. and Ludders, P. (1990) Gas exchange of *Ficus carica* in response to salinity. In: *Plant Nutrition- Physiology and Applications* (ed. Beusichem, M. L.) Pp. 487-493. Kluwer Academic Publishers, Dordrecht. The Netherlands.
- Grattan, S. R. and Grieve, C. M. (1999) Mineral nutrient acquisition and response by plants grown in saline environments. In: *Handbook of Plant and Crop Stress* (ed. Pessarakli, M.) Pp. 203-229. Marcel Dekker, New York.
- Hopkins, W. (2008) *Introduction to plant physiology*. Wiley.
- Iezzoni, A. F. and Pritts, M. P. (1991) Applications of principal component analysis to horticultural research. *HortScience*, 26: 334-338.
- João-Correia, M., Leonor-Osorio, M. J. O., Barrote, I., Martins, M., David, M. (2006) Influence of transient shade periods on the effect of drought on photosynthesis, carbohydrate accumulation and lipid peroxidation in sunflower leaves. *Environmental and Experimental Botany*. 1: 75-84.
- Karnosky, D. F., Gagnon, Z. E., Dickson, R. E., Coleman, M. D., Lee, E., Lsebrands, J. (1996) Changes in growth, leaf abscission, and biomass associated with seasonal tropospheric ozone exposures of *Populus tremuloides* clones and seedlings. *Canadian Journal of Forest Research* 26: 23-37.

- Luachli, A. and Wteneke, J. (1979) Studies on growth and distribution of Na, K and CL soybean varieties differing in salt tolerance. *Z. pflanenernacher. Bodenkd* 142: 3-13.
- Marschner, H. (1995) Functions of Mineral Nutrients: Micronutrients. *Mineral Nutrition of Higher Plants*. 2<sup>nd</sup> Ed. Academic Press Limited. San Diego.
- Mathaba, N., Bertling, I. and Bower, J. P. (2013) Chilling injury in citrus fruit: a holistic view. *Acta Horticulturae*. 1007: 103-110.
- Metwali, E. M. R., Hemaïd, I. A. S., Al-Zahrani, H. S., Howladar, S. M. and Fuller, M. P. (2014) Influence of different concentration of salt stress on in vitro multiplication of some fig (*Ficus carica* L.) cultivars. *Life science Jurnal* 11:386-397.
- Munns, R. and Tester, M. (2008) Mechanisms of salinity tolerance. *Annual Review of Plant Biology* 59: 651-81.
- Munns, R. (2002) Comparative physiology of salt and water stress. *Plant Cell Environment* 25: 239-250.
- Pourghayoumi, M., Bakhshi, D., Rahemi, M., Noroozisharaf, A., Jafari, A., Salehi, M., Chamane, R. and Hernandez, F. (2016) Phytochemical attributes of some dried Fig (*Ficus carica* L.) fruit cultivars grown in Iran. *Agricluturæ Conspectus Scientificus* 81: 161-166.
- Qasim, M., Ashraf, M., JAamil, M., Ashraf, M., Rahman, S. and Rha, E. (2003) Water relations and leaf gas exchange properties in some elite canola (*Brassica napus*) lines under salt stress. *Annals of Applied Biology* 142: 307-316.
- Ritchie, S., Nguyen, A. S. and Holaday, H. T. (1990) Leaf water content and gas-exchange parameters of two wheat genotypes differing in drought resistance. *Crop Science* 30.
- Rodriguez-Dominguez, C. M., Buckley, T., Egea, G., de Cires, A., Hernandez-Santana, V., Martorell, S. and Diaz-Espejo, A. (2016) Most stomatal closure in woody species under moderate drought can be explained by stomatal responses to leaf turgor. *Plant Cell Environment* 39: 2014-2026.
- Ruiz-Sanchez, M. C., Domingo, R., Torrecillas, A. and Perez-Pastor, A. (2000) Water stress preconditioning to improve drought resistance in young apricot plants. *Crop Science* 156: 245-251.
- SAS (2002) Institute Inc. Cary, NC, USA,.
- Shabala, S. and Pottosin, I. (2014) Regulation of potassium transport in plants under hostile conditions : implications for abiotic and biotic stress. *Physiologia Plantarum* 151: 257-279.
- Soliman, H. I. A. and Abd Alhady, M. R. A. (2017) Evaluation of salt tolerance ability in some fig (*Ficus carica* L.) cultivars using tissue culture technique. *Journal of Applied Biology and Biotechnology*.5: 29-39.
- Syvrtsen, J., Melgar, J. and Garcia-Sanchez, F. (2010) Salinity tolerance and leaf water use efficiency in Citrus. *Journal of the American Society for Horticultural Science* 135: 33-39.
- Szczerba, M. W., Britto, D. T., Balkos, K. D. and Kronzucker, H. J. (2008)  $\text{NH}_4^+$ -stimulated and -inhibited components of  $\text{K}^+$  transport in rice (*Oryza sativa* L.). *Journal of Experimental Botany* 59: 3415-3423.
- Szczerba, M. W., Britto, D. T. and Kronzucker, H. J. (2009)  $\text{K}^+$  transport in plants: physiology and molecular biology. *Plant Physiology* 166: 447-466.
- Tester, M. and Davenport, R. (2003)  $\text{Na}^+$  tolerance and  $\text{Na}^+$  transport in higher plants. *Annales Botanici Fennici* 91: 503-527.
- Toribio, F. and Montes, P. (1996) Variedades de la higuera. *Ciencia Rural* 27: 92-96.
- Walker, R. R., Torokfalvy, E., Steele Scott, N. and Kriedemann, P. E. (1981) An analysis of photosynthetic reponse to salt treatment in *Vitis vinifera*. *Australian Journal of Plant Physiology* 8: 359-374.
- Walker, R. R., Torokfalvy, E. and Downton, W. J. S. (1983) Photosynthetic responses of the citrus varieties rangpur lime and etrog citron to salt treatment. *Australian Journal of Plant Physiology*. 9: 783-790.
- Zarei, M., Azizi, M., Rahemi, M. and Tehranifar, A. (2016) evaluation of nacl salinity tolerance of four fig genotypes based on vegetative growth and ion content in leaves, shoots, and roots. *HortScience* 51: 1427-1434.
- Zarei, M., Azizi, M., Rahemi, M., Tehranifar, A. and Davarpanah, S. (2017) Effect of salinity stress on some physiological and biochemical responses of four fig (*Ficus carica* L.) hybrids. *Iranian Journal of Horticultural Science and Technology*. 18: 143-158.