International Journal of Horticultural Science and Technology
Vol. 1, No. 2; December 2014, pp 121-129

Salt stress-induced changes in leaf antioxidant activity, proline and protein content in 'Shah Anjir' and 'Anjir Sabz' fig seedlings

Rohollah Abdoli Nejad and Akhtar Shekafandeh*
Department of Horticultural Sciences, College of Agriculture, University of Shiraz, Shiraz, Iran

(Received: 16 February, 2014, Accepted: 19 April 2014)

Abstract
A major portion of the Iranian fig (*Ficus carica*) industry is located in high-salinity regions, and salinity is an important limiting factor in the production of this fruit. The present study was conducted to investigate the changes of leaf antioxidant-enzyme activity, proline and total protein content in two fig cultivars with two leaf shapes: 'Anjir Sabz' with lobate and palmate leaves, and 'Shah Anjir' with lobate and palmate leaves, under salt stress condition. The seedlings were exposed to different NaCl concentrations so that six different electrical conductivity levels of 0.6, 3, 6, 9, 12 and 15 dSm^{-1} were achieved in pots. This experiment was performed as factorial based 6×2(2) in a completely randomized design with four replications and two seedlings in each replicate. The results showed that as the soil salinity increased, the proline and protein contents of both cultivars were increased. However, palmate leaves of both cultivars accumulated more proline and protein than those of their lobate leaves. The activities of antioxidant enzymes increased in seedlings of both cultivars; however, superoxide desmutase and catalase showed more activity in palmate leaves than lobate leaves. There were no significant differences between the two leaf shapes in relation to peroxidase. The results seem to suggest that seedlings with palmate leaves are more salt tolerant than seedlings with lobate leaves.

Keywords: electrical conductivity, ficus carica, sodium chloride.

Introduction

Salinity is one of the major environmental constraints on plant growth and productivity. The deleterious effects of salinity on plant growth are associated with low osmotic potential of soil solution (water stress), nutritional imbalance, ion toxicity and oxidative stress (Cavagnaro *et al*., 2006). Under optimal conditions reactive oxygen species (ROS) including superoxide radicals (O_2^{*-}), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (-OH), which are continuously produced at low levels mainly in chloroplast, mitochondria and peroxisomes of plant cells (Gill and Tuteja, 2010). The balance between production and removal of ROS is controlled by different cellular mechanisms (Uchida *et al*., 2002), whereas, under severe abiotic stress condition, such as salinity, the production of ROS increases. ROS destabilize a host of cellular functions by damaging nucleic acids, oxidizing proteins, and causing lipid peroxidation (LPO) (Cicek and Cacirlar, 2008). Stress-induced ROS accumulation is defused by compatible osmolytes (such as sugar and proline) and enzymatic antioxidant

* Corresponding author
Email: shekafan@shirazu.ac.ir

systems. In general, plants have a system of antioxidant enzymes, including superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POD) as well as an antioxidant non-enzymatic system that comprises ascorbic acid and glutathione. It is known that salt-tolerant genotypes of most plant species have higher activities or levels of antioxidant enzyme than those of salt-sensitive ones (Sorkheh *et al*., 2012).

Fig (*Ficus carica* L.) is a fruit tree native to Asia and the eastern Mediterranean region and is a member of the Moraceae family (Duenas, 2008). World fig production per annum is about one million tons. Iran stands fourth in annual production, with a national yield of 76,414 t (FAO, 2010). The province of Fars is particularly renowned for its dried figs, producing 29,000 t in 2010.

The usual propagation method of fig trees is hardwood cutting. Nowadays, due to the lack of water and increasing soil salinity, using grafted plants (desire scion on tolerant rootstock) can be a good option to confront the adverse condition of soil salinity. In addition, using seedlings, due to their deep root system in comparison to the shallow roots of stem cuttings, also can help. To our knowledge, there are no published papers on salt tolerance of the fig seedlings for use as rootstock.

Soil salinity has been intensified by consecutive years of drought, which is a major constraint for fig production in Iran. As a consequence, the salinity problem has received notable attention. Till now, figs have not been properly evaluated for salt tolerance. In recent decades, most selection procedures in fig breeding for salinity tolerance have been based on differences in agronomic character, such as survival, plant height, leaf area, relative growth, etc. The selection will be more convenient and practical if distinctive indicators against salt tolerance can be suggested at whole-plant, tissue or cellular levels (Ashraf and Harris, 2004).

The objective of this research was to evaluate the changes of antioxidant enzymes, proline and protein in relation to leaf shape of two fig cultivars under various levels of salt stress.

Materials and Methods

The mature fruit of two fig cultivars, ‘Anjir Sabz’ and ‘Shah Anjir’, were collected from open-pollinated fig trees from the Estahban Fig Research Station in Fars Province, Iran. Geographically, the Station is situated at 29° and 28' North latitude and 54° and 29' East longitude and its average elevation from sea level is about 1767 metres. Fruits were stored in deionized water for 12 hours and seeds were separated and rinsed several times and then dried with a towel. A total of 1,500 seeds of ‘Anjir Sabz’ and ‘Shah Anjir’ were sown in 200 ml plastic drinking cups (three seeds in each cup) containing a mixture of sand, soil and perlite (1:1:1, v/v/v). After three months, seedlings were transferred to plastic pots (7 kg) filled with a mixture of soil, sand and leaf mould (1:1:1, v/v/v), with a gravel layer at the bottom without drainage. One seedling was planted per pot. At that time, the experimental seedlings were collected for their phenotypic homogeneity. Due to the distinctive differences between leaves of seedlings in both cultivars, they were divided into four groups: ‘Anjir Sabz’ lobate and palmate leaves; ‘Shah Anjir’ lobate and palmate leaves (Fig. 1).

The field capacity of the soil used for potting was determined according to the protocol described by Richards (1949). Potted seedlings were irrigated for one year to field capacity level with deionized water. In order to achieve optimum seedling vegetative growth, Kristalon complete fertilizer (pH 6.7) (Behrooyesh, Co.) was applied to each pot with irrigation water each fortnight. The seedlings were grown in a greenhouse under natural sunlight. The average day and night temperatures were 30 ± 2°C and 21 ± 2°C

with relative humidity of 40% and 60%, respectively. After one year's growth in pot, salinity treatments were applied. The experiment comprised six levels of salinity: 0.0, 0.75, 1.68, 2.52, 3.30 and 4.50 g of NaCl Kg^{-1} soil, which created electrical conductivities (EC) of 0.6, 3, 6, 9, 12 and 15 dSm^{-1} in the pots, respectively. The salts were applied to pots by irrigation water in a step-wise way until the appropriate concentrations were attained. Leaf samples were harvested six weeks after salt treatments to determine proline and protein contents and antioxidant activities.

Fig. 1. Differences in leaf shapes of two fig cultivars. Top left: 'Anjir Sabz' lobate leaf; top right: 'Anjir Sabz' palmate leaf; bottom left: 'Shah Anjir' lobate leaf; bottom right: 'Shah Anjir' palmate leaf.

Proline and protein content

Proline was extracted and its concentration determined by the method of Bates *et al*. (1973). Leaf tissues were homogenized in 3% sulpho-salicylic acid and the homogenate was centrifuged at 3,000×*g* for 20 minutes. The supernatant was treated with acetic acid and ninhydrin, boiled for one hour, and then the absorbance was determined at 520 nm. Proline (Sigma™) was used for a standard curve. The protein concentration of leaf crude extract was determined according to Bradford (1976) using bovine serum albumin (BSA) as a protein standard. Fresh leaf samples (1 g) were homogenized with 4 mL Na-Phosphate buffer (pH 7.2) and then centrifuged at 4°C. Supernatants and dye were transferred to spectrophotometer cuvettes with a pipette and absorbance was measured using a spectrophotometer (Model UV-120-20, Japan) at 595 nm.

Antioxidant enzyme activity

Frozen leaf samples (0.5 g) were used for enzyme extraction. Samples were ground with 2 mL of 50 mM phosphate buffer (pH 7.2) using pre-chilled mortar and pestle. The phosphate buffer contained 1 mM EDTA, 1 mM PMSF and 1% PVP-40. Then, the extract was centrifuged at 4°C at 17,000×g for 10 minutes. The supernatant was used for measurements of enzyme activity. A photochemical method by Giannopolitis and Reis (1977) was used to determine superoxide dismutase (SOD EC 1.15.1.1) activity. The reaction solution (3 mL) contained 50 µM NBT, 1.3 µM riboflavin, 13 mM methionine, 75 µM EDTA, 50 mM phosphate buffer (pH 7.8), and 20-50 µL of the enzyme extract. The test tubes containing the reaction solution were irradiated under artificial light (15 fluorescent lamps) at 78 µ mol $m^{-2}s^{-1}$ for 15 minutes. The absorbance of the irradiated

solution was read at 560 nm using a spectrophotometer (Model Cary 50). One unit of SOD activity was defined as the amount of enzyme that inhibited 50% ρ-nitro blue tetrazolium chloride (NBT) photo-reduction. Catalase (CAT, EC 1.11.1.6) activity was assayed by using a spectrophotometer that monitored the decrease in the absorbance of H_2O_2 at 240 nm using the method of Chance and Maehly (1955). The assay solution contained 50 mM potassium phosphate buffer (pH 7.0) and 15 mM H_2O_2. The reaction was started by the addition of 100 µL of enzyme extract to the reaction mixture and the change in absorbance ensued one minute after the start of the reaction. One unit of activity was considered as the amount of enzyme decomposing 1 mM of H_2O_2 in one minute. Peroxidase (POD, EC 1.11.1.7) activity was determined by measuring the oxidation of guaiacol in the presence of H_2O_2, and following an increase in absorbance at 470 nm over a one-minute interval. The enzyme was assayed in a solution containing 50 mM phosphate buffer (pH 7.0), 0.3% H_2O_2 and 1% guaiacol. The reaction started by the addition of 20 µL enzyme extract at 25°C. One enzyme unit was calculated on the basis of the formation of 1 mM tetra-guaiacol for one minute.

Statistical analysis

The experiment was performed as factorial based 6×2(2) in a completely randomized design with four replications and two seedlings in each replicate. A total of 192 seedlings (in each cultivar, 48 seedlings for each leaf form) were used. Variance of the data was analysed with the GLM procedure of the SAS software (version 9.1). Means were separated by LSD ($P \leq 0.05$).

Results and Discussion

Symptoms of salt injury started to appear on the leaf tips of lobate leaves of both cultivars receiving high-salinity treatment (12-15 dSm^{-1}) after five days of application. In control and lower levels of salinity treatments (0.6 and 3 dSm^{-1}), the leaves of seedlings were vigorous. In moderate levels of salinity (6 and 9 dSm^{-1}), the symptoms of leaf necrosis appeared two weeks after salt treatment.

In relation to proline content, as the soil salinity increased in amount, the proline content was increased in both cultivars regardless of leaf shapes. At low levels of salinity (0.6. 3, and 6 dSm^{-1}), there was no significant difference between two leaf shapes of both cultivars. However, in 15 dSm^{-1}, the proline contents of palmate leaves of both cultivars were significantly greater than that of the lobate leaves (Table 1).

This increased level of proline might have a protective effect on cell membrane of fig leaves due to its osmo-protectant nature. Free proline has been also known to act as a metal chelator, a protein stabilizer, an inhibitor of LPO, and as an OH^* and $^1O_2$ scavenger (Ashraf and Foolad, 2007). These results are in agreement with those previously reported by Karimi *et al*. (2009) in pistachio and Saeed (2005) in pomegranate.

As the total soluble leaf protein content increased up to the 6 dSm^{-1} treatment, soil salinity level increased regardless of leaf shape and cultivar, whereas the protein content decreased gradually at the higher concentrations. In EC 9 dSm^{-1} NaCl, 'Shah Anjir' accumulated significantly more protein than 'Anjir Sabz' (Table 2). Initial increase of protein may be due to synthesis of new stress proteins. This is similar to the results of Khani and Heidari (2008), who showed that drought stress caused initial increase in protein level due to the expression of new stress proteins and subsequent decrease due to severe decrease in photosynthesis in two different maize cultivars.

Table 1. Effects of NaCl concentrations on proline content (µmol g^{-1} F.W) of two fig cultivars and two leaf forms in each cultivar.

	'Anjir Sabz'		'Shah Anjir'		
NaCl (dSm^{-1})	lobate leaf	palmate leaf	lobate leaf	palmate leaf	Means †
0.6	7.06 h	8.21 h	7.59 h	7.61 h	7.56 F
3	7.97 h	7.87 h	7.86 h	8.12 h	7.95 E
6	12.67 g	13.44 g	13.13 g	13.68 fg	13.23 D
9	15.57 f	16.52 e	15.78 e	16.71 e	16.14 C
12	19.24 d	20.17 cd	19.93 cd	20.65 cd	20.65 B
15	21.10 cd	23.6 ab	21.87 bc	24.64 a	22.82 A
Means	13.93C	14.95AB	14.36BC	15.23A	
	Significance			proline (µmol g^{-1} F.W)	
	NaCl			**	
	leaf form			*	
	NaCl×leaf form			**	

† Means with the same letters (small for interactions and capital for main effects), are not significantly different at $P \leq 0.05$ using LSD test. * $P \leq 0.05$. ** $P \leq 0.01$.

Table 2. Effects of NaCl concentrations on total protein (mg g^{-1} Fw) of two fig cultivars and two leaf forms in each cultivar.

Cultivar	'Anjir Sabz'		'Shah Anjir'		
NaCl (dSm^{-1})	lobate leaf	palmate leaf	lobate leaf	palmate leaf	Means †
0.6	32.75 ghi	35.50 efg	35.25 fg	33.75 fgh	34.31 C
3	33.75 fgh	34.00 fgh	33.75 fgh	35.25 fg	34.18 C
6	44.50 ab	45.25 a	41.50 bc	44.75 ab	44.00 A
9	39.50 cd	39.00 cde	42.50 abc	42.50 abc	40.87 B
12	29.25 ijk	27.25 k	35.75 efg	36.75 def	32.25 D
15	23.50 l	26.75 kl	31.00 hij	28.25 jk	27.37 D
Means	33.87 B	34.62 B	36.62 A	36.87 A	
	Significance			protein (mg g^{-1}Fw)	
	NaCl			**	
	Leaf form			*	
	NaCl×leaf form			**	

† Means with the same letters (small for interactions and capital for main effects), are not significantly different at $P \leq 0.05$ using LSD test. * $P \leq 0.05$. ** $P \leq 0.05$.

By increasing soil salinity, superoxide dismutase (SOD) was highly activated, showing the highest peak at 9 dSm^{-1} regardless of leaf shape and cultivar. Generally, SOD was more active in the seedlings with the palmate leaves than those of lobate leaf forms. The highest SOD activity was recorded in 'Shah Anjir' seedlings at 9 dSm^{-1}: regardless of leaf shape, 819.52 Ug^{-1}F.W. for lobate and 830 Ug^{-1} F.W for palmate; this is compared to 'Anjir Sabz' at the same NaCl concentration: 742.87 Ug^{-1} F.W for lobate, 753.81 Ug^{-1} F.W for palmate (Table 3).

Table 3. Effects of NaCl concentrations on SOD activity (Ug^{-1} F.W) of two fig cultivars and two leaf forms in each cultivar.

Cultivar	'Anjir Sabz'		'Shah Anjir'		
NaCl (dSm^{-1})	lobate leaf	palmate leaf	lobate leaf	palmate leaf	Means †
0.6	248.02 m	251.54 m	252.65 m	257.23 m	253.31 E
3	356.7 jk	432.43 i	311.93 l	368.54 j	366.93 D
6	543.45 g	587.78 e	625.67d	687.78 c	610.31 B
9	742.87 b	753.81 b	819.52 a	830.54 a	786.18 A
12	490.32 h	572.59 ef	561.84 g	552.67 fg	521.62 C
15	348.00 jk	440.75 i	334.89 kl	336.12 kl	369.93 D
Means	453.91 C	512.00 A	482.83 B	506.12 A	
Significance				SOD (Ug^{-1} F.W)	
NaCl				**	
Leaf form				**	
NaCl×leaf form				**	

† Means with the same letters (small for interactions and large for main effects), are not significantly different at $P \leq 0.05$ using LSD test. * $P \leq 0.05$. ** $P \leq 0.05$.

Considerable inequality between the synthesis of ROS and the antioxidant defence action in any cell compartment can cause oxidative stress and damage (Mittler, 2002). Plants have evolved in a way that has led them to possess the scavenging mechanism of ROS by enzymatic pathways (Demiral and Turkan, 2005). It is well known that environmental stresses trigger the generation of ROS, whereas SOD is known to play a vital role when plants are under stress condition and provides the first defence barrier against the lethal effects of high levels of ROS, which can bring chain reactions (Gill and Tuteja, 2010). At this stage, superoxide dismutase (SOD) catalyses the dismutation of $O_2^{\bullet -}$ radicals to molecular O_2 and H_2O_2 (Meloni *et al*., 2003).

In both cultivars, the maximum catalase (CAT) activity was observed in palmate leaves at 9 dSm^{-1} NaCl (84.42 Ug^{-1}F.W in 'Shah Anjir' and 76.77 Ug^{-1}F.W in 'Anjir Sabz'); greater activity of catalase was observed at 12 dSm^{-1} NaCl in lobate leaves (84.81 in 'Shah Anjir' and 76.42 in 'Anjir Sabz') (Table 4). These results are similar to the previous observations that CAT activity plays a central protective role during salt stress (Weibing *et al*., 1993; Vaidyanathan *et al*., 2003). It also coincides with the salt tolerance in other plants, such as mulberry (Sudhakar, 2001). CAT is a super-active molecule in the world of enzymes; one molecule of CAT can convert six million molecules of H_2O_2 to H_2O and O_2 per minute (Gill and Tuteja, 2010). With increasing salinity levels, the POD activity significantly increased (Table 5). No significant differences were observed between the two cultivars. POD scavenges the accumulated H_2O_2 to non-toxic levels by converting it into water and oxygen (Apel and Hirt, 2004) by means of several reductants available in the cells (Mittler, 2002).

Regarding seedling growth (for example, stem length), there was a high interaction between leaf shape and levels of salinity. In both cultivars, at each level of salinity, the stem length of palmate leaf seedlings was higher than of lobate leaf seedlings (Table 6). As a consequence, 'Shah Anjir' accumulated more proline and protein under salt stress; antioxidant enzyme activities were more prominent than those for the 'Anjir Sabz' cultivar. In addition, in both cultivars, the antioxidant enzyme activity of palmate leaf seedlings was more prominent than for lobate leaf seedlings. The highest antioxidant enzyme activity was observed in 'Shah Anjir'

palmate leaf seedlings. Our results are in agreement with the findings of Weibing *et al*. (1993), who showed a positive relation between fig salt tolerance and free proline in leaves and concluded that this physiological parameter could be used as an index for the evaluation of salt tolerance.

According to our observations and data, both cultivars with palmate leaves were healthier and taller than those with lobate leaves (Fig. 2). Therefore, it can be stated that they are more tolerant to salt stress than lobate leaf seedlings.

Acknowledgement

The authors wish to express their appreciation to Dr H. Salehi for providing laboratory facilities for this study.

Table 4. Effects of NaCl concentration on CAT activity (Ug^{-1} F.W) of two fig cultivars and two leaf forms in each cultivar.

Cultivar	'Anjir Sabz'		'Shah Anjir'		
NaCl (dSm^{-1})	lobate leaf	palmate leaf	lobate leaf	palmate leaf	Means †
0.6	50.34 i	54.35 hi	52.93 hi	54.62 hi	52.83 D
3	54.56 hi	54.18 hi	52.10 i	55.47 hi	54.69 D
6	58.45 h	68.91 f	60.12 h	68.06 f	64.13 C
9	72.34 d	76.77 b	70.25 e	84.42 a	76.43 B
12	76.42 bc	74.43 c	84.81 a	82.72 a	78.45 A
15	74.23 c	72.63 d	74.16 c	78.53 b	76.85 B
Means	64.39 C	65.06 BC	65.72 BC	70.63 A	
Significance				SOD (Ug^{-1} F.W)	
NaCl				**	
Leaf form				*	
NaCl×leaf form				**	

† Means with the same letters (small for interactions and capital for main effects), are not significantly different at $P \leq 0.05$ using LSD test. * $P \leq 0.05$. ** $P \leq 0.05$.

Table 5. Effects of NaCl concentrations on POD activity (Ug^{-1} F.W) of two fig cultivars and two leaf forms in each cultivar.

Cultivar	'Anjir Sabz'		'Shah Anjir'		
NaCl (dSm^{-1})	lobate leaf	palmate leaf	lobate leaf	palmate leaf	Means †
0.6	12.01 f	10.12 f	12.34 f	12.21f	12.19 F
3	12.65 f	14.14 f	12.93 f	14.67 f	14.00 E
6	22.03 e	22.46 e	22.07 e	24.15 e	11.43 D
9	34.34 d	34.81d	36.23 d	36.76 d	35.44 C
12	40.64 c	42.29 b	44.37 b	44.91 b	44.07 B
15	48.98 a	52.05 a	50.34 a	52.12 a	50.62 A
Means	28.62 A	30.14 A	28.14 A	30.60 A	
Significance				SOD (Ug^{-1} F.W)	
NaCl				**	
Leaf form				ns	
NaCl×leaf form				**	

† Means with the same letters (small for interactions and capital for main effects), are not significantly different at $P \leq 0.05$ using LSD test. ns = non-significant. * $P \leq 0.05$. ** $P \leq 0.05$.

Table 6. Effects of NaCl concentrations on shoot length (cm) of two fig cultivars and two leaf forms in each cultivar.

Cultivar	'Anjir Sabz'		'Shah Anjir'		
NaCl (dSm^{-1})	Lobate leaf	Palmate leaf	Lobate leaf	Palmate leaf	Means †
0.6	59.9 cd	66.8 a	57.8 cde	68.8 a	62.8A
3	58.5 cde	66.1 a	61.4 bc	64.5 ab	62.6A
6	54.8 ef	64.6 bc	56.3 de	64.6 ab	59.3B
9	48.3gh	54.1ef	52.9 fg	54.2 ef	52.4C
12	43.3 ij	48.6 gh	47.5 hij	43.5 Hij	46.4D
15	42.6 i	46.4 hi	44.4 hij	45.4 hij	45.0D
Mean	51.0B	57.5A	53.1B	57.2A	

Significance	shoot length (cm)
NaCl	**
Leaf form	*
NaCl×leaf form	**

† Means with the same letters (small for interactions and capital for main effects), are not significantly different at $P \leq 0.05$ using LSD test. * $P \leq 0.05$. ** $P \leq 0.05$.

'Shah Anjir' 'Anjir Sabz'

Fig. 2. In each photo: right seedling: palmate leaf; left seedling: lobate leaf.

References

1.Apel, K. and H. Hirt. 2004. Reactive Oxygen Species: Metabolism, Oxidative Stress, and Signal Transduction, Annu. Rev. Plant Biol. 55:373-399.

2.Ashraf, M. and M.R. Foolad. 2007. Roles of Glycine Betaine and Proline in Improving Plant Abiotic Stress Resistance. Env. Exp. Bot. 59:206-216.

3.Ashraf, M. and P.J.C. Harris. 2004. Potential Biochemical Indicators of Salinity Tolerance in Plants. Plant Sci. 166:3-16.

4.Bates, L.S., R.P. Waldern and I.D. Teave. 1973. Rapid Determination of Free Proline for Water Stress Studies. Plant Soil. 39:205-107.

5.Bradford, M. M. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principles of

Protein Dye Binding. Anal. Biochem. 72:248-254.

6.Cavagnaro, J., M. Ponce., J. Guzman and M. Cirrincione. 2006. Argentinean Cultivars of *Vitis vinifera* Grow Better than European Ones when Cultured *in Vitro* under Salinity. Bio cell. 30:1-7.

7.Chance, B. and A.C. Maehly. 1955. Assay of Catalases and Peroxidases, P. 764-755. In: Colowick & Kaplan (eds.), Methods in Enzymology. Academic Press, New York.

8.Cicek, N. and H. Cacirlar. 2008. Changes in Some Antioxidant Enzyme Activities in Six Soybean Cultivars in Response to Long-Term Salinity at Different Temperatures. Gen. Appl. Plant Physiol. 34:267-280.

9.Demiral, T. and I. Turkan. 2005. Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. Environ. Exp. Bot. 53:247-257.

10.Duenas, M., J.J. Pérez-Alonsoa, C. Santos-Buelgaa and T. Escribano-Bailona, 2008. Anthocyanin Composition in Fig (*Ficus carica* L.) J. Food Compos. Ana. 21:107-115.

11.FAO. 2010. FAO STAT Agricultural Statistics Database. Retrived from http://www.fao.org

12.Giannopolitis, C.N. and S.K. Ries. 1977. Superoxide dismutases: I. Occurrence in higher plants. Plant Physiol. 59(2):309-314.

13.Gill, S.S. and N. Tuteja. 2010. Reactive Oxygen Species and Antioxidant Machinery in Abiotic Stress Tolerance in Crop Plants. Plant Physiol. Biochnol. 48:909-930.

14.Karimi, S., M. Rahemi., M. Maftoun., S. Eshghi and V. Tavallali. 2009. Effects of Long-Term Salinity on Growth and Performance of Two Pistachio *(Pistacia vera* L.) Rootstocks. Aust. J. Basic Appl. Sci. 3(3): 1630-1639.

15.Meloni, D.A., M.A. Oliva, C.A. Martinez and J. Cambraia. 2003. Photosynthesis and Activity of Superoxide Dismutase, Peroxidase and Glutathione Reductase in Cotton under Salt Stress. Environ. Exp. Bot. 49:69-76.

16.Mittler, R. 2002. Oxidative Stress, Antioxidants and Stress Tolerance. Trends Plant Sci. 7:405-410.

17.Mohammadkhani, N. and R. Heidari. 2008. Effects of Drought Stress on Soluble Proteins in Two Maize Varieties. Turk. J. Biol., 32: 23-30.

18.Richards, L.A., 1949. Methods of Measuring Soil Moisture Tension. Soil Sci. 68: 95-112.

19.Saeed, W. T. 2005. Pomegranate Cultivars as Affected by Paclobutrazol, Salt Stress and Change in Fingerprints. Bull. Faculty Agr. 56:581-616.

20.Sorkheh, K ., B. Shiran, V. Rouhi, M. Khodambashi and A. Sofo. 2012. Salt Stress Induction of some key Antioxidant Enzymes and Metabolites in Eight Iranian Wild Almond Species. Acta Physiol. Plant. 34:203-213.

21.Sudhakar, C., A. Lakshmi and S. Giridarakumar. 2001. Changes in the Antioxidant Enzyme Efficacy in Two High Yielding Genotypes of Mulberry (*Morus alba* L.) under Nacl Salinity. Plant Sci. 161:613-619.

22.Uchida, A., A.T. Jagendorf., T. Hibino and T. Takabe. 2002. Effects of Hydrogen Peroxide and Nitric Oxide on Both Salt and Heat Stress Tolerance in Rice. Plant Sci. 163:515-523.

23.Vaidyanathan, H., P. Sivakumar., R. Chakrabary and G. Thomas. 2003. Scavenging of Reactive Oxygen Species in Nacl–Stressed Rice (*Oryza sativa* L.) Differential Response in Salt–Tolerant and Sensitive Varieties. Plant Sci. 165:1411-1418.

24.Weibing, J., M. Kai and Y. Wang. 1993. A Preliminary Study on Physiological Indexes of Salinity Tolerance in Fig. Jiangsu J. Agr. Sci. (Abstract).