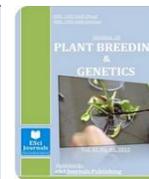




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## BREEDING DROUGHT RESISTANCE IN FIG VIA CONTROLLED HYBRIDIZATION

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### ABSTRACT

This study as a part of a fig drought tolerance breeding program, was conducted to evaluate the effects of pollen sources and maternal plants on drought tolerance of first generation seedlings. Three Iranian cultivars were selected as maternal plants from hot dry lands of central Iran and cross pollinated with four Iranian caprifigs. The offsprings were subjected to drought stress by stopping irrigation for 15 days and reirrigated for 10 days to screen drought tolerance. The results indicated hybrids 1, 7, 8, 9 and 11 were able to preserve water status and growth rate at higher levels during the water stress period. These hybrids were able to accumulate higher concentrations of proline and soluble carbohydrates for osmoregulation and prevent structural damages under water stress. It was concluded that drought tolerance in fig offsprings is highly related to drought tolerance of their parents, and using a highly drought tolerant parent in controlled pollinations may result in a drought tolerant hybrid. Hence, using caprifig 'Khormaei' as paternal parent and fig 'Siah' or 'Sabz' as maternal parent in controlled pollinations to obtain drought tolerant fig hybrids was suggested.

**Keywords:** Caprifig, Controlled Pollination, Growth Indices, Membrane Stability index, Proline, Soluble Carbohydrates.

### INTRODUCTION

At least one third of the earth surface is classified as arid and semi-arid. Such lands are prone to incidence of periodic droughts during growing season which affect growth and production of crops. Using drought tolerant cultivars is a sustainable approach to preserve crop production under such circumstances (Zamani *et al.*, 2002). Fig is one of the most important crops of arid and semi-arid lands of the world, however, global warming and incidence of extended drought condition significantly affected its production during the last decade. The extent of the drought damages to fig orchards of the Middle East is as much as high which fig production using the existing cultivars seems impossible.

Gholami *et al.* (2012) and Rostami and Rahemi (2013) reported genetic differences in drought tolerance of fig and caprifigs, respectively. Hence there is a hope to use such drought tolerant plants in replacing cultivar of the existing orchards and or use as rootstock for establishing new fig orchards. On the other hand, such

genotypes are a valuable source to be involved in the classic breeding programs as pollen sources or maternal plants, to produce high yield cultivars suitable for drought prone conditions.

There are limited reports on evaluating drought tolerance of Iranian caprifigs to utilize drought tolerant genotypes in controlled cross pollinations, however, our previous researches on Iranian caprifigs showed that there are valuable drought tolerant genotypes adoptable to hot dry lands of central Iran (Rostami and Rahemi, 2013) which potentially can be used in fig drought breeding programs. Hence, this study was conducted to evaluate the effect of pollen source from Iranian caprifigs with known range of drought tolerance on drought tolerance of the first generation offsprings.

### MATERIALS AND METHODS

**Plant material, experimental conditions and treatments:** This study was conducted as a complete randomized design with 20 replications per treatment (one pot per replication) at the experimental greenhouse of the Department of Horticultural Science of Shiraz University, Iran during March to September, 2012. Plant material used in this study was involved 12 first generation seedlings obtained from controlled

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pollinations. Maternal plants were involved three high yield fig cultivars adapted to hot dry climate of central Iran ('Sabz', 'Siah', and 'Shah Anjir'). Four Iranian male caprifig genotypes namely 'Danesh Sephid', 'Pouz Donbali', 'Shah Anjiri', and 'Khormaei' were used as pollen sources. These genotypes generally are distributed in the southern mountains of Iran and widely used as pollen source for caprifig. Controlled pollinations were performed on trees of Iranian Fig Research Center, Istahban, Iran. Branches of the female parents were isolated using cloth bags at four directions (North, East, West, and South) at beginning of fruit formation stage. Some fruits of the male parents containing *Blastophaga* bees were placed into the bags

and cross pollination was done by the natural act of the *Blastophaga* bees. The bags were removed about three weeks after the reception period of the female flowers. Seeds from the controlled pollinations were extracted from the fruits at the end of growing season 2011 and kept in dry and cold condition. The seeds planted in jiffy pots at the beginning of winter 2012 and kept under greenhouse condition till formation of 2 leaves, and then the jiffy pots placed into plastic bags containing 5 kg of sand, leafmould and loamy soil (1:1:1, v/v/v). Table 1 represent the physic-chemical properties of the soil used in the media. Three months later at the beginning of summer 2012, drought stress was applied to the seedlings during active growth of the seedlings.

Table 1. Physic-chemical properties of the soil used in the media.

Zn (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)	K (ppm)	P (ppm)	N (%)	OC (%)	pH	EC
1.50	7.16	21.14	1.76	400	23.8	0.094	1.54	7.9	1.93
Clay (%)				Silt (%)			Sand (%)		
34.4				44.2			21.4		

**Application of drought stress:** Drought stress was applied by withholding irrigation for 15 days. The plants in the control treatment were irrigated every day to keep water content of the pots at field capacity (FC) level. After the experimental period, the drought-stressed plants irrigated to FC level and recovery rate of the genotypes was evaluated after 10 days. The experiment repeated twice. The following observations were made at three steps involving first day of the experiment, at the end of the water stress, and after the recovery period.

**Shoot length and trunk diameter measurements:** Shoot length and trunk diameter were measured by a ruler and a digital caliper, respectively. Number of leaves were also counted before and after the drought period and the rewatering period. Relative shoot length and relative leaf number were calculated using the following formula:

$$\text{Relative shoot length} = \frac{\text{secondary shoot length} - \text{initial shoot length}}{\text{initial shoot length}}$$

$$\text{Relative leaf number} = \frac{\text{secondary leaf number} - \text{initial leaf number}}{\text{initial leaf number}}$$

In the above formula, initial shoot length and initial leaf number were recorded just before starting the experiment.

**Water content of the plants, relative water content (RWC) and water potential of the leaves ( $\Psi_{\text{Leaf}}$ ) determinations:** To evaluate the effect of the treatments

on water content of the plants, relative water content (RWC) and water potential of the leaves ( $\Psi_{\text{Leaf}}$ ) were determined. RWC was determined by using ten 7 mm diameter leaf discs. The leaf discs of each treatment were weighed (FW). They were then hydrated until saturation (constant weight) for 48 h at 5°C in darkness (TW). The leaf discs were dried in an oven at 105°C for 24 h (DW). Relative water content was calculated according to the following expression (Filella *et al.*, 1998):

$$\text{RWC}\% = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

$\Psi_{\text{Leaf}}$  was measured with a pressure chamber at 2 o'clock. After excising the fully expanded leaves, they were let stop bleeding and then  $\Psi_{\text{Leaf}}$  was measured. Electrolyte leakage was used to assess membrane permeability. Electrolyte leakage was measured using an electrical conductivity meter by using the method described by Lutts *et al.* (1995).

**Photosynthesis pigments and carotenoids measurement:** Photosynthesis pigments and carotenoids were measured in leaf discs with known weight during the experiment. The discs were cut into smaller pieces and extracted with 5 mL of cold acetone. Absorbance of the extract was measured by a spectrophotometer at 470, 646 and 663 nm. Concentration of chlorophylls and the carotenoids were determined following the equation proposed by Wellburn (1994). Five hundred mg leaf material was

homogenized in 1 ml of acidified (1% HCl) methanol and maintained at 4°C for 24 h. The absorption of anthocyanins at 550 nm was measured by a spectrophotometer UV-120-20 (Japan)). Concentration of anthocyanins was determined by using the extinction coefficient (Wagner, 1979):

$$\epsilon_{550} = 33,000 \text{ (cm}^2\text{/moll.)}$$

**Proline content determination:** Proline content was determined in 300 mg of leaf material via the method described by Bates *et al.* (1973). The absorbance was measured at 520 nm with a spectrophotometer (UV-120-20 (Japan)). L-Proline (SIGMA™) was used as standard. To evaluate soluble carbohydrates in the leaves, 200 mg of oven-dried leaves grounded and extracted by ethanol 80%. Concentration of soluble sugars was measured by the method described by Buysee and Mekex (1993).

Statistical differences between measurements were analyzed following the analysis of variance ANOVA using SPSS 16.0 software. Differences of means were considered significant using Duncan’s multiple range test at a probability level of  $P < 0.05$ .

**RESULTS**

**Relative growth of the hybrids height:** We obtained 12 F1 fig seedlings from controlled pollinations (Table 2). Drought tolerance of the parents was previously evaluated (Gholami *et al.*, 2012; Rostami and Rahemi, 2013) and is represented in table 2. Figure 1A shows relative growth of the hybrids height during the drought stress period. Drought stress reduced height of the plants and the lowest growth rates were found in hybrids No. 3, 4, and 11. Table 2. Cross pollinations and drought tolerance of the paternal plants.

Table 2. Cross pollinations and drought tolerance of the paternal plants.

Maternal ♀	Female Parent Drought Tolerance	Paternal ♂	Male Parent Drought Tolerance	Hybrid No.
Siah	Tolerant	Pouzdonbali	Semi-tolerant	1
Shah Anjir	Sensitive	Pouzdonbali	Semi-tolerant	2
Shah Anjir	Sensitive	Shah Anjiri	Tolerant	3
Sabz	Tolerant	Pouzdonbali	Semi-tolerant	4
Siah	Tolerant	Daneh Sefhid	Sensitive	5
Siah	Tolerant	Khormaei	Tolerant	6
Shah Anjir	Sensitive	Khormaei	Tolerant	7
Sabz	Tolerant	Khormaei	Tolerant	8
Siah	Tolerant	Shah Anjiri	Tolerant	9
Sabz	Tolerant	Shah Anjiri	Tolerant	10
Sabz	Tolerant	Daneh Sefhid	Sensitive	11
Shah Anjir	Sensitive	Daneh Sefhid	Sensitive	12

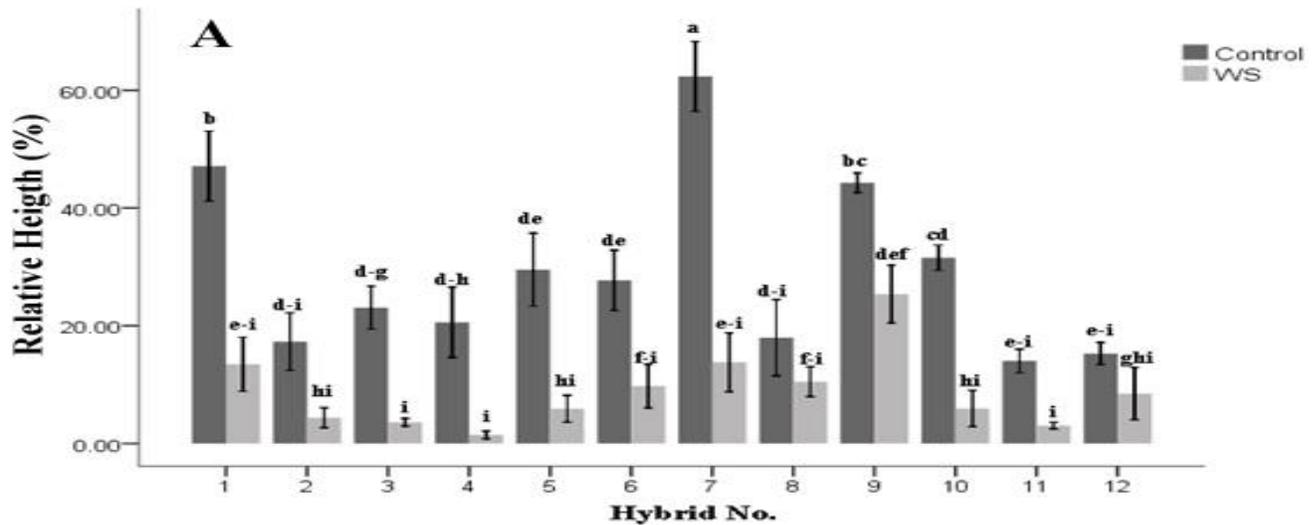


Figure 1A. Relative growth of the seedlings height during water stress periods in control and water stressed (WS) plants. †. Means with the same letters did not showed significant differences in Duncan’s Multiple Range test at  $P < 0.05$ .

Hybrid No. 9 had the highest rate of height increase during the water stress. Relative growth of the seedlings increased after the rewatering period, however, it did not recover to the respective control treatment in any of the hybrids (Figure 1B). During the rewatering period, the highest relative growth of seedlings height was found in hybrid No. 8, however hybrid No. 12 had the lowest height growth.

**Relative leaf number:** Water stress significantly

reduced relative leaf number of the seedlings (Figure 2A). At the end of the water stress period, the lowest relative leaf numbers were found in hybrids No. 2, 3, 4, 5, and 12, however, hybrid No. 1 had the highest relative leaf number at this stage. After the rewatering period relative leaf number of the seedlings significantly increased and the highest value was found in hybrid No. 2 and hybrid No. 12 had the lowest leaf number (Figure 2B).

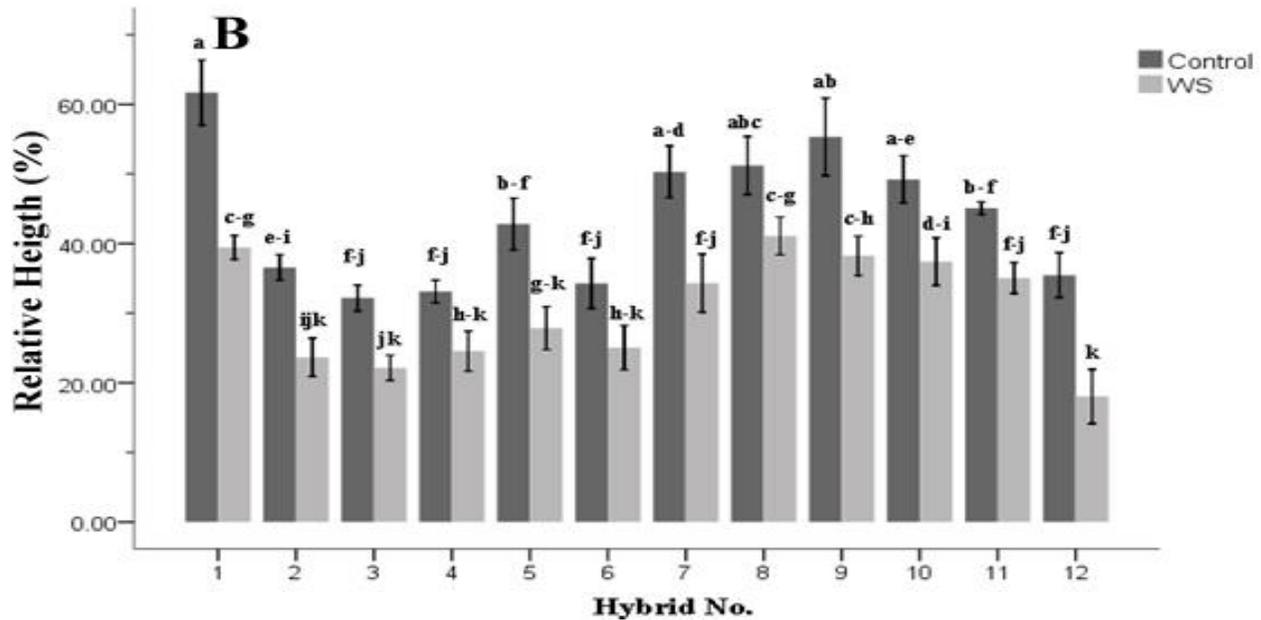


Figure 1B. Relative growth of the seedlings height during re-watering periods in control and water stressed (WS) plants. †. Means with the same letters did not showed significant differences in Duncan's Multiple Range test at P<0.05.

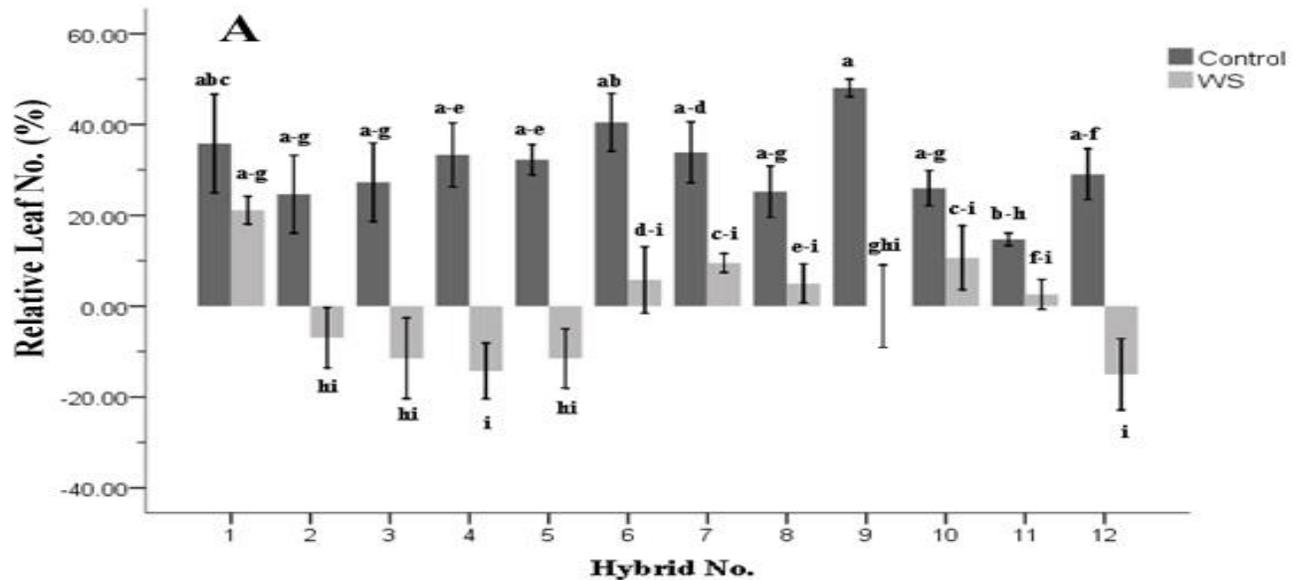


Figure 2A. Relative leaf number of the seedlings during water stress periods in control and water stressed (WS) plants. †. Means with the same letters did not showed significant differences in Duncan's Multiple Range test at P<0.05.

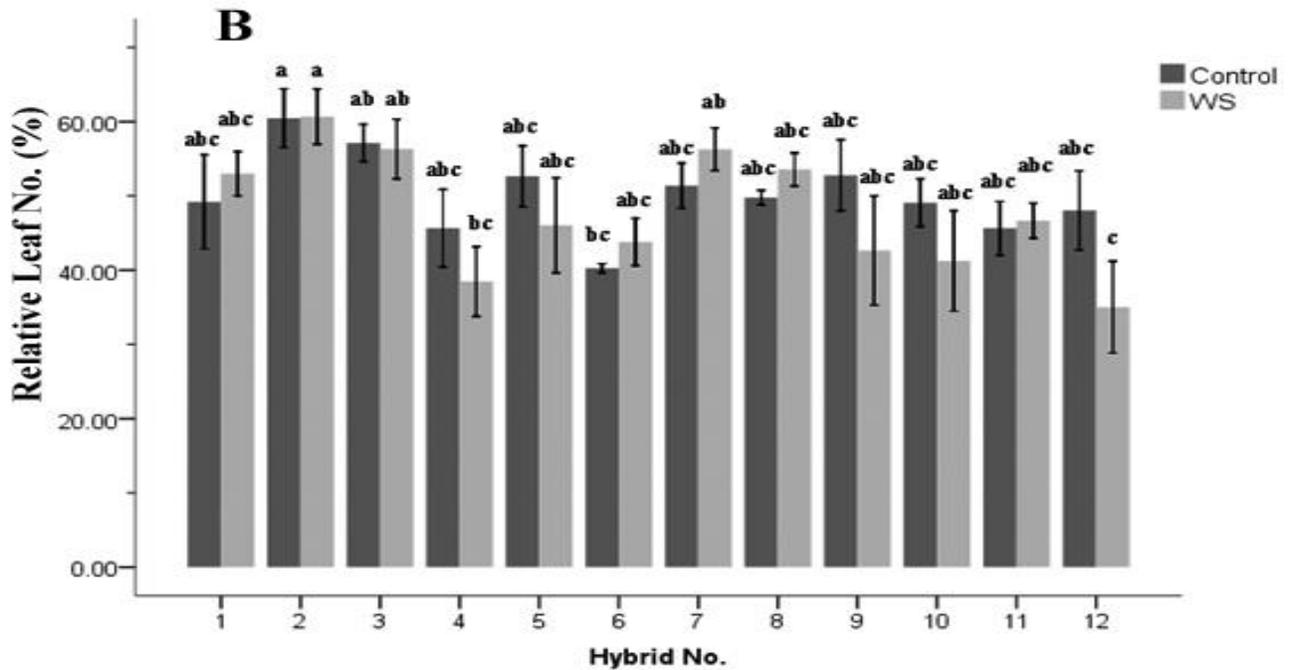


Figure 2B. Relative leaf number of the seedlings during re-watering periods in control and water stressed (WS) plants. †. Means with the same letters did not showed significant differences in Duncan’s Multiple Range test at P<0.05.

**Relative diameter:** Preventing irrigation significantly reduced relative diameter of the seedlings trunk (Figure 3A). At the end of the water stress period, the lowest relative trunk diameters were found in hybrids No. 2 and 4, and water stressed plants of hybrid No. 1 had the

highest trunk diameter growth. Trunk diameter of the seedlings was recovered to the respective control treatments after the rewatering period, however hybrids No. 1, 2, and 3 were exceptions (Figure 3B).

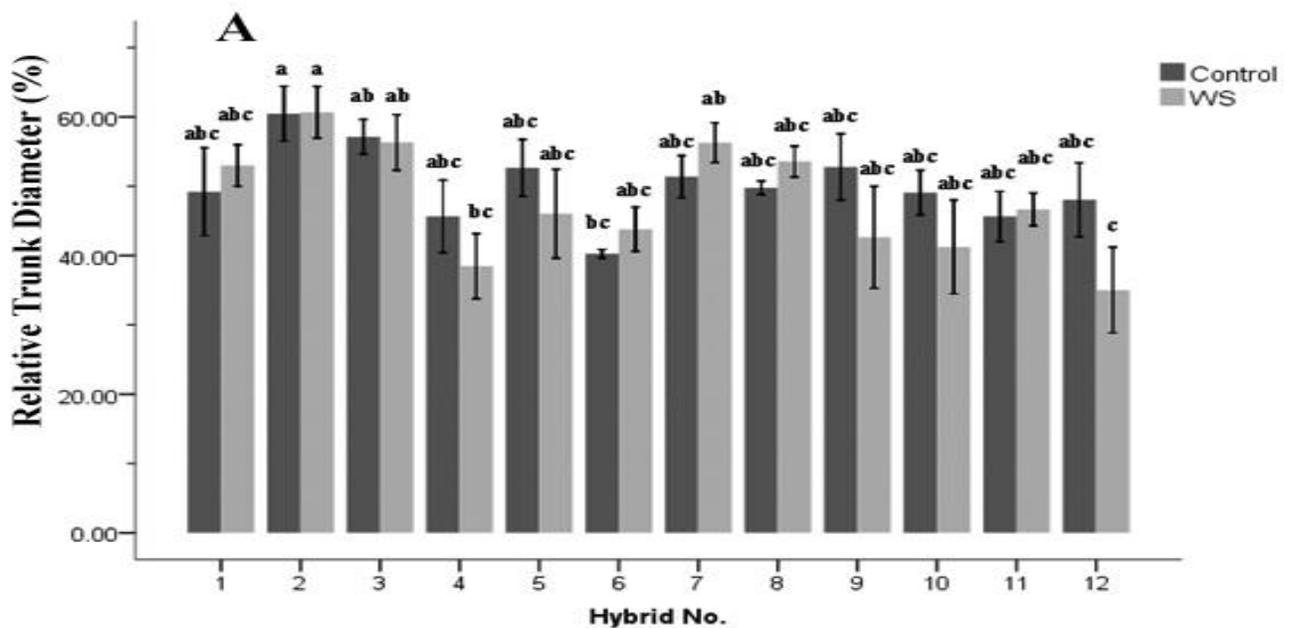


Figure 3A. Relative trunk diameter of the seedlings during water stress periods in control and water stressed (WS) plants. †. Means with the same letters did not showed significant differences in Duncan’s Multiple Range test at P<0.05.

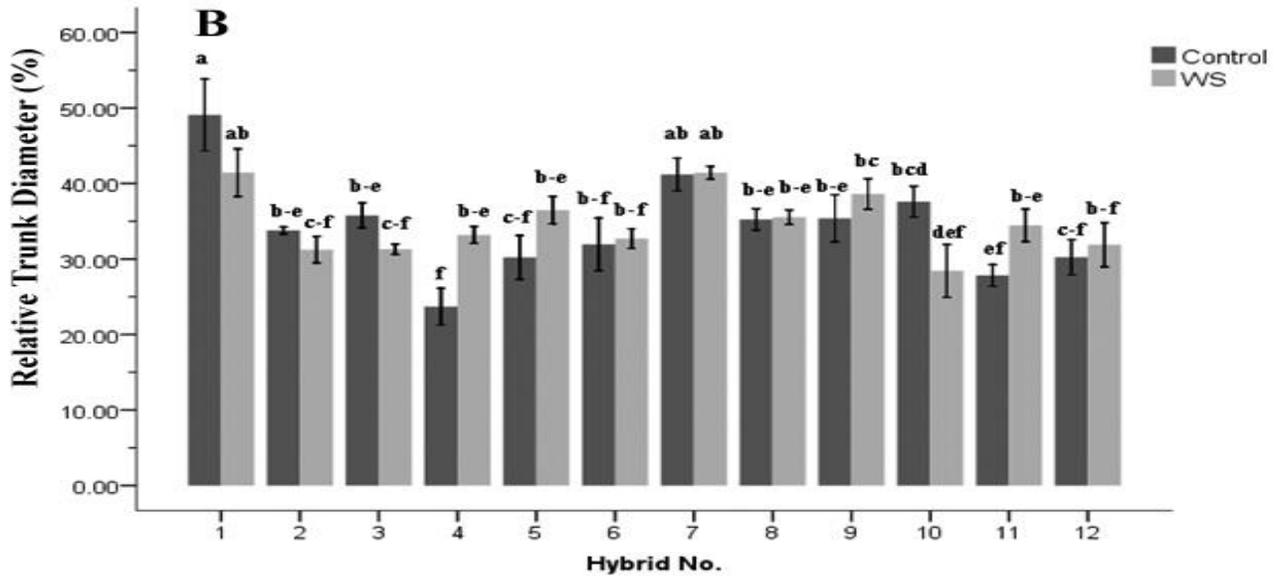


Figure 3B. Relative trunk diameter of the seedlings during re-watering periods in control and water stressed (WS) plants. †. Means with the same letters did not showed significant differences in Duncan’s Multiple Range test at P<0.05.

**leaf relative water content:** Water stress significantly reduced leaf relative water content (RWC) of the fig hybrids (Figure 4). The lowest RWC was found in hybrid No. 3, however hybrids No. 1 and 8 were able to preserve RWC at higher level till the end of the water stress period. RWC was significantly increased in all hybrids after the rewatering period. Leaf water potential

( $\Psi_w$ ) significantly reduced in the leaves of water stressed plants (Figure 5) and the lowest value was found in hybrid No. 3, however hybrids No. 1 and 9 had the highest  $\Psi_w$  at the end of the water stress period.  $\Psi_w$  significantly increased in the leaves of the fig hybrids after the rewatering period.

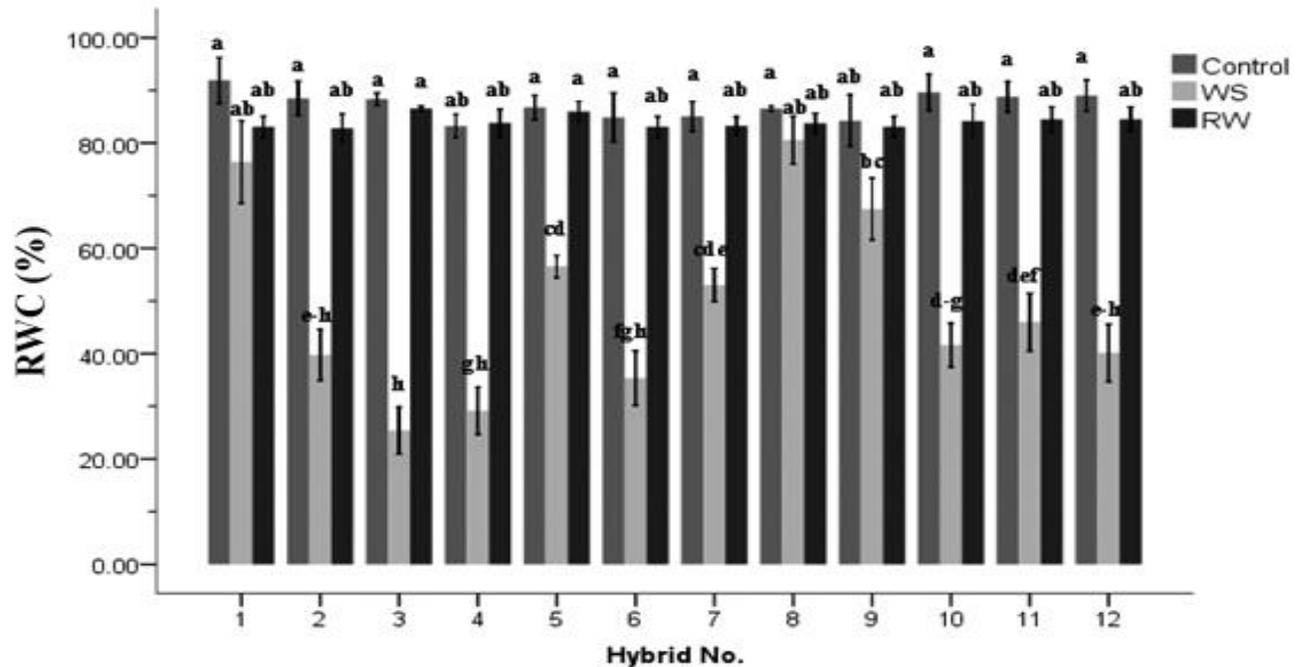


Figure 4. Leaf relative water content of the seedlings after water stress (WS) and rewatering (RW) periods. †. Means with the same letters did not showed significant differences in Duncan’s Multiple Range test at P<0.05.

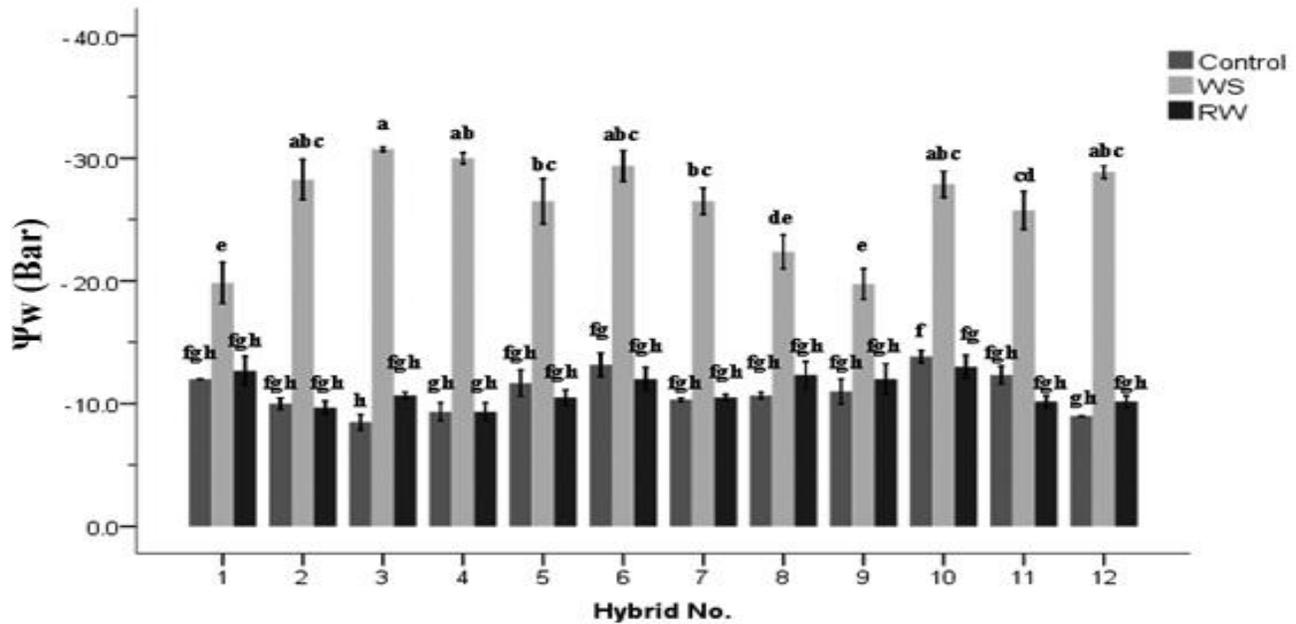


Figure 5. Leaf water potential ( $\Psi_w$ ) of the seedlings after water stress (WS) and rewatering (RW) periods. †. Means with the same letters did not showed significant differences in Duncan’s Multiple Range test at  $P < 0.05$ .

**Leaf membrane stability index:** Leaf membrane stability index (MSI) was significantly reduced at the end of the water stress period (Figure 6) and the lowest values were found in hybrids No. 4 and 6, however, MSI preserved at the highest levels in hybrids 1 and 8. Rewatering significantly increased MSI in the leaves of water stressed plants. Water stress had

different effects on leaf pigments involving carotenoids and chlorophylls (Figure 7). Leaf pigments significantly reduced in the leaves of hybrids No. 2, 3, 4, 5, 6, 10 and 12 after the water stress period, however, they increased in the leaves of the other hybrids. The same trends were found in the case of carotenoids to chlorophylls ratio (Figure 8).

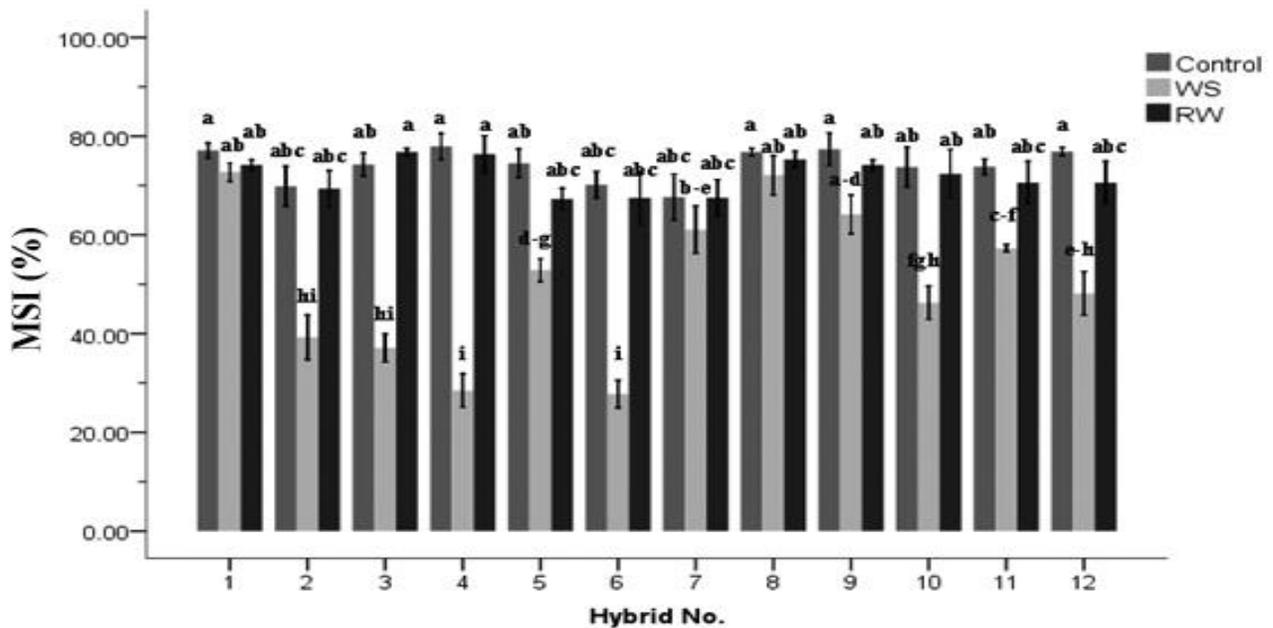


Figure 6. Leaf membrane stability (MSI) of the seedlings after water stress (WS) and rewatering (RW) periods. †. Means with the same letters did not showed significant differences in Duncan’s Multiple Range test at  $P < 0.05$ .

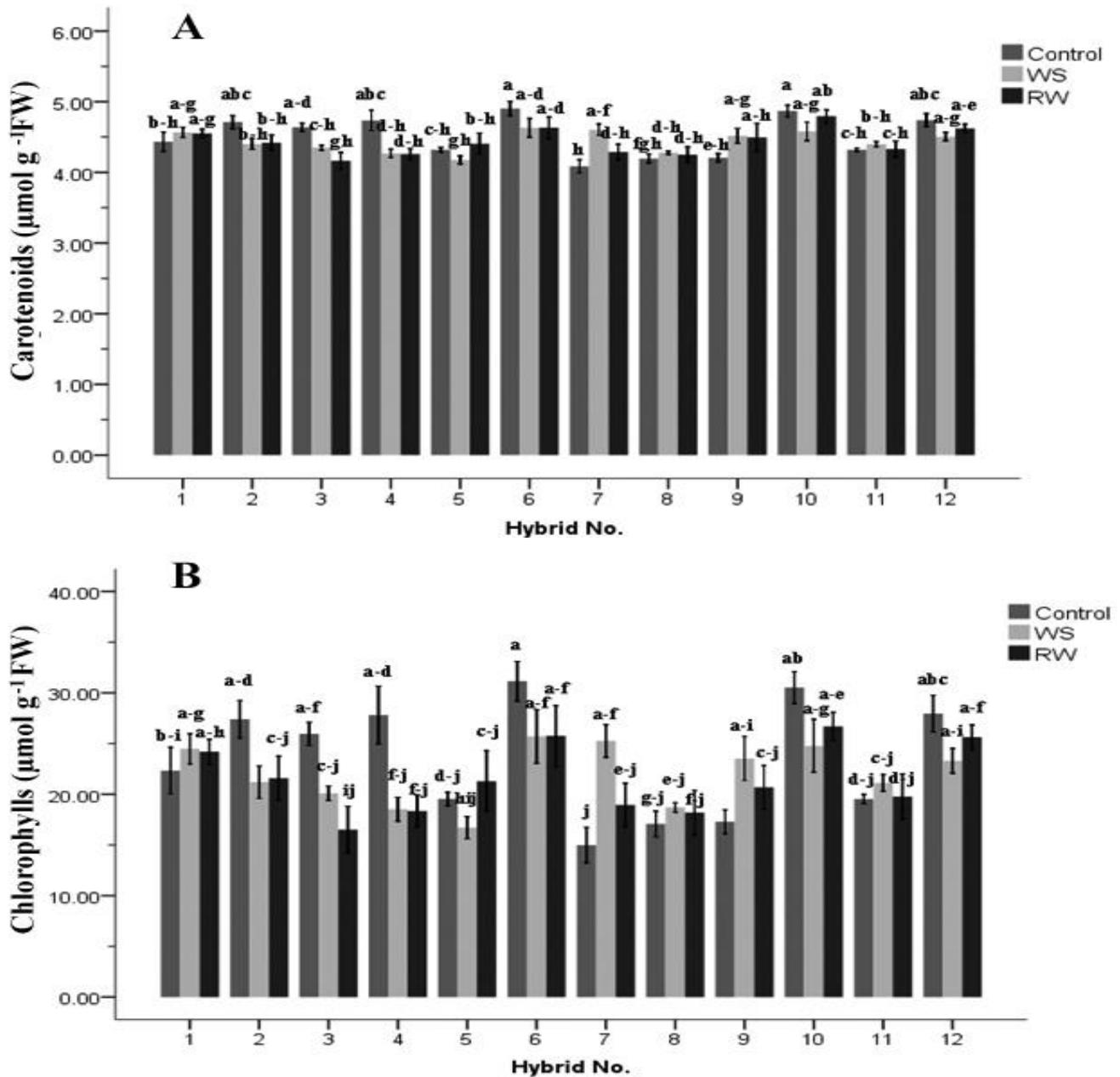


Figure 7. Carotenoids (A) and chlorophylls (B) content in the leaves of the seedlings after water stress (WS) and rewatering (RW) periods. †. Means with the same letters did not showed significant differences in Duncan’s Multiple Range test at P<0.05.

**Soluble carbohydrates:** Water stress significantly increased soluble carbohydrates in the leaves of hybrids No. 1, 7, 8, 9, and 11, however, with the exception of hybrid No. 2 which its soluble carbohydrates was remained unchanged the other hybrids showed significant reduction in the soluble carbohydrates (Figure 9). By the way, the highest soluble carbohydrates content was found in the leaves

of hybrid No. 1 and the lowest value was found in the leaves of hybrid No. 6. Rewatering period increased soluble carbohydrates content in the leaves of water stressed plants, however it did not fully recovered in the hybrids leave to their respective control treatments. Water stress period had no significant effects on reserve carbohydrates content in the leaves of the seedlings (data not shown).

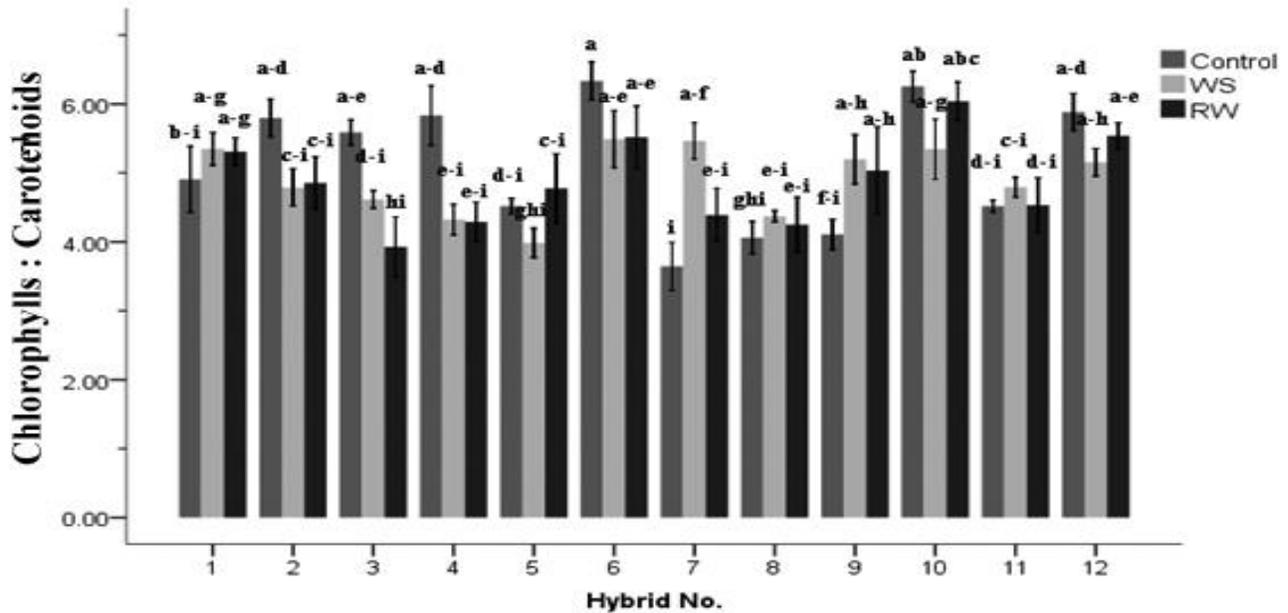


Figure 8. Carotenoids to chlorophylls content in the leaves seedlings after water stress (WS) and rewatering (RW) periods. †. Means with the same letters did not showed significant differences in Duncan’s Multiple Range test at P<0.05.

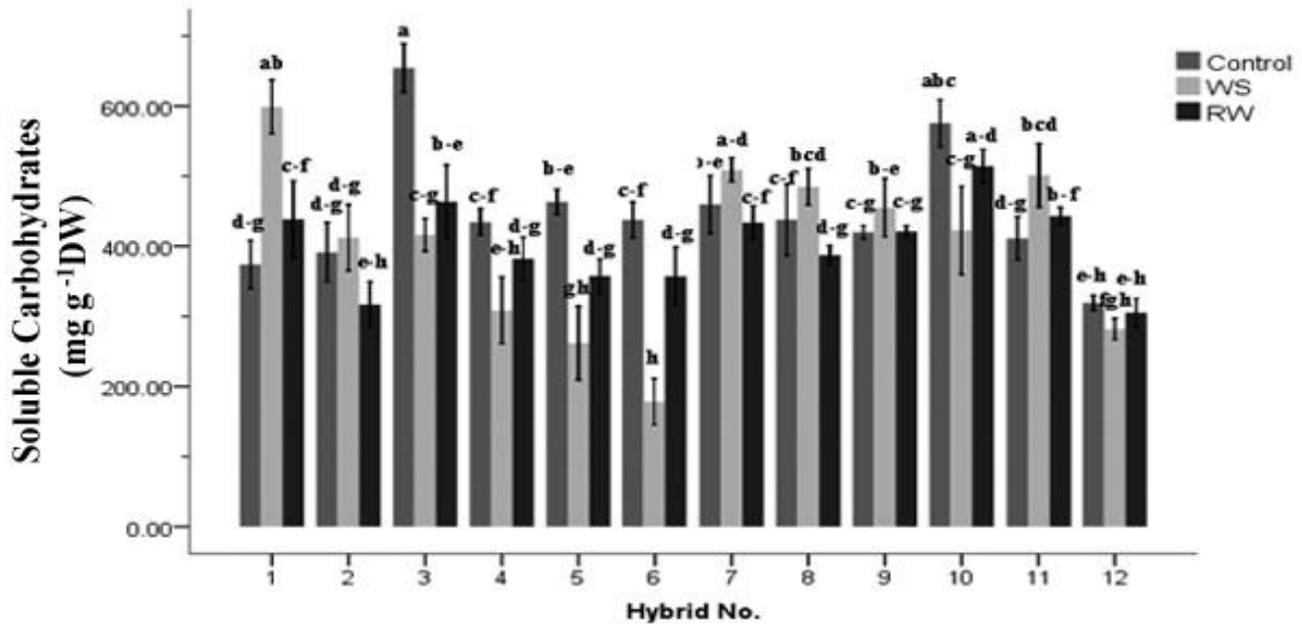


Figure 9. Soluble carbohydrates content in the leaves of the seedlings after water stress (WS) and rewatering (RW) periods. †. Means with the same letters did not showed significant differences in Duncan’s Multiple Range test at P<0.05.

**Proline concentration:** Proline concentration was significantly increased in the leaves of the seedling at the end of the water stress period (Figure 10). The highest proline concentrations were found in the leaves of hybrid No. 1, 8, and 10 and the lowest

concentration was found in the leaves of hybrid No. 4. Proline concentration significantly reduced in the leaves of water stressed plants after the rewatering period.

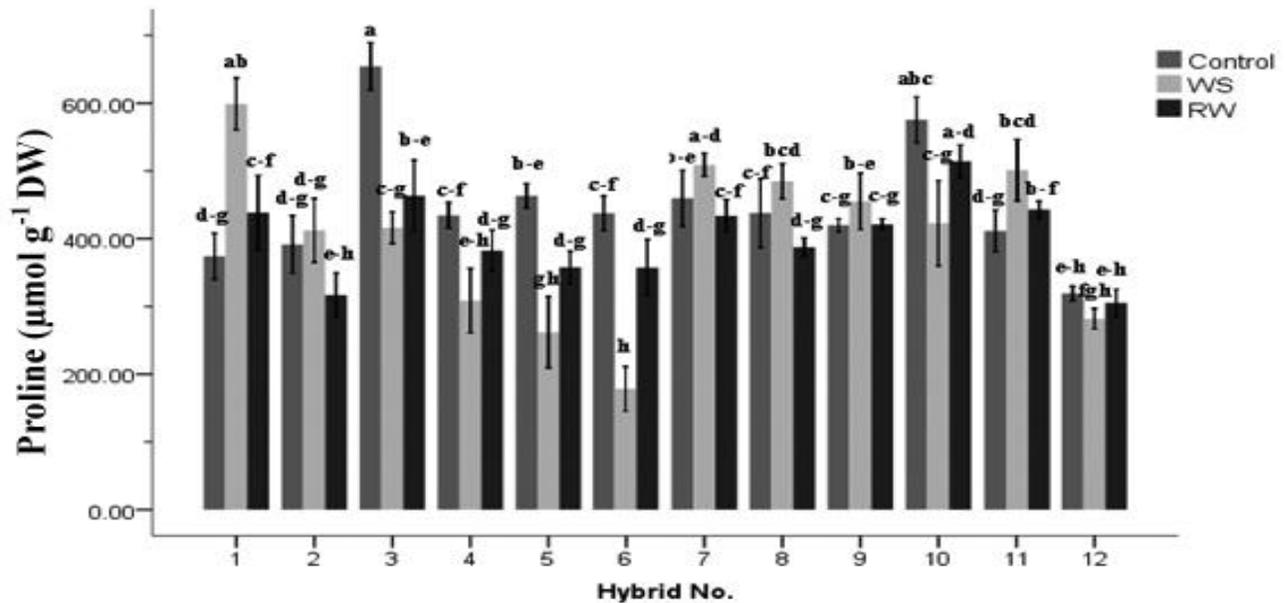


Figure 10. Proline content in the leaves of the seedlings after water stress (WS) and rewatering (RW) periods. †. Means with the same letters did not showed significant differences in Duncan’s Multiple Range test at P<0.05.

**DISCUSSION**

RWC and  $\Psi_w$  are the most parameters which widely used to evaluate water relation of plants under water stress (Pérez-Pérez, 2009). In this study hybrids 1, 5, 7, 8, and 9 were able to preserve RWC at higher level till end of the drought period. Teulate *et al.* (1997) introduced RWC as a reliable indicator of water status in plants which can be used in screening drought tolerance. On the other hand, Chartzoulakis *et al.* (2002) showed  $\Psi_w$  is more sensitive to water stress than RWC. Our data showed reduced  $\Psi_w$  in the leaves of all hybrids, however it remained at higher level in the leaves of hybrids 1, 5, 7, 8, and 9. It was probably because of ability of hybrids 1, 5, 7, 8, and 9 in preserving RWC at higher levels in their leaves and preventing turgor loss. Active osmoregulation which is a characteristic of drought tolerant plants is a key factor in preserving water status during soil water deficit. periods conditions.

Plants with the ability of preserving water content during water stress periods are more tolerant against water stress. Irigoyen *et al.* (1992) stated that higher RWC during water stress may represent higher consistency of cell wall against mechanical injuries and destructions under water stress Hybrids 1, 5, 7, 8, and 9 showed such an ability to preserve water status under water deficit condition, hence it may be used in drought tolerance screening of fig seedlings. However, relative

leaf changes data showed its better to consider other physiological responses in determining drought tolerance of plant, too. High RWC observed in hybrid No. 5 probably was correlated to high leaf shedding of the plant, an effort to prevent water loss during water deficit condition (Levitt, 1980; Blum, 1997). Such characteristic is not acceptable for cultivated crops, hence this hybrid classified as a non- drought tolerant plant.

In addition to leaf formation, shoot height, and trunk diameter growth of the fig hybrids also limited under water stress period. It was an obvious response to drought and there are many reports available showing effects of *in vitro* and *in vivo* water deficit on growth limitation. . Salisbury and Ross (1992) stated that cell growth limitation is one of the most sensitive responses to drought, because of need to high turgor for cell expansion. In addition to loss of turgor, limitation in photosynthesis and availability of minerals also may affect growth parameters of plants (Syvertsen, 1985). Our data revealed that preserving growth rate under water deficit condition is a characteristic of drought tolerant fig hybrids, which was probably due to their ability to preserve water status under drought condition. As noted before, limitation in leaf formation and shedding some leaves under water deficit helps plant to preserve water status by reducing light absorbing and transpiration surface during water limitation (Blum,

1997). The phenomenon was found in the fig hybrids, however, intense leaf abscission observed in hybrids No. 2, 3, 4, 5, and 12 was a result of severe drought stress injuries. As Manivannan *et al.* (2007) mentioned before, such a loss in leaves probably hasten the reduction in growth of the other organs by reducing photosynthesis capacity of the drought sensitive plants.

Plant growth parameters involving relative shoot length and relative leaf number were fully recovered to the respective control level in the drought tolerant fig hybrids. However, the sensitive hybrids were not able to get recovered after the rewatering period, probably because of severe constructional damages to the growing points of shoot. Such damages were obvious as necrosis parts of young shoot apices. MSI data also proved incidence of constructional damages to tissues during the water stress period. The highest MSI values were found in hybrids 1 and 8 which was in coincidence to the highest RWC level under drought stress. Severe water loss causes malfunction in physiological processes of plant which results in accumulation of reactive oxygen species (ROS). Previous studies have showed that ROS formation under drought stress extensively damage internal organelles, enzymes, and nucleic acids (Herbinger *et al.*, 2002). Our previous studies on caprifig genotypes revealed that in addition to ability of preserving water status under water deficit condition, presence of an effective antioxidant system involving enzymes and non-enzyme compounds is essential for preventing constructional damages of drought condition (Gholami *et al.*, 2012; Rostami and Rahemi, 2013).

There are many reports on chlorophylls decline under drought stress. Structural damages of chloroplasts due to ROS formation and/or photo degradation of the pigments probably led to loss of chlorophylls under drought stress (Anjum *et al.*, 2011). Chlorophyll concentration significantly reduced in the leaves of hybrids No. 2, 3, 4, 5, 6, 10 and 12 under drought stress. Chlorophyll maintenance under drought stress in tolerant genotypes has been reported by Kraus *et al.* (1995) and Sairam *et al.* (1998). Preserving level of chlorophylls under water stress treatments in the leaves of the other hybrids indicates the lower constructional damage to photosynthesis apparatus in their leaves.

The obtained data revealed that concentration of carotenoids remains higher in the leaves of drought tolerant fig hybrids under drought stress conditions.

Carotenoids have critical role as photoprotective compounds by quenching triplet chlorophyll and scavenging singlet oxygen derived from excess light energy may limit structural damages to chlorophylls under drought stress (Nishida *et al.*, 2007), thus higher carotenoids in the leaves of the tolerant hybrids may explain less structural damage and higher chlorophyll concentration in their leaves. The higher ratio of chlorophyll to carotenoids indicates the capacity of higher carotenoid concentration to protect the photosynthetic apparatus (Loggini *et al.*, 1999).

Proline is the most important organic molecule widely used in osmoregulation in plant during osmotic stress (Turkan *et al.* 2005). Osmoregulation retards leaf senescence and increase plant yield under water deficit condition (Liu and Stutzel, 2002). In this study higher levels of RWC were in coincidence with higher proline accumulation in the leaves of hybrids No. 1, 7, 8, 9, and 11, which suggests that proline maybe play a critical role in water absorption or preservation in fig seedlings during water stress. Proline accumulation was also noted in previous studies on fig under drought stress (Karimi *et al.*, 2012). Sivritepe *et al.* (2008) reported a positive correlation between higher proline accumulation and drought tolerance in plants, which is in agreement with our data. In this study accumulated proline in the leaves vanished rapidly during the rewatering period. It can be concluded that proline turnover may have a role in recovery of water stress plants. Kuznetsov and Shevyakova (1991) stated that proline is used by the stressed plants as source of nitrogen after drought stress.

In this study water stress induced significant soluble carbohydrates accumulation in the leaves of hybrids No. 1, 7, 8, 9, and 11 which was related to better water preservation and MSI in their leaves. Reduction in growth rate and increased hydrolysis of reserve carbohydrates cause soluble carbohydrates accumulation under drought stress (Levit, 1980; Rieger, 2003; Perez-Perez *et al.*, 2007). In this study reserve carbohydrates did not reduced significantly under the water stress period. Hence it can be concluded that increase in soluble carbohydrates observed under drought stress probably is due to limitation of growth because of water deficiency. On the other hand, the data suggest that photosynthesis of hybrids No. 1, 7, 8, 9, and 11 unlike the other hybrids has not reduced during the

stress period. Based on the results it concluded that soluble carbohydrates accumulation in the leaves of water stressed fig seedlings maybe involved in their drought tolerance mechanism and also may be used as a physiological marker in evaluating photosynthesis capacity of the plants during drought stress. Based on the results of the current study, we classified the fig hybrids as drought tolerant (1, 7, 8, 9, and 11), Semi-tolerant (5, 6, 10, and 12), and sensitive (2, 3, and 4) (Figure 11). Our previous studies have revealed that caprifigs 'Khormaei' and 'Shah Anjiri' are very drought tolerant, 'Pouz Donbali' is semi-tolerant and 'Dane Sefid' is a sensitive plant (Rostami and Rahemi, 2013). Gholami *et al.* (2012) studies on the maternal parents have showed that figs 'Sabz' and 'Siah' are drought tolerant and fig 'Shah Anjir' is a sensitive cultivar. Figure 11 shows that using caprifig 'Khormaei' as paternal material and fig 'Siah' or 'Sabz' as maternal parent in controlled pollinations bring hope in obtaining drought tolerant fig hybrids.

#### CONCLUSION

It can be concluded that drought tolerance of offsprings is highly related to drought tolerance of their parents, and using a highly drought tolerant parent in controlled pollinations may result in a drought tolerant hybrid. Hence, in drought tolerance breeding programs of fig, it is suggested to evaluate drought tolerance of parents before making hybridizations for saving energy, time, and resources. Further investigation are needed to release hybrids No. 1, 7, 8, 9 and 11 as drought tolerant rootstock.

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#### REFERENCES

Anjum, S.A., L. C. Wang, M. Farooq, M. Hussain, L. L. Xue, and C. M. Zou. 2011. Brassinolide application improves the drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange. *J. Agro. Crop. Sci.* 197:177-185.

Bates, L.S.R., P. Waldren and I. D. Tear. 1973. Rapid determination of free proline for water-stress. *Plant Soil.* 39, 205-207.

Blum, A. 1997. Crop responses to drought and the interpretation of adaptation., In: Drought Tolerance in Higher Plants, Genetical,

Physiological and Molecular Biological Analysis. (Ed), Belhassen, E., Kluwer, Dordrecht., pp. 57-70.

Chartzoulakis, K., A. Patakas, G. Kofidis, A. Bosabalidis and A. Nastou. 2002. Water stress affects leaf anatomy, gas exchange, water relations and growth of two avocado cultivars. *Sci. Hort.* 95:39-50.

Filella, I., J. Llusia, J.O. Pin, J.U. and Pen. 1998. Leaf gas exchange and fluorescence of *Phillyrea latifolia*, *Pistacia lentiscus* and *Quercus ilex* saplings in severe drought and high temperature conditions. *J. Envir. Exper Bot.* 39: 213-220.

Gholami, M., M. Rahemi, and S. Rastegar. 2012. Use of rapid screening methods for detecting drought tolerant cultivars of fig (*Ficus carica* L.). *Scientia.*143: 7-14.

Herbinger, K., M. Tausz, A. Wonisch, G. Soja, A. Sorger and D. Grill. 2002. Complex interactive effects of drought and ozone stress on the antioxidant defense systems of two wheat cultivars. *Plant Physiol. Biochem.* 40:691-696.

Irrigoyen, J.J., D.W. Emerich and M. Sanchez-Diaz. 1992. Water stress induced changes in concentrations of praline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. *Plant Physiol.*84:55-60.

Karimi, S., S. Hojati, S. Eshghi, R. Nazari-Moghadam, S. Jandoust. 2012. Magnetic field treatment improves fig 'Sabz' explants tolerance to *in vitro* induced drought stress. *Sci.Hort.*137: 95-100.

Kuznetsov, V.I.V. and N.I. Shevyakova. 1999. Proline under stress: Biological role, Metabolism and regulation. *Russ. J. Plant physiol.* 46(2): 274-287.

Levitt, J., 1980. Responses of Plants to Environmental Stress. Vol. 2, Academic Press. New York.

Liu, F. and H. Stutzel. 2002. Leaf water relations of vegetable Amaranth (*Amaranthus spp.*) in response to soil drying. *Euro. J. Agro.* 16(2):137-150.

Loggini, B., A., Scartazza, E. Brugnoli and F. Navari-Izzo. 1999. Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. *Plant Physiol.* 119: 1091-1099.

Lutts, S., J. M. Kinet and J. Bouharmont. 1995. Changes in plant response to NaCl during development of rice varieties differing in salinity resistance. *J. Exper. Bot.* 46:1843-1852.

- Nishida, Y. and W. YamashitaMiki. 2007. Quenching activities of hydrophilic and lipophilic antioxidants against singlet oxygen using chemiluminescence detection system. *Caroten. Sci. Hort.* 11: 16-20.
- Perez-Perez, J.G., J.P. Syversten, P. Botia and F. Garcia-Sanchez. 2007. Leaf water relations and net gas exchange responses of salinized *carrizo citrange* seedling during drought stress recovery. *Ann. Bot.* 100: 335-345.
- Rieger, M.L, R. and W.R. Bianco Okie. 2003. Responses of *Prunus ferganensis*, *P. persica* and two hybrids to moderate drought stress. *Tree Physiol.* 23: 51-58.
- Sairam, R. K., D. S. Shukla and D. C. Saxena. 1998. Stress induced injury and antioxidant enzymes in relation to drought tolerance in wheat genotypes. *Biol. Plant.* 40: 357-364.
- Salisbury, B.S. and C. Ross. 1992. *Plant physiology*, Forth ed, Wadsworth Publishing Company. Belmont. 682 p.
- Sivritepe, N., N. U. SivritepeErturk, C. Yerlikaya, I. Turkan, M. and F. Bor, Ozdemir. 2008. Response of the cherry rootstock to water stress induced *in vitro*. *Biol.Plant.* 52: 573-576
- Slavin, J.L. 2006. Figs: Past, Present and Future. *Nut. Today.* 41:180-184.
- Syversten, J.P., 1985. Integration of water stress in fruit trees. *Hort Sci.* 20(6), 1039-1043.
- Turkan. I., M. Bor, F. Ozdemir and H. Koca. 2005. Differential responses of lipid peroxidation and antioxidants in the leaves of drought - tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. *Plant Sci.* 168: 223-231.
- Wagner, G. J. 1979. Content and vacuole/extravacuole distribution of neutral sugars, free amino acids, and anthocyanin in protoplasts. *Plant Physiol.* 64:88-93.
- Wellburn, A.R., 1994. The spectral determination of chlorophyll a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Plant Physiol.* 144, 307-313.
- Zamani, Z., A. Taheri, A. Vezaei and K. Poustini. 2002. Proline content and stomatal resistance of almond seedlings as affected by irrigation intervals. *Acta Hort.* 591: 411-416.