Adv. Hort. Sci., 2012 26(3-4): 131-137

In vitro drought effects on morphological and physiological indices of two fig (*Ficus carica* L.) cultivars

A. Shekafandeh(*), **S. Hojati**
Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz, Iran.

Key words: drought, growth parameters, polyethylene glycol, proline.

Abstract*: In vitro* responses of two fig cultivars, 'Sabz' and 'Siah', were evaluated in MS media containing four levels of polyethylene glycol (PEG) (0, 2, 4, 6%) as a simulation of water stress. The results showed that in Sabz cultivar, shoot length, shoot fresh and dry weights were 43, 36 and 25%, respectively, lower than control in drought treatments caused by 4% PEG, while the leaf area and specific leaf area were not significantly ($P>0.05$) affected. In Siah cultivar, shoot length, fresh and dry weights were 57, 58 and 40%, respectively, lower in stressed media in comparison to control. In contrast to Sabz cultivar, leaf area and specific leaf area of Siah cultivar were significantly reduced by addition of 6% PEG (56 and 21.5%, respectively). Naturally, the amount of proline in 'Sabz' was higher than in 'Siah' (81.8 µmole g^{-1} versus 16.7 µmole g^{-1}). However, in both cultivars, with addition of PEG in culture media, leaf proline content was increased, in comparison to control. With increasing PEG% in culture media, the amount of leaf soluble sugars content increased and the amount of starch decreased. The result show that 'Siah' is more sensitive to drought than 'Sabz' and that *in vitro* culture can be used to evaluate drought tolerance of cultivars.

1. Introduction

Despite considerable advances in technology, agriculture is exposed to climate changes in all parts of the world. Among climatic factors, rainfall is the most critical because 70% of the main areas under cultivation are still without irrigation (Wilhite, 2001).

Water shortage is the main characteristic of agriculture in Mediterranean regions, inducing water stress during spring and summer. Fruit trees survive in this situation because they are prone to physiological or morphological changes which enable them to avoid drying damage, cast it back, or tolerate it (Torrecillas *et al*., 1999). Drought tolerance is observed with different rates in almost all plant species. Understanding plant responses to the external environment is an essential component for selection stress tolerance (Reddy *et al*., 2004). In recent years, in southern Iran, low and poor distribution of rainfall has caused great damage to plants. Water stress, particularly in rain-fed fig production areas (e.g. Estahban) has become a big issue. In the Estahban region not only the annual production has decreased, but also the highly productive trees are in danger of destruction.

One strategy to confront this problem is the identification and selection of more tolerant genotypes. Traditional breeding approaches for selection of tolerant plants are time consuming and complex processes. Imposing water stress in field-grown crops is difficult because of the unpredictability of rainfall and the possibility of seepage from adjoining plots and drought escape of deep roots from osmotic stress. Since field evaluation of drought effects is highly correlated with environmental conditions, *in vitro* screening techniques allow for a better control of culture conditions. Tissue culture also offers the possibility of screening many plants in limited space and time, assuming that there is a correlation between cellular, tissue, organ and *in vivo* plant responses (Mohammad *et al*., 2000).

Polyethylene glycol (PEG) has been used to simulate water stress in plants. PEG of high molecular weight is a non-ionic osmoticum lowering the water potential of nutrient solution without being taken up or being phytotoxic (Hassan *et al*., 2004). It has been shown that the shoot length decreases with increasing water stress via *in vitro* culture in cherry (Sivritepe *et al*., 2008) and mulberry (Tewary *et al*., 2000). In a study on mulberry, leaf relative water content (RWC) decreased with increasing water stress (Ramanjulu *et al*., 1998).

Accumulation of proline is the most common plant response to decreasing water potential (Helal Ragab and Samir Moustafa, 2008). It has been reported that with increasing PEG in date palm seedling culture, the rate of proline was increased in tolerant cultivar (Djibril *et al*., 2005).

Carbohydrates have an important role in osmotic regulation in different plant parts (Masoudi-Sadaghiani *et al*., 2011). Simple sugars such as glucose and fructose were increased and the rate of sucrose and starch decreased under water stress (Sharp and Davies, 1979; Munns and Weir, 1981; Wang and Stutte, 1992; Nawar and Ezz, 1993; Clif-

(*) Corresponding author: shekafan@shirazu.ac.ir.

Received for publication 26 April 2012
Accepted for publication 12 September 2012

ford *et al*., 1998; Perez-Perez *et al*., 2007; Mafakheri *et al*., 2010). In Iran, Fars province with about 36,000 hectares under fig cultivation is the primary fig production area. Estahban district with 20,000 hectares under fig cultivation is the center of fig cultivation in Fars province, as well as in Iran. As mentioned above, due to drought in recent years, highly productive trees are in danger of destruction. The cultivars Sabz and Siah are the most desirable cultivars in Iran; there has not been report on water stress tolerance of these cultivars. Selection of more drought tolerant cultivars can be used to establish of new orchards and a promising rootstock for other interested cultivars.

The aim of this investigation was to evaluate the effects of drought created by polyethylene glycol (PEG) on morpho-physiological changes of two important fig cultivars ('Sabz' and 'Siah') and leading to selection of more drought tolerant varieties using *in vitro* culture.

2. Materials and Methods

Plant material and explant decontamination

In this research, the *in vitro*-derived micro-shoots of two cultivars of *Ficus carica* L. 'Sabz' and 'Siah' were used. The branches (20~30 cm long) were cut from rain-fed, mature mother trees established in Estanban Fig Research Station located at 29° 07' N latitude and 54° 02' E , 197 km southeast of Shiraz. Freshly grown stems with 2~6 nodes were cut from the above branches and washed in a solution of water-detergent (three drops of detergent in 100 ml of water) for 30 min, then placed in a solution of benomyl (3%) for 1 h (Torres, 1998). To prevent the secretion of phenolic compounds into the culture media, explants were treated in a solution of 2% ascorbic and citric acid for 45 min. At this point, the stems were transferred to a laminar air flow cabinet. They were first dipped in 70% (V/V) ethanol for 45 s, then treated with 15% (V/V) Chlorox solution (a household bleach containing 5.25% sodium hypochlorite) for 15 min and rinsed three times with sterilized distilled water. Nodal segments of 1~1.5 cm long were cut and used as explants. The basal medium was MS (Murashige and Skoog, 1962), containing 3% sucrose (MERCK, LGaA 64271Darmstadt, Germany) and 1mg l^{-1} (4.4 µM) benzyl adenine (BA) and solidified by 0.8% agar-agar (MERCK, LGaA 64271Darmstadt, Germany). The pH was adjusted to 5.7±0.05 prior to autoclaving at 1.2 atm pressure and 121˚C for 20 min. Cultures were maintained in a growth chamber at 25±2˚C, 58% relative humidity and a photon flux density of 40 µmol m^{-2} s^{-1} was provided by white fluorescent tubes, with a 16 h photoperiod.

PEG treatments

After four weeks, the micro-shoots (2 cm length) from the above cultures were transferred into 500 ml culture vessels containing 100 ml MS basal media supplemented with different levels of PEG (0, 2, 4 and 6%) as selective agent and maintained under the environmental conditions described above.

Growth measurements

Seven weeks after the beginning of experiments, shoot length and shoot fresh and dry weight were evaluated.

Leaf area was measured by leaf area meter (Delta-Tdevices England) and specific leaf area was calculated as follows:

specific leaf area = leaf area (cm^2)/leaf dry weight (g)

Relative water content

Leaf relative water content (RWC) was estimated according to the method described by Whetherley (1950). Twenty healthy leaf discs of 0.7 cm diameter were cut from plants, using a leaf punch and washed three times with double distilled water. Leaf discs were weighed (FW), then placed into a 10 ml conical flask, immersed in 10 ml distilled water for 4 h in dark. Turgid weight (TW) of leaf discs were then measured and samples were dried in an oven (80ºC) until constant weight (DW) was achieved. RWC was calculated from the following equation:

Relative water content % = FW - DW/TW - DW x 100

Chlorophyll content

For chlorophyll determination, prior to extraction fresh leaf samples were washed with deionized water to remove any surface contamination. One g leaf samples were ground in 80% acetone using a pestle and mortar. The mixture was centrifuged at 4800 rpm for 20 min. The optical density of the supernant was measured at 663 and 645 nm wavelengths and chlorophyll content was calculated using the following equation:

Mg Chl g FW = [20.2 (OD645 nm) + 8.02 (od663 nm)] x V/FW x 1000

where V is final volume of solution (ml) and fw, leaf fresh weight (mg).

Proline contents

To determine proline content of the leaves, 0.5 g of plant material was homogenized in 10 ml of 3% aqueous of sulfosalcylic acid and the homogenate filtered through Whatman # 2 filter paper. Two ml of filterate was reacted with 2 ml acid ninhydrin and 2 ml of glacial acetic acid in a test tube for 1 h at 100°C and the reaction terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene, mixed for 15~20 seconds. Chromophore containing toluene was aspirated from the aqueous phase and the absorbance was read at 520 nm. The proline concentration was determined based on standard curve and calculated as follows:

$$\mu \text{ moles proline/g FW} = \frac{(\mu g \text{ proline/ml x ml toluene})}{115.5 \ \mu g / \mu mole} \Big/ \text{g sample/5}$$

Electrolyte leakage

Electrolyte leakage was measured as an assessment of cell wall permeability. Electrolyte leakage was measured using an Electrical Conductivity Meter. Two mature leaves per plant were taken and cut into 1 cm segments. After three washes to remove surface contamination, leaf samples were placed in individual vials containing 10 ml of

distilled water. The samples were incubated at room temperature on a shaker (100 rpm) for 24 h. Electrical Conductivity (EC) of bathing solution (EC1) was read after incubation. Samples were then placed in an autoclave at 120°C for 20 min and the second reading (EC2) was determined after cooling the solution to room temperature. The electrolyte leakage was calculated as EC1/EC2 and expressed as percent.

Soluble sugars content

Soluble sugars were extracted from 0.1 g fresh leaves by heating with 5 ml of 80% ethanol in a water bath at 70°C for 30 min. The insoluble residue was removed by centrifuging at 5000 g for 10 min. One ml of the resulting extract was mixed with 1 ml of 5% phenol solution and 5 ml of sulfuric acid. The mixed solution was permitted to cool to room temperature, then vortexed. The absorbance was read at 490 nm using a spectrophotometer. The soluble sugar content of each sample was determined using standard curve for glucose and expressed as mg glucose g^{-1} FW (McCready *et al*., 1950; DuBois *et al*., 1956).

Starch content

The solid residue remaining in the centrifuge tube after removal of all soluble sugars in the previous section was washed, re-extracted and re-centrifuged four times using 80% (V/V) ethanol. Starch content in the samples was determined colorimetrically using anthrone method (McCready *et al*., 1950). The absorbance was determined at 630 nm in a digital spectrophotometer (Spectronic 20 D+; Spectronic Instruments Inc., New York, USA.) as described by López *et al*. (2002).

Statistical analysis

The experiment was arranged as a 2×4 factorial experiment in completely randomized design (CRD) with 10 replicates, each consisting of three plants. Thus, there were 30 plants in each treatment and a total of 240 plants in the experiment. Data were subjected to analysis of variance using the SPSS software (ver. 13.0) SPSS Inc. Mean differences were determined by Duncan's multiple range tests at $p \leq 0.05$.

3. Results and Discussion

Water stress caused a reduction in micro-shoot growth of the two fig cultivars (Table 1). In 'Sabz', shoot fresh and dry weights and shoot length were 36, 25, and 43% lower, respectively, in drought treatments caused by 4% PEG compared with the control, while in 'Siah', these traits were 58, 40 and 57% lower respectively, in the same stressed media (4% PEG), than the control. In this latter cultivar, in contrast to 'Sabz', leaf area and specific leaf area were significantly affected by water deficit (56 and 21.5%, respectively) (Table 2). The findings of this study showed that the growth rate of 'Siah' in optimum conditions (control) was the same as Sabz cultivar, but with increasing intensity of water stress, the growth reduction rate in 'Siah' was more than in 'Sabz'. The main effect of cultivars showed that without respect to different levels of PEG, 'Sabz' had a significantly higher leaf area (8.2 cm) than 'Siah' (5.51) (Table 2). Water stress significantly reduced vegetative growth indices of fig micro-shoots. This phenomenon has been previously reported (Oukabli *et al*., 2008).

Table 1 - Interaction of water stress and cultivar on micro-shoot length, fresh and dry weight

	Drought stress (PEG%)				
	0	2	4	6	
Cultivar	Average fresh weight (g)				Mean
Siah	3.80 a [z]	2.1 bc	1.6 c (58)	2.10 bc	2.4 A
Sabz	3.00 ab	2.3 bc	1.9 bc (36)	2.10 bc	2.3 A
Mean	3.41 A	2.2 B	1.8 B	2.08 B	
	Average dry weight (g)				
Siah	0.50 a	0.30 bc	0.3 bc (40)	0.30 bc	0.34 A
Sabz	0.40 ab	0.40 ab	0.3 bc (25)	0.30 bc	0.36 A
Mean	0.46 A	0.34 B	0.3 B	0.31 B	
	Shoot length (cm)				
Siah	2.60 a [z]	1.4 b	1.1 b (57)	1.20 b	1.57 A
Sabz	2.33 a	1.4 b	1.3 b (43)	1.30 b	1.59 A
Mean	2.47 A	1.4 B	1.2 B	1.25 B	

[z] In each row and column, means with similar letters (small letters for interaction and big letters for main effects) are not significantly different using Duncan's multiple rang test P≥0.05.
The percentage of reduction with respect to control is reported in parentheses.

Table 2 - Interaction of water stress and cultivar on leaf area (cm^2) and specific leaf area (g cm^{-2}) of micro shoots

	Drought stress (PEG%)				
	0	2	4	6	
Cultivar	Leaf area (cm^2)				Mean
Siah	8.2 a [z]	6.9 ab	3.4 b	3.6 b (56)	5.51 B
Sabz	12.4 a	8.2 a	6.9 ab	6.6 ab	8.52 A
Mean	10.0 A	7.6 AB	5.4 B	5.4 B	
	Specific leaf area (g cm^{-2})				
Siah	14.4 a	14.6 a	13.4 a	11.3 b (21.5)	13.4 A
Sabz	16.8 a	15.3 a	15.1 a	13.1 a	15.1 A
Mean	15.6 A	14.9 A	14.4 A	12.2 A	

[z] In each row and column, means with similar letters (small letters for interaction and big letters for main effects) are not significantly different using Duncan's multiple rang test P≥0.05.
The percentage of reduction with respect to control is reported in parentheses.

The long-term use of PEG *in vitro* on growth reduction and shoot regeneration in other plants has been well documented (Bressan *et al*., 1982; Handa *et al*., 1982; Handa *et al*., 1983; Dami and Hughes, 1995; Al-Khayri and Al-Bahrany, 2004). In most cases, PEG has been used to stimulate

water stress in plants. PEG with high molecular weight is a non-penetrating inert osmoticum which lowers the water potential of nutrient solutions without being taken up or being phytotoxic (Hassan *et al*., 2004). It has been shown that *in vitro* growth reduction of apple (Molassiotis *et al*., 2006) and cherry (Sivritepe *et al*., 2008) was due to the decrease in water potential created by PEG.

Under water stress conditions, the reduction of mineral absorption has resulted in limited leaf growth and development and decreased plant water transpiration. Therefore, producing smaller leaves can be the first plant defense mechanism against water deficit. Hsiao (1973) reported that a decrease in leaf area results in lower light absorption and photosynthetic capacity; thereby reducing photosynthetate and plant growth. This can be true with plants *in vivo*, but probably this is less important *in vitro*, because *in vitro* plants are more heterotrophic, so decreases in their growth cannot be due to a deficiency of carbohydrates. In the present work it was clearly shown that the decrease in water availability is the main cause of decreasing growth. Specific leaf area (SLA) is a function of leaf area. The reduction of SLA in water deficit conditions is due to the fact that leaf area development is more affected than deposition of dry matter (Blum and Pneul, 1990).

In both cultivars with increasing PEG% in culture media, leaf relative water content (RWC) decreased and an increase in ion leakage was recorded (Table 3, Fig. 1). These findings are in agreement with the results obtained by other researchers *in vitro* (Sawwan *et al*., 2000; Al-Khayri and Al-Bahrany, 2004; Chai *et al*., 2005). Leaf yellowing and chlorosis were the consequence of structural damage to cell membranes in explants. Interestingly, in 6% PEG, although the rate of relative water content reduction in leaves of Sabz cultivar (50%) was more than 'Siah' (20%), the difference in their ion leakage was not significant.

Leaves accumulated significant quantities of proline and, in contrast, the amount of chlorophyll was decreased with increasing PEG percentage in culture media (Fig. 2).

Table 3 - Interaction of water stress and cultivar on leaf relative water content (%) and leaf ion leakage (%)

	Drought stress (PEG%)				
	Leaf relative water content (%)				
Cultivar	0	2	4	6	Mean
Siah	69.2 a [z]	68.6 a	59.3 ab	54.9 ab (20)	63.0 A
Sabz	73.6 a	51.4 ab	61.7 a	36.7 b (50)	55.8 A
Mean	71.4 A	60.6 AB	58.8 AB	45.7 B	
	Leaf ion leakage (%)				
Siah	50.6 d	57.1 bcd	55.5 bcd	64.2 abc	56.9 B
Sabz	54.1 cd	57.0 bcd	68.7 a	66.2 a	61.5 A
Mean	52.3 C	57.0 BC	62.1 AB	65.2 A	

[z] In each row and column, means with similar letters are not significantly different using Duncan's multiple rang test P≥0.05.
The percentage of reduction with respect to control is reported in parentheses.

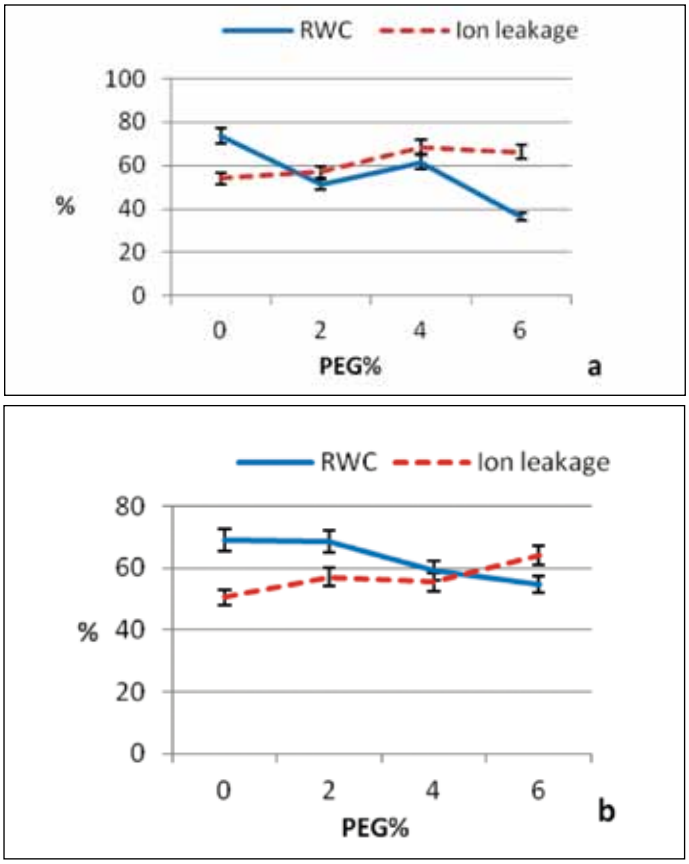

Fig. 1 - Changes in leaf water content (RWC) and ion leakage in two fig cultivars: 'Sabz' (a) and 'Siah' (b).

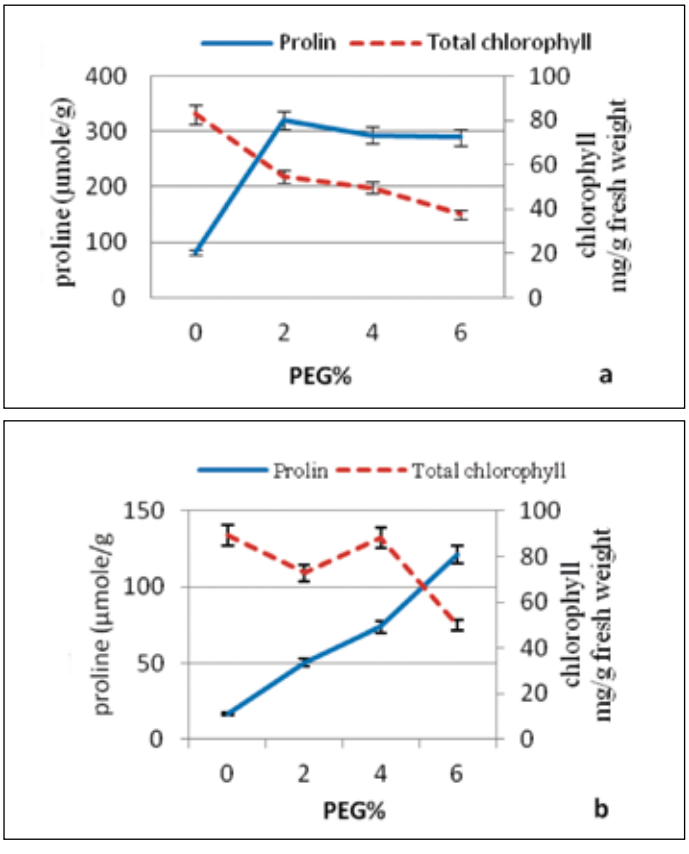

Fig. 2 - Changes in proline and total chlorophyll in two fig cultivars: Sabz (a) and Siah (b).

Naturally, the amount of proline in 'Sabz' was higher than in 'Siah' (81.8 µmole g^{-1} versus 16.7 µmole g^{-1}) (Table 4). In both cultivars, with increasing PEG % in media, proline content increased. However, the results indicated that, in all levels of PEG treatments, the amount of proline in 'Sabz' was higher than in 'Siah'. Plants produce and accumulate such compounds compatible to their metabolism to overcome adverse effects of drought stress (Zhu, 2001). Accumulation of such substances would cause more negative water potential in plants, a necessary condition to absorb and keep the water in plant tissues (Sivritepe *et al*., 2008).

Table 4 - Interaction of water stress and cultivars on leaf proline content (µm g^{-1} fresh weight)

	Drought stress (PEG%)				
Cultivar	0	2	4	6	Mean
Siah	16.8 c [z]	50.5 b	73.7 b	121.0 b	65.5 B
Sabz	81.8 b	320.7 a	293.6 a	289.5 a	246.4 A
Mean	42.8 B	170.6 A	199.4 A	205.2 A	

[z] In each row and column, means with similar letters are not significantly different using Duncan's multiple rang test P≥0.05

With increasing the severity of water stress, proline and soluble sugars (as compatible substances) accumulated significantly in explant leaves of both cultivars. The accumulation of these substances has been well documented in field (Clifford *et al*., 1998; Zamani *et al*., 2002; Mafakheri *et al*., 2010) and *in vitro* conditions (Handa *et al*., 1982; Brito *et al*., 2002; Al-Khayri and Al-Bahrany, 2004; Molassiotis *et al*., 2006; Sivritepe *et al*., 2008). A notable point in our results was the significant increase in proline accumulation in leaves of both cultivars. It has been reported that there is a direct positive relationship between drought tolerance and proline concentration in plant tissues (Al-Khayri and Al-Bahrany, 2004; Sivritepe *et al*., 2008). The role of proline in destroying reactive oxygen species (ROS) in water stress conditions has been reported in different plant species (Turkan *et al*., 2005; Verslues *et al*., 2006). Therefore, it can be expected that plants which accumulate more proline under water stress are more tolerant. Thus, it may be concluded that 'Sabz' is more tolerant than 'Siah', although in some plants a relationship was not found between proline accumulation and drought tolerance.

The changes in leaf sugar and starch contents under different levels of PEG are shown in Table 5 and figure 3. In both cultivars, with increasing PEG%, the amount of soluble sugar content increased and the amount of starch deceased. In drought conditions, soluble sugar can act as osmoticum and also osmoprotectant. In addition, accumulation of sugar may partly protect protein against the oxidative damage created by free radicals (Bohnert and Shen, 1999). In the present work, soluble sugars content in 'Sabz' was greater than in 'Siah', but it did not have significant effect on water absorption. Accumulating soluble sugars can be due to starch hydrolysis, which is in agreement with the results obtained by Shawky *et al*. (1997). Taylor *et al*. (1982) also reported that carbohydrates (reducing and non-reducing sugars) are the most abundant component in osmotic adjustment in tomato seedlings.

Table 5 - Interaction of water stress and cultivar on leaf TSS and starch (mg g^{-1} dry weight)

	Drought stress (PEG%)				
	0	2	4	6	
Cultivar	TSS (mg g^{-1} dry weight)				Mean
Siah	170.3 c	168.9 c	256.6 bc	287.0 b	220.69 B
Sabz	276.4 bc	344.5 b	357.5 b	522.2 a	375.2 A
Mean	215.8 C	256.7 BC	311.6 B	404.6 A	
	Starch (mg g^{-1} dry weight)				
Siah	133.5 ab	103.8 bc	73.7 bc	65.3c	94.1 A
Sabz	167.0 a	124.2 abc	76.3 bc	80.3 bc	111.9 A
Mean	152.56 A	112.83 B	75.4 B	73.9 B	

[z] In each row and column, means with similar letters (small letters for interaction and big letters for main effects) are not significantly different using Duncan's multiple rang test P≥0.05

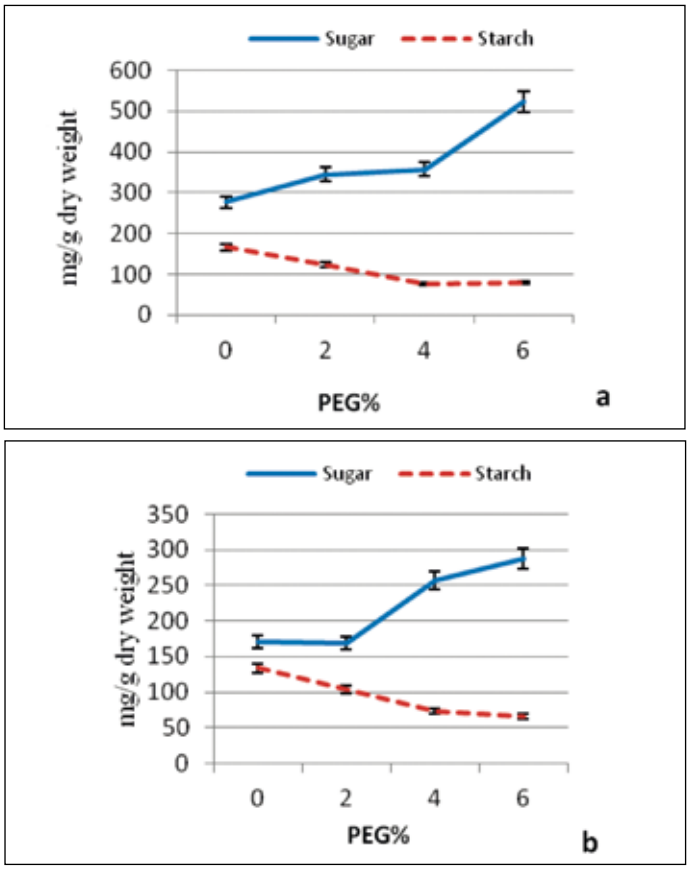

Fig. 3 - Changes in leaf sugar and starch content in two fig cultivars: 'Sabz' (a) and 'Siah' (b).

In this study, with increasing PEG in culture media, the amount of total chlorophyll declined in leaves of the two fig cultivars. Effect of drought stress on reduction of chlorophyll content of other plants *in vitro* has previously

been reported (Hernández-Sebastià *et al*., 2000; Brito *et al*., 2002; Molassiotis *et al*., 2006). Under field conditions, also chlorophyll content was reduced with increasing drought (Munne-Bosch and Penuelas, 2004). In the present experiment, with increasing water deficit (with addition of PEG), chlorophyll content was decreased along with accumulation of leaf proline (Fig. 3). This may be due to the fact that chlorophyll and proline are synthesized through glutamate pathway, causing an increase in synthesis of proline under drought conditions, which resulted in reduction of chlorophyll synthesis (Aspinall and Paleg, 1981).

4. Conclusions

The assessment of drought tolerance of two fig cultivars showed that under water stress conditions in both cultivars, leaf fresh and dry weight, total chlorophyll and starch contents decreased, but proline and soluble sugar increased. Finding of this research showed that Siah cultivar was more vigorous than Sabz cultivar in control treatment, even if the differences were not statically significant. In stress conditions, the growth rate reduction in 'Siah', was more obvious than in 'Sabz', and this latter cultivar was more drought tolerant, possibly due to accumulation of proline and soluble sugars. The results of our work show that it is possible to use *in vitro* culture as a successful method for selection of tolerant varieties.

References

AL-KHAYRI J., AL-BAHRANY A., 2004 - *Growth, water content and proline accumulation in drought stressed callus of date palm*. - Biol Plant, 48: 105-108.

ASPINALL D., PALEG L.G., 1981 - *Proline accumulation. Physiological aspects,* pp. 215-226. - In: PALEG L.G., and D. ASPINALL (ed) *Physiology and biochemistry of drought resistance in plants*. Academic Press, Sydney Australia, pp. 492.

BLUM A., PNEUL Y., 1990 - *Physiological attributes associated with drought resistance of wheat cultivars in a Mediterranean environment*. - Aust. J. Agric. Res., 41: 799-810.

BOHNERT H., SHEN B., 1999 - *Transformation and compatible solutes*. - Sci. Hort., 78: 237-260.

BRESSAN R., HANDA A., HANDA S., HASEGAWA P., 1982 - *Growth and water relations parameters of cultured tomato cells after adjustment to low external water potentials*. - Plant Physiol., 70: 1303-1309.

BRITO G., COSTA A., FOSECA H., SANTOS C., 2002 - *Response of* Olea europea *ssp. maderensis* in vitro *shoots exposed to osmotic stress*. - Sci. Hort., 97: 411-417.

CHAI T., FADZILLAH N., KUSNAN M., MAHMOODI M., 2005 - *Water stress induced oxidative damage and antioxidant responses in micropropagated banana plantlets*. - Biol. Plant., 49: 153-156.

CLIFFORD S.C., ARNDT S.K., CORELLT J.E., JOSHI S., SANKHLA N., POPP M., JONES H.G., 1998 - *The role of solute accumulation, osmotic adjustment and changes in cell wall elasticity in drought tolerance in* Ziziphus mauritiana *(Lamk)*. - J. Exp. Bot., 49(323): 967-977.

DAMI I., HUGHES H., 1995 - *Leaf anatomy and water loss of* in vitro *PEG-treated 'Valiant' grape*. - Plant Cell Tiss. Org. Cult., 42: 179-184.

DJIBRIL S., OULD KNEYTA M., DIOUF D., BADIANE F., SAGNA M., BORGEL A., 2005 - *Growth and development of date palm* (Phœnix dactylifera L.) *seedling under drought and salinity stresses*. - African J. Biotech., 4(9): 968-972.

DUBOIS M., GILLES K.A., HAMILTON J.K., REBERS P.A., SMITH F., 1956 - *Colorimetric method for determination of sugars and related substances*. - Anal. Chem., 28(3): 350-356.

HANDA A.K., BRESSAN R.A., HANDA S., HASEGAWA P.M., 1982 - *Characteristics of cultured tomato cells after prolonged exposure to medium containing polyethylene glycol*. - Plant Physiol., 69: 514-521.

HANDA A.K., BRESSAN R.A., HANDA S., HASEGAWA P.M., 1983 - *Clonal variation for tolerance to polyethylene glycol-induced water stress in cultured tomato cells*. - Plant Physiol., 72: 645-653.

HASSAN N., SHAABAN L., HASHEM E., SELEEM E., 2004 - In vitro *selection for water stress tolerant callus line of* Helianthus annus *L. cv. Myak*. - Inter. J. Agri. Biol., 6(1): 13-18.

HELAL RAGAB M., SAMIR MOUSTAFA A., 2008 - *Comparative response of drought tolerant and drought sensitive maize genotypes to water stress*. - Aust. J. Crop. Sci., 1(1): 31-36.

HERNÁNDEZ-SEBASTIÀ C., SAMSON G., BERNIER P., PICHÉ Y., DESJARDINS Y., GLOMUS Y., 2000 - I*ntra radices causes differential changes in amino acid and starch concentrations* of in vitro *strawberry subjected to water stress*. - New Phytol., 148: 177-186.

HSIAO T.C., 1973 - *Plant responses to water stress*. - Annu. Rev. Plant Physiol., 24: 519-570.

LÓPEZ S., MAROTO J.V., SAN BAUTISTA A., PASCUAL B., ALAGARDA J., 2002 - *Differences in carbohydrate content of waiting-bed strawberry plants during development in the nursery*. - Sci Horti., 94: 53-62.

MAFAKHERI A., SIOSEMARDEH A., BAHRAMNEJAD B., STRUIK P., SOHRABI Y., 2010 - *Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars*. - Aust. J. Crop Sci., 4(8): 580-585.

MASOUDI-SADAGHIANI F., ABDOLLAHI MANDOULAKANI B., ZARDOSHTI M., RASOULI-SADAGHIANI M., TAVAKOLI A., 2011 - *Response of proline, soluble sugars, photosynthetic pigments and antioxidant enzymes in potato* (Solanum tuberosum L.) *to different irrigation regimes in greenhouse condition*. - Aust. J. Crop Sci., 5(1): 55-60.

McCREADY R.M., GUGGOLZ J., SILVIERA V., OWENS H.S., 1950 - *Determination of starch and amylose in vegetables*. - Anal. Chem., 22 (9): 1156-1158.

MOHAMMAD A., HUSSAIN I., RASHID H., QURAISHI A., 2000 - *Enhanced* in vitro *multiplicationof banana* (Musa sp.). - Plant Tissue Cult., 10(2): 111-117.

MOLASSIOTIS A.N., SOTIROPOULOS T., TANOU G., KOFIDIS G., DIAMANTIDIS G., THERIOS I., 2006 - *Antioxidant and anatomical responses in shoot culture of the apple rootstock MM 106 treated with NaCl, KCl, mannitol or sorbitol*. - Biol. Plant., 50: 61-68.

MUNNE-BOSCH S., PENUELAS J., 2004 - *Drought-induced*

oxidative stress in strawberry tree (Arbutus anedo L.) *growing in Mediterranean Field Conditions*. - Plant Sci., 166: 1105-1110.

MUNNS R., WEIR R., 1981 - *Contribution of sugars to osmotic adjustment in elongation and expanded zones of wheat leaves during moderate water deficits at two light levels*. - Aust. J. Plant. Physiol., 8: 93-105.

MURASHIGE T., SKOOG F., 1962 - *A revised medium for rapid growth and bioassays with tobacco tissue culture*. - Physiol. Plant., 15: 473-497.

NAWAR A., EZZ T., 1993 - *Leaf relative content, growth and carbohydrate metabolism in apricot seedlings grown under different soil moisture levels*. - Alexandria. J. Agric. Res., 38(1): 337-353.

OUKABLI A., MEKAOUI A., IBNOUALI-EL-ALOUI M., BARI A., 2008 - *Contribution to identification of fig* (Ficus carica) *genotypes tolerant to drought*. - Acta Horticulturae, 798: 382-388.

PEREZ-PEREZ J., SYVERSTEN J., BOTINA P., GARCIA-SANCHEZ F., 2007 - *Leaf water relations and net gas exchange responses of salinized Carrizo citrange seedling during drought stress recovery*. - Ann. Bot., 100: 335-345.

RAMANJULU S., SREENIVASALU N., KUMAR S.G., SUDHAKAR C., 1998 - *Photosynthetic characteristic in mulbery during water stress and rewatering*. - Photosynthetica, 35: 259-263.

REDDY A.R., CLAITANYA K.V., VIVEKANADAN M., 2004 - *Drought-induced responses of photosynthesis and antioxidant metabolism in higher plan*. - Plant Physiol., 161: 1189-1202.

SAWWAN J., SHIBLI R., SWAIDAT I., TAHAT M., 2000 - *Phosphorus regulates osmotic potential and growth of African violet under* in vitro-*induced water deficit*. - J. Plant Nutr., 23: 759-771.

SHARP R., DAVIES W., 1979 - *Solute regulation and growth by roots and shoots of water-stressed maize plants*. - Planta, 147: 43-49.

SHAWKY I., RAWASH M., BEHAIRY Z., 1997 - *Growth and chemical composition of grape transplants as affected by some irrigation regims*. - Acta Horticulturae, 441: 439-447.

SIVRITEPE N., SIVRITEPE N., ERTURK U., YERLIKAYA C., TURKAN I., BOR M., OZDEMIR F., 2008 - *Response of the cherry rootstock to water stress induced* in vitro. - Biol. Plant, 52: 573-576.

TAYLOR A., MOTES J., KIRKHAM M. 1982 - *Osmotic regulation in germinating tomato seedling*. - J. Amer. Soc. Hort. Sci., 107(3): 387-390.

TEWARY P., ARDHANA S., RAGHUNATH M., SARKAR A., 2000 - In vitro *response of promising mulberry* (Morus *sp*.) *genotypes for tolerance to salt and osmotic stresses*. - Plant Growth Reg., 30: 17-21.

TORRECILLAS A., GALEGO R., PREZ-PASTOR A., RUIZ-SANCHEZ M., 1999 - *Gas exchang and water relations of young apricot plants under drought conditions*. - J. Agri. Sci., 132: 445-452.

TORRES K.C., 1998 - *Tissue culture techniques for horticultural crops*. - Van Nostrand Reinhold, New York, USA, pp. 285.

TURKAN I., BOR M., OZADEMIR F., KOCA H., 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(1): 223-231.

VERSLUES P., AGARAAL M., KATIYAR-AGARAAL S., ZHU J., ZHU J., 2006 - *Methods and concepts in quantifying resistance to drought salt and freezing, abiotic stressed that affect plant after status*. - The Plant J., 45: 523-539.

WANG Z., STUTTE G., 1992 - *The role of carbohydrate in active osmotic adjustment in apple under water stress*. - J. Amer. Soc. Hort. Sci., 117(5): 816-823.

WHETHERLEY P., 1950 - *Studies in the water relations of the cotton plants. I. The field measurement of water deficit in leaves*. - New Phytol., 49: 81-97.

WILHITE D., 2001 - *Drought (a global assessment)*. - Routledge, London, UK, pp. 752.

ZAMANI Z., TAHERI A., VEZAEI A., POUSTINI K., 2002 - *Prolinee content and stomatal resistance of almond seedlings as affected by irrigation intervals*. - Acta Horticulturae, 591: 411-416

ZHU J., 2001 - *Plant salt tolerance*. - Trends Plant. Sci., 6: 66-71.