Scholarly Journal of Agricultural Science Vol. 3(11), pp. 473-479 November, 2013
Available online at http:// www.scholarly-journals.com/SJAS
ISSN 2276-7118 © 2013 Scholarly-Journals

*Full Length Research Paper*

# Physiological and biochemical responses of four edible fig cultivars to water stress condition

**Somayeh Shirbani[1], Jalil Abdolahi Pour Haghighi[2], Moslem Jafari[3*] and Gholam Hossein Davarynejad[4]**

[1,4]Department of horticulture, Ferdowsi University of Mashhad, Mashhad, Iran.
[2]Department of Water Engineering, College of Agriculture, Shiraz University, Shiraz, Iran.
[3]Fig Research Station, Estahban,

Accepted 23 October, 2013

**To evaluate the resistance of different cultivars of Estahban edible fig to water stress, the study was carried out in 2009 at Ferdowsi University of Mashhad, Khorasan-e-Razavi, Iran. Four various fig cultivars (Sabz, Siah, Matti and Shah-anjir) were studied through a factorial experiment (4 × 4) in a Completely Randomized Design (CRD) in three replications under water stress treatments including severe stress (25% of field capacity), moderate stress (50% of field capacity), mild stress (75% of field capacity), and control treatment (100% of field capacity). The effect of moisture stress on physiological and biochemical characteristics of plants was investigated by application of ANOVA test. The results showed drought stress decreases the chlorophyll and leaf Relative Water Content (RWC) and increases the proline and electrolyte leakage. Although, Siah showed the highest tolerance according to most determined parameters, it did not provide favourable results in electrolyte leakage.**

**Key words:** Chlorophyll, Estahban, fig, proline, siah, water stress.

## INTRODUCTION

The fig (*Ficus carica*) probably originated in Western Asia and spread to the Mediterranean (Tous and Ferguson, 1996). Iran is the fourth largest producer of fig with more than 76,414 tons production in 2010 (FAO, 2012). Most of the fig trees in Iran are cultivated in Estahban, a semi-arid region in the south-east region of Fars province (Javanmard, 2010).

There are more than ten different cultivars of fig in Estahban. These fig cultivars belong to the Smyrna type fig and Sabz is the major economic cultivar among them (Rahemi and Jafari, 2008). The amount of precipitation in Estahban in typical years is about 350 mm and the amount of fig production is about 17000 tons (Jafari et al., 2012).

Last drought periods in the region confirmed that resistance varies among fig cultivars, though all of them are rain-fed trees. Zare et al. (2009) reported 50% reduction in the yield and the price of figs in the damaged rain-fed orchards due to the continuous drought conditions in the region.

Water stress has been defined as the induction of turgor pressure below the maximal potential pressure (Fitter and Hay, 1987; Osmond et al., 1987).

Cell membranes are one of the first targets of many plant stresses and it is generally accepted that the maintenance of their integrity and stability under water stress condition is a major component of drought tolerance in plants. The degree of cell membrane injury induced by water stress may be easily estimated through measurements of electrolyte leakage from the cells (Bajji, 2002).

Under drought, the maintenance of leaf turgor may be achieved by the way of osmotic adjustment in response to the accumulation of proline, sucrose, soluble carbohydrates, glycine betaine, and other solutes in cytoplasm improving water uptake from drying soil (Anjum et al., 2011). Proline acts as a reserve source of carbon, nitrogen and energy during recovery from stress (Zhang et al., 1997).

Relative water content is considered a measure of plant water status, reflecting the metabolic activity in tissues and used as a most meaningful index for dehydration

*Corresponding author. E-mail: jafarim@farsagres.ir.

tolerance (Anjum et al., 2011). RWC is affected by the interaction of severity, duration of the drought event and species (Yang and Miao, 2010).

Decreased or unchanged chlorophyll level during drought stress has been reported in many species, depending on the duration and severity of drought (Kyparissis et al., 1995; Zhang and Kirkham, 1996). Exposure of two olive cultivars to reduced irrigation led to lower Chl (a + b) contents. These reductions were 29 and 42% for Chemlali and Chetoui, respectively (Guerfel et al., 2009).

Based on observations and studies done by local experts, four main cultivars spread in the region were selected to be studied. Our aim is to find the more resistant cultivar in water stress condition through observing chlorophyll, leaf relative water content (RWC), proline and electrolyte leakage levels of four edible fig cultivars in order to apply it as a stock for the commercial cultivar.

## MATERIALS AND METHODS

This study was conducted in the factorial experiment through completely randomized design in three replications in the University of Mashahd in 2009. At the beginning of February, cuttings of different cultivars of Estahban's edible fig including Sabz, Siah, Shah-anjir and Matti were collected from Fig Research Station of Estahban. The cuttings were rooted in perlite and coco peat medium. Then, the healthy and uniformed plants were transferred to the 10 L pots containing clay, sand and peat in the ratio of 1:1:1. Three similar pots with equal weight and three samples for each replication were considered in each experimental unit. The plants were being irrigated until the water drainage from the pots got visible during the establishment time (90 days). Water stress was imposed on the samples in gravimetric method as follow:

By using a pressure plate method, the Permanent Wilting Point (PWP) was determined for four accidental soil samples. To find out the Field Capacity (FC), three similar pots in size and weight were completely saturated and left them to exit gravitational water.

Gravimetric soil water content at FC and PWP were obtained equal to 0.032 and 0.075, respectively. Available water (AW) determined as 24.5% or 2.45 kg for $W_s$=10 kg, by using the following equation:

$$AW = (W_{fc}-W_{pwp}) \times Ws$$

Through the application of weighing method and 2 days interval irrigation, the following treatments were provided in the study: 100% of FC (control), 75% of FC (mild stress), 50% of FC (moderate stress) and 25% of FC (severe stress).

By using the following equation, leaf relative water content (RWC) was found for young fully expended leaves:

$$RWC\% = (FW–DW) / (TW–DW) \times 100$$

Where FW and DW are fresh and dry weight of leaf samples and TW is the weight of leaves after floating in distilled water for 24 h. To measure the chlorophyll content, after weighing 0.2 g fresh tissue of leaves, it was cut into small pieces of appropriate size (8 to 10 mm) and then ground with a pestle and mortar in 5 ml of distilled water in cool temperature of about 10°C and dim light condition to change into unified mass. The volume in the flask was made up to 25 ml with distilled water. 4.5 ml of 80% Aston was added to 0.5 ml of the mixture and centrifuged at 3500 (rpm) for 15 min. Then, the absorbance was measured at 645 and 663 nm for this supernatant solution in a BioQuest CE 2502 spectrophotometer. The chlorophyll concentration was obtained by the following equations:

Chl a (microgram/ml) = (12.5 OD663) - (2.55 OD645)
Chl b (microgram/ml) = (18.29 OD645) - (2.58 OD663)
Chl total = Chl a + Chl b

To measure the proline amino acid in the leaves, 0.1 g fresh leaves was ground well with pestle and mortar in 10 ml of 3.3% sulphosalicylic acid. After filtering, the filtrate was collected in a test tube and kept in an ice and water mixture. In the next step, 2 ml of ninhydrin (1.25 ninhydrin + 20 ml phosphoric acid 6 molar + 30 ml pure acetic acid) and 2 ml acetic acid were added to every test tube containing the extract.

The tubes were placed in a boiling water bath maintained at 100°C for one hour and the mixture cooled. Under the hood, 6 ml toluene was added to each tube and shook well for 15 to 20 s. In this condition, two different phases were made. The absorbance was measured at 520 nm by spectrophotometer for 1 ml of the upper phase containing proline (Bates et al., 1973).

The electrolyte leakage index which is used to determine the leaf cell membrane stability was evaluated based on Blum and Eberkon (1981) method modified by Marcum et al. (1995). At first, the leaves were cut into 2 cm pieces, and put in the tubes after washing by 10 ml distilled water. Then, the test tubes placed in an orbital shaker (90 rpm) for 17 to 18 h.

After that, the initial electrical conductivity ($EC_i$) was measured by JenWay conductivity meter model 4310. The cells of leaf tissues were killed by autoclaving for 15 min at 120°C. Maximum electrical conductivity ($EC_m$) was also measured after cooling the mixture of tubes. At the end, electrolyte leakage was calculated through the following equation:

$$EL = (ECi/ECm) \times 100$$

**Table 1.** Analysis of variance for the effects of cultivar and stress level on physiological and biochemical characteristics of four cultivars fig plants.

| S.O.V | df | Mean of squares | | | |
|---|---|---|---|---|---|
| | | Proline | Chlorophyll | Electrolyte leakage | RWC |
| Cultivar | 3 | 0.1$^{ns}$ | 0.3** | 670** | 80.9* |
| Stress level | 3 | 15.2** | 4.1** | 5072** | 1031** |
| Cultivar × Stress level | 9 | 0.6$^{ns}$ | 0.2** | 68** | 31$^{ns}$ |
| Experimental error | 32 | 0.3 | 0.01 | 23 | 24.7 |

ns, * and **: Not significant, significant at 5 and 1% levels, respectively.

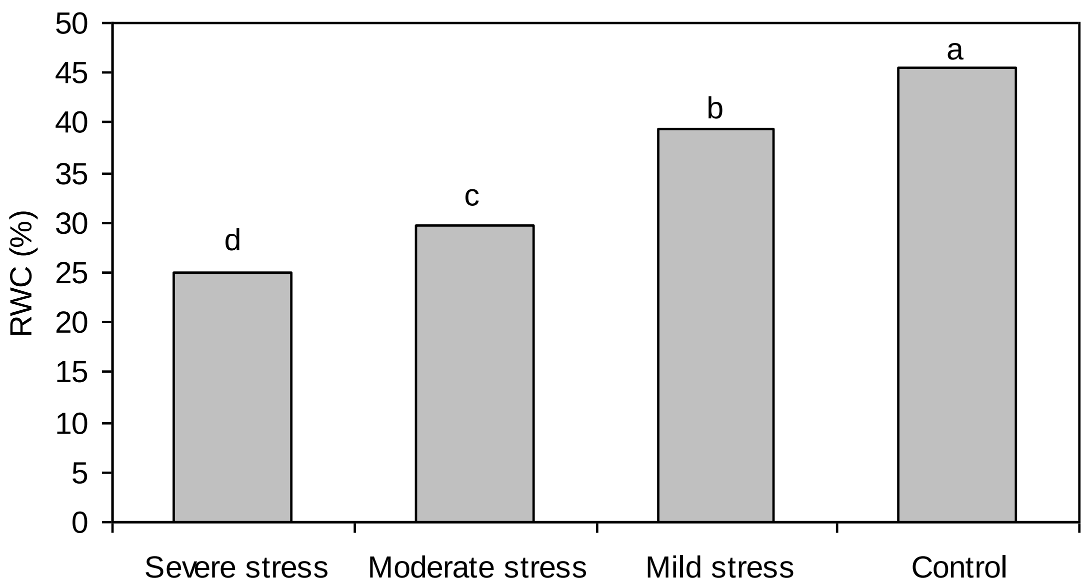


**Figure 1.** Leaf relative water content (RWC) (%) for different water stress treatments ($p \leq 0.01$).

Data analyses were analyzed by using MSTAT software. The LSD was used to compare the means at 5% of significant.

## RESULTS AND DISCUSSION

### Relative water content (RWC)

The results of ANOVA test showed a significant difference of leaf relative water content (RWC) in both the levels of water stress ($p \leq 0.01$) and fig cultivars ($p \leq 0.05$) (Table 1).

The least and the most RWC were observed in severe stress (24.9%) and control treatment (45.48%), respectively (Figure 1). Siah cultivar had the most RWC among all cultivars, followed by Matti, Shah-anjir and it was minimum in Sabz (Figure 2). Exposure of plants to drought stress substantially decreased the leaf water potential, relative water content and transpiration rate, with a concomitant increase in leaf temperature (Siddique et al., 2001).

### Electrolyte leakage

The electrolyte leakage feature in the interaction effect between cultivar and stress level is significant ($p \leq 0.01$) (Table 1). The electrolyte leakage of all cultivars increased in the treatments following the order: severe stress> moderate stress> mild stress> control treatment, while Siah cultivar has the most electrolyte leakage. There are no significant differences among the other three. In severe stress condition, adversely to the other treatments, the electrolyte leakage of Sabz cultivar is slightly (4.1%) more than Shah-anjir (Figure 3).

According to several reports, the production and storage of toxic groups of free oxygen increased when the water stress is along with an oxidative stress (Hill et al., 1986; Foyer et al., 1994). The activity of antioxidant enzymes such as superoxide dismutase (SOD, EC 1.15.1.1), which restrain this type of oxygen was highly reduced. Hence, their destructive effect with rapid peroxide of membrane fat under the water stress condition ruins the cell membrane stability (Wang and Huang, 2004).

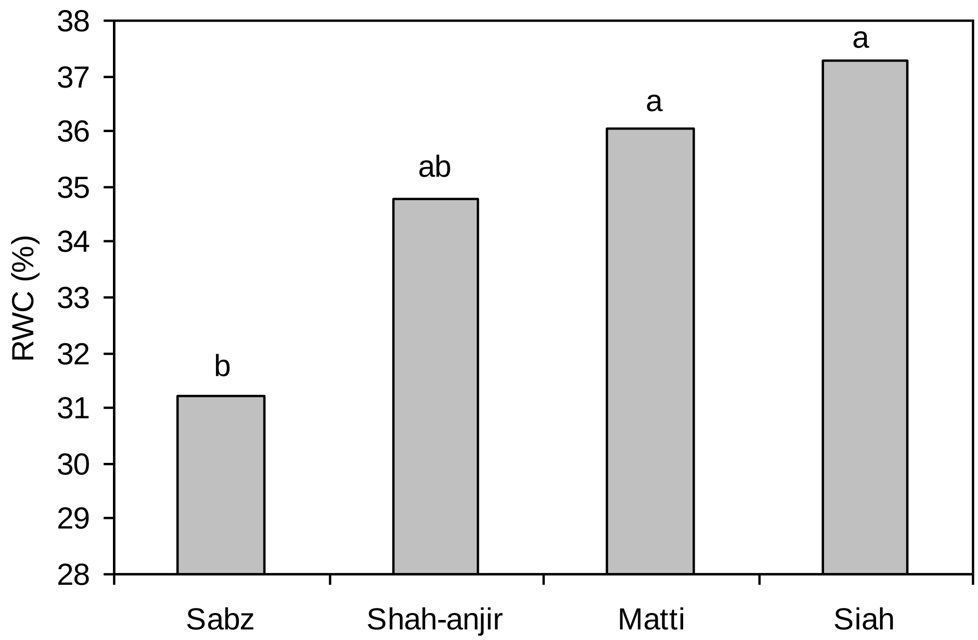


**Figure 2.** Leaf relative water content (RWC) (%) of fig cultivars (p≤0.05).

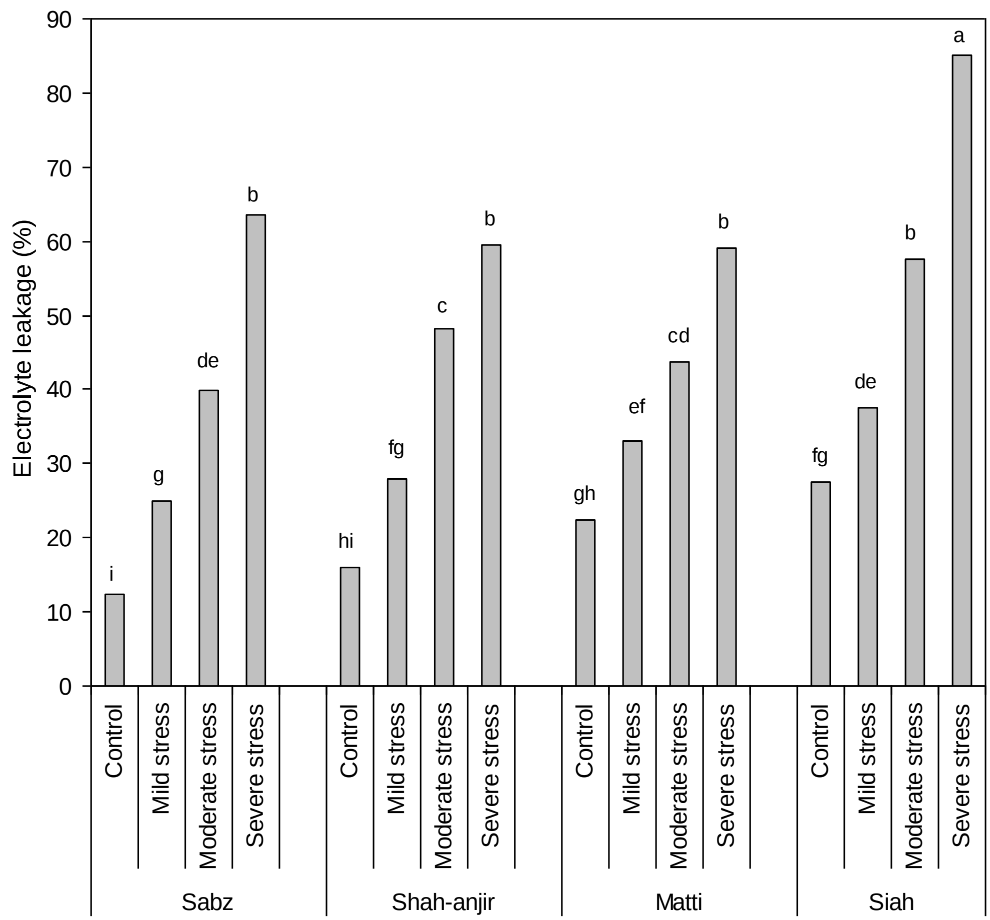


**Figure 3.** Interaction effect of cultivar and water stress on electrolyte leakage (%) (p≤0.01).

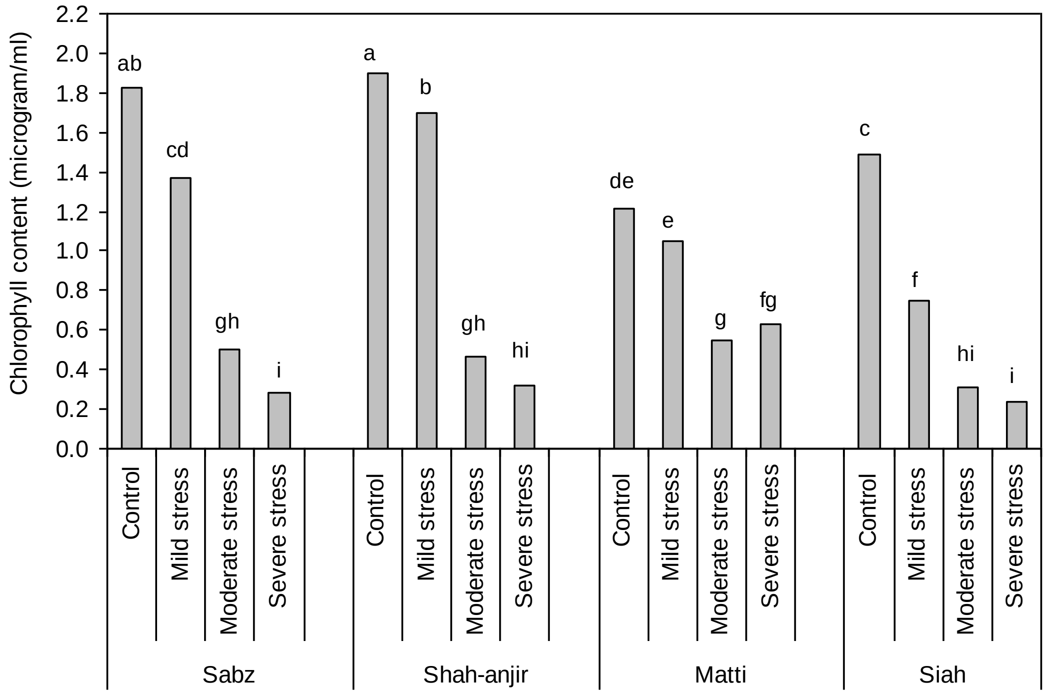


**Figure 4.** Interaction effect of cultivar and water stress on chlorophyll content (microgram/ml) (p≤0.01).

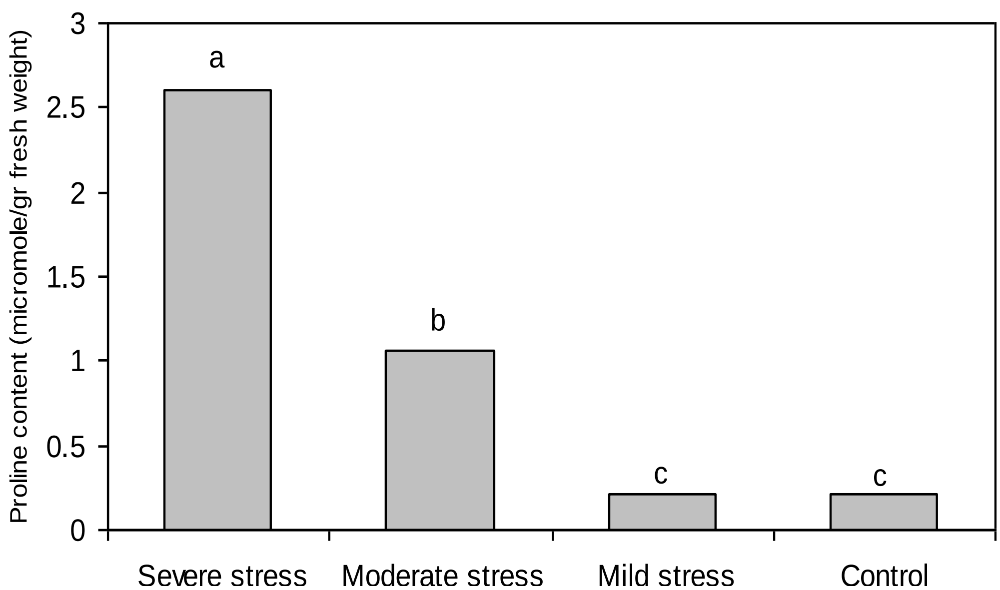


**Figure 5.** Proline content (micromole/g fresh weight) for different water stress treatments (p≤0.01).

Retaining physiological functions like photosynthesis and growth activities rely on more stability in cell membrane under the sever water stress treatments (Bewley, 1979). A study on the durum wheat by Bajji et al. (2002) indicated that the keeping membrane healthy under the water stress is one of the most important components of plant tolerance. Membrane injury was observed in bean plants due to water reduction (Kocheva et al., 2004).

## Chlorophyll content

Early studies on different plants like Arabidopsis and wheat indicated water stress condition is accompanied by reducing of the chlorophyll concentration (Jung, 2004; Ahmadi and Sio-se mardeh, 2004).

The results in Table 1 showed that the chlorophyll content of leaf in the interaction effect between cultivar and stress level was significant (p≤0.01). Fig plants under sever stress and moderate stress displayed the least amount of chlorophyll content. Whereas, mild stress and control treatment have the most chlorophyll content in the treatments, the control treatment has the highest leaf chlorophyll content significantly (1.61 microgram/ml) (Figure 4).

There was a significant difference of chlorophyll content among the fig cultivars such that Shah-anjir (1.1 microgram/ml) which is in the top level was followed by Sabz (0.99 microgram/ml), Matti (0.86 microgram/ml) and Siah (0.7 microgram/ml). This trend is different in some cases as in severe stress condition; Sabz (0.28) and Siah (0.24) had less chlorophyll content in comparison with Matti (0.63) and Shah-anjir (0.32).

## Proline content

Proline content was significantly different among the treatments in p≤0.01 (Table 1). According to Figure 5, while severe stress treatment had the most proline content (2.6 micromole/g fresh weight), mild stress and control treatment had the least one (0.21 micromole/g fresh weight). Under osmotic or dehydration stress conditions, membrane integrity must be maintained to prevent protein denaturation. Proline may interact with enzymes to preserve protein structure and activities (Kavi et al., 2005).

## CONCLUSIONS

The purpose of the current study was to evaluate physiological and biochemical reactions of four edible fig cultivars to water stress. The result showed that in the stress condition, among all trial cultivars, Siah and Sabz had the most and least amount of RWC, respectively. Water stress decreased the leaf water potential tangibly due to reduction in RWC (Chartzoulakis et al., 2002).

It was found that the electrolyte leakage increased under severe stress treatment. Siah had the most electrolyte leakage, while there were no significant differences among the other cultivars.

A consequence of the altered membrane integrity is the increase of the cell permeability accompanied by electrolyte leakage from the cell (Blum and Ebercon, 1981). An important strategy for the development of drought resistance in plants is the maintenance of cell membrane integrity after the imposition of water stress (Vasquez-Tello et al., 1990).

The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation.

Both the chlorophyll a and b are prone to soil dehydration (Farooq et al., 2009). Chlorophyll content declined under drought stress in the experiment. Matti had significantly the least chlorophyll content and Shah-anjir had the most one under control treatment, indicating its validity in the normal condition of field.

Corresponded to the previous studies on the plants like eggplant, alfalfa, wheat and sweet corn (Sarker et al., 2005; Akhondi et al., 2006; Ahmadi and Sio-se mardeh, 2004; Mohammadkhani and Heidari, 2008), proline increased in water stress condition in the experiment. Proline seems to have diverse roles under osmotic stress conditions, such as stabilization of proteins, membranes and subcellular structures (Vanrensburg et al., 1993).

In general, evaluating of various features of major fig cultivars in Estahban area showed Siah is a more adaptable cultivar under water stress condition than the other tested ones. This is in agreement with the local knowledge and experience of fig growers.

## REFERENCES


Ahmadi, A. and Sio-se mardeh, A. (2004). The effects of water stress on soluble carbohydrates, chlorophyll and proline contents of four Iranian wheat cultivars under different moisture regimes. Iranian Journal of Agriculture Sciences. 35(3): 753-763.

Akhondi, M., Safarnejad, A. and Lahouti, M. (2006). Effect of drought stress on proline accumulation and mineral nutrients changes in Alfalfa (Medicage Sativa L.). Journal of Science and Technology of Agriculture and Natural Resources. 10(1): 165-175.

Anjum, S.A., Xie, X., Wang, L.C., Saleem, M.F., Man, C. and Lei, W. (2011). Morphological, physiological and biochemical responses of plants to drought stress. African Journal of Agricultural Research. 6(9): 2026–2032.

Bajji, M., Kinet, J.M. and Lutts, S. (2002). The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. Plant Growth Regulation. 36: 61-70.

Bates, L.S., Waldren, R.P. and Teare, L.D. (1973). Rapid determination of free proline for water stress studies. Plant soil. 39: 205-207.

Bewley, J. D. (1979). Physiological aspects of desiccation tolerance. Annual Review of Plant Physiology. 30: 195-238.

Blum, A. and Ebercon, A. (1981). Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Science. 21:43-47.

Chartzoulakis, K., Patakas, A.G., Kofidis, A. and Nastou, A. (2002). Water stress effects leaf anatomy, gas exchange, water relations and growth of two avocado cultivars. Scientia Horturae. 95: 39-50.

FAO. (2012). FAOSTAT-Agriculture. Food and Agricultural Organization of the United Nations. Accessed April 14, 2012. http://faostat. fao. org.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D. and Basra, S.M.A. (2009). Plant drought stress: effects, mechanisms and management. Agronomy for Sustainable Development. 29: 185-212.

Fitter, A. H. and Hay, R. K. M. (1987). Environmental Physiology of Plants. London: Academic Press.

Foyer, C.H., Leandais, M. and Kunert, K.J. (1994). Photo oxidative stress in plants. Plant Physiology. 92: 696-717.

Guerfel, M., Baccouri, O., Boujnah, D., Chaibi, W. and Zarrouk, M. (2009). Impacts of water stress on gas exchange, water relations, chlorophyll content and leaf structure in the two main Tunisian olive (Olea europaea L.) cultivars. Scientia Horticulturae. 119: 257-263.

Hill, J., verheggen, F.P., fernssen, H., Vankammen, A. and Zabel, K. (1986). Bleomycin resistance: a new dominant selectable marker for plant cell transformation. Plant Molecular Biology. 7: 171-176.

Jafari, M., Abdolahi Pour Haghighi, J. and Zare, H. (2012). Mulching impact on plant growth and production of rainfed fig orchards under drought conditions. Journal of Food, Agriculture and Environment. 10(1): 428-433.

Javanmard, M. (2010). Occurrence of mould counts and aspergillus species in Iranian dried figs at different stages of production and processing. Journal of Agricultural Science and Technology. 12: 331-338.

Jung, S. (2004). Variation in antioxidant metabolism of young and mature leaves of Arabidopsis thaliana subjected to drought. Plant Science. 166:459-466.

Kavi Kishor, P.B., Sangam, S., Amrutha, R.N., Sri Laxmi, P., Naidu, K.R., Rai, K.R.S.S., Rao, S., Reddy, K.J., Theriappan, P. and Sreenivasulu, N. (2005). Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. Current Science. 88: 424-438.

Kocheva, K., Lambrev, P., Georgiev, G., Goltsev, V. and Karabaliev, M. (2004). Evaluation of chlorophyll fluorescence and membrane injury in the leaves of barley cultivars under osmotic stress. Bioelectrochemisty. 63: 121-124.

Kyparissis, A., Petropoulun, Y. and Manetas, Y. (1995). Summer survival of leaves in a soft-leaved shrub (Phlomis fruticosa L., Labiatae) under Mediterranean field conditions: avoidance of photoinhibitory damage through decreased chlorophyll contents. Journal of Experimental Botany. 46: 1825-1831.

Marcum, K.B., Engelke, M.C., Morton, S.J. and White, R.H. (1995). Rooting characteristics and associated drought resistance of zoysiagrass. Agronomy Journal. 87: 534-538.

Mohammadkhani, N. and Heidari, R. (2008). Drought-induced accumulation of soluble sugars and proline in two Maize cultivars. World Applied Sciences Journal. 3(3): 448-453.

Osmond, C.B., Austin, M.P., Billings, J.A., Boyer, J.S., Dacey, W.H., Nobel, P.S., Smith, S.D. and Winner, W.E. (1987). Stress physiology and the distribution of plants. BioScience. 37(1): 38-48.

Rahemi, M. and Jafari, M. (2008). Effect of caprifig type on quantity and quality of Estahban dried fig Ficus carica, cv. Sabz. Acta Horticulturae. 798: 249-252.

Sarker, B.C., Hara, M. and Uemura, M. (2005). Proline synthesis, physiological responses and biomass yield of eggplants during and after repetitive soil moisture stress. Scientia Horturae. 103: 387-402.

Siddique, M.R.B., Hamid, A. and Islam, M.S. (2001). Drought stress effects on water relations of wheat. Botanical Bulletin of Academia Sinica. 41: 35-39.

Tous, J. and Ferguson, L. (1996). Mediterranean fruits. In: J. Janick (eds), Progress in New Crops. Arlington: ASHS Press.

Vanrensburg, L., Kruger, G. H. J. and Kruger, R. H. (1993). Proline accumulation as drought tolerance selection criterion: Its relationship to membrane integrity and chloroplast ultra structure in Nicotiana tabacum L. Journal of Plant Physiology. 141: 188–194.

Vasquez-Tello, A., Zuily-Fodil, Y., Thi, A. T. P. and Vieira Da Silva, J. B. (1990). Electrolyte and Pi leakages and soluble sugar content as physiological tests for screening resistance to water stress in Phaseolus and Vigna species. Journal of Experimental Botany. 41(228): 827–832.

Wang, Z. and Huang, B. (2004). Physiological recovery of Kentucky bluegrass from simultaneous drought and heat stress. Crop Science. 44: 1729-1736.

Yang, F. and Miao, L.F. (2010). Adaptive responses to progressive drought stress in two poplar species originating from different altitudes. Silva Fennica. 44: 23-37.

Zare, H., Zare, E., Pir Moradiyan, N., Joukar, L., Mobayen, K.h., Nazari, A., Golkar, G.h. and Kamkar Haghighi, A.A. (2009). Reducing damages caused by drought on the rainfed fig cultivation and its economic-social impacts. Seed and Plant Improvement Institute, Karaj, p 40.

Zhang, C-S., Lu, Q. and Verma, D. P. S. (1997). Characterization of Δ1-pyrroline-5-carboxylate synthetase gene promoter in transgenic Arabidopsis thaliana subjected to water stress. Plant Science. 129: 81–89.

Zhang, J. and Kirkham, M.B. (1996). Antioxidant response to drought in sunflower and sorghum seedlings. New Phytologist. 132: 361-373.