

Effects of Drought Stress on Some Anatomical Characteristics of Barley Leaves

Farshad Sorkhi

Received: December 30, 2015 Accepted: June 1, 2017

Assistant Professor, Department of Agriculture, Miandoab Branch, Islamic Azad University, Miandoab, Iran

*Corresponding author; E-mail: farsorkhy@yahoo.com

Abstract

In this research, effect of drought stress on leaf characteristics was investigated in the experimental field of Miandoab Azad University, Iran using four facultative barley cultivars. Two separate experiments were conducted in pot and field conditions. In both experiments a factorial arrangement was used and the treatments were completely randomized in four replications. In each experiment half of the experimental units were drought stressed and the other half were irrigated normally. For the pot experiment, plants were sampled at fourth leaf stage in order to obtain winter leaves. For the field experiment, when plants reached at late stem elongation stage, penultimate leaves were sampled and used for preparing microscopic slides.

Xylem and phloem diameter and mesophyll, bundle sheath and epidermal cells area were measured in the transverse sections prepared from middle parts of the leaves. Significant differences were observed among genotypes under drought stress in terms of leaf characteristics. Results also showed that drought stress changes the diameter and the surface area of the cells. However, the changes were not the same in the winter and spring leaves. The changes in some cases such as diameter of xylem vessels were considerable. For example, in the cultivars Sahra and Jonob, the winter leaves had bigger xylem vessels under drought stress as compared to the normal condition while in the spring leaves the xylem diameter was smaller under the same condition.

Keywords: Anatomical characteristics; Barley; Drought; Leaf

Introduction

Barley (*Hordeum vulgare* L.) is a major crop ranked fourth in the world among cereals (Baik and Ullrich 2008). Barley is typically cultivated in the arid and semi-arid regions of Iran generally in areas with low precipitation that is not suitable for wheat (Talame *et al.* 2007). Drought is a significant limiting factor for agricultural productivity and generally inhibits plant growth through the reduction in water absorption and nutrient uptake. Different plant features such as leaf anatomy have been considered as characters useful for increasing drought stress tolerance (Lee *et al.* 2011). It has been shown that anatomical changes in leaf may help plants to maintain high levels of photosynthetic rates and high

transpiration efficiencies (Evans *et al.* 1994). Cuticle thickness (Bohnert and Jensen 1996) and stomatal frequency (Rebetzke *et al.* 2010) are anatomical characteristics which are believed to be useful for breeding drought stress tolerant genotypes. Leaf morphological characters including leaf area (Zagdanska and Kozdoj 1994), shape (Reddy *et al.* 2004) and developing behavior (Hu *et al.* 2000) are also considered as effective characters for environmental stress tolerance.

In wheat leaves the venation network consists of a series of large or lateral and small or intermediate longitudinal veins which are connected to each other by transverse veins (Zagdanska and Wisniewski 1996). The diameter of

the lateral veins decreases toward the leaf tip while the number and the size of the intermediate veins do not change along the length of the leaf and their water conductivity remains constant (Hallik *et al.* 2009). However, the xylem vessel diameter is considered as the main factor limiting flow rate though the changes of the xylem conductivity along the leaf axis also depends on the different stages of xylem maturation (Payvandi *et al.* 2014) and apoplastic and symplastic movement of water to the evaporation sites inside the leaf at mesophyll cells surface (Cochard *et al.* 2004).

Anatomical changes of the leaf are used as indicators of stress symptoms (Niinemets and Sack 2006). In the developing leaves these changes have significant effects on photosynthesis. For example, palisade mesophyll cells length and number in leaves are shown to be correlated with photosynthetic capacity (Syvertsen *et al.* 1995). Morphological and anatomical modifications under drought stress condition are associated with leaf structure (Niinemets and Sack 2006). Transpiration rate of plants growing in dry regions is under the control of leaf size (Dias *et al.* 2007), epidermal cells and cuticle thickness (Press 1999) and stomatal pore area (Drake *et al.* 2013). Dehydration tolerance also has been shown to improve by other characteristics such as increased mechanical resistance of the cell walls by increasing the lignification level (Moore *et al.* 2008), increased succulence, increased water storage capacity and accumulation of mucilage (Harb *et al.* 2010).

Growth responses of plants to drought stress are the result of changes in cell division, enlargement and deposition of cell wall materials (Fricke and

Flowers 1998). It has been shown that the suberised lamellae of the mestome sheath cells form an incomplete barrier near the xylem to keep separate the oppositely directed fluxes of water and assimilates through the sheath (Verma *et al.* 2004). Drought stress has shown to prevent cell division and growth (Zagdanska and Kozdoj 1994). Tissues exposed to environments with low water availability have generally shown reduction in cell size and increase in vascular tissue and cell wall thickness (Guerfel *et al.* 2009). Mesophyll cells are more vulnerable to water stress damages compared to the bundle sheet cells (Mansoor *et al.* 2002). In the water stress tolerant sugarcane genotypes cell wall thickness increased under stress condition (Vasantha *et al.* 2005). Smaller epidermal cells were found in *Lolium perenne* under drought stress condition (Japp and Newman 1987). Epidermal and mesophyll cell sizes were shown to reduce under water stress environment (Arteimos *et al.* 2002).

Reduction in cross sectional area was attributed to a decrease in the size of the vein segments and a reduced number of medium and small veins (Hallik *et al.* 2009; Payvandi *et al.* 2014). Reduced area of protoxylem and metaxylem in midrib and large vein segments in growing tissues may be responsible for lower water deposition into the growth zone under saline conditions (Hu *et al.* 2005; Talame *et al.* 2007).

Barley leaves can be classified as winter leaves which are usually narrow, small and thin with small sheaths growing very close to each other and spring leaves which are wide, long and thick with large sheath. Winter leaves are cold stress tolerant and do their metabolism under low

temperature conditions while spring leaves do their best performance under higher temperature levels. However, in the growing leaves there are three distinct regions. The first is up to 30 mm from ligule in which cells are dividing. In the second region, 30-60 mm from ligule, newly produced cells are enlarging and cell wall is developing. The last region which expands up to the leaf tip is photosynthetically active (Kazemi Arbat 2005). Little information exists regarding the effects of environmental factors on the anatomy of these leaves. The aims of this study was, therefore, to determine the effects of drought stress on the size of epidermal, mesophyll and bundle sheath cells and vascular tissues of barley leaves.

Materials and Methods

In this research, two separate experiments were conducted in pot and field conditions at the experimental field of Miandoab Azad University, Iran. The pot experiment was factorial based on completely randomized design with four replications in which half of the pots were drought stressed under a rain shelter. Plants were sampled at fourth leaf stage in order to obtain winter leaves. Four barley facultative cultivars including Sahra and Jonob as drought tolerant, Zarjo as semi-tolerant and Valfajr as a sensitive genotype were used. Seeds were germinated on wet tissue papers and were then sown at Nov 22, 2014 in pots containing a mixture of vermiculite and peat (1:1). Soil water holding capacity (FC) was determined (McKim *et al.* 1980) before planting and the amount of water applied to the pots was adjusted at 100% and 60% of FC for normal and drought stressed pots, respectively. In each replication

there were two pots for each genotype, one was drought stress treatment and the other kept at normal condition and all were randomly arranged. During the growth period pots were weighted every day using an electronic balance (precision=1 gram) and irrigated up to their initial weight to maintain the growing condition constant. One week after full expansion of the fourth leaf, leaf blades of the same size from each pot were sampled. From each, small segments at 60-65 mm from the blade base were again sampled.

The genotypes were sown at the same time in the 2 × 3 meter square experimental plots in the field condition in order to obtain typical spring leaves. The experiment was factorial and the treatments were completely randomized using four replications. In the drought stressed plots, irrigation withheld before booting stage so that when flag leaves emerged, the soil water content was at about 60 percent of soil water holding capacity. In the normal condition, plots were irrigated normally every seven days. When plants reached at late stem elongation stage, penultimate leaves were sampled and used for preparing microscopic slides.

Samples from both leaf types were immediately transferred into 10% formalin solution for 48 hours. To prevent leaf curling, samples were fixed on small pieces of cardboards by nips. Samples were then processed as follows to be prepared for taking transverse sections. First, they transferred to a 1:1 mixture of 96% ethanol and 10% formalin for 60 minutes. Then, they were immersed step by step in 50%, 70%, 80%, 90% and 96% ethanol solutions each for 60 minutes. The samples were then immersed two times in

100% ethanol and two times in 100% xylol. Finally, samples were submerged in melted paraffin inside the blocking cassettes. Paraffin blocks were then fixed in the microtome clump and were transversely sectioned while the blade was adjusted at 5 μ m. Sections were then transferred on microscopic slides and incubated into an electric oven adjusted at 70 °C for 20 minutes. After that, sections were stained using hematoxylin and eosin.

Anatomical examinations were performed on five images randomly taken from slides using an eye-piece digital camera fixed on a light microscope at 10 \times 40 magnification. Scion image analysis software was used to measure the area of epidermal, bundle sheath and mesophyll cells and the diameter of phloem sieve tubes and xylem vessels. In each case mean values of 10 random observations were used for data analysis.

All data were subjected to analysis of variance using the corresponding linear additive model. MSTATC and SPSS software were used to analyze the data obtained. Duncan's multiple range test was used to compare means, and Excel software was used to construct diagrams.

Results

Winter leaves characteristics

Xylem vessel diameter: The effect of genotype, drought stress and interaction of these two factors on xylem vessel diameter was significant (Table 1). Under drought stress condition xylem vessels diameter was increased in Sahra, Jonob and Zarjo while decreased in Valfajr as compared to the normal condition (Figure 1A).

Phloem sieve tubes diameter: Phloem sieve

tubes' diameter was significantly affected by genotype, drought stress and their interaction (Table 1). Drought stress decreased phloem sieve tubes diameter in Valfajr and Jonob while increased in Sahra and Zarjo as compared to the normal condition (Figure 1B).

Bundle sheath cells area: Genotype did not have significant effect on bundle sheath cells area. However, the effect of drought stress and interaction of genotype by drought stress was significant on this trait (Table 1). Highest and lowest bundle sheath cell areas were found in Valfajr and Zarjo under normal condition, respectively. Compared to the control condition bundle sheath cells area decreased in Valfajr and Jonob under drought stress condition while it was increased in Zarjo and Sahra under the same condition (Figure 1C).

Mesophyll cells area: Mesophyll cells area was significantly affected by genotype, drought stress and genotype by drought stress interaction (Table 1). Under drought stress condition mesophyll cells area decreased in Valfajr, Jonob and Zarjo while increased in Sahra. Highest and lowest mesophyll cells area were found in Sahra and Zarjo under drought stress condition, respectively (Figure 1D).

Upper epidermal cells area: Upper epidermal cells area was also significantly affected by genotype, drought stress and genotype by drought stress interaction (Table 1). Epidermal cells area decreased in Valfajr, Zarjo and Sahra under drought stress condition while it was increased in Jonob. Highest and lowest epidermal cell areas were found in Valfajr and Zarjo under stress condition (Figure 1E).

Lower epidermal cells area: Drought stress and genotype by drought stress interaction effects on the lower epidermal cells area were significant (Table 1). In the Jonob cultivar lower epidermal cells area increased significantly under drought stress condition while in others the changes were not significant (Figure 1F).

Spring leaves characteristics

Xylem vessel diameter: Xylem vessel diameter was significantly affected by genotype, drought stress and their interaction (Table 2). Xylem vessel diameter in Valfajr significantly decreased under drought stress condition. Meanwhile, there were no changes in the xylem diameter in Zarjo, Sahra and Jonob under stress as compared to the normal condition (Figure 2A).

Phloem sieve tubes diameter: Genotype had significant effect on phloem sieve tubes diameter (Table 2). Generally, phloem sieve tubes diameter was significantly higher in the Jonob cultivar as compared to Valfajr and Sahra while the difference between Jonob and Zarjo was not significant (Figure 2B).

Bundle sheath cells area: Data analysis showed that genotype, drought stress and their interaction were not significant on the bundle sheath cells area (Table 2).

Mesophyll cells area: Genotype, drought stress and their interaction on the mesophyll area were significant (Table 2). In Valfajr, mesophyll cells area significantly increased under drought stress condition while it decreased in the Jonob cultivar. Changes in the mesophyll cells area of genotypes Zarjo and Sahra under drought stress condition were not significant (Figure 2C).

Upper epidermal cells area: Drought stress, genotype and their interaction on the upper epidermal cells area were significant (Table 2). Results showed that upper epidermal cells area increased in Sahra, Jonob and Valfajr under drought stress condition while it was decreased in the cultivar Zarjo as compared to the normal condition (Figure 2D).

Lower epidermal cells area: This trait was significantly increased in Valfajr under drought stress condition and decreased in Zarjo. There was no change in the epidermal cells area of Sahra (Figure 2E).

Discussion

In this experiment xylem vessel diameter was increased under drought stress condition in the winter leaves except in the Valfajr cultivar; xylem vessel diameter in Valfajr reduced significantly under drought stress condition. Furthermore, reductions in other cultivars were not considerable. Therefore, lower xylem conductivity in the spring leaves is expected to cause less water movement under higher evaporating demands of their growing period. Decreasing xylem diameter may play a role in adaptation of plants to drought stress condition since smaller diameter decreases the hydraulic conductivity of the xylem (Fitter and Hay 2002). It was shown that in the water stressed susceptible winter wheat cultivars xylem diameter is greater compared to the tolerant ones (Verma *et al.* 2004).

It has been shown in spring wheat leaves

that the largest lateral vessels decrease in diameter with distance along the leaf towards the tip, resulting in the decreased hydraulic conduction (Verma *et al.* 2004; Talame *et al.* 2007) which in turn may decrease the rate of water movement. This may help plants to use the available water slowly and as a result for a longer period of time. On the other hand, increasing the xylem vessel diameter in some plant parts may also be beneficial under water stress condition as may provide plants with a water reservoir which can be used at day times of high water demand. A bottleneck is shown to exist at the basal region of the leaf which could limit water transport (Fitter and Hay 2002).

The existence of genetic variation in this case shows that changing the capacity of the phloem sieve tubes can be targeted in the breeding of cultivars for higher assimilate translocation to the grains. Values of phloem cross-sectional area of different vein types along the barley leaf blade supported the idea that lateral veins are responsible for translocation of assimilates while intermediate veins are working as collecting reservoirs (Minchin *et al.* 2002). It has been shown that phloem sieve tubes area depends on the sink with which they are related (Fitter and Hay 2002). This is in contrast with what is expected from the phloem sieve tubes diameters implying that there may be other limiting factors affecting grain size in these cultivars.

Since bundle sheath cells are not photosynthetically active, increasing their size in cost of reducing mesophyll cell numbers may decrease the photosynthetic capacity of the leaf. It has been shown that increasing the number of the

bundle sheath cells extensions reduces leaf photosynthetic capacity per unit area (McClendon 1992). Bundle sheath cells extensions can prevent effective lateral diffusion among surrounding mesophyll cells. On the other hand, they can work as a light penetration system helping the light to penetrate into the deep cell layers and increase photosynthetic capacity (Niinemets and Sack 2006). There is, however, no report about the effect of the size of the bundle sheath cells on the rate of photoassimilates and solutes movement through plasmodesmata connecting xylem and phloem to them.

It has been reported that reduction in the size is the major response of the cells to drought stress condition. It was shown that mesophyll cells size decreased in olive plants leaves under drought stress condition while the number of mesophyll cells and as a result the number of chloroplasts and CO₂ fixation increased (Culter *et al.* 1977). Water stress decreased the size of the mesophyll cells and their intercellular spaces (Bongi *et al.* 1987; Mediavilla *et al.* 2001). This may help plants to have higher photosynthesis rates. There is no report about the effect of drought stress on barley leaf mesophyll cells size. Environmental stresses, however, were shown to change mesophyll cells dimensions in other crop plants. For example, high temperature stress increased the thickness of palisade and spongy cell layers and lower epidermal cells in soybean leaves. Decreased levels of photosynthesis are shown to be mediated through anatomical changes in soybean leaves under high temperature stress (Djanaguiramaana *et al.* 2011). It has been found in wild and transgenic tobacco plants that

irrespective of genotype or environment, CO₂ transfer conductance varied in proportion to the surface area of chloroplasts exposed to intercellular airspaces (Evans *et al.* 1994). In avocado changes in mesophyll cell structure was shown to be the cause of low photosynthetic rate (Chartzoulakis *et al.* 2002; Chaves and Oliveira 2004).

Changes in the size and shape of the epidermal cells in both winter and spring leaf types can facilitate the penetration of light into the mesophyll cells. Reductions of the epidermal cell size in the winter and spring leaves in cultivar Zarjo and also in the winter leaves of Valfajr are in accordance with the findings of Bosabalidis and

Kofidis (2002) in olive and Jones *et al.* (1980) in ryegrass.

Results obtained from this experiment showed that anatomical changes of the winter and spring leaves are not the same under drought stress condition. Different responses of the two types of leaves were not unexpected since they grow under different environmental conditions. However, in some cases such as xylem vessels diameter responses were considerably different. For example, in cultivars Sahra and Jonob winter leaves had larger xylem vessel diameter under drought stress compared to the normal condition while in spring leaves they had smaller vessels under drought stress condition.

Table 1. Analysis of variance of winter leaf traits of barley varieties under normal and drought stress conditions

SOV	df	Xylem vessel diameter	Phloem sieve tubes diameter	Bundle sheath cells area	Mesophyll cells area	Upper epidermal cells area	Lower epidermal cells area
Genotype	3	171.96*	12.02*	12.20 ^{ns}	1367.25*	1030.85*	293.46 ^{ns}
Water condition	1	654.85**	28.80*	20.96*	2152.37*	2532.22**	1038.54*
Genotype× Water condition	3	236.50*	19.52*	80.38**	2797.98**	1133.48*	1319.86*
Error	28	55.83	4.37	6.94	452.28	320.74	233.27
C.V. (%)		10.2	11.7	4.5	5.9	6.0	4.1

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

Table 2. Analysis of variance of spring leaf traits of barley varieties under normal and drought stress conditions

SOV	df	Xylem vessel diameter	Phloem sieve tubes diameter	Bundle sheath cells area	Mesophyll cells area	Upper epidermal cells area	Lower epidermal cells area
Genotype	3	13.40*	4.89*	6.02 ^{ns}	342.80*	199.96*	87.38*
Water condition	1	18.67*	0.09 ^{ns}	15.79 ^{ns}	181.64 ^{ns}	318.03*	231.04**
Genotype× Water condition	3	26.40**	1.02 ^{ns}	2.18 ^{ns}	462.13**	137.68*	119.49*
Error	28	2.98	1.13	4.91	90.19	45.90	27.17
C.V. (%)		9.8	6.3	2.1	9.0	13.4	14.6

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

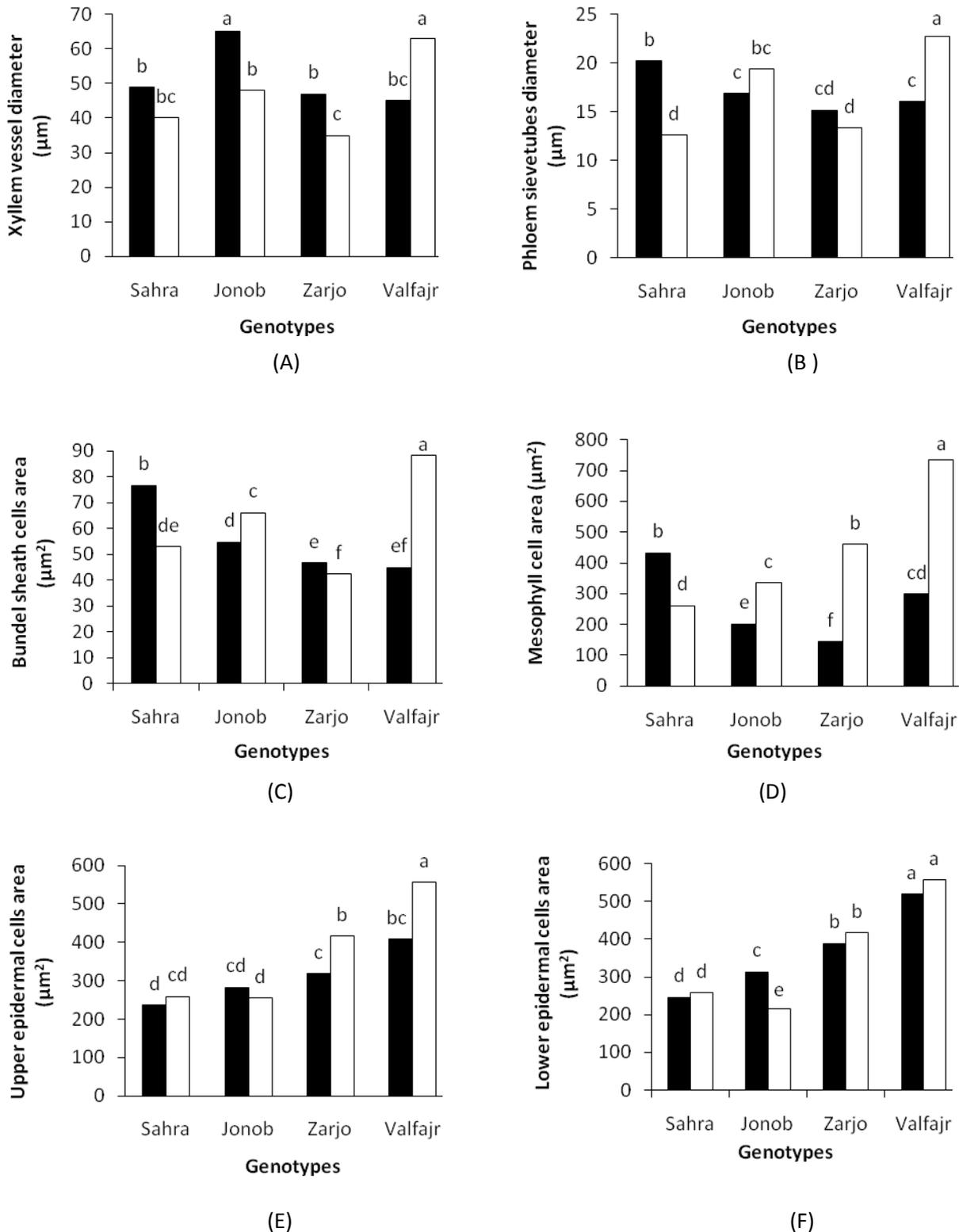


Figure 1. Means of barley genotypes for winter leaf characteristics grown under normal (white) and drought stress (black) conditions; A) Xylem vessel diameter, B) Phloem sieve tubes diameter, C) Bundel sheath cells area, D) Mesophyll cell area, E) Upper epidermal cells area, F) Lower epidermal cells area. Means with different letters are significantly different at 0.05 probability level based on Duncan's multiple range test.

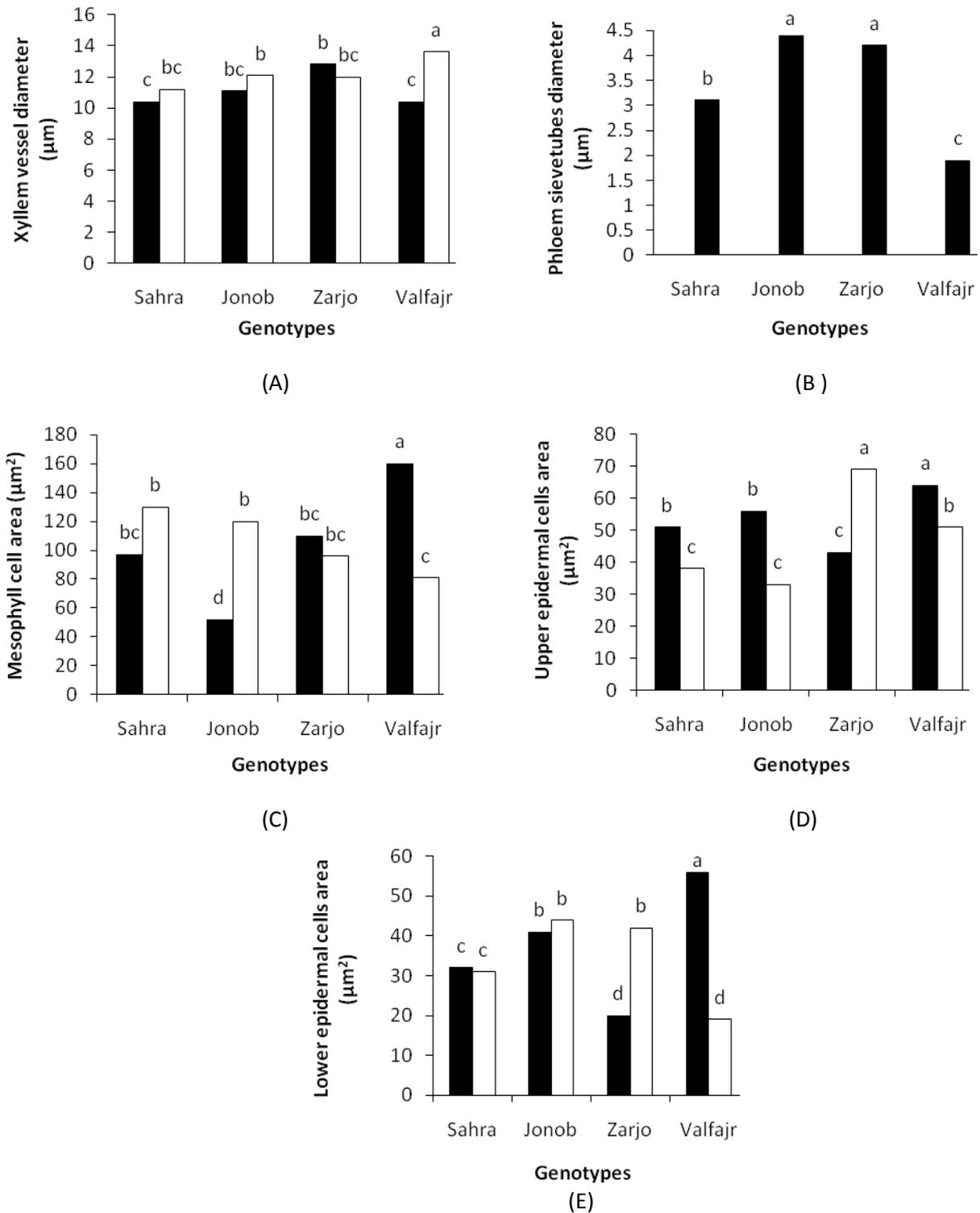


Figure 2. Means of barley genotypes for spring leaf characteristics grown under normal (white) and drought stress (black) conditions; A) Xylem vessel diameter, B) Phloem sieve tubes diameter, C) Mesophyll cell area, D) Upper epidermal cells area, E) Lower epidermal cells area. Means with different letters are significantly different at 0.05 probability level based on Duncan's multiple range test.

References

- Baik BK and Ullrich SE, 2008. Barley for food: characteristics, improvement and renewed interest. *Journal of Cereal Science* 48: 233-242.
- Bohnert HJ and Jensen RG, 1996. Strategies for engineering water stress tolerance in plants. *Trends in Biotechnology* 14: 89-97.
- Bongi G, Mencuccini M and Fontanazza G, 1987. Photosynthesis of olive leaves: effect of light flux density, leaf age, and temperature and H₂O vapor pressure deficit on gas exchange. *Journal of the American Society for Horticultural Science* 112: 143-148.
- Bosabalidis AM and Kofidis G, 2002. Comparative effects of drought stress on leaf anatomy of two olive cultivars. *Plant Science* 163(2): 375-379.
- Chartzoulakis K, Patakas A, Kofidis G, Bosabalidis A and Nastou A, 2002. Water stress affects leaf anatomy, gas exchange, water relations and growth of two avocado cultivars. *Scientia Horticulturae* 95: 39-50.
- Chaves MM and Oliveira MM, 2004. Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. *Journal of Experimental Botany* 55: 2365-2384.
- Cochard H, Nardini A and Coll L, 2004. Hydraulic architecture of leaf blades: where is the main resistance? *Plant, Cell and Environment* 27: 1257-1267.
- Culter JM, Rains DW and Loomis RS, 1977. The importance of cell size in the water relations of plants. *Physiologia Plantarum* 40: 255-260.
- Dias J, Pimenta JA, Medri ME, Boeger MRT and de Freitas CT, 2007. Physiological aspects of sun and shade leaves of *Lithraea molleoides* (Vell.) Engl. (Anacardiaceae). *Brazilian Archives of Biology and Technology* 50(1): 91-99.
- Djanaguiramana M, Prasada PVV, Boyle DL and Schapaugh WT, 2011. High-temperature stress and soybean leaves: leaf anatomy and photosynthesis. *Crop Science* 51: 2125-2531.
- Drake PL, Froend RH and Franks PJ, 2013. Smaller, faster stomata: scaling of stomatal size, rate of response and stomatal conductance. *Journal of Experimental Botany* 64: 495-505.
- Evans JR, Caemmerer SV, Setchell BA and Hudson GS, 1994. The relationship between CO₂ transfer conductance and leaf anatomy in transgenic tobacco with a reduced content of rubisco. *Australian Journal of Plant Physiology* 21(4): 475-495.
- Fitter A and Hay R, 2002. *Environmental Physiology of Plants*. Academic Press. 367 pages.
- Fricke W and Flowers TJ, 1998. Control of leaf cell elongation in barley. Generation rates of osmotic pressure and growth-associated water potential gradients. *Planta* 206: 53-65.
- Guerfel M, Baccouri O, Boujnah D, Chaidi W and Zarrouk M, 2009. Impact 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.
- Hallik L, Niinemets U and Wright IJ, 2009. Are species shade and drought tolerance reflected in leaf-level structural and functional differentiation in Northern Hemisphere temperate woody flora? *New Phytologist* 184: 257-274.
- Harb A, Krishnan A, Madana M, Ambavaram R and Pereira A, 2010. Molecular and physiological analysis of drought stress in *Arabidopsis* reveals early responses leading to acclimation in plant growth. *Journal of Plant Physiology* 154: 1254-1271.
- Hu Y, Fromm J and Schmidhalter U, 2005. Effect of salinity on tissue architecture in expanding wheat leaves. *Planta* 220: 838-848.
- Hu Y, Schnyder H and Schmidhalter U, 2000. Carbohydrate accumulation and partitioning in elongating leaves of wheat in response to saline soil conditions. *Australian Journal of Plant Physiology* 27: 363-370.
- Japp AP and Newman I, 1987. Morphological and anatomical effects of severe drought on the roots of *Lolium perenne* L. *New Phytologist* 105: 393-402.
- Jones MB, Leafe LE and Stiles W, 1980. Water stress in field grown perennial ryegrass. Its effects on leaf water status, stomatal resistance and leaf morphology. *Annals of Applied Biology* 96: 103-110.
- Kazemi Arbat H, 2005. *Morphology and Anatomy of Cereal Crops*. Volume 2. University of Tabriz Press, Tabriz, Iran (In Persian).
- Kiseleva LS and Kaminskaya DA, 2002. Hormonal regulation of assimilate utilization in barley leaves in relation to the development of their source function. *Russian Journal of Plant Physiology* 49(4): 535-540.

- Kreck M, Slamka P, Olsovska K, Brestic M and Bencikova M, 2008. Reduction of drought stress effect in spring barley (*Hordeum vulgare* L.) by nitrogen fertilization. *Plant, Soil and Environment* 54(1): 7-13.
- Lee KW, Choi GJ, Kim KY, Ji HC, Zaman R and Lee SH, 2011. Identification of drought induced differentially expressed genes in barley leaves using the annealing control-primer-based GeneFishing technique. *Australian Journal of Crop Science* 5(11): 1364-1369.
- Mansoor U, Ashraf M and Rao AR, 2002. Variation in leaf anatomy in wheat germplasm from varying drought-hit habitats. *International Journal of Agriculture and Biology* 4: 12-16.
- McClendon JH, 1992. Photographic survey of the occurrence of bundle sheath extension in deciduous dicots. *Plant Physiology* 99: 1677-1679.
- McKim HL, Walsh JE and Arion DN, 1980. Review of Techniques for Measuring Soil Moisture in Situ. United States Army Corps of Engineers. Cold Regions Research and Engineering Laboratory, Hanover, New Hampshire, USA. Report 80-31.
- Mediavilla S, Escudero A and Heilmeyer H, 2001. Internal leaf anatomy and photosynthetic resource-use efficiency: interspecific and intraspecific comparisons. *Tree Physiology* 21: 251-259.
- Minchin PEH, Thorpe MR, Farrar JE and Koroleva OA, 2002. Source-sink coupling in young barley plants and control of phloem loading. *Journal of Experimental Botany* 53(374): 1671-1676.
- Niinemets U and Sack L, 2006. Structural determinants of leaf light harvesting capacity and photosynthetic potentials. In: Esser K, Luttge UE, Beyschlag W and Murata J (Eds). *Progress in Botany*, Vol. 67. Pp. 385-419. Springer-Verlag, Berlin.
- Payvandi S, Daly KR, Joes BL, Talboys P and Roose T, 2014. A mathematical model of water and nutrient transport in xylem vessels of a wheat plant. *Bulletin of Mathematical Biology* 76: 566-596.
- Press MC, 1999. The functional significance of leaf structure: a search for generalizations. *New Phytologist* 143: 213-219.
- Rebetzke GJ, Condon AG and Richards RA, 2010. Genomic regions for canopy temperature and their genetic association with stomatal conductance and grain yield in bread wheat (*Triticum aestivum* L.). *Functional Plant Biology* 8: 150-154.
- Reddy AR, Chiatanya KV and Vivekanandan M, 2004. Drought induced responses of photosynthesis and antioxidant metabolism in higher plants. *Journal of Plant Physiology* 161: 1189-1202.
- Syvetsen JP, Lloyd J, Mcconchie C, Kriedemann PE and Farquhar GD, 1995. On the relationship between leaf anatomy and CO₂ diffusion through the mesophyll of hypostomatous leaves. *Plant, Cell & Environment* 18: 149-157.
- Talame V, Ozturk NZ, Bohnert HJ and Tuberosa R, 2007. Barley transcript profiles under dehydration shock and drought stress treatments: a comparative analysis. *Journal of Experimental Botany* 58(2): 229-240.
- Vasanth S, Alarmelu S, Hemaprabha G and Shanthi RM, 2005. Evaluation of promising sugarcane genotypes for drought. *Journal of Sugar Technology* 7(2): 82-83.
- Verma V, Foulkes MJ, Worland AJ, Sylvester-Bradley R, Caligari PDS and Snape JW, 2004. Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought-stressed environments. *Euphytica* 135: 255-263.
- Zagdanska B and Kozdoj J, 1994. Water stress-induced changes in morphology and anatomy of flag leaf of spring wheat. *Acta Societatis Botanicorum Poloniae* 63(1): 61-66.
- Zagdanska B and Wisniewski K, 1996. Endoproteinase activities in wheat leaves upon water deficit. *Acta Biochimica Polonica* 43(3): 515-520.