Journal of Plant Physiology and Breeding
2018, 8(1): 57-73 ISSN: 2008-5168

Effects of drought stress on some physiological variables and grain yield of different wheat varieties

Mahdiye Askary[1*], Aliakbar Maghsoudi Moud[2], Vahid Reza Saffari[2] and Afsoun Askari[3]

Received: February 15, 2016 Accepted: June 14, 2017
[1]PhD student of Agronomy and Plant Breeding, University of Birjand, Birjand, Iran and former MSc student, Shahid Bahounar University, Kerman, Iran
[2]Associate Professor of Agronomy and Plant Breeding, Shahid Bahounar University of Kerman, Kerman, Iran
[3]Former MSc student of Agronomy and Plant Breeding, University of Birjand, Birjand, Iran
*****Corresponding author; Email: Mahdiye.askary@yahoo.com

Abstract
This study was conducted to determine the effect of drought stress on yield and some physiological characteristics of wheat cultivars. Six cultivars were grown under normal and drought stress in greenhouses and field conditions. Leaf samples were taken for physiological measurement including relative water content, transpiration rate, membrane stability, chlorophyll content and chlorophyll fluorescence parameters and stomatal frequency and length. Grain yield was determined for plants grown under field condition. Results showed that treatments have a significant impact on plant traits. Drought stress decreased leaf chlorophyll content and photochemical efficiency of PSII due to increasing F0 and decreasing Fm and increased ion leakage. Drought stress also decreased grain yield and the highest yield was obtained in plots with normal condition. Cultivars Alvand and Chamran showed the highest level of photochemical efficiency of PSII, membrane stability and grain yield under drought stress and were considered as the more tolerant cultivars to drought stress than other cultivars under conditions of this investigation.

Keywords: Chlorophyll fluorescence; Drought stress; Photosystem II efficiency; Wheat; Yield

Citation: Askary M, Maghsoudi Moud A, Saffari VR and Askari A, 2018. Effects of drought stress on some physiological variables and grain yield of different wheat varieties. Journal of Plant Physiology and Breeding 8(1): 57-73.

Introduction

The global human population is expected to grow from a current 6 to 9 billion people by the year 2050 (United Nations Population Division 2000). Presently, more than 10% of the population of the world is undernourished while global production of cereal crops is regarded as the most important food crops (FAO 2012). Universally, wheat, rice and maize account for 58% of the global area of annual crops and provide about 50% of food calories (Fischer *et al*. 2009).

Wheat is the most important cereal in the human diet worldwide (Richards 2000). However, concerns have increased over the past decade regarding that wheat production was almost stopped rising in farmers' fields (Lin and Huybers 2012). Many factors have been cited as explanations for the slowdown in cereals yield. Among these factors, soil and atmospheric water deficit restrict growth through photosynthesis decline. In particular, the arid and semi-arid areas are subjected to variability in soil moisture dynamics, especially during the summer period, characterized by low air humidity, high solar radiation and a high rate of evapotranspiration (Galmés *et al*. 2007).

The occurrence of morphological and physiological responses, which may lead to some

adaptation to drought stress, may vary considerably among species. In general, strategies of drought avoidance or drought tolerance can be recognized, both involving diverse mechanisms that provide the plants ability to respond and survive in drought condition (Levitt 1980). The photosynthetic apparatus, especially photosystem II (PSII), may be temporarily affected by environmental stresses before an irreversible morphological damage is observed (Flexas and Medrano 2002). This early detection of stress could identify the physiological condition of plants at larger spatial and temporal scales before visible effects are apparent (Zarco-Tejada *et al.* 2002). Non-invasive remote sensing techniques, such as chlorophyll fluorescence and plant reflection, are being developed to monitor plant stress and photosynthetic status and also to detect and predict changes in the natural environment (Baker and Rosenqvist 2004). Stressed plants use less radiant energy for photosynthesis and have evolved numerous mechanisms that safely dissipate excess light to avoid photo inhibition (Flexas and Medrano 2002). On the whole, the excited chlorophyll molecule is subjected to various competing de-excitation reactions including photosynthesis, heat loss and chlorophyll fluorescence which has been successfully used as a non-invasive method to detect plant stress (Baker and Rosenqvist 2004). Stomatal closure may lead to increased susceptibility to photo-damage (Powles 1984). Since the chlorophyll fluorescence can give insights into the ability of a plant to tolerate environmental stresses and the extent to which those stresses have damaged the photosynthetic apparatus (Maxwell and Johnson 2000), it can be an excellent tool to study stress-induced changes in PSII (Naumann *et al.* 2008), which is believed to play a key role in the response of leaf photosynthesis to environmental stresses (Baker 2004). However, in this strategy, wheat by involving stomatal closure may lead to decrease in CO_2 assimilation and hence in growth and yield (Chaves 1991). Stomatal regulation of photosynthesis during drought stress has been well documented (Chaves 1991). At least under mild drought conditions, it has been shown that stomata play the dominant role in controlling the decline of net CO_2 uptake, by leading to decrease in leaf internal CO_2 concentration (Cornic 2000). Nonetheless, the limitations to CO_2 assimilation imposed by stomatal closure may promote an imbalance between photochemical activity at PSII and electron requirement for photosynthesis, leading to an over excitation and subsequent photo-inhibitory damage of PSII reaction centers (Krause 1991). Currently, chlorophyll fluorescence is widely employed as a rapid, efficient and non-invasive tool for detecting functional changes of photosynthetic apparatus under abiotic or biotic stresses, such as temperature stress in maize (Sowinski 2005) and salinity in barley (Belkhodja 1994). The ratio of Fv/Fm (variable fluorescence/maximum fluorescence) gives an estimate of the maximum quantum efficiency of PSII photochemistry (Dai 2007), which has been widely used to detect stress-induced perturbation in the photosynthetic apparatus (Rizza 2001).

The negative effect of drought on photosynthesis is well-documented which decreases carbon assimilation progressively due to increasing water deficit as a result of stomatal closure (Cornic 2000; Flexas and Medrano 2002). Under drought stress conditions, the possibility of over-excitation of PSII increases, which reduces the photosynthetic rate leading to an increase in the dissipation of absorbed energy through non-radiative processes (Baker 2008). Since the

capacity for photo-protection is limited, certain conditions can lead to damage and loss of active PSII reaction centers. This means that any small change in the values of fluorescence will provide information about a reduction in controlled emission of energy and concomitantly about an unfavorable situation for the plant (Demmig-Adams *et al.* 1992). Therefore, non-invasive measurement of photosynthesis by chlorophyll a fluorometry may potentially provide a means to determine plant viability and performance in response to drought (Woo *et al.* 2008).

The plant plasma membrane plays an important role in the growth and development of plants and controls many cellular processes in plants, such as secondary active transport, cell PH and turgor (Chen *et al*. 2012). Recent studies have shown that membrane stability is very sensitive to abiotic stress, such as drought stress (Chen *et al.* 2012). Therefore, environmental stress can cause damage to plant cells; the membrane system is particularly sensitive to such environmental factors.

The aim of this study was to investigate the physiological behavior of wheat under drought stress conditions. For this purpose, we measured chlorophyll fluorescence, chlorophyll content, relative water content, stomatal conductance, membrane stability, ion leakage, transpiration rate and economic yield. To this end, the choice of a measurable parameter as an indicator of the entire wheat yield under drought stress is important to provide a technique for selection of cultivars with different drought tolerances.

Materials and Methods

Two separate experiments were conducted under controlled and field conditions in the Faculty of Agriculture of Shahid Bahounar University of Kerman, Kerman, Iran.

Experiment A

This experiment was conducted in the greenhouse. Mean daily maximum temperature, mean daily minimum temperature and relative humidity of the air were 30±2 ◦C, 18±2 ◦C and 40%, respectively. The experiment was carried out as factorial based on completely randomized design with three replications in 2011. Experimental factors were: water at two levels (100% of field capacity as the control and 60% of field capacity) and six cultivars including Alvand, Chamran, Omid, Roshan, Pishtaz and Gaspard. Seeds were surface sterilized and germinated on wet tissue papers at 27 ◦C for two days. Seedlings were then transferred to 3-L pots filled with 1800 g washed sand. Soil surface of the pots was covered with a layer of perlite in order to prevent evaporation. During the growth period pots were weighted every day to the nearest gram and irrigated up to their initial weight to maintain the constant growing condition. Seedlings were subsequently thinned to one per pot. In order to avoid nutrient deficiency, each pot was fertilized with Hoagland nutrient solution. Two weeks after the plant establishment, portions of similar size from each plant were sampled and following variables were measured.

Chlorophyll fluorescence

Chlorophyll fluorescence was measured between 9:30 a.m. and 11:00 a.m. using a pulse amplitude modulation fluorimeter (Junior PAM, Walz, Germany). Leaves selected previously for measurement of stomatal length were used for fluorescence measurements. In general, minimal fluorescence, F0, was measured on 30-min dark-

adapted leaves using weak modulated light (approximately 6 mol m^{-2} s^{-1} at 660 nm), and maximal fluorescence, Fm, was measured after 0.8 s saturating white light pulse (10000 mol m^{-2} s^{-1}) on the same leaves. Maximal variable fluorescence (Fv= Fm–F0) and the photochemical efficiency of PSII (Fv/Fm= (Fm−F0)/Fm)) for dark adapted leaves were calculated. Effective quantum yield of photochemical energy conversion in PSII, PSII= (Fm−Fs)/Fv was also calculated, where Fs represents fluorescence yield (Genty *et al*. 1989).

Leaf pigments (Chl a, Chl b)

0.25g of each sample was extracted by 80% acetone and put in the freezer at −5 ˚C for 24 h. For each replicate (plant), chlorophyll content was the average of five measurements on the same leaf. Pigments were determined according to Lichthenthaler (1987).

Relative water content (RWC)

0.1 g from each plant's leaves (FW) was measured using a digital scale. Then, each sample was placed inside a Petri dish and after adding 10 ml of distilled water, was placed in the dark for 24 hours to be completely saturated. In following, the saturated weight (SW) of the samples was measured after removing from the distilled water. Next, samples were dried in an oven at 75 ˚C for 24 hours and were weighed (DW). Leaf relative water content was calculated from the following equation (Gonzalez 2003):

$$\mathrm{RWC} = \frac{(\mathrm{FW}-\mathrm{DW})}{(\mathrm{SW}-\mathrm{DW})} \times 100$$

Stomatal length

A thin layer of transparent nail polish that was diluted with acetone was drawn on the surface of the leaf. After drying the nail polish, a thin layer of the leaf which was covered by the transparent nail polish was taken with banderole and was put on the slides for observing with microscope. This process led to the leaf epidermis image to be seen under the microscope. The prepared slides were observed with an optical microscope, magnification x10 × x40. To measure the length of the stomata a micrometer eyepiece was used and the size of stomata in the 0.234 mm^2 of the leaf area was measured.

Transpiration rate

Leaf samples were selected from the same plants and immediately placed into a digital scale to the nearest gram and a camera recorded the consequent reduction of leaf weight for three minutes while a time recorder was active on it. It was assumed that the weight loss of leaves was due to transpiration during this time. The data obtained were arranged in a file in Microsoft Excel and then linear charts were drawn between time and weight loss of the leaves for all three replications and hybrids. Finally, the data obtained from the diagrams were analyzed according to the factorial experiment used in this study.

Percentage of ionic leakage

The method of Hu *et al.* (2009) was used for measuring ion leakage. For this purpose, 0.2 gr of healthy and fresh aerial parts of the plants, after washing the potential ions from their surface, was put into a test tube with lid and 10 ml of distilled water was added. The test tubes were placed in a hot water bath at a temperature of 32 ˚C for 2 hours and then, the electrical conductivity of the sample (EC_1) was measured using an EC meter. Then, test tubes were autoclaved for 20 minutes and after cooling the pipes to the temperature of 25 ˚C, the

maximum electrical conductivity of samples (Ec_2) was measured and the percentage of ionic leakage was obtained from the following formula:

$$\frac{Ec1}{Ec2} \times 100$$

Stability of the membrane

From each experimental unit a leaf in the same position was separated and washed thoroughly with distilled water and dried by drying paper. Then, circular pieces were cut by punch and immediately were kept in a lidded tube containing 5 ml of distilled water. Electrical conductivity meter was adjusted for two hours to record and store every three minutes the electrical conductivity of the solution. The gained data were then regressed on the relevant time period. Finally, all data which were obtained from the graphs were analyzed according to the experimental design utilized in this investigation. It is assumed that an increase in the electrical conductivity of the solution was due to the continuous ion leakage from the leaf samples and the amount of leakage reflected the membrane stability against ion leakage (Maghsoudi Mud, 2008).

Experiment B

The same genotypes were sown in a field (latitude 30° N, longitude 57 °E and altitude 1754 m above sea level) using a split plot design based on randomized complete block design with three replications. Water levels (60 and 100% of the field capacity) were arranged in the main plots and six wheat cultivars in the subplots. There were four rows in each subplot and inter- and intra-row spacing was 75 and 25 cm, respectively. Before preparing the seed bed, soil samples of the test location were taken from the 0-120 cm depth and several physical and chemical properties were measured (Table 1).

Grain yield

After removing the corn kernels from the cobs, they were placed in envelopes and dried at 75 °C in in the oven. Then, the grain weight was measured and converted to kilograms per hectare.

Table 1. Physical and Chemical properties of the field soil (0-120 cm depth).

Depth (cm)	Clay (%)	Silt (%)	Sand (%)	Texture	pH	EC ($ds.m^{-1}$)
0-30	18	33.6	48.4	Loam	9.1	4.64
30-60	18	45.6	36.4	Loam	8.7	8.75
60-90	19	18.6	62.4	Sandy loam	8.1	5.27
90-120	18	29.6	53.4	Sandy loam	8.43	4.83

Statistical analysis

After analysis of variance, means were compared using LSD test at 5% probability level. The SAS (V9.1) and Excel software were used to analyze the data and draw the figures.

Results

Leaf chlorophyll fluorescence

The effects of drought stress and cultivar were significant for F0, Fm and Fv/Fm at both stem and spike stages. At the spike stage, the interaction of

water level × cultivar was significant for F0 but was not significant for Fm and Fv /Fm. However, at the stem stage the water level × cultivar interaction was significant for F0, Fm and Fv/Fm (Table 2). The amount of F0 was higher under drought stress than the normal irrigation condition at both stages (Figure 1). The highest F0 belonged to Roshan cultivar at both stages (Figure 2). Fm was significantly lower under drought stress condition at the spike stage but not at the stem stage (Figure 1). At the stem stage, the highest Fm was obtained from the Omid cultivar along with Alvand, Roshan and Chamran cultivars (Figure 2). Omid had also the highest Fm at the spike stage but was not significantly different from Roshan, Chamran and Pishtaz cultivars (Figure 2). Fv/Fm decreased significantly under drought stress at both stages (Figure 1). Alvand showed the highest Fv/Fm at the spike stage. Alvand had also the highest Fv/Fm at the stem stage but was not significantly different from Roshan and Chamran cultivars (Figure 2). At both stem and spike stages, the highest and lowest PSII were obtained from the normal and drought stress conditions, respectively (Figure 1). Furthermore, Alvand showed the highest PSII at both the stem and spike stages, but its value was not significantly different from Omid and Roshan cultivars at the stem stage (Figure 2). PSII estimates directly the efficiency of light use for electron transport. F_V/F_m and F_m showed very high and significant positive correlation (0.92^{**} and 0.90^{**}, respectively) with grain yield (Table 6).

Leaf pigments

Analysis of variance for Chl a and Chl b showed that the main effects of water level and cultivar and also water level × cultivar interaction were significant (Table 2). There was a significant difference between the normal and drought stress conditions for Chl a and Chl b and their value decreased by reducing the moisture at the drought stress condition (Figure 3, left). The highest Chl a was obtained from Chamran cultivar but it was not significantly different from Roshan (Figure 3, right). Chamran had also the highest Chl b, but it was not significantly different from the cultivars of Roshan and Omid (Figure 3, right). Table 6 shows the correlation coefficients among the traits under study. There was a significant positive correlation (0.54^{*}) between chlorophyll b and grain yield but the correlation coefficient of chlorophyll a with grain yield (0.48) was not significant.

Carotenoid content increased significantly under drought stress condition (Figure 3, left). Among cultivars, Pishtaz and Gaspard showed the highest and the lowest carotenoid content, respectively. However, the value for Pishtaz was not significantly different from those of Roshan and Chamran (Figure 3, right).

Relative water content

The results showed that drought stress had a significant effect on RWC of the wheat plant (Table 2). Drought stress reduced RWC about 13% as compared to the normal condition (Table 3). Although no statistically significant difference was observed between cultivars, but the highest RWC was obtained for Chamran (Table 3). RWC of the leaf is one of the indicators to measure the tolerance of plants to drought stress. The correlation coefficient of RWC and grain yield was positive and significant (0.81^{**}) (Table 7).

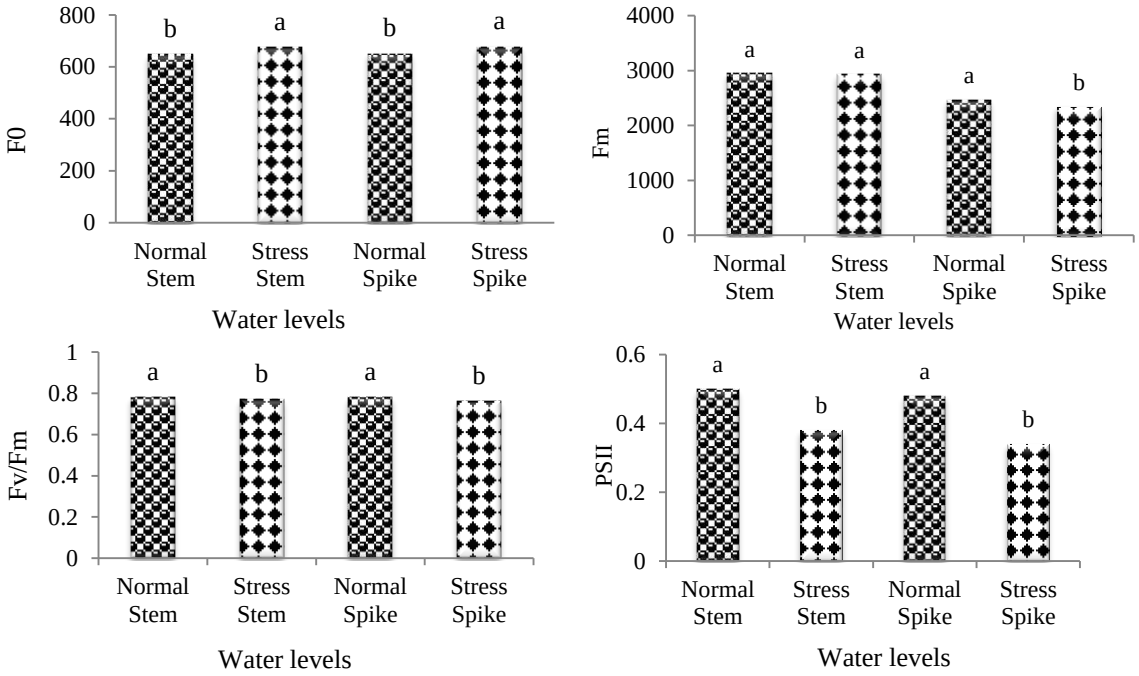

Figure 1. Mean values of F0 (minimal fluorescence of dark-adapted leaves), Fm (maximum fluorescence of dark-adapted leaves), Fv/Fm (maximum quantum efficiency of photosystem II) and PSII at different water levels. Means that have the same letters within each stage are not significantly different at 5% level of probability based on LSD test.

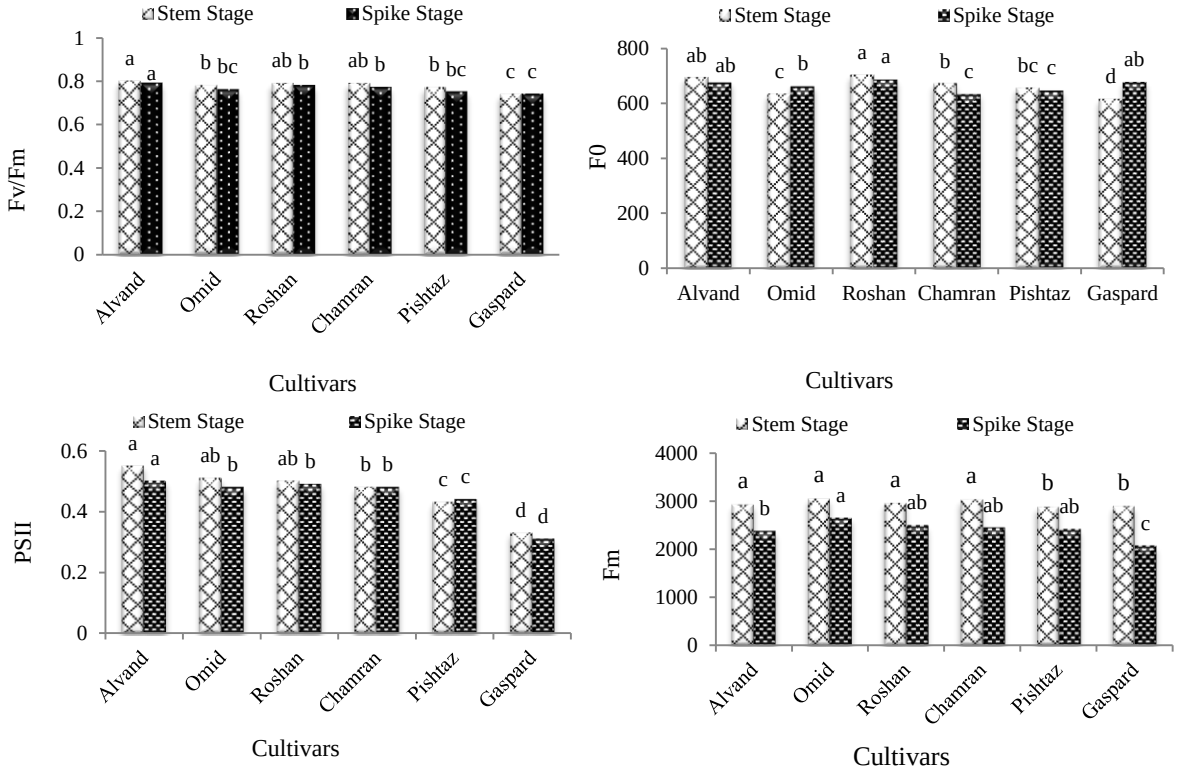

Figure 2. Mean values of F0 (minimal fluorescence of dark-adapted leaves), Fm (maximum fluorescence of dark-adapted leaves), Fv/Fm (maximum quantum efficiency of photosystem II) and PSII in wheat cultivars. Means that have the same letters within each stage are not significantly different at 5% level of probability based on LSD test.

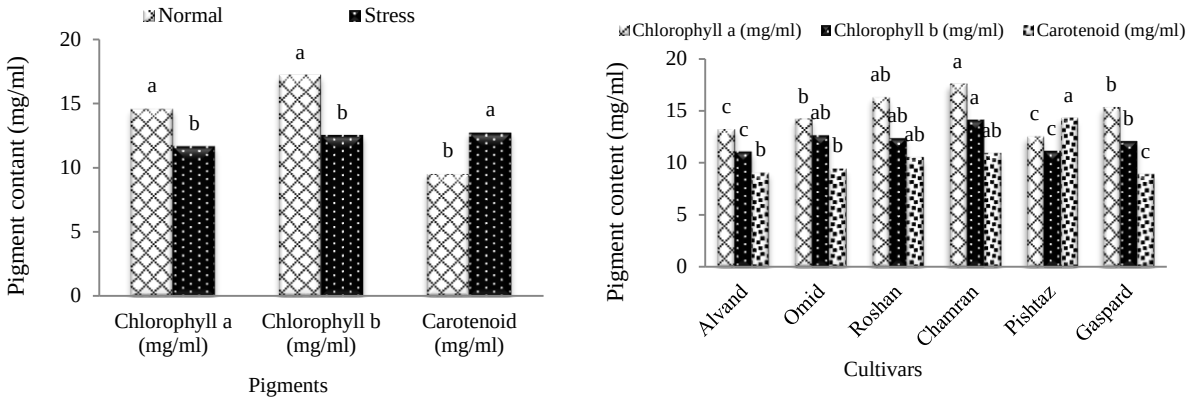

Figure 3. Pigment content of wheat cultivars (right) and different water levels (left). Means that have the same letters within each trait are not significantly different at 5% level of probability based on LSD test.

Stomatal length

The results indicated the significant effect of drought stress and cultivar on stomatal length (Table 2). However, the interaction of water level × cultivar was not significant for this characteristic (Table 2). Stomatal length decreased under drought stress at both abaxial and adaxial surfaces. Among cultivars, Gaspard acquired the lowest stomatal length at both surfaces. The highest stomatal length on the adaxial surface belonged to Chamran and Alvand, however, on the abaxial surface, the highest stomatal length was observed for Alvand and Omid cultivars (Table 3). The correlation of stomatal length with grain yield was significant at 5% probability level (0.58* and 0.51* for adaxial and abaxial surfaces, respectively).

Table 2. Analysis of variance of the effect of drought stress and cultivar on wheat traits under study.

SOV	df	Chl a	Chl b	Carotenoid	Transpiration $\times 10^5$	Stomata length (adaxial)	Stomata length (abaxial)	Ion leakage	Membrane stability (60 min)	RWC
Water level (W)	1	47.9**	37.2**	5.28**	0.008ns	70.1**	31.7**	93.9*	102.4*	1656.0**
Cultivar (C)	5	43.3**	49.4**	2.88**	140**	23.5**	52.37**	600.8**	592.0**	101.1ns
W × C	5	59.1**	20.3**	0.09 ns	0.013*	1.1ns	0.9ns	1.9ns	2.15ns	33.3*
Error	36	21.2	16.6	0.27	0.053	5.86	3.2	17.0	17.7	69.1
CV (%)		12.9	13.1	9.3	1.02	18.9	12.8	6.0	13.6	10.0

		Spike stage			Stem stage		
SOV	df	F0	Fm	Fv/Fm	F0	Fm	Fv/Fm
Water level (W)	1	813.0**	190386.0**	0.002*	7828.5**	2883.0**	0.001*
Cultivar (C)	5	3287.5**	290616.0**	0.005**	9305.1**	72833.2**	0.001**
W × C	5	1905.0**	16577.0ns	0.0004ns	7351.7**	170625.4*	0.006**
Error	36	256.0	24150.7	0.0007	3097.5	6008.3	0.0006
CV (%)		2.4	6.5	3.4	8.4	8.4	3.2

*and**: significant at 5% and 1% probability levels, respectively, ns: not Significant; RWC: relative water content; F0: minimal fluorescence of dark-adapted leaves; Fm: maximum fluorescence of dark-adapted leaves; Fv/Fm: maximum quantum efficiency of photosystem II; For membrane stability, data were not shown for other time points because the results were similar.

Table 3. Means of stomatal length and relative water content (RWC) for normal and drought stress conditions and wheat cultivars.

Water levels	Stomata length adaxial	Stomata length abaxial	RWC
Normal	14.04a	14.91a	89.22a
Drought Stress	11.62b	13.29b	77.47b
Cultivars			
Alvand	14.00a	17.78a	85.63a
Omid	13.00ab	16.25a	79.29a
Roshan	13.37ab	13.87b	84.21a
Chamran	15.00a	13.62b	88.34a
Pishtaz	11.25bc	11.62c	83.23a
Gaspard	10.50c	11.37c	79.37a

In each column and for each factor, means that have the same letters are not significantly different at 5% level of probability based on LSD test.

Transpiration rate

Analysis of variance showed the significant effect of cultivar and the interaction of water level × cultivar (Table 2). The main effect of drought stress was not significant on transpiration rate. Although the interaction term was significant, but Chamran and Roshan showed the highest and the lowest transpiration rates at both conditions (0.093 and 0.056 mm^{-3} s^{-1}, respectively) (Figure 4). There was a highly significant positive correlation (0.89**) between grain yield and transpiration rate (Table 7).

Ion leakage

Effect of drought stress and cultivar on ion leakage was significant, however, the interaction of water level × cultivar was not significant (Table 2). The reduction of moisture level from normal (100% of field capacity) to drought stress (60% of field capacity) increased ion leakage significantly. The ion leakage of Gaspard was significantly higher than other cultivars. The lowest amounts of ion leakage were obtained for Alvand, Omid and Chamran cultivars (Figure 5).

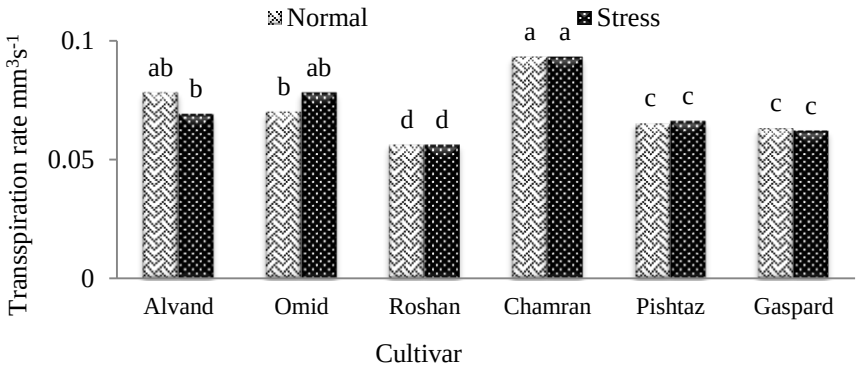

Figure 4. Mean transpiration rate (mm^3s^{-1}) of wheat cultivars at different levels of water. Means that have the same letters are not significantly different at 5% level of probability based on LSD test.

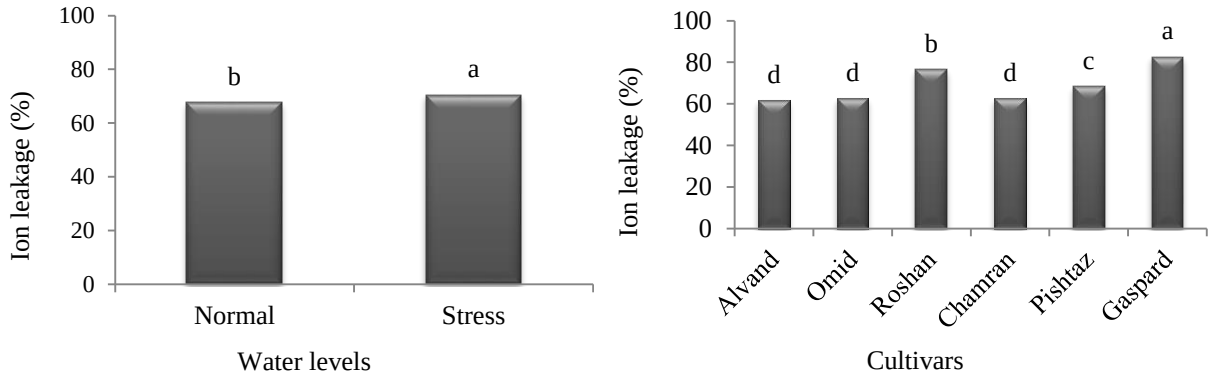

Figure 5. Ion leakage of wheat cultivars (right) and different water levels (left). Means that have the same letters are not significantly different at 5% level of probability based on LSD test.

Membrane stability

Although the main effects of drought stress and cultivar on membrane stability at the 60 min time point were significant at 5% and 1% probability levels, respectively, the interaction of water level × cultivar was not significant (Table 2; data were not shown for other time points due to similarity of the results). Comparison of the means between drought stress and normal conditions showed a significant difference between these two water levels at all three times (15 min, 60 min, 120 min). By reducing the amount of soil moisture from 100% of field capacity to 60% of field capacity, the membrane stability decreased at all three times (Figure 6, left). On the whole, the highest and lowest membrane stability were obtained from the time points of 15 minutes and 120 minutes, respectively (Figure 6, left). Therefore, with the passage of time which increased the effect of drought stress, membrane stability decreased.

A statistically significant difference was observed among cultivars at all three times (Figure 6, right). At 15 min, the highest membrane stability was obtained from Alvand and its value was significantly different from other cultivars, except Chamran. After 60 minutes, Alvand, Omid and Chamran had the highest membrane stability but their values were not significantly different from Roshan. At the 120th minute, the membrane stability of Alvand was significantly higher than other cultivars. On the other hand, Gaspard and Roshan showed the lowest amount of membrane stability (Figure 6, right). Furthermore, as seen from Table 6, although the correlation coefficient of membrane stability with grain yield was positive at all three times (0.19 for 15 min; 0.41 for 60 min; 0.63** for 120 min), this correlation was only significant for the longest time point (120 min). Therefore, the results showed that as the time gets longer, the effect of drought stress on cell membrane stability increased, and have resulted in a significant correlation coefficient between membrane stability and grain yield.

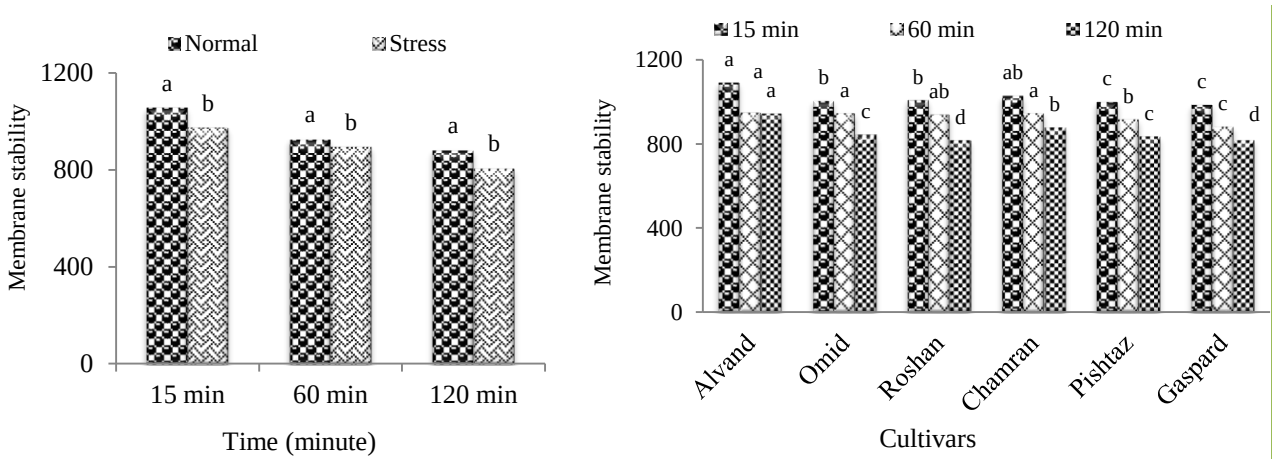

Figure 6. Membrane stability of wheat cultivars (right) and different water levels (left) at three time points. Means that have the same letters at each time point are not significantly different at 5% level of probability based on LSD test.

Table 4. Analysis of variance of grain yield for water levels and wheat cultivars.

SOV	df	Mean squares
Replication	2	13214.9ns
Drought stress	1	213195.5**
Ea	2	335.1
Cultivar	5	294534.9**
Drought stress × Cultivar	5	3221.5ns
Eb	20	4473.6
CV (%)		10.2

**Significant at 1% probability level; ns: not significant

Table 5. Grain yield for normal and drought stress conditions and wheat cultivars.

Water levels	Grain yield ($Kg.ha^{-1}$)
Normal	734.28a
Stress	580.37b
Alvand	1025.78a
Omid	753.96b
Roshan	674.22b
Chamran	571.81c
Pishtaz	543.21c
Gaspard	374.97d

For each factor, means with different letters are significantly different at 5% probability level based on LSD test.

Grain yield

The results indicated the significant effect of drought stress and cultivar at 1% probability level on grain yield (Table 4), but the interaction of water level × cultivar was not significant. Comparison of the means showed that the decrease of moisture level has led to the reduction of grain yield (Table 5). Reduction of grain yield from normal to water stress condition was about 21% (Table 5). Among cultivars, the highest grain yield was obtained for Alvand which was significantly different from other cultivars (Table 5).

Discussion

In this investigation drought stress increased F0 and decreased fluorescence chlorophyll, Fm and Fv/Fm ratio. The reduction in chlorophyll a, b content indicates possible changes in PSII as the consequence of an adaptive mechanism in chloroplasts due to exposure to stress. The increase in F0 and the decrease in Fv/Fm values may be the result of damage to the PSII reaction center which reduces the ability to transfer energy from the LHCII antenna complex to the reaction center (Ouzounidou 1993). The decrease in Fm and also fluorescence chlorophyll under stress may be due to two reasons. First, by inhibition of electron transport at the donor side of the PSII which results in the accumulation of P680 (Govindjee 1995). Second, due to the decrease in the pool size of quinone A (QA). Area over the fluorescence induction curve between F0 and Fm is proportional to the pool size of the electron acceptor QA on the reducing side of PSII. Fv/Fm has been used extensively as a method of early stress detection (Baker and Rosenqvist 2004).

Drought stress decreased stomatal length in our study. These results agree with the previous studies indicating that stomata play central role in plant physiology, because they can adapt to environmental changes (Cornic 2000). Everad *et al.* (1994) reported that under drought stress there was a significant correlation between grain yield and stomata size.

Drought stress decreased membrane stability but increased ion leakage. These results indicate that drought stress has an adverse effect on the stability of the membrane and the cells have consequently higher ion leakage due to damage to their membranes.

Drought stress reduced RWC in the wheat cultivars under study. RWC is considered as an alternative measurement of plant water status and drought resistance of the plant is related to its ability to maintain high RWC in the leaves under stress condition (Nautiyal *et al*. 2002; Faraloni *et al*. 2011). A reasonable assumption might be that the PSII efficiency is related to the leaf water status. The cultivars that maintain high RWC under stress, sustain a high Fv/Fm ratio, and experience a lower injury to PSII performance. In most species, photosynthesis becomes irreversibly depressed when leaf RWC falls to around 60-70% (Lawlor and Cornic 2002). The ability to maintain high leaf water status under drought stress conditions is considered as a parameter to evaluate the dehydration tolerance level (Hoekstra *et al*. 2001). Quisenberry and Ritz (1987) have reported a correlation between leaf water content of wheat and grain yield and stated that cultivars which retain water in their tissues have higher tolerance to stress, and consequently have higher grain yield.

The useful utilization of chlorophyll fluorescence in testing the tolerance to dehydration by means of measurements carried out on detached leaves, was reported for woody perennials (Percival and Sheriffs 2002). Measurement of fluorescence parameters under drought stress was focused on the survival characteristics of the plants in this investigation, thus it provides an effective method to study the effect of environmental stress on plants' performance.

Conclusion

The main goal of this investigation was to find out whether the use of chlorophyll fluorescence measurements carried out on dehydrated detached leaves in the greenhouse could be used as a valid tool for the rapid screening of wheat plants tolerant to drought stress. Drought stress showed reduction of Fv/Fm at central leaf areas surrounding vascular bundles, especially of young leaves. In this study, RWC and stomatal size decreased under drought stress which consequently reduced the grain yield because there was a significant and positive correlation of these traits with grain yield. Gaspard had the lowest RWC, stomatal length and grain yield in this study. On the other hand, Alvand and Chamran cultivars showed the highest RWC, photochemical efficiency of photosystem II (Fv/ Fm) as well as the lowest ion leakage, the most stable membrane and the highest grain yield among the cultivars under study which can be regarded as tolerant cultivars to drought stress under environmental conditions of this investigation.

Table 6. Correlation coefficients among membrane stability index (MSI) at three time points, chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoid, F_0, F_m, Fv/Fm and grain yield (GY) of wheat cultivars.

Traits	MSI15 min	MSI60 min	MSI120 min	Chl a	Chl b	Carotenoid	F0	Fm	Fv/Fm	GY
MSI15min	-									
MSI60 min	0.36ns	-								
MSI120 min	0.44ns	0.55*	-							
Chl a	0.15ns	-0.42ns	0.46ns	-						
Chl b	-0.41ns	-0.41ns	-0.65**	0.44ns	-					
Carotenoid	0.36ns	0.56*	0.61**	-0.56*	0.49ns	-				
F0	-0.41ns	-0.41ns	-0.43ns	-0.64**	-0.76**	-0.63**	-			
Fm	0.19ns	0.15	0.68**	0.41ns	0.56*	0.62**	-0.24ns	-		
Fv/Fm	0.46ns	0.51*	0.67**	0.55*	0.78**	0.92**	0.38ns	0.54*	-	
GY	0.29ns	0.41ns	0.63**	0.48ns	0.54*	31/0-ns	-0.41ns	0.90**	0.92**	-

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

Table 7. Correlation coefficients among relative water content (RWC), stomata length on adaxial surface of the leaves (SLadaxial), stomata length on the abaxial surface (SLabaxial), transpiration rate (TR) and grain yield (GY) of wheat cultivars.

Trait	RWC	SLadaxial	SLabaxial	TR
RWC	-			
SLadaxial	0.66**	-		
SLabaxial	0.54*	0.72**	-	
TR	0.64**	0.26ns	0.56*	-
GY	0.81**	0.58*	0.51*	0.89**

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

References

Baker NR and Rosenqvist E, 2004. Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. Journal of Experimental Botany 55: 1607-1621.

Baker NR, 2008. Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annual Review of Plant Biology 59: 89-113.

Belkhodja R, Morales F, Abadia A, Go´mez-Aparisi J and Abadia J, 1994. Chlorophyll fluorescence as a possible tool for salinity tolerance screening in barley (*Hordeum vulgare* L.). Plant Physiology 104: 667-673.

Chaves MM, 1991. Effects of water deficits on carbon assimilation. Environmental and Experimental Botany 94: 33-45.

Cornic G, 2000. Drought stress inhibits photosynthesis by decreasing stomatal aperture-not by affecting ATP synthesis. Trends in Plant Science 5(5): 187-188.

Dai F, Zhou M and Zhang G, 2007. The change of chlorophyll fluorescence parameters in winter barley during recovery after freezing shock and as affected by cold acclimation and irradiance. Plant Physiology and Biochemistry 45: 915-921.

Demmig-Adams B and Adams III WW, 1992. Photo-protection in plants: a role for xanthophyll zeaxanthin. Annual Review of Plant Physiology and Plant Molecular Biology 1020: 1-24.

Everad JD, Gucci R, Kang SC, Flore JA and Leoscher WH, 1994. Gas exchange and carbon partitioning in the leaves of celery (*Apium graviolens* L.) at various levels of root zone salinity. Plant Physiology 106: 281-292.

Faraloni C, Cutinob I, Petruccelli R, Leva AR, Lazzeri S and Torzillo G, 2011. Chlorophyll fluorescence technique as a rapid tool for in vitro screening of olive cultivars (*Olea europaea* L.) tolerant to drought stress. Environmental and Experimental Botany 73: 49-56.

Fischer RA, Byerlee D and Edmeades GO, 2009. Can technology deliver on the yield challenge to 2050? In: FAO Expert Meeting on How to Feed the World in 2050, June 24-26, Rome.

Flexas J, Escalona JM, Evain S, Gul´ıas J, Moyam I, Osmond CB and Medrano H, 2002. Steady-state chlorophyll fluorescence (Fs) measurements as a tool to follow variation of net CO_2 assimilation and stomatal conductance during water-stress in C_3 plants. Physiologia Plantarum 114: 231-240.

Food and Agricultural Organization (FAO), 2012. FAOSTAT. Production; Crops. http://faostat.fao.org/site/567/default.aspx#ancor.

Galmés J, Medrano H and Flexa J, 2007. Photosynthetic limitation in response to water stress and recovery in Mediterranean plants with different growth forms. New Phytologist 175: 81-93.

Genty B, Briantais JM and Baker NR, 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta (BBA) 990: 87-92.

Gonzalez L and Gonzalez-Vilar M, 2003. Determination of relative water content. Handbook of Plant Ecophysiology Techniques. Kluwer Academic Publishers, London.

Govindjee A, 1995. Sixty-three years since Kautsky: chlorophyll a fluorescence. Australian Journal of Plant Physiology 22: 131-160.

Hoekstra FA, Golovina EA and Buitink J, 2001. Mechanisms of plant desiccation tolerance. Trends in Plant Science 6(9): 431-438.

Hu L, Wang Z, Du H and Huang B, 2009. Differential accumulation of dehydrins in response to water stress for hybrid and common Bermuda grass genotypes differing in drought tolerance. Journal of Plant Physiology 167: 103-109.

Krause GH and Weis E, 1991. Chlorophyll fluorescence and photosynthesis: the basics. Annual Review of Plant Physiology and Plant Molecular Biology 42: 313-349.

Lawlor DW and Cornic G, 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant, Cell and Environment. 25: 275-294.

Levitt J, 1980. Responses of Plants to Environmental Stresses. Water, Radiation, Salt and Other Stresses. Vol. II. Academic Press, New York.

Lichthenthaler HK, 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In: Colowick SP and Kaplan NO (Eds.). Methods in Enzimology. Vol. 148. Pp. 350-382. Academic Press, San Diego, USA.

Lin M and Huybers P, 2012. Reckoning wheat yield trends. Environmental Research Letters 7(2): 024016. doi:10.1088/1748-9326/7/2/024016.

Maghsoudi-Mud AA, 2008. Physiological, morphological and anatomical basis of drought tolerance in wheat. Shahid Bahounar University Publication, Kerman, Iran.

Maxwell K and Johnson GN, 2000. Chlorophyll fluorescence a practical guide. Journal of Experiment Botany 51: 659-668.

Naumann JC, Young DR and Anderson JE, 2008. Leaf chlorophyll fluorescence, reflectance and physiological response to freshwater and saltwater flooding in the evergreen shrub, *Myrica cerifera*. Environmental and Experimental Botany 63: 402-409.

Nautiyal PC, Ravindra V and Joshi YC, 1995. Gas exchange and leaf water relations in two peanut cultivars of different drought tolerance. Biologia Plantarum 37: 371-374.

Nautiyal PC, NageswaraRao RC and Joshi YC, 2002. Moisture-deficit induced changes in leaf water content, leaf carbon exchange rate and biomass production in groundnut cultivators differing in specific leaf area. Field Crops Research 74: 67-79.

Ouzounidou G, 1993. Changes in variable chlorophyll fluorescence as a result of Cu-treatment: dose–response relations in Silene and Thlaspi. Photosynthetics 29: 455-462.

Percival GC and Sheriffs CN, 2002. Identification of drought-tolerant woody perennials using chlorophyll fluorescence. Journal of Arboriculture 28(5): 215-223.

Powles SB, 1984. Photoinhibition of photosynthesis induced by visible light. Annual Review of Plant Physiology 35: 15-44.

Quisenberry KS and Reitz LP, 1987. Wheat and Wheat Improvement. American Society of Agronomy Incorporation, Madison, WI, USA.

Richards RA, 2000. Selectable traits to increase crop photosynthesis and yield of grain crops. Journal of Experimental Botany 51: 447-458.

Rizza F, Pagani D, Stanca AM and Cattivelli L, 2001. Use of chlorophyll fluorescence to evaluate the cold acclimation and freezing tolerance of winter and spring oats. Plant Breeding 120: 389-396.

Sowinski P, Rudzin´ska-Langwald A, Adamczyk J, Kubica I and Fronk J, 2005. Recovery of maize seedling growth, development and photosynthetic efficiency after initial growth at low temperature. Journal of Plant Physiology 162: 67-80.

United Nations Population Division, 2000. World population prospects the 2000 revision highlights. Population Division, Department of Economic and Social Affairs, United Nations, NY, USA.

Woo NS, Badger MR and Pogson BJ, 2008. A rapid, non-invasive procedure for quantitative assessment of drought survival using chlorophyll fluorescence. Plant Methods 4: 27. doi: 10.1186/1746-4811-4-27.

Zarco-Tejada PJ, Miller JR, Mohammed GH, Noland TL and Sampson PH, 2002. Vegetation stress detection through chlorophyll a + b estimation and fluorescence effects on hyper spectral imagery. Journal of Environmental Quality 31(5): 1433-1441.

بررسی اثرات تنش خشکی بر ارقام مختلف گندم توسط برخی متغیرهای فیزیولوژیکی و عملکرد دانه

مهدیه عسکری[1*]، علی اکبر مقصودی مود[2]، وحیدرضا صفاری[2] و افسون عسگری[3]

۱-دانشجوی دکتری زراعت دانشگاه بیرجند، بیرجند و فارغ التحصیل کارشناسی ارشد از دانشگاه شهید باهنر کرمان، کرمان
۲- دانشیار گروه زراعت و اصلاح نباتات، دانشکده کشاورزی، دانشگاه شهید باهنر کرمان، کرمان
۳-دانش آموخته کارشناسی ارشد اصلاح نباتات، گروه زراعت و اصلاح نباتات، دانشکده کشاورزی، دانشگاه بیرجند، بیرجند
*مسئول مکاتبه؛ Mahdiye.askary@yahoo.com

چکیده

این تحقیق به منظور بررسی اثر تنش خشکی بر عملکرد و برخی خصوصیات فیزیولوژیک ارقام گندم انجام پذیرفت. شش رقم مورد مطالعه در شرایط گلخانه و مزرعه تحت تنش خشکی رشد یافتند. نمونه‌های برگی برای اندازه‌گیری خصوصیات فیزیولوژیک از قبیل طول روزنه سطوح بالایی و زیرین برگ، محتوی نسبی آب، سرعت تعرق، نشت یون، پایداری غشای کلروفیل و فلورسانس کلروفیل از گیاهان رشد یافته در شرایط گلخانه گرفته شدند. عملکرد دانه از گیاهان رشد یافته در شرایط مزرعه اندازه‌گیری گردید. نتایج نشان داد که تنش خشکی اثرات معنی‌داری بر صفات اندازه‌گیری شده داشت. بین ارقام گندم نیز از نظر اکثر صفات مورد نظر اختلاف معنی‌دار به دست آمد. تنش خشکی باعث افزایش F0 و کاهش Fm شد. همچنین تنش خشکی سبب کاهش عملکرد دانه گردید به طوری‌که بالاترین عملکرد از شرایط بدون تنش خشکی به‌دست آمد. ارقام الوند و چمران بالاترین شاخص کلروفیل، راندمان کوانتومی و عملکرد دانه را تحت شرایط تنش خشکی دارا بودند و به عنوان ارقام متحمل به تنش در این مطالعه شناسایی شدند.

واژه‌های کلیدی: تنش خشکی؛ عملکرد؛ فلورسانس کلروفیل، کارایی فتوسیستمII، گندم