

ARTICLE

# Evaluation of drought stress at vegetative growth stage on the grain yield formation and some physiological traits as well as fluorescence parameters of different bread wheat cultivars

Mohsen Saeidi<sup>1</sup>, Shiva Ardalani<sup>1</sup>, Saeid Jalali-Honarmand<sup>1</sup>, Mohammad-Eghbal Ghobadi<sup>1</sup>, Majid Abdoli<sup>2\*</sup>

<sup>1</sup>Department of Agronomy and Plant Breeding, Campus of Agriculture and Natural Resources, Razi University, Kermanshah, Iran

<sup>2</sup>Young Researchers and Elite Club, Zanjan Branch, Islamic Azad University, Zanjan, Iran

**ABSTRACT** The effects of water stress on grain yield, its components, and physiological traits during the vegetative stage of wheat have been evaluated. Greenhouse trials were carried out by using factorial experiment based on randomized complete block design (RCBD) in three replications. Moisture stress was applied at vegetative growth stage (soil moisture was around 50% of field capacity from the beginning of stem elongation to flowering stage) and different wheat cultivars (such as Pishtaz, DN-11, Sivand and Marvdasht) were evaluated as the second factor. Water stress significantly decreased grain yield by decreasing the number of grains per spike. Under water stress from the beginning of stem elongation to flowering stages, Sivand and DN-11 cultivars had the lowest grain yield. The lowest and the highest reductions in grain yield and biological yield were detected in Marvdasht and DN-11, respectively. Results show that Marvdasht had the highest, while DN-11 had the lowest relative water constant (RWC) and performance index (PI) values. Water stress significantly decreased the chlorophyll content, PI and RWC values, at the same time significantly increased the carotenoid concentration, whereas the maximum quantum yield of photosystem II (Fv/Fm) did not change. According to the results, Pishtaz and Marvdasht cultivars are tolerant against drought stress and can recover very fast after stress is eliminated.

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**KEY WORDS**

wheat  
drought stress  
grain yield  
chlorophyll  
photosystem II

## Abbreviations

RWC: Relative water content; RCBD: Randomized complete block design; Fv/Fm: Maximum quantum yield of PSII; PI: Performance index; PSII: Photosystem II; Chl: chlorophyll.

## Introduction

Plants under natural and agricultural conditions are exposed to stress constantly. Drought limits plant growth and field crops production more than any other environmental stress (Zhu 2002). Drought is the most common environmental stress affecting about 32% of the 99 million hectares under wheat cultivation in developing countries and at least 60 million hectares under wheat cultivation in developed countries (Rajaram 2000). Iran is one of the countries where abiotic stresses like drought, salinity, heat and cold result in yield decrease,

soil fertility destruction and also cessation of farming. In Iran, about 67% of the wheat cultured area is devoted to dry farming lands, which are exposed to drought stress during the growth season (Galeshi and Oskouie 2002).

Drought and high temperature (heat) stress are considered to be the two major environmental factors limiting crop growth and yield (Wang et al. 2003; Prasad et al. 2008). For example, the combined effects of heat and drought on yield are more detrimental than the effects of each stress factor alone, as seen in barley (Savin and Nicolas 1996) and wheat (Prasad et al. 2011). These two stress factors induce many biochemical, molecular, and physiological changes and responses that influence various cellular and whole plant processes that affect crop yield and quality. Some studies suggest that drought stress influences the thermal tolerance of photosynthesis (Havaux 1992; Lu and Zhang 1999). In contrast, some studies have reported that drought greatly exacerbates the effects of heat stress on plant growth and photosynthesis (Xu and Zhou 2005; 2006). Stomatal closure is one of the earliest responses of plants to water deficit that

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\*Corresponding author. E-mail: majid.abdoli64@yahoo.com

limits transpiration water loss and helps plants to retain water status under drought. However, closure of stomata in turn, results in reduction of CO<sub>2</sub> availability for photosynthetic carbon metabolism, depresses net CO<sub>2</sub> assimilation rate and inhibits plants ability for dry matter accumulation (Chaves et al. 2009; Hajiboland et al. 2014). In addition, declines in the CO<sub>2</sub> availability to the Calvin cycle enzymes result in lower regeneration of NADP<sup>+</sup> and production of excess excitation energy that damages photosystems (Hajiboland 2014).

The impacts of environmental stress, particularly those of drought and heat, have been studied independently (Prasad et al. 2008). Mirzaei et al. (2011) reported that drought stress at all growth stages induced reducing grain yield and yield components. Drought stress at stages of stem elongation, flowering and grain filling stages induced 32%, 32% and 35% reduce in grain yield, respectively. Also, sensitivity for stress is highest in stem elongation stage than in other growth stages. In a study on wheat, Day and Intalap (1970) declared that decrease of grain yield relate to drought stress in the stem elongation stage it results decreased number of spike per unit area and grain yield on spike. Drought stress during maturity resulted in about 10% decrease in yield, while moderate stress during the early vegetative period had essentially no effect on yield (Bauder 2001). Gupta et al. (2001) studied physiological and yield attributes of two wheat genotypes with stress at boot and anthesis. They reported that number of grains, grain yield, biological yield and harvest index decreased to a greater extent when water stress was imposed at anthesis stage. The quality of wheat can be affected by water stress; in this relationship Noorka et al. (2009) reported that quality traits of wheat grain were significantly affected under water stress conditions. Although stress typically depresses grain yield (Hsiao 1973), it can elevate the value of other components of the economic yield, such as quality of grain protein (Guttieri et al. 2000; Pompa et al. 2009). Aslani et al. (2013) reported that an increase in protein content, gluten index, dry gluten and SDS sedimentation volume, consistent with a decrease in grain yield, 1000 grains weight, bread volume and moisture content was observed when a terminal water stress happened. Keyvan (2010) and Abdoli et al. (2013) reported that, there was a decrease in relative water content, total chlorophyll

content, photosynthesis rate and increased proline content with the increase in the intensity of drought stress on wheat cultivars, but was not observed on trend relating to soluble carbohydrates content. Matin (1989) studying barley, reported that drought tolerant cultivars usually maintained higher leaf RWC under stress. Changes in the RWC of leaves are considered as a sensitive indicator of drought stress and more useful integrator of plant water balance than the leaf water potential (Strauss and Agenbag 2000; Clavel et al. 2005).

Understanding the biochemical and physiological basis of water stress tolerance in plants is vital to select and breed plants for improving crop water stress tolerance (Chaves et al. 2003). Historically, research on physiological and biochemical changes that occur during leaf senescence (drought stress) focused on loss of photosynthetic pigments, degradation of protein, and re-absorption of mineral nutrients (Saeidi et al. 2010; Hajiboland 2014). Taking the above facts into consideration, this project was designed to determine the effect of water stress on some physiological traits of wheat and to characterize genotypic yield response to water stress conditions. Such study will provide valuable information that can be used for the genetic basis of improvement of wheat to enhance yield under stress conditions.

## Materials and Methods

### Experimental procedure and design

The pot experiments were conducted during the growing season from 2011 to 2012 in the greenhouse of Campus of Agricultural and Natural Resource, Razi University in Kermanshah state in the west of Iran (47° 9'E; 34° 21' N), with 1319 meter elevated from sea level. The experiment was laid out in a randomized complete block design (RCBD) in a factorial arrangement with three replications. It comprised of four wheat cultivars *i.e.*, Pishtaz, Sivand, Marvdasht and DN-11 and two water stress treatments *i.e.*, control or well water (irrigation at field capacity in all stages of plant growth normally), water stress at vegetative growth stage (soil moisture was around 50% of field capacity from

**Table 1.** Characteristics of cultivars used in the experiments.

Characteristics	Pishtaz	Sivand	Marvdasht	DN-11
Grain yield	High	High	Medium	High
Growing type	Spring	Spring	Spring	Spring
Physiological maturity	Late maturing	Late maturing	Late maturing	Early maturing
Plant height	Tall	Tall	Tall	Medium
Grain weight	High	High	Low	Medium
Grain color	Yellow	Yellow	Yellow	Yellow

Source: Abdoli and Saeidi 2012

**Table 2.** Physico-chemical properties of the soil used in the experiment.

Physical property		Chemical property (Saturation extract)	
Sand (%)	17	K (mg kg <sup>-1</sup> )	329
Silt (%)	39	Available P for plant (mg kg <sup>-1</sup> )	8.0
Clay (%)	44	N (%)	0.098
Field capacity (0.033 MPa, cm <sup>3</sup> cm <sup>-3</sup> )	0.35	pH	7.41
Bulk density (g cm <sup>-3</sup> )	1.23	Organic C (g kg <sup>-1</sup> )	1.14

**Table 3.** Minimum, maximum and mean of temperature, relative humidity and precipitation at the site of experiment during 2011-2012.

Month	Precipitation (mm)	Temperature (°C)			Evaporation (mm)
		Minimum	Maximum	Mean	
Oct.	0	2.5	32.4	18.7	205.3
Nov.	131	-1.9	24.6	10.6	57.5
Dec.	0.8	-8.7	18.8	3.1	0.2
Jan.	10.4	-9	15.8	4.4	-
Feb.	68.2	11.2	14.9	3	-
Mar.	34.3	-11.2	22	4.4	-
Apr.	35.4	4.7	19.2	11.9	82.2
May.	25.2	19.3	26.5	17.8	120.5
Jun.	0	14.2	23.7	23.9	304.6
Jul.	0	17	36.9	27	361.2
Aug.	0	19	39	29	367.8
Sep.	0	15	36	25.5	284.6

Source: Meteorological Office, Iran.

the beginning of stem elongation to flowering stages – 31 to 59 of the Zadoks's scale) (Zadoks et al. 1974). These four wheat cultivars were chosen because they have the highest area under cultivation in the Kermanshah province and they are new cultivars with unknown physiological characteristics. Some growing characteristics of cultivars used in the experiments are shown in Table 1. The seeds of wheat cultivars were obtained from Seed and Plant Improvement Institute, Agricultural and Natural Resources Research Center of Kermanshah, Iran. Seeds were sown in plastic pots (PVC) with a diameter of 20 cm and height of 30 cm which were filled with 2.5 kg of fertilized peat and soil in 1:1 ratio. Ten grains per pot were sown at distances and depth and one week after their emergence, the number of the seedlings was reduced to 5 per pot. A 0-30 cm layer of the soil was collected from the top and its physicochemical properties were evaluated (Table 2). Humidity and mean temperatures during the crop season are presented in Table 3.

### Sampling

20 uppermost leaves of 5 plants per pot (4 leaves per plant) were harvested at 10, 20 and 40 days after water stress at vegetative growth stage. Samples were frozen in liquid ni-

trogen for 1 min and stored at -80 °C till the investigation of biochemical and physiological characteristics.

### Chlorophyll and carotenoid content measurements

The leaves were homogenized in ice cold 80% acetone (1.5 ml for 250 mg sample) and extracted for 24 h. Samples were centrifuged at 6000 g for 15 min at 4 °C and the supernatants were collected. The pigment composition was measured using a double-beam spectrophotometer according to the method described by Lichtenthaler and Wellburn (1983) and Arnon (1949). This method involves measurement of the light absorbed in the plant extract at 663, 645 and 470 nm.

Chl a (mg g<sup>-1</sup>.fw) = [(12.7 × A<sub>663</sub>)-(2.6 × A<sub>645</sub>)] × ml acetone/mg

Chl b (mg g<sup>-1</sup>.fw) = [(22.9 × A<sub>645</sub>)-(4.68 × A<sub>663</sub>)] × ml acetone/mg

Chl total (mg g<sup>-1</sup>.fw) = Chl a + Chl b

### Relative water content measurements

Leaf relative water content (RWC) was estimated according to Henson et al. (1981) and Castillo (1996) for each drought

**Table 4.** Analysis of variance and mean comparison of the effect of irrigation regimes and cultivars on grain yield and its components and some agronomic characteristics in different wheat cultivars under water stress.

Treatments	Grain yield (g/plant)	Biomass (g/plant)	Harvest index (%)	1000 grain weight (g)	Number of grains per spike
Irrigation levels					
Well water	3.76 a	7.76 a	48.5 a	27.3 a	44 a
Water stress	1.72 b	4.30 b	40.0 b	25.3 a	28 b
Decrease (%)	-54	-45	-18	-7	-36
Cultivars					
Pishtaz	2.86 a	6.20 a	46.1 a	29.7 a	34 b
DN-11	2.52 ab	5.42 ab	46.9 a	23.0 b	32 b
Sivand	2.36 b	4.82 b	48.9 a	33.7 a	22 c
Marvdasht	2.56 ab	5.94 a	43.1 a	20.2 b	47 a
Irrigation levels (I)	**	**	*	ns	**
Cultivars (C)	ns	*	ns	**	**
I × C	**	*	*	ns	*
CV (%)	12.6	13.9	18.3	12	15.9

ns, \* and \*\*: Non significant, significant at 5% and 1% levels of probability, respectively. Means followed by the same letters in each column are not significantly different at 5% level, according to Duncan's Multiple Range test.

period. After 10, 20 and 40 days of water stress RWC values were measured in the morning from 8.00 am to 10.00 am. For this 10 fully matured leaves of 5 plants per pot (2 leaves per plants) were selected from the same heights and their fresh weights were recorded. The leaves were soaked into distilled water under low lighting conditions for 24 h to measure their saturated weight. After recording turgescence weight, leaves were dried at 75 °C for 48 h and their dry weights were measured. RWC was calculated using the following formula:

$$\text{RWC} = \frac{[(\text{fresh weight}) - (\text{dry weight}) / (\text{bulge weight}) - (\text{dry weight})] \times 100}{}$$

### Chlorophyll fluorescence parameters measurements

Chlorophyll fluorescence parameters were quantified using a portable Plant Efficiency Analyser type MK2 (PEA, Hansatech Instruments, UK). Ten leaves were adapted to darkness for 30-45 min by attaching light exclusion clips to the surface of two topmost full-blown leaves in situ from five plants in each pot. The fluorescence responses were induced by flash exposure to saturated white light with a photon flux density of about 3500 mmol m<sup>-2</sup> s<sup>-1</sup>. Among the chlorophyll fluorescence parameters given by the equipment, only two, Fv/Fm and SFI were taken into consideration because these parameters showed significant differences after treatments and/or line in the analysis of variance. The Fv/Fm showed that the maximal quantum yield of photochemistry in dark-adapted state and SFI or Vitality Index expresses an ability of the plant to avoid drought and to maintain its physiological activity at a certain level. This Vitality Index combines criteria of structure and function: it reflects the fraction of

non-photochemical phenomenon (fluorescence and heat dissipation) when the majority of the PSII reaction centers are open for maximal photon absorption (Strasser and Strasser 1995; Strasser et al. 1999).

### Measuring grain yield and its components

For measuring the number of grains per spike and 1000 grain weight (grain yield), 10 plants harvested from each of the treatments (two pots) were measured. Harvest index was calculated by dividing grain yield to biomass production.

### Statistical analysis

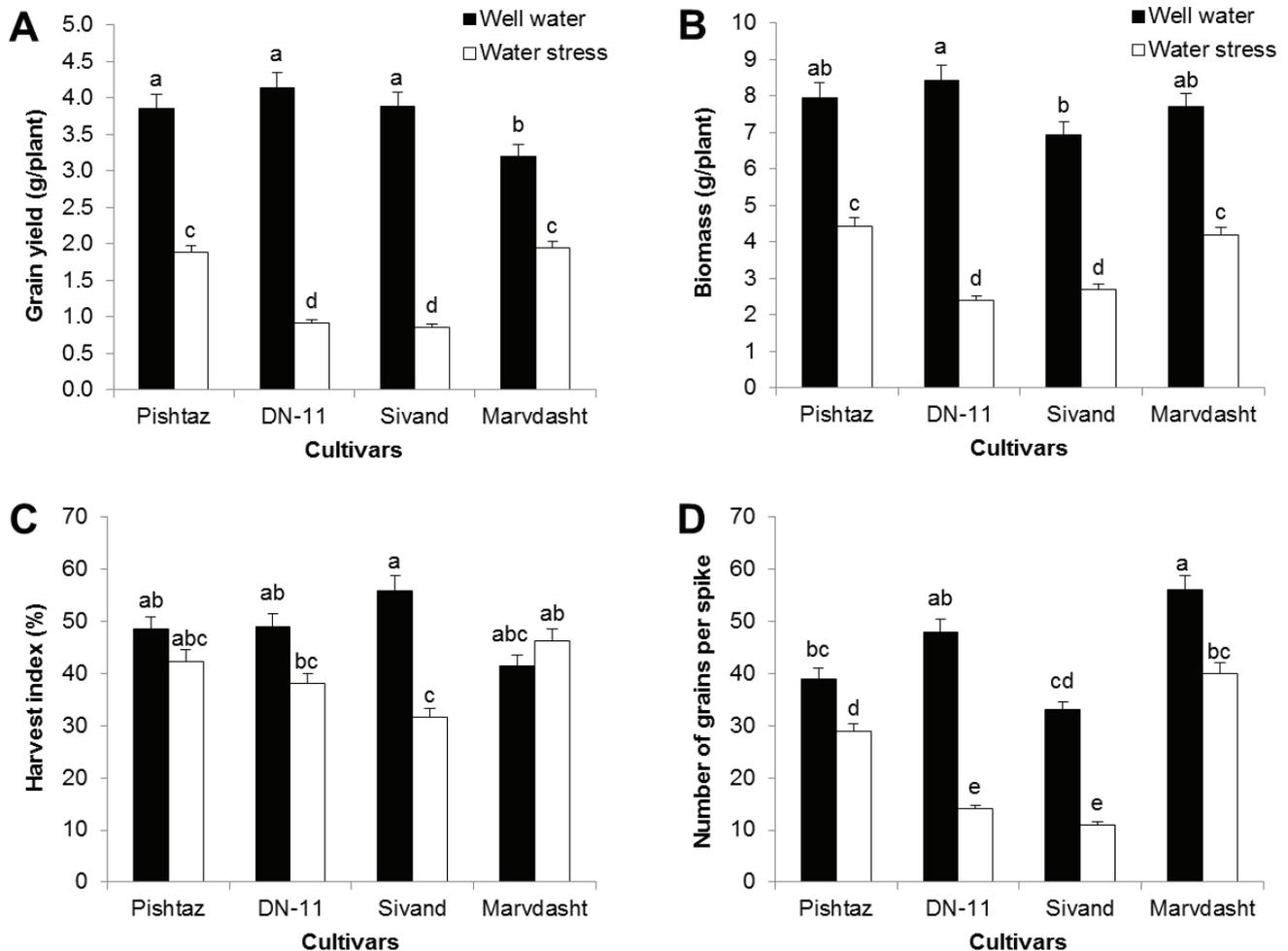
The obtained data were subjected to analysis of variance (ANOVA) using Duncan's multiple range test (DMRT) by using Statistical Analysis System software (version 9.1, SAS Institute). Differences were considered statistically significant when P<0.05 (Duncan 1955). The figures were drawn using Microsoft Excel (version 10.0).

## Results and Discussion

### Grain yield and its components

The results obtained from mean comparison analysis of grain yield and its components are shown in Table 4. Water stress at the vegetative growth stage caused 54%, 45%, 18% and 36% reduction in grain yield, biomass, harvest index and the number of grains per spike in average, respectively (Table 4). It had no significant effect on 1000 grain weight.

**Figure 1.** Influence of water stress at vegetative growth stage on grain yield (A), biomass (B), harvest index (C) and the number of grains per spike (D) of different wheat cultivars. Means followed by the same letters in each trait are not significantly different at 5% level, according to Duncan's Multiple Range test.



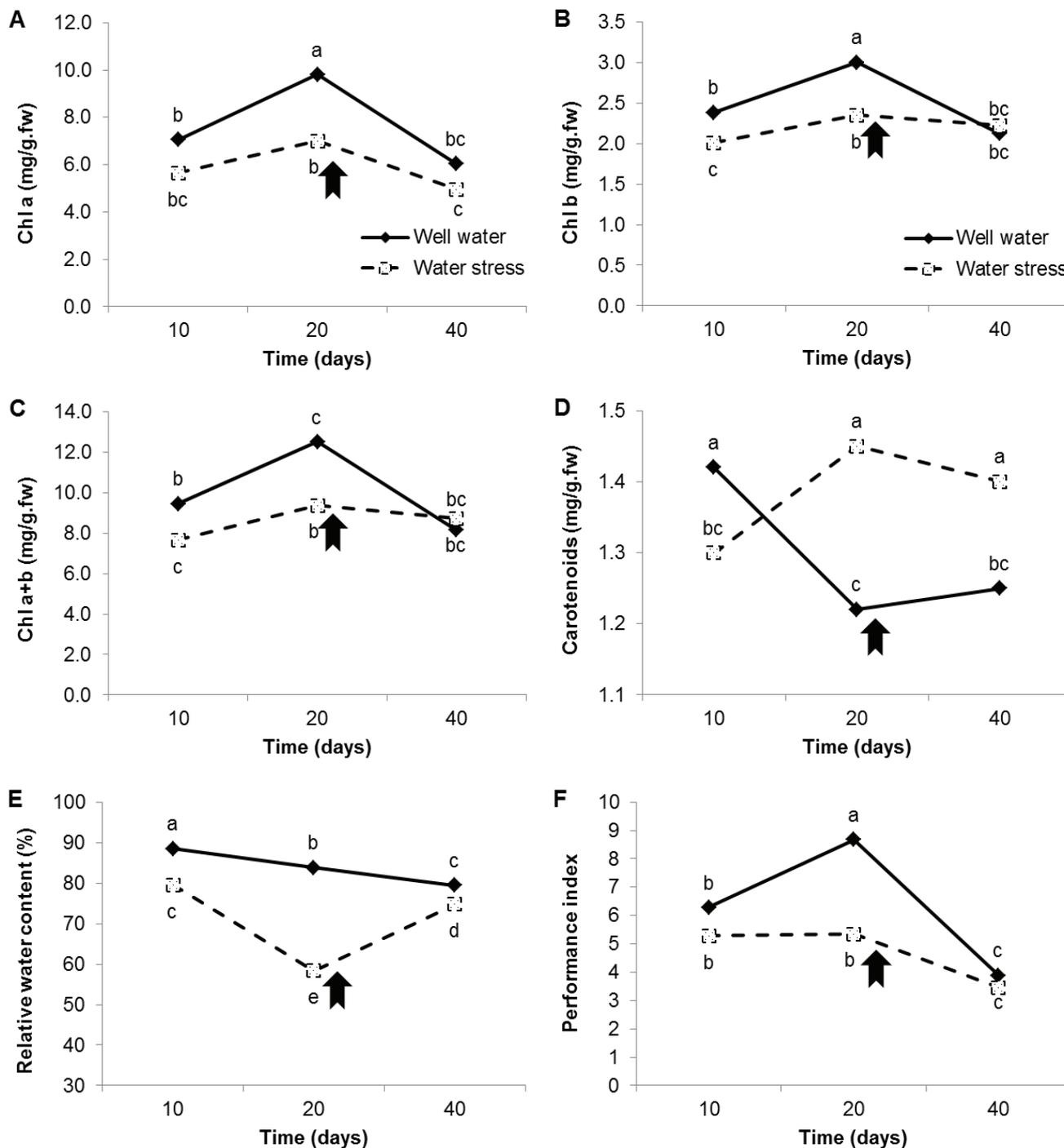
Average grain yield and the number of grains per spike of different cultivars in controlled condition were 3.76 g plant<sup>-1</sup> and 44 grains, respectively. Under water stress these values significantly reduced to 1.72 g plant<sup>-1</sup> and 28 grains. Sivand (2.36 g plant<sup>-1</sup>) had the lowest and Pishtaz (2.86 g plant<sup>-1</sup>) had the highest grain yield (Table 4). Under water stress, the lowest and the highest reduction in grain yield were seen in Marvdasht and DN-11, respectively. DN-11 and Sivand had the lowest grain yield production under water stress at the vegetative growth stage (0.91 and 0.85 g plant<sup>-1</sup>, respectively) (Fig. 1A). Maralian et al. (2010) reported that tillering and heading stages were sensitive to water stress and grain yield of wheat decreased more than 37% compared with well-watered conditions.

Under well-watered conditions, Marvdasht and DN-11

had the highest number of grains per spike (56 and 48 grains spike<sup>-1</sup>) while Sivand had the lowest values (33 grains spike<sup>-1</sup>). After water stress, Marvdasht had the highest (40 grains spike<sup>-1</sup>) and Sivand had the lowest (11 grains spike<sup>-1</sup>) values, respectively (Fig. 1D). Mirbahar et al. (2009) declared that water stress significantly reduced the spike length, spikelets per spike, grains per spike and 1000 grain weight of all 25 wheat varieties. The highest reduction in all parameters was found in terminal drought, while post flowering drought and pre-flowering drought affected the 1000 grain weight significantly.

Under well-watered conditions, DN-11 cultivars had the highest (8.44 g plant<sup>-1</sup>) and Sivand had the lowest (6.94 g plant<sup>-1</sup>) biomass, respectively. Under water stress from the beginning of stem elongation to flowering stage Pishtaz and

**Figure 2.** Changes in Chl a (A), Chl b (B), total Chl (C) and carotenoid (D) content as well as RWC (E) and PI (F) values of leaves in well watered and water stress at the vegetative growth stage. Means followed by the same letters in each trait are not significantly different at 5% level, according to Duncan's Multiple Range test. Arrows indicate the re-irrigation in the water stress.



Marvdasht had the highest (4.44 and 4.20 g plant<sup>-1</sup>, respectively) and Sivand and DN-11 the lowest (2.70 and 2.40 g plant<sup>-1</sup>) biological yield (Fig. 1B). Under water stress at

vegetative growth stage, the lowest reduction in biomass was noted in Marvdasht and the highest in DN-11.

Harvest index can be expressed as the ability of plants

**Table 5.** Analysis of variance and mean comparison of the effect of irrigation regimes, cultivars and sampling procedures on some physiological traits in different wheat cultivars under water stress.

Treatments	Chl a (mg g <sup>-1</sup> fw)	Chl b	Chl total	Carotenoids	RWC (%)	PI	Fv/Fm
Irrigation levels							
Well water	7.54 a	2.50 a	10.0 a	1.33 b	83.9 a	6.28 a	0.751 a
Water stress	6.37 b	2.19 b	8.57 b	1.60 a	70.9 b	4.70 b	0.735 a
Decrease (%)	-15	-12	-15	17	-15	-25	-2
Cultivars							
Pishtaz	6.98 ab	2.38 a	9.37 a	1.51 a	78.8 a	4.93 b	0.735 a
DN-11	7.12 ab	2.41 a	9.53 a	1.47 a	73.9 b	5.64 b	0.733 a
Sivand	6.43 b	2.21 a	8.65 a	1.36 a	78.1 a	4.79 b	0.738 a
Marvdasht	7.29 a	2.39 a	9.69 a	1.51 a	78.8 a	6.59 a	0.763 a
Sampling procedures							
10	6.36 b	2.19 b	8.56 b	1.27 b	84.0 a	5.79 b	0.774 a
20	8.25 a	2.68 a	10.9 a	1.79 a	71.1 c	7.01 a	0.766 a
40	6.23 b	2.17 b	8.43 b	1.33 b	77.1 b	3.76 c	0.688 b
Irrigation levels (I)	**	**	**	**	**	**	ns
Cultivars (C)	ns	ns	ns	ns	*	**	ns
Sampling procedures (S)	**	**	**	**	**	**	**
I × C	ns	ns	ns	ns	ns	ns	ns
I × S	**	**	**	**	**	**	ns
S × C	ns	ns	ns	ns	ns	ns	ns
I × C × S	ns	ns	ns	ns	ns	ns	ns
CV (%)	16.7	14.1	15.9	18.7	6.58	23.4	5.59

ns, \* and \*\*: Non significant, significant at 5% and 1% levels of probability, respectively. Means followed by the same letters in each column are not significantly different at 5% level, according to Duncan's Multiple Range test.

to allocate photosynthetic assimilates to produce economic yield. A significant variation was noted for this trait among cultivars under both well-watered and water stress conditions. Water stress significantly decreased harvest index in most cultivars (Table 4). Under well-watered conditions, Sivand had the highest (55.9%) and Marvdasht cultivars had the lowest (41.6%) harvest index. However, under water stress Marvdasht had the highest (46.3%) and Sivand had the lowest (31.6%) harvest index (Fig. 1C). Significant reduction in harvest index occurred under water stress (Table 4) due to a higher reduction in grain yield than in biomass production (Shafazadeh et al. 2004; Abdoli et al. 2013). Richards et al. (2002) also reported that high harvest index under control treatment can be accompanied with high grain yield under water stress.

### Physiological traits

The results obtained from mean comparison analysis of some physiological traits are shown in Table 5. A reduction in the chlorophyll a and b content occurred during drought stress. So that, water stress at the vegetative growth stage caused 15%, 12% and 15% reduction in Chl a, Chl b and total Chl concentration in average, respectively (Table 5). Ashraf et al. (1994) and also Reddy and Vora (1986) related the decrease in chlorophyll concentration under drought stress to the increase

in activity of the enzyme chlorophyllase. A drought stress induced decrease in pigment content was previously reported in several plant species, including durum wheat (Loggini et al. 1999) and bread wheat (Saeidi et al. 2010).

In well-watered condition, Chl a, Chl b, total Chl and PI in the leaves reached a maximum within 20 day. After this time a reduction could be observed (Fig. 2A, B, C, F). In generally, the amount of carotenoids was increased with time in water stress condition (Fig. 2D). Danda and Behl (2004) also reported a four-unit increase in SPAD as relative water content decreased from 94% to 87% (under water stress), although, there are many reports showing the decrease in leaf chlorophyll under drought stress conditions (Ashraf et al. 1994; Sairam et al. 1997; Rahimi et al. 2010; Abdoli et al. 2013).

Analysis of variance showed that cultivars significantly differed for RWC and PI (Table 5). Sivand, Pishtaz and Marvdasht cultivars possessed the highest and DN-11 cultivar had the lowest RWC under both conditions (Table 5). Schonfeld et al. (1988) observed a decline in the amount of RWC in wheat due to drought stress and reported the highest RWC in the tolerant genotype. Many important physiological and morphological processes such as leaf enlargement, stomatal opening and associated leaf photosynthesis can be directly affected by the reduction of leaf turgor potential, which accompanies the loss of water from leaf tissue (Jones and Turner

1978). These researchers reported that with a decrease in RWC, leaf osmolality increased and the slow development of water deficits resulted not only in osmotic adjustment, but also in a decrease in leaf tissue elasticity. There is a similar trend in the results of other authors (Bhutta 2011). After a few days of withholding water, an RWC decrease was observed in leaves indicating the onset of water stress. RWC improved after eliminating stress (Fig. 2E).

In the current study, water stress and its interaction with cultivars had no significant effect on the maximum efficiency of PSII (Fv/Fm) (Table 5). Drought stress reduced the PI from 6.28 in the control plants to 4.70 in stressed plants (Table 5). The results show that, Marvdasht had the highest PI (6.59) while DN-11, Pishtaz and Sivand had the lowest PI values (5.64, 4.93 and 4.79) (Table 5). PI is found to be a very sensitive parameter in different crops and in most of environmental stress situations (Strasser et al. 1999; Jiang et al. 2006; Christen et al. 2007), which is in accordance with our results achieved on winter wheat plants under drought stress. Van Heerden et al. (2007) observed also a very good positive correlation between CO<sub>2</sub> assimilation capacity and PI values under water stress.

## Conclusions

Our results indicated that water stress during the vegetative stage did not change 1000 grain weight significantly but decreased some agronomy traits such as grain yield, biomass and the number of grains per spike. Also, water stress at the vegetative growth stage significantly decreased some physiological parameters such as chlorophyll a, b and total chlorophyll, PI and RWC and significantly increased carotenoids concentration in the leaves. The maximal quantum yield of PSII (Fv/Fm) did not change with water stress. According to the results, Pishtaz and Marvdasht cultivars are tolerant against drought stress and can recover very fast after removing stress. These plants could improve membrane integrity, relative water content and antioxidant level after releasing the stress.

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