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Post-anthesis Drought Stress Effects on Photosynthesis Rate and Chlorophyll Content of Wheat Genotypes

Mohammad Javad Ahmadi-Lahijani[1] and Yahya Emam[2*]

[1]Former MSc Student of Crop Production and Plant Breeding, Faculty of Agriculture, Shiraz University, Shiraz, Iran, presently PhD student of Agronomy, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
[2]Department of Crop Production and Plant Breeding, Faculty of Agriculture, Shiraz University, Shiraz, Iran
[*]Corresponding author; Email: yaemam@shirazu.ac.ir

Abstract

Water stress is one of the major abiotic stresses in agriculture worldwide. In order to assess photosynthesis response and grain yield of 25 wheat genotypes under water deficit (post-anthesis stress) conditions, a 2-year study (2010-12) was carried out as a split-plot arrangement using randomized complete block design with three replications. The most sensitive gas exchange variable to water deficit was found to be mesophyll conductance (g_m) (62% reduction), followed by photosynthesis rate (Pn) (42% reduction). Water deficit also reduced grain yield by an average of 45%. Pn and g_m were significantly correlated with grain yield under both conditions. Higher chlorophyll content was associated with higher Pn under water deficit conditions. Maintenance of greater green leaf area during grain filling period was related to greater grain yield. Genotypes with higher Pn and g_m were those with optimum grain yield (i.e. *cvs*. Zarrin and Darya), hence, Pn and g_m were found to be the appropriate indices for screening wheat genotypes under the terminal water deficit conditions.

Keywords: Canopy temperature; Gas exchange variables; Grain filling period; SPAD; Terminal water deficit, Water use efficiency.

Abbreviations: Net Photosynthesis rate (Pn), Sub-stomatal CO_2 concentration (Ci), Mesophyll conductance (g_m), Stomatal conductance (g_s), Transpiration rate (E), Water use efficiency (WUE), Leaf temperature (LT), Canopy temperature (CT), Chlorophyll content (Chl), Grain filling period (GFP), Grain yield (GY).

Introduction

Wheat (*Triticum aestivum* L.) is the most important field crop in the world (Emam 2011), which supplies food for more than 35% of the world population (Amiri *et al.* 2005). Wheat plays an essential role to decrease the gap between food production and importation in developing countries (Alam *et al.* 2008). Food security highly depends on continuous crop improvement, particularly with increased resistance to such abiotic stresses as drought and salinity (Denby and Gehring 2005). Along with rapid population growth and increase in food demand, the pressure

on untouched lands and waters also increases (Araus *et al.* 2008). Wheat production is adversely affected by water stress from 50% to 70% around the world (Zhao *et al.* 2008). Approximately, 32% of the wheat-growing areas in developing countries experience some degrees of water stress during the growing season (FAO 2009). According to climate predictions, drought will become worse in the coming years of the present century in Mediterranean areas. Late-season water stress is one of the major problems contributing to wheat yield reduction in the Mediterranean areas. Thus, it would be vital to identify physiological

mechanisms leading to screen more water-efficient cultivars. A better perception of drought tolerance mechanisms of wheat could also help breeders to select drought-tolerant genotypes.

Water stress directly reduces plant productivity by lowering the net CO_2 assimilation rate (A) (Taiz and Zeiger 2010). Water stress limits leaf expansion, stomatal conductance and primary processes of photosynthesis (Passioura 1994). Under water stress conditions, photosynthesis is reduced mainly due to limitation of CO_2 diffusion into the leaf as a result of limited mesophyll and stomatal conductance (Flexas *et al*. 2004). This photosynthesis depression directly affects crop growth and yield (Dubey 2005). The decline in CO_2 assimilation of plants under water stress has been attributed to stomatal and/or non-stomatal limitations to photosynthesis (Chaves 1991). Reynolds *et al*. (2007) and Fischer *et al*. (1998) reported that increase in photosynthesis capacity could result in yield improvement. Changes in the functionality of leaf photosynthesis and leaf senescence during grain filling period have been noted as two major factors that affect the rate of dry matter accumulation (Tollenaar and Lee 2006).

There is a strong link between dry matter accumulation, grain yield and water use. The results of some experiments suggested that variability in WUE among species and cultivars is genetically controlled (Hall *et al*. 1990). Both stomatal and non-stomatal factors are thought to affect WUE. Clearly, stomata are important regulators of transpiration rate and any change in the stomatal conductance affects transpiration rate more than Pn (Martin and Ruiz-Torres 1992). Canopy temperature (CT) can be used as an indicator of crop water status (Gardner *et al*. 1992). Blum *et al*. (1989) suggested canopy temperature as a potential tool to select drought-tolerant genotypes. According to Fischer *et al*. (1998), greater stomatal conductance, higher photosynthesis rate and cooler canopies could be potential indirect selection indices to screen cultivars with greater yields.

Leaf chlorophyll content is an important factor determining the photosynthesis rate and dry mater accumulation (Ghosh *et al*. 2004). SPAD (Soil Plant Analysis Development) values can be used as an indicator of chlorophyll content status of plant leaves (Dong *et al*. 2005). Portable chlorophyll meters estimate the amount of chlorophyll pigments by measuring the amount of light transmitted through a leaf. Easily and rapidly measurement, non-destructive feature and computation of the average value of several samples are among the advantages of using SPAD (Hoang and Kobata 2009). Drought speeds up senescence by expediting the chlorophyll degradation, nitrogen loss and lipid peroxidation (Yang *et al*. 2001). Kassahun *et al*. (2010) suggested that higher SPAD values of sorghum were associated with higher relative grain yield under post-flowering drought stress conditions. Liu *et al*. (2006) observed significant increase in electrolyte leakage and reduction in chlorophylls *a* and *b* of wheat cultivars subjected to water stress.

Despite being a major selection index used in drought prone environments, because of low heritability of grain yield, selection just based on grain yield might not be appropriate (Ashkani *et al*. 2007). Hence, physiological parameters such as photosynthesis rate, stomatal conductance and green leaf area duration could be used to identify genotypes better adapted to and provide higher yields under post anthesis drought stress conditions (Labuschagne *et al*. 2008). Therefore,

the present study was conducted to examine the possibility of gas exchange variables as well as grain yield as reliable indices to screen drought tolerant wheat genotypes under post-anthesis drought stress conditions.

Materials and Methods
Site and experimental procedure
Twenty-five wheat genotypes were evaluated during 2010-11 and 2011-12 growing seasons at the Experimental Field of College of Agriculture, Shiraz University (52º53´N, 29º36´W, 1180 m above sea level). A split plot arrangement based on randomized complete block design with three replications was used. Irrigation treatments, including well-watered condition for which the plots were fully irrigated until maturity, and water deficit condition for which irrigation was cut-off following heading/anthesis at ZGS 62 (Zadoks *et al.* 1974) in mid-April to maturity, were assigned to main plots, and 25 bread wheat genotypes including seven breeding lines (C-85-14, C-85-5, C-85-7, C-85-9, C-86-2, C-86-4, M-73-6) and 18 cultivars (Line A, Star, Omid, Azar, Agosta,

Bahar, Parsi, Pishtaz, Jonz, Chamran, Darya, Zarrin, Shiraz, Shiroodi, Tabasi, Falat, Karaj2, Kavir) to sub-plots. Genotypes were chosen from a preliminary experiment carried out at the same place comparing a large number of genotypes to select the more adapted ones during 2008-2010 growing seasons (unpublished data). Uniform wheat seeds were hand-sown on November 2010 and 2011 in rows 30 cm apart giving 320 plants/m^2 density in the plots of 2×1 m.

Soil texture was clay loam. Some physiochemical properties of the soil were as follow: pH= 7.6, total organic carbon (%)= 1.26, total nitrogen (%)= 0.12, EC (dS m^{-1})= 1.0, potassium (mg kg^{-1})= 450, phosphorus (mg kg^{-1})= 21.5. Experimental plots were fertilized with nitrogen as urea (46% N) at the rate of 150 kg ha^{-1}. Urea was applied at two stages, half before planting and the other half at the stem elongation. Temperature, rainfall and relative humidity of field experiment during the 2-year of growing seasons are presented in Table 1. There was no rainfall from flowering to the end of the growing season.

Table 1. Means of temperature, relative humidity and rainfall of experimental field during the growing season

		Nov.	Dec.	Jan.	Feb.	March	April	May	June
2010-2011	Temperature (ºC)	10.7	5.6	3.3	4.6	8.8	11.8	17.7	23.9
	Rainfall (mm)	0	0	48.5	107.5	71.8	30.5	0	0
	RH (%)	38	31	43	49	50	50	48	24
2011-2012	Temperature (ºC)	11.2	4.6	4.4	3.9	5.7	11.1	17.2	21.5
	Rainfall (mm)	23.5	79.5	61	127	27	45	0	0
	RH (%)	35.5	52.6	58.3	55.4	42.8	69.3	35.1	26.1

Note: Data were obtained from Climate Research Center, Shiraz University, Shiraz, Iran

Physiological measurements

Gas exchange variables

Gas exchange variables including net assimilation rate (Pn) (μmolm^{-2}s^{-1}), stomatal conductance (g_s) (molm^{-2}s^{-1}), transpiration rate (E) (mmolm^{-2}s^{-1}), sub-stomatal CO_2 concentration (Ci) (μmolmol^{-1}) and leaf temperature (LT) (°C) were measured on flag leaves using an infra-red gas analysis system (ADC BioScientific Lci Analyser, UK). The measurements were taken on three young fully expanded flag leaves exposed directly to the sunlight when solar radiation levels were between 1200 and 1800 μmolm^{-2}s^{-1} in the two inner rows of each plot at midday (10-14) during grain filling period (ZGS 75). Average of three measurements was taken as gas exchange variable. Mesophyll conductance (g_m) was determined by dividing A/Ci (Fischer *et al.* 1998). Water use efficiency (WUE) was calculated as the ratio between photosynthesis rate (A) and transpiration rate (E) (mmol CO_2 assimilated / mol H_2O transpired) (Mateos-Naranjo *et al.* 2010).

Leaf and canopy temperature

Leaf temperature (LT) was recorded concurrently with the gas exchange variable measurements. Canopy temperature (CT) was measured using an infrared thermometer (TIR 8861, Terminator, China) after cutting-off irrigation at ZGS 75. Three readings at the angle of approximately 30˚ to the horizon were taken from the same side of each sub-plot at midday (11-13 PM) in a cloudless condition, and the mean of these readings were taken as CT.

Chlorophyll content (SPAD)

Total chlorophyll content was measured on intact flag leaves using a portable chlorophyll meter (CCM-200, Opti-Science, USA). At least three flag leaves per replicate were measured on the same leaves during grain filling period (ZGS 75). Readings were taken from three plants per replicate at the middle of leaf lamina and averaged. CCM-200 estimates chlorophyll at two (653 nm and 931 nm) wavelengths in 0.71 mm^2.

Agronomic characters

Grain yield was measured after physiological maturity by harvesting the central 1 m^2 of each subplot, and then were threshed and weighed. The grain filling period (GFP) was measured as the number of days from ear emergence to maturity.

Statistical analysis

The collected data were subjected to combined analysis of variance using SAS (*v*. 9.1) software (SAS Institute, Cary, NC, USA). Since the year × cultivar and year × cultivar × irrigation regime interactions were not significant, means of two years were considered. The relationships between parameters were analyzed by determining Pearson's correlation coefficients using MINITAB (*v*. 16) software. Significance of means was determined using the LSD test (P≤0.01).

Results

Gas exchange variables of different genotypes

The effect of genotype and water deficit stress was significant on all gas exchange variables. The genotype by water deficit interaction was also significant for all of these variables, except for WUE which indicate that the differences between

genotypes were not similar at different water deficit levels (Table 2).

Generally, exposure of wheat genotypes to water deficit significantly reduced the rate of photosynthesis and stomatal conductance (42% and 39% compared to the control, respectively (Table 3). Genotypes responded differently to terminal water deficit (Table 3). In the well-watered plants, the highest Pn was recorded in Zarrin cultivar (20.5 $\mu molm^{-2}s^{-1}$), whereas, *cv.* Darya showed the highest photosynthesis rate (11.9 $\mu molm^{-2}s^{-1}$) under water deficit conditions. These genotypes also showed the highest grain yield under well watered and stress conditions, respectively. The lowest and the highest reduction

of Pn was observed by 14% and 60% in *cv.* Falat and line C-85-7, respectively. Significant correlations between photosynthesis rate and grain yield were observed for both water regimes (Table 4). *Cv.* Bahar and to a lesser extent, line C-85-5 and *cv.* Parsi showed the highest g_s under well-watered conditions. Under terminal water deficit conditions, *cv.* Tabasi recorded the highest g_s, while, *cvs.* LineA and Karaj2 had the poorest performance. The mean g_s of *cv.* Falat showed the least differences between normal and stress conditions (4.5%), while the stomata closure in other genotypes were higher and ranged from 12 to 64 percent.

Table 2. Variance analysis of genotypes, irrigation treatments and their interactions on gas exchange variables of wheat genotypes

Source of variance	df	Pn	Ci	gs	gm	E	WUE
							Mean squares
Year (Y)	1	0.026ns	4.563ns	0.0008ns	0.00007ns	0.142*	28.619*
Rep. (Y)	4	0.370ns	1127.846*	0.011**	0002**	0.191**	44.117**
Irrigation (I)	1	3296.369**	425256.750**	0.471**	0.391**	16.732**	18.580*
Y×I	1	20.425ns	57.203ns	0.00003ns	0.0007ns	0.036ns	15.467ns
Error a	4	29.685	3966.886	0.0017	0.005	0.117	93.187
Genotype (G)	24	35.534**	2090.182**	0.013**	0.003**	0.167**	15.796**
I×G	24	24.084**	1563.194**	0.009**	0.002**	0.123**	9.481ns
Y×G	24	0.406ns	24.855ns	0.000056ns	0.000069ns	0.005ns	1.905ns
Y×I×G	24	0.566ns	30.286ns	0.000052ns	0.000060ns	0.007ns	2.201ns
Error b	192	2.958	344.516	0.002	0.0003	0.032	6.796
(CV) (%)		14.03	10.48	25.84	20.60	16.57	22.46

Notes: Pn: Net photosynthesis rate, Ci: Sub-stomatal CO_2 concentration, gs: Stomatal conductance, gm: Mesophyll conductance, E: Transpiration rate, WUE: Water use efficiency.
ns, *, and **: non-significant and significant at 5% and 1% probability levels, respectively.

Mesophyll conductance (g_m) also decreased significantly when exposed to terminal water deficit (Table 3). On the average, under normal conditions, genotypes showed 62% higher g_m compared to water deficit conditions. Among genotypes, *cv.* Zarrin showed significantly higher g_m than that of other genotypes under normal

conditions (Table 3), while, under water deficit conditions, *cv.* Darya exhibited the highest g_m, though it was not statistically different from *cvs.* Shiraz and Tabasi. The lowest mesophyll conductance was observed in LineA under stress condition. The effect of water deficit on sub-stomatal CO_2 concentration (Ci) of wheat

genotypes is shown in Table 3. Genotypic differences in the sub-stomatal CO_2 concentration were also significant (Table 2). The mean Ci for the stressed plants was 54% higher than those of well-watered environment. Line C-85-9 showed the highest Ci followed by LineA under water deficit conditions.

Transpiration rate (E) was significantly reduced when exposed to water deficit conditions (Tables 2 and 3). There were also obvious differences in transpiration rate between wheat genotypes (Table 3). The effect of water deficit on transpiration rate was similar to that of stomatal conductance. The highest E under water deficit conditions was recorded in *cv*. Tabasi, while, under normal conditions, *cv*. Bahar showed the highest E. Plants grown under normal condition showed higher water use efficiency than those grown under water deficit conditions. In both irrigation treatments, breeding lines showed higher WUE. Under well-watered condition, line C-85-7 and under water deficit conditions, line C-85-14 showed the highest WUE. However, no significant relationship was found between grain yield and WUE in water deficit condition (Figure 3B).

Total chlorophyll content (SPAD)

Water deficit significantly decreased chlorophyll content of all genotypes but to a different extent (Figure 1 and Table 5). Zarrin cultivar showed the highest chlorophyll content under well-watered and water deficit conditions by 50.6 and 40.6, respectively. *Cvs*. LineA and Falat by 32.1 and 38.9 recorded the lowest chlorophyll content in water-deficit and well-watered conditions,

respectively. The least reduction in the chlorophyll content was observed in *cv*. Falat by 7.6% followed with *cv*. Shiraz by 9.9% when exposed to water deficit condition. Significant positive correlation was observed between total chlorophyll content and grain yield under well-watered (r=0.91, P≤0.01) and water deficit (r=0.53, P≤0.01) conditions (Table 4).

Leaf and canopy temperature

Water deficit had significant effect on flag leaf temperature (Table 5). Water stressed plants had always higher flag leaf temperature than that of the well-watered plants during grain filling period (Figure 5). Genotypes with higher leaf temperature showed lower photosynthesis rate and stomatal conductance. Under water deficit conditions, *cv*. Karaj2 had the highest leaf temperature (37.5°C), whereas, the leaf temperature of *cv*. Bahar (35.1°C) were the lowest. Highly negative correlation coefficient was found between LT and Pn in both irrigation treatments (r=-0.72, P≤0.01 and r=-0.60, P≤0.01; under normal and water deficit conditions, respectively). Canopy temperature was also increased by water deficit during grain filling period (Figure 5). Water deficit condition increased CT between 0.4 to 3.7 °C in different genotypes. Under normal conditions, *cv*. Bahar showed the lowest CT (16.4°C) as well as the highest g_s, while, *cv*. Kavir (22.1°C) followed by *cv*. Darya (22.4°C) exhibited the lowest CT in the water deficit condition.

Table 3. Mean net photosynthesis rate (Pn), sub-stomatal CO_2 concentration (Ci), mesophyll conductance (g_m), stomatal conductance (g_s), transpiration rate (E) and water use efficiency (WUE) under well-watered and water deficit conditions

Genotype	Well-watered						Water deficit					
	Pn μmolm⁻²s⁻¹	Ci μmolm⁻²s⁻¹	g_m molm⁻²s⁻¹	g_s molm⁻²s⁻¹	E mmolm⁻²s⁻¹	WUE μmolm⁻²s⁻¹	Pn μmolm⁻²s⁻¹	Ci μmolm⁻²s⁻¹	g_m molm⁻²s⁻¹	g_s molm⁻²s⁻¹	E mmolm⁻²s⁻¹	WUE μmolm⁻²s⁻¹
Line A	11.63±1.15	144.8±8.84	0.079±0.01	0.124±0.014	1.014±0.12	11.4±1.53	6.49±0.49	245.7±4.43	0.026±0.002	0.067±0.016	0.530±0.04	13.1±2.33
C-85-14	14.32±0.71	142.3±5.03	0.094±0.007	0.166±0.03	1.173±0.11	11.5±1.13	7.49±0.71	235.7±2.23	0.031±0.002	0.105±0.02	0.493±0.05	15.3±2.62
C-85-5	18.76±0.58	130.4±6.46	0.145±0.01	0.285±0.01	1.534±0.11	12.4±1.05	10.08±1.26	201.3±14.8	0.052±0.008	0.143±0.016	0.770±0.13	13.6±3.27
C-85-7	17.95±1.06	135.7±2.13	0.135±0.01	0.252±0.04	1.323±0.05	13.6±0.96	7.37±0.63	239.0±11.1	0.031±0.004	0.092±0.01	0.587±0.07	12.6±1.73
C-85-9	16.52±1.61	138.6±10.4	0.124±0.01	0.174±0.04	1.328±0.05	12.4±0.7	7.63±1.06	247.7±13.8	0.032±0.006	0.079±0.02	0.927±0.13	10.0±2.34
C-86-2	13.12±0.78	139.3±8.04	0.095±0.009	0.161±0.04	1.243±0.05	10.6±0.58	10.74±1.16	209.0±8.05	0.052±0.007	0.135±0.006	0.823±0.21	11.8±3.45
C-86-4	18.84±0.93	134.8±8.16	0.142±0.01	0.275±0.006	1.415±0.07	13.4±0.99	8.29±0.44	231.7±17.0	0.037±0.004	0.115±0.02	0.897±0.19	10.2±3.21
M-73-6	16.93±1.40	133.3±4.67	0.128±0.01	0.234±0.06	1.447±0.09	11.7±0.79	8.10±0.73	244.7±17.5	0.034±0.005	0.087±0.01	0.770±0.13	9.6±1.71
Star	15.06±2.15	143.8±7.56	0.106±0.01	0.185±0.05	1.384±0.09	11.2±2.37	8.87±0.95	228.7±21.6	0.040±0.007	0.149±0.02	0.837±0.10	11.6±1.18
Omid	12.58±1.60	157.6±9.80	0.083±0.01	0.129±0.03	1.262±0.02	10.1±1.44	8.06±0.03	238.3±8.21	0.034±0.001	0.090±0.007	0.903±0.12	9.9±1.65
Azar	14.93±1.06	135.6±3.61	0.110±0.004	0.222±0.05	1.277±0.11	11.7±0.23	8.63±0.34	217.3±7.88	0.040±0.001	0.127±0.009	0.993±0.19	9.6±2.83
Agosta	18.39±1.06	138.3±3.71	0.133±0.01	0.259±0.01	1.421±0.05	12.95±0.35	10.12±0.71	203.7±2.50	0.050±0.004	0.130±0.01	0.937±0.05	10.3±1.15
Bahar	19.89±0.72	126.3±6.77	0.159±0.01	0.296±0.009	1.567±0.05	12.7±0.26	10.21±0.36	191.3±13.1	0.054±0.004	0.125±0.009	0.890±0.08	11.0±0.64
Parsi	19.52±1.11	124.0±12.8	0.162±0.02	0.285±0.01	1.460±0.05	13.1±0.90	10.13±0.44	202.3±5.69	0.050±0.002	0.141±0.002	0.953±0.05	11.6±1.54
Pishtaz	12.57±0.74	146.5±12.0	0.086±0.004	0.129±0.03	1.133±0.12	11.3±1.42	9.23±1.77	217.7±19.2	0.045±0.01	0.105±0.02	0.803±0.004	9.8±1.95
Jonz	16.43±1.16	133.5±12.0	0.125±0.01	0.232±0.02	1.393±0.03	11.8±1.11	8.32±0.34	220.3±5.94	0.038±0.002	0.145±0.02	1.073±0.17	10.5±2.09
Chamran	12.83±0.62	146.1±5.79	0.088±0.003	0.156±0.005	1.269±0.07	10.2±1.06	9.83±0.53	204.3±4.83	0.048±0.002	0.139±0.02	1.117±0.05	9.3±1.08
Darya	16.22±0.75	135.9±25.8	0.129±0.02	0.191±0.02	1.287±0.14	12.8±0.99	11.62±1.59	177.7±11.9	0.067±0.01	0.163±0.01	0.903±0.09	10.6±0.87
Zarrin	20.54±0.34	119.7±10.8	0.175±0.01	0.273±0.01	1.439±0.02	14.2±0.05	10.83±0.27	189.7±6.26	0.057±0.002	0.140±0.01	0.790±0.13	12.1±1.78
Shiraz	13.38±0.52	151.6±12.8	0.089±0.009	0.173±0.01	1.286±0.03	10.4±0.13	10.83±0.78	180.0±8.50	0.061±0.005	0.137±0.01	1.113±0.08	9.9±1.03
Shiroodi	13.45±2.43	145.8±12.6	0.096±0.02	0.157±0.03	1.200±0.10	11.6±2.78	8.12±1.41	231.3±14.9	0.036±0.008	0.112±0.04	0.843±0.09	9.6±0.82
Tabasi	17.39±0.35	126.2±5	0.138±0.004	0.214±0.01	1.350±0.09	13.0±0.94	11.00±1.09	189.0±10.3	0.059±0.009	0.177±0.005	1.150±0.05	9.6±1.18
Falat	11.97±0.48	149.4±1.88	0.082±0.002	0.137±0.006	1.179±0.12	10.5±1.25	10.14±0.72	192.7±19.4	0.055±0.008	0.127±0.017	0.927±0.18	11.0±2.07
Karaj 2	13.92±1.22	147.3±7.93	0.096±0.01	0.224±0.01	1.383±0.02	10.1±1.04	7.23±0.68	237.0±3.83	0.031±0.002	0.101±0.01	0.740±0.18	9.8±2.09
Kavir	13.30±1.55	154.8±12.9	0.089±0.01	0.189±0.03	1.234±0.04	10.8±1.28	10.43±0.91	182.7±11.2	0.058±0.008	0.161±0.01	1.050±0.06	10.0±0.84
LSD	4.12	25.12	0.04	0.11	0.30	3.94	3.13	49.3	0.020	0.076	0.283	4.5

LSD is given at P≤0.01

Grain yield and grain filling period

Irrigation treatments significantly affected both grain yield and GFP in all genotypes (Table 5). On the average, under water deficit condition grain yield and GFP were 45% and 23% lower than those of well-watered condition. The highest grain yield under normal conditions was obtained from *cv*. Zarrin (not significantly different from *cvs*. Parsi and Bahar), while, in water deficit condition, *cv*. Darya had the highest grain yield followed by *cv*. Tabasi (Table 6). Indeed, positive correlation was found between grain yield and photosynthesis rate under irrigated (r=0.90, P≤0.01) and water deficit (r=0.81, P≤0.01) conditions (Table 4). Under well-watered conditions, line C-85-5 and *cv*. Parsi had the longest GFP (59 d) followed by *cv*. Bahar (58 d), whereas, *cv*. Kavir showed the longest GFP (47 d)

Table 4. Pearson correlation coefficients between physiological traits and grain yield in 25 wheat genotypes under well-watered and water deficit stress conditions

	$Pn^†$	Ci	g_m	g_s	E	WUE	LT	CT	GFP	Chl
GY										
Well-watered	0.90^{**}	-0.91^{**}	0.91^{**}	0.83^{**}	0.72^{**}	0.84^{**}	-0.81^{**}	-0.82^{**}	0.72^{**}	0.91^{**}
Water deficit	0.81^{**}	-0.74^{**}	0.80^{**}	0.68^{**}	0.63^{*}	0.40^{ns}	-0.37^{ns}	-0.69^{**}	0.61^{**}	0.53^{**}

^{ns}, * and **: Non-significant and significant at 0.05 and 0.01 probability levels, respectively

† Pn: Net photosynthesis rate, Ci: Sub-stomatal CO_2 concentration, g_m: Mesophyll conductance, g_s: Stomatal conductance, E: Transpiration rate, WUE: Water use efficiency, LT: Flag leaf temperature, CT: Canopy temperature, GFP: Grain filling period, GY: Grain yield, Chl: Chlorophyll content

Table 5. Analysis of variance of the effect of genotypes, irrigation treatments and their interactions on grain yield, grain filling period, leaf and canopy temperatures and chlorophyll content of wheat genotypes

Sources of variance	df	Mean squares				
		GY	GFP	LT	CT	Chl
Year (Y)	1	6655.230*	161.333**	92.629**	8.500**	164.872**
Rep/Year	4	1237.067ns	18.706ns	17.423**	18.324**	23.747*
Irrigation (I)	1	7289260.563**	10443.00**	722.300**	1235.860**	5687.194**
Y×I	1	3260.403ns	0.0001ns	1.569ns	0.032ns	34.816*
Error a	4	54359.013	0.210	13.590**	13.171**	6.123ns
Genotype (G)	24	37129.701**	16.830**	6.970**	10.849**	86.849**
I×G	24	35405.987**	8.070**	4.238**	10.151**	24.876**
Y×G	24	1054.029ns	0.080ns	0.592ns	0.207ns	14.095*
Y×I×G	24	2037.924ns	0.090ns	0.461ns	0.227ns	8.713ns
Error b	192	1380.943	9.467	1.287	0.881	7.697
(CV) (%)		7.04	6.66	3.27	4.28	6.74

Notes: GY: Grain yield, GFP: Grain filling period, LT: Leaf temperature, CT: Canopy temperature, Chl: Chlorophyll content

ns, *, and **: Non-significant and significant at 5% and 1% probability levels, respectively

Table 6. Grain filling period and grain yield of 25 wheat genotypes grown under well-watered and water deficit conditions

Cultivar	Grain yield (gm^{-2})		GFP (Day)	
	Well-watered	Water deficit	Well-watered	Water deficit
LineA	587	280	47	32
C-85-14	698	315	55	36
C-85-5	792	379	59	41
C-85-7	760	340	47	35
C-85-9	754	334	56	39
C-86-2	658	397	54	45
C-86-4	768	306	54	39
M-73-6	702	371	50	41
Star	660	346	50	32
Omid	566	347	51	43
Azar	753	423	57	46
Agosta	735	385	55	41
Bahar	821	365	58	38
Parsi	820	351	59	42
Pishtaz	577	367	47	38
Jonz	720	338	48	37
Chamran	613	443	44	33
Darya	668	499	49	45
Zarrin	826	382	57	41
Shiraz	525	407	52	46
Shiroodi	584	384	49	37
Tabasi	768	486	55	46
Falat	534	385	49	45
Karaj 2	603	277	49	36
Kavir	588	413	47	47
LSD	77.62	67.13	6.39	6.60

LSD is given in P≤ 0.01

followed by *cv*. Shiraz (46 d) under water deficit condition. There was positive significant correlation between GFP and Chl content (0.43, P≤ 0.01), grain yield (0.61, P≤0.01) and Pn (0.64, P≤0.001) under water deficit condition.

Discussion

The results of the present work showed that there was large variability among wheat genotypes with respect to physiological traits. Post-anthesis drought stress decreased Pn and gs of 25 genotypes during grain filling period. Many researchers reported that drought stress decreases photosynthesis rate, stomatal conductance and transpiration rate during grain filling period (Subrahmanyam *et al.* 2006; Wang *et al.* 2010; Vassileva *et al.* 2011). Reduction in Pn could be attributed to both stomatal and non-stomatal limitations under drought stress condition (Misson *et al.* 2010). Stomatal closure and limitation of CO_2 diffusion into chloroplast has been reported to be the main cause of photosynthesis reduction

under mild to moderate drought stress (Flexas and Medrano 2002). Nevertheless, impairment of ATP synthesis is also reported to be a likely limitation to photosynthesis under severe water stress (Lawlor 2002).

Researchers claimed that the first response of plants to water scarcity is stomatal closure and the main limitation to photosynthesis is through such closure (Flexas and Medrano 2002). Tabasi, followed by Darya and Kavir cultivars performed better in all traits recorded under water deficit condition. High sensitivity of g_s to drought stress in wheat genotypes has also been reported by Ritchie *et al.* (1990). The close relationship between Pn and g_s (R^2=0.65) indicated the important role of stomata in photosynthesis regulation under water deficit environment (Figure 2A). We observed positive relationship between grain yield and g_s under water deficit condition (Figure 3A). Our results were in agreement with Cornish *et al.* (1991) who

reported that the high yielding cultivar of cotton showed greater Pn and g_s. There was a strong relationship between g_m and Pn (R^2=0.96) under water deficit condition (Figure 2B). While stomatal closure is the main reason for decreasing photosynthesis rate under mild to moderate drought stress, (Flexas *et al.* 2004), severe drought stress impairs photosynthesis apparatus and causes increase in sub-stomatal CO_2 concentration due to low consumption of CO_2 (Iturbe Ormaetxe *et al.* 1998). Damage to biomembrane structure was reported to be the main non-stomatal limitation to photosynthesis (Shao *et al.* 2007).

The significant negative correlation between sub-stomatal CO_2 concentration and photosynthesis rate (r=-0.93, P≤0.001) under water deficit condition suggests the impairment of such major metabolic activities as photosynthesis and reduction in CO_2 consumption. The increase in Ci under water deficit condition indicates the
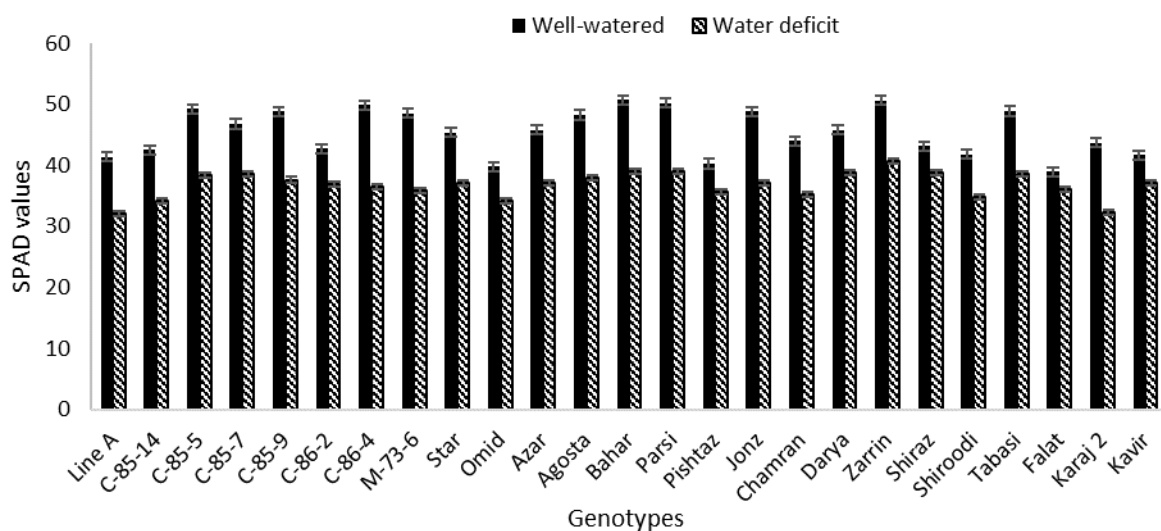

Figure 1. Total chlorophyll content (SPAD values) of genotype during grain filling period. Genotypes are arranged on the x-axis in order of overall mean values. The bar above each column is the SE (*n=9*) for comparing irrigated and non-irrigated means in each genotype
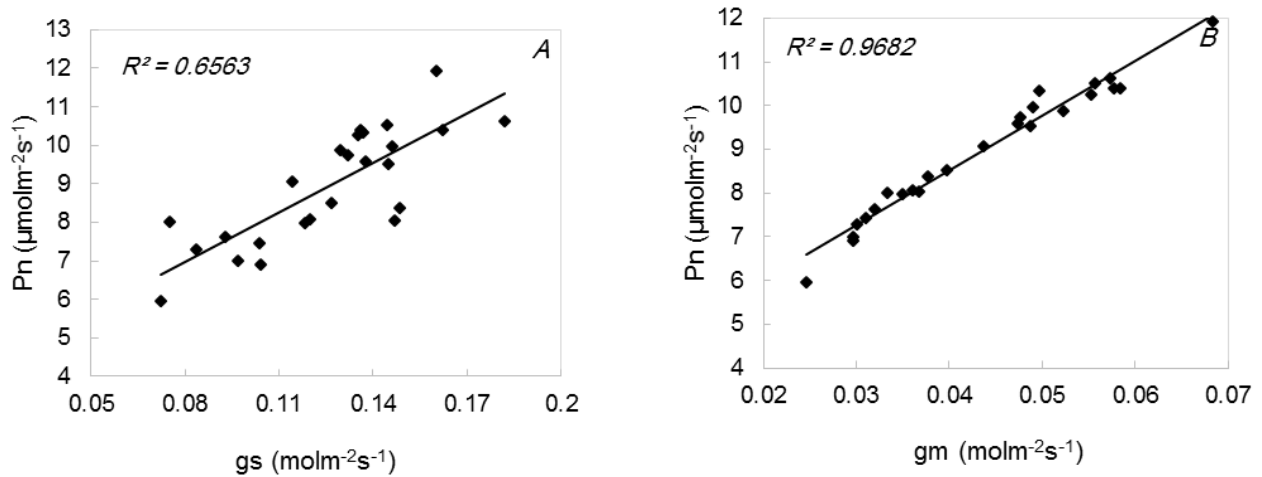
Figure 2. Scattered diagram showing the relationship between net photosynthesis rate (Pn) [μmolm^{-2}s^{-1}] and stomatal (g$_s$) [molm^{-2}s^{-1}] (*A*) and mesophyll (g$_m$) [molm^{-2}s^{-1}] (*B*) conductance of 25 wheat genotypes in response to drought stress. Values represent the means for nine measurements (three measurements in each replication) made on flag leaf

predominance role of non-stomatal limitations (mesophyll resistance) to photosynthesis (El Hafid *et al.* 1998). According to Flexas and Medrano (2002), plants generally demonstrate greater non-stomatal limitations to Pn under severe water stress. Reduction of chloroplast activity due to lower RuBP carboxylation efficiency, RuBP regeneration and Rubisco content has also been noted by some authors as possible reasons of non-stomatal limiting factors to net photosynthesis rate (Mediavilla *et al.* 2004; Demirevska *et al.* 2008; Misson *et al.* 2010). According to our results, higher reduction in g$_m$ (62%) compared to g$_s$ (39%) under water deficit environment indicates that g$_m$ was likely the main factor in photosynthesis regulation, as also previously being noted by other workers (Siddique *et al.* 2000; Mafakheri *et al.* 2010).

Water deficit resulted in lower stomatal conductance, lower transpiration rate and lower photosynthesis rate. Higher grain yield was associated with higher stomatal conductance and higher transpiration rate. The major role of transpiration is leaf cooling, therefore, higher transpiration rate supplies higher stomatal conductance and results in greater photosynthesis rate and longer crop growth duration (Fischer *et al.* 1998). Water deficit led to WUE depression, but not necessarily in all genotypes. Some genotypes such as C-85-14 and C-85-5 showed improved WUE under water deficit condition which could be related to lower transpiration rate under such condition (Table 3). In the present investigation, no significant correlation was obtained between grain yield and WUE in water stress environment (Table 4). Higher transpiration
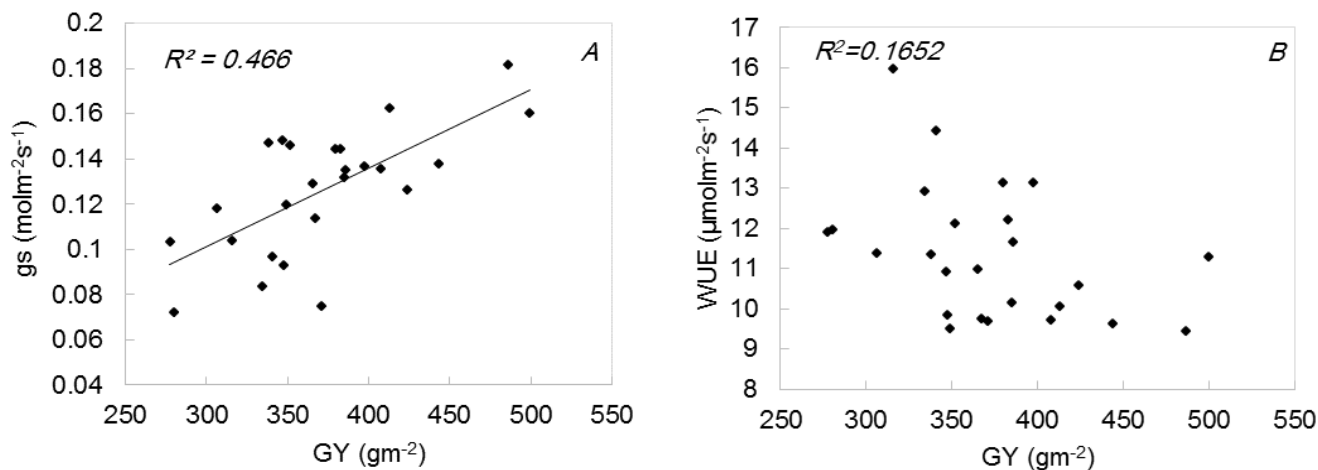
Figure 3. Scattered diagram showing relationship between grain yield (GY) [gm^{-2}] and stomatal conductance (g$_s$) [molm^{-2}s^{-1}] (*A*) and water use efficiency (WUE) [µmolm^{-2}s^{-1}] (*B*) of 25 wheat genotypes in response to drought stress. Values represent the means for nine measurements (three measurements in each replication) made on flag leaf

rate is associated with higher water uptake from the soil and better crop water relations, which could be a positive selection tool for higher yield potential and better adaptation for stressed-prone environments (Reynolds *et al.* 2001).

It is often thought that a better WUE results in higher grain yield under water stress conditions, however, it might not be true in many cases. Blum and Sullivan (1983) [unpublished data, reviewed in Blum (2005)] found that higher biomass production under water stress condition was associated with relatively lower WUE. Similarly, Munoz *et al.* (1998) reported that higher yield production under water deficit condition was mainly associated with higher water use and therefore, lower WUE. WUE is often considered as parallel to drought resistance without bearing in mind that this is a ratio between two physiological

(net photosynthesis and transpiration rate) parameters (Blum 2005). Lower g$_s$ under water deficit conditions could be beneficial due to higher transpiration efficiency to decrease water use and to store water for such critical stages as grain filling period (Kirkegaard *et al.* 2007). Higher WUE could be achieved by lowering stomatal conductance, however, Fischer *et al.* (1998) reported that increase in photosynthesis rate of new wheat cultivars was in the expense of higher stomatal conductance. Kobata *et al.* (1996) demonstrated that higher WUE in rice was associated with lower transpiration rate. According to Araus *et al.* (2007), it seems that increase in WUE is more associated with reduction in stomatal conductance, under which the growth rate might be reduced and may result in grain yield reduction.

Post-anthesis drought stress accelerated chlorophyll degradation in wheat genotypes. 'Stay-green' during the post-anthesis period is an efficient drought-tolerance criterion in crops. Genotypes having the ability to maintain greater green leaf area duration (stay green traits) during grain filling are potential candidates to assure yield production in semi-arid regions (Hoang and Kobata 2009). Many studies have demonstrated a genetically determined correlation between yield and flag leaf area duration (Gregersen *et al.* 2008). In this study, we also observed highly positive correlation between grain yield and total chlorophyll content under both conditions. Borrel *et al.* (2000) concluded that higher leaf chlorophyll concentration was associated with postponed senescence in sorghum. Xu *et al.* (2000) suggested that under severe post-anthesis drought conditions, the stay green lines of sorghum would have higher SPAD values and higher total chlorophyll concentration. They also reported that total chlorophyll per leaf area (mg cm^{-2}) showed a near linear relationship with the SPAD values ($R^2=0.91$), suggesting the profitability and accuracy of SPAD values to determine the total chlorophyll content of leaves.

Maintenance of greater green leaf area during mid to late grain filling period was related to a greater grain yield (Figure 4A) and higher net photosynthesis rate (Figure 4B) under post-anthesis water deficit conditions. Generally, senescence is characterized by chlorophyll loss and a progressive decline in photosynthetic capacity (Xu *et al.* 2000). Early onset of senescence affects assimilation rate and grain filling in crops, therefore, genotypes with late-

onset of chlorophyll degradation might have potential to produce higher yield under drought prone environments.

There was significant correlation between LT and g_s under well-watered and water deficit conditions (r=-0.74, P≤0.001; r=-0.43, P≤0.01, respectively). As a consequence of stomatal closure, leaf temperature would be increased due to less transpiration rate. Winter *et al.* (1988) also found significant differences in term of leaf temperature among drought stressed and well-watered plants. Increased leaf temperature as a result of stomatal closure is mainly due to radiation load on the canopy (Condon *et al.* 2004). When stomata are closed, leaf temperature would be increased and higher canopy temperature impairs photosynthesis rate (Reynolds *et al.* 2012). It is reported that high yielding genotypes have cooler canopies under stress conditions conditions (Pinter *et al.* 1990). In the present study, we also found that high-yielding genotypes under water deficit condition had cooler canopies. Indeed, positive correlation between GY and g_s (r= 0.68**) under water deficit condition indicates the importance of these variables in the selection of high-yielding and drought-tolerant genotypes. This is in agreement with Fischer *et al.* (1998) which found positive correlation between grain yield and g_s under different irrigation conditions in wheat plants. Siddique *et al.* (2000) reported that the increase in leaf and canopy temperature exposed to water deficit is likely due to higher respiration and lower transpiration rate resulting from stomatal closure.

Genotype × irrigation interaction had significant effect on grain yield and GFP. This

interaction was more pronounced in some genotypes such as *cv*. Shiraz which had short GFP under normal irrigation, while, under water deficit condition showed the greatest GFP. The opposite result was observed for the *cv*. Bahar that showed

the longest GFP (together with line C-85-5 and cv. Parsi) under normal condition and the lower than average GFP under water deficit condition. A positive significant correlation between GFP and GY (r=0.61, P≤0.01) (Table 4) and Pn (r=0.64,
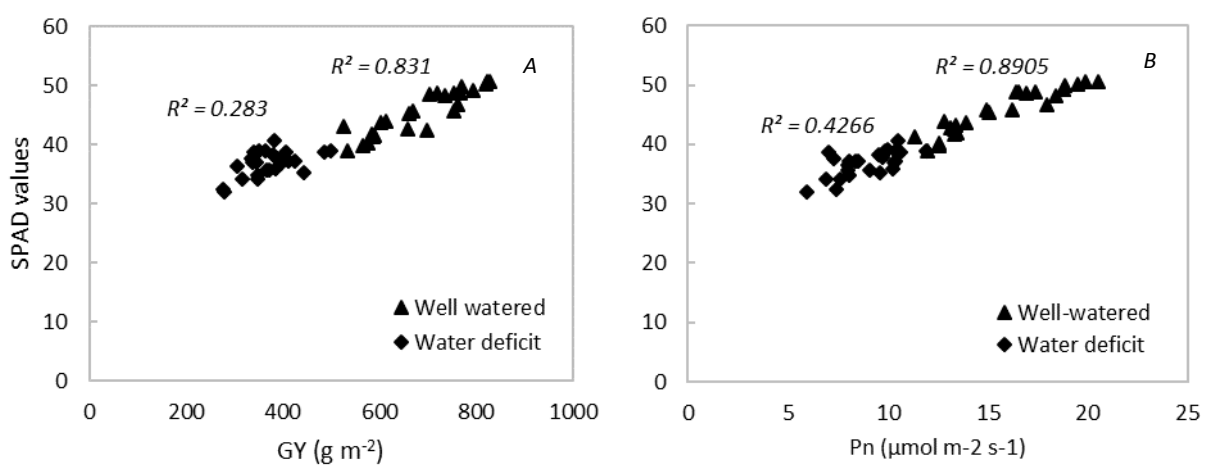

Figure 4. Scattered diagram showing relationship between SPAD values and grain yield (GY) [g m^{-2}] (*A*) and net photosynthesis rate (Pn) [μmolm^{-2}s^{-1}] (*B*) of 25 wheat genotypes under well-watered and water deficit conditions. Values represent the means for nine measurements (three measurements in each replication) made on flag leaf
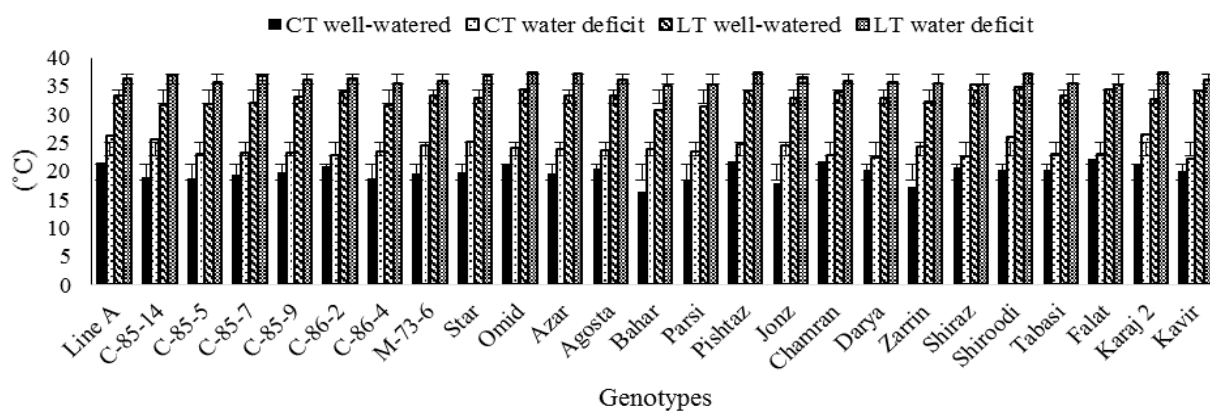

Figure 5. Leaf temperature (LT) [℃] and canopy temperature (CT) [℃] for genotypes during grain filling period. Genotypes are arranged on the x-axis in order of overall mean values. The bar above each column is the SE (*n=9*) for comparing irrigated and non-irrigated means in each genotype

P≤0.01) under water deficit condition shows the importance of longer photosynthesis duration under drought stress, which can lead to increase in grain weight as well as grain yield. Our results for wheat agree with those reported by Gonzalez *et al.* (2010) for barley which found that genotypes with greater photosynthetic activity were those better tolerated to drought and produced higher yields under water deficit conditions. The least differences in GFP between normal and water deficit conditions in such cultivars as Darya and Kavir might be beneficial to produce reliable yield under such conditions. Indeed, maintaining the photosynthetic activity during GFP allowed these cultivars to stabilize their yields under stress condition. Grain yield reduction due to post-anthesis water deficit has been previously reported by Gooding *et al.* (2003) and Ahmadi-Lahijani and Emam (2013). Higher yield of new maize hybrids has been reported to be due to longer photosynthetic activity over the GFP (Ding *et al.* 2005). Maintaining photosynthetic activity postpones senescence and increases GFP and grain yield (Reynolds *et al.* 2000; Ding *et al.* 2005).

One of the efficient ways to reduce negative impacts of water scarcity on crop productivity and function is to compare genotypes based on physiological parameters under both stress and non-stress conditions. In the present study, wheat genotypes were examined using physiological parameters under two water regimes during two growing seasons. Genotypes with higher chlorophyll content under water deficit condition showed higher photosynthetic activity and grain yield, indicating the importance of lengthening of grain filling period in translocation of higher assimilates from photosynthetic parts to reproductive organs. Net photosynthesis rate, stomatal and mesophyll conductance showed highly positive correlations with grain yield. Indeed, genotypes with higher grain yield under water deficit condition exhibited greater photosynthesis rate along with higher stomatal and mesophyll conductance. Therefore, based on these results, higher values of these criteria under water deficit conditions could be appropriate physiological indices to screen drought-tolerant wheat genotypes. The present work highlighted the potential of physiological parameters to select wheat genotypes best fit to water deficit conditions. Furthermore, *cvs.* Tabasi and LineA were identified as the most tolerant and sensitive genotypes to post anthesis water deficit stress, respectively.

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