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Morphological and physiological attributes associated to drought tolerance of Ethiopian durum wheat genotypes under water deficit condition

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Abstract

The experiment was conducted to assess the differential morpho-physiological response to stimulated water deficit and to determine the relationship between some of these morphological and physiological traits and yield components of eighteen durum wheat genotypes grown in pots under lathhouse condition. Water deficit significantly affected gas exchange and chlorophyll fluorescence parameters. It reduced the net photosynthesis rate (P_n), transpiration rate (E) and stomatal conductance (g_s) measured both at anthesis and grain-filling stages. Similarly, the value of initial fluorescence (Fo) was increased while variable fluorescence (Fv), maximum fluorescence (Fm) and optimum quantum yield fluorescence (Fv/Fm) were decreased under water deficit. RWC of the leaves was decreased by 36.7% while SLA increased by 12.6% due to moisture stress relative to the well-watered control. No significant correlations were found between chlorophyll fluorescence parameters and grain yield under water deficit condition. Similarly, no significant correlations were found between leaf gas exchange parameters and grain yield. On the other hand, peduncle length and excursion were positively correlated with grain yield while negatively correlated with drought susceptibility index under water deficit condition. Leaf posture and rolling had also a profound effect on grain yield and other attributes. Erect-leaved genotypes had more grain yield, HI, kernel numbers per spikelet and grain-filling rate but had lower kernel weight than droopy leaved. Similarly, genotypes exhibited strong leaf rolling under water deficit condition had more grain yield, kernel numbers per spike and water use efficiency. The genetic variability found for leaf posture, leaf rolling, peduncle length and excursion among the Ethiopian durum wheat genotypes suggests the opportunity for selection superior and adapted genotype in water-limited environments. These can be achieved by integrating these morphological traits as indirect selection in conjunction with other yield components.

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Introduction

Drought is one of the most common environmental stresses that limit durum wheat production in drought-prone areas of Ethiopia. Changes in global climate are forecast to increase the extension of drought-prone areas. A viable solution for crop production in these areas is to develop drought tolerant varieties. A physiological approach would be the most attractive way to develop new varieties (Araus et al., 2008), but breeding for specific, sub-optimal environments involves a deeper understanding of yield-determining process.

Photosynthesis, which is the most significant process influence crop production, is also inhibited by drought stress. Studies have shown that the photosynthetic rate (P_n) of leaves of both C_3 and C_4 plants decreases as relative water content (RWC) and water potential (Ψ) decrease (Cornic and Massacci, 1996). Limitation of net photosynthetic rate in low moisture stressed plant is mainly through stomatal closure (Cornic and Massacci, 1996; Cronic, 2000) and/or by metabolic impairment (Flexas and Medrano, 2002). The relative magnitude of stomatal and non-stomatal factors limiting photosynthesis depends on the severity of drought. High photosynthetic rate is considered to be one of the most important breeding strategies for crop improvement (El Hafid *et al.*, 1998; Richards, 2000). However, selection for higher rates of leaf photosynthesis has not generally improved the yield in favorable environments, most probably because the source is less limiting than the sink (Abbad *et al.*, 2004) and greater success might be expected for higher rates under water stress.

Chlorophyll fluorescence analysis may also provide a sensitive indicator of stress condition in plants. It can also be used to estimate the activity of the thermal energy dissipation in photosystem II, which protects photosytems from the adverse effect of light and heat stress. The measurement of chlorophyll fluorescence *in situ* is a useful tool to evaluate the tolerance of the photosynthetic apparatus to environmental stress (Maxwell and Johnson, 2000). Dark-adapted values of Fv/Fm reflect the potential quantum efficiency of PSII and are used as a sensitive indicator of photosynthetic performance, with optimal values of around 0.832 measured from most plant species (Johnson *et al.*, 1993). Values lower than this are measured when the plant is exposed to stress, indicating a particular phenomenon of photo-damage to PSII reaction centers, and the development of slowly relaxing quenching process (Maxwell and Johnson, 2000; Baker and Rosenqvist, 2004) which reduce the maximum efficiency of PSII photochemistry.

There are also several approaches to investigate morphological traits for the purpose of increasing yield under water-limited conditions. Leaf rolling, induced by loss of turgor and poor osmotic adjustment represents an important drought-avoidance mechanism (Richards, 1996). Under drought condition, leaf rolling decreased stomatal closure (O'Toole *et al.*, 1979). The erectophile leaf canopy has been also proposed as a trait that could increase crop yield potential by improving radiation use efficiency in high radiation environments (Reynolds *et al.*, 1999). Peduncle length has been also suggested as useful indicator of yield capacity in dry environments. Kaya *et al.* (2002) have been found a strong positive correlation between peduncle length and grain yield. In other cases, such relationship has been found inverse (Briggs and Aytenfisu, 1980) or no relationship (Villegas *et al.*, 2006) depending on the environment.

The effect of water stress on the yield and yield components of durum wheat at different growth stages have been the subject of many studies (Simane *et al.*, 1993; Solomon *et al.*, 2003). However, no study had been conducted on the physiological and morphological response of diverse Ethiopian durum wheat genotypes to water deficit conditions.

Therefore, the present study aims to determine water deficit effects on leaf gas exchange and chlorophyll fluorescence parameters in leaves of eighteen durum wheat (*Triticum tugidium L. durum*) genotypes and to determine the relationship between some morphological and physiological traits and yield under water deficit conditions.

Materials and methods

Plating materials

The study was conducted in a lathhouse at Sinana Agricultural Research Center (SARC) during the 2006/07 main season. It is located at 7° 7'N latitude, 40° 10' E longitude and 2400 m.a.s.l altitude in Bale Zone of Oromia Region, Ethiopia. To embrace the variability existing among the Ethiopian durum wheat genotypes, three landrace [B5-5B, S-17B, and WA-13], thirteen commercial cultivars [Asassa, Bekelcha, Boohai, Egersa, Foka, Gerardo, Ilani, Kilinto, Obsa, Oda, Quamy, Tob-66 and Yeror] and two advanced lines from the breeding program [CDSS93Y107 and CD94523] were used for this study. The examined genotypes are different in genetic background, origin and several characteristics.

Experimental design

Plants were grown in 21 cm diameter and 18 cm length plastic pots filled with a textural class of clay (49.7% clay, 27.3% silt and 23% sand). Each pot was filled with 4 kg uniformly air-dried soil (17.1% moisture). The field capacity and permanent wilting point of the soil were 47.8% and 11.5%, respectively. Pots were arranged in Randomized Complete Block Design (RCBD) in factorial combination of the eighteen genotypes and four water regimes with three replications. A total of 216 pots, 12 pots were assigned to each genotype. 2g N and 2 g P_2O_5 fertilizers were applied to each pot during planting and additional 0.5 g N was applied at the first tillering. Planting was done on August 10, 2006. Eight seeds were sown per pot and the seedlings were thinned to four at two leaf growth stages. Five hundred ml of water was added to each pot every other day for a period of a month until the plants reach four leaf growth stages.

Water deficit treatment

Following the Zadock's scale [Zadock *et al.*, 1974], plants were subjected to water stress at different growth stages: stress continuously from tillering to physiological maturity (M1), stress from anthesis to physiological maturity (M2), and stress from grain-filling stage to physiological maturity (M3) and well-watered control (C) treatments. The water levels were maintained in the range of 35-50% field capacity in the stress treatments while above 75% in the control treatment. These water stress conditions are designed to simulate the environments that experience very low water supply after crop establishment in different parts of the country. During the stress period, plants were left without water for 12 days by withholding irrigation until early morning wilting is observed. Then pots were weighted and irrigated until the weight of every pot became equal to the weight of the predetermined water level. The amount of water depleted from pots was obtained by weighing pots every two to three days, and the loss in weight was restored by watering pots with the amount of water equal to the loss in weight.

Gas exchange parameters

Gas exchange was measured on the flag leaves of fully-grown stressed and unstressed of the main tiller of three plants per pot using a portable photosynthesis system (CI-301PS CO_2 Gas Analyzers, CID Inc., 1996, USA). The traits measured were net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (E), and internal carbon dioxide concentration (Ci). In addition, apparent mesophyll conductance (the conductance of CO_2 from the stomatal cavity to the chloroplast) was determined as the ratio P_n and Ci following the procedure of Fischer *et al.* (1998) to produce information on the relative importance of the mesophyll limitation to P_n. Instantaneous water use efficiency (iWUE) was also calculated as the ratio of net photosynthesis rate to transpiration rate. Measurements were made on non-overcast days at 9:00 - 11:00 am at anthesis and grain filling stages.

Relative water content (RWC)

RWC was measured from flag leaves at anthesis. Immediately after cutting at the base of lamina, leaves were sealed within plastic bags and transferred to the laboratory. Fresh weight (FW) was measured after excision and the full turgid weight (TW) after the dehydration of the leaves by placing them in a test tube containing 100 ml distilled water for 24 h at room temperature (about 21°C). After soaking, leaves were carefully blotted dried with tissue paper. Dry weight (DW) was measured after oven drying at 70 °C for 72 hrs. The RWC was calculated from the equation given by Schonfeld *et al.* (1988) as: RWC = (FW-DW)/ (TW- DW) x 100, where FW, TW and DW are fresh, turgid, and dry weight of the leaf, respectively.

Specific leaf area (SLA) and kernel ash content (G_aM_a)

SLA of the flag leaf was also determined as the ratio of leaf area (cm^2) to dry mass (g). Kernel ash content which is expressed in dry weight basis (%), was determined from the kernel after complete combustion of the grain powder at 575 °C for 16 h (until light gray ash was obtained) in a muffle electric furnace and then the weight the residue was recorded.

Chlorophyll fluorescence

The polyphasic rise of fluorescence transients was measured at tillering, anthesis and grain filing stage using a portable Plant Efficiency Analyzer (PEA, Hansanthech, UK) from three leaves per pot. The initial fluorescence (Fo) and maximal fluorescence (Fm), variable fluorescence (Fv) and maximal photochemical efficiency of PSII (Fv/Fm) were measured immediately after dark-adapted the leaves for 30 minute using leaf clips provided with PEA. The transients were induced by red light of 3000 μmol m^{-2} s^{-1} provided by an array of six light emitting diodes (peak 650 nm). Measurement was performed during day time (between 10:00 and 4:00).

Morphological parameters

Rolling of leaves under stressed condition was also assessed visually using 1 to 3 scale (1 = no rolling of leaf, 2 = intermediate rolling and 3 = complete rolling) and leaf posture was assessed visually using 1 to 3 scale (1 = erect, 2 = semi-erect and 3 = droopy). Peduncle length (cm) was determined as average height of peduncle from the last node of the main stem to the initial tip of the spike and peduncle excursion was measured as the distance from the flag leaf ligule to the base of spike from four plants per pot.

Yield and yield component

Data were also collected for number of kernels per spike, 100 kernel weight, spike length, air-dried aboveground biomass and grain yield per plant. Harvest index was determined as the proportion of grain yield to the overall aboveground biomass per plant.

Crop water use efficiency

WUE was determined as the ratio of grain yield to seasonal plant water use. The seasonal water use was obtained by summing up the difference in soil water between measurements just before and after irrigation of each pot.

Statistical data analysis

The data were subject variance analysis using SAS GLM procedure release 8.02 (SAS Institute Inc., 2001). Means comparisons were carried out to estimate the differences between water deficit treatments and genotypes using Duncan's Multiple Range Test. Linear correlation analyses were used to determine the association between grain yields, physiological and morphological traits using SPSS.

Results

Leaf gas exchange parameters

The photosynthetically active radiation (PAR) level during measurement both at anthesis and grain-filling stages was greater than the saturating photosynthetic capacity of wheat crop (i.e. 1500 µmol m^{-2} s^{-1}) (Blum, 1990). Water deficit treatment both at anthesis and grain-filling stages significantly affected the gas exchange parameters. The reduction was more pronounced at grain-filling stage. P_n and E were reduced by 30.2% and 52.3% due to the stress induced at anthesis as compared to the control treatment, respectively. It reduced by 59.0% and 71.7% as compared to the control treatment at grain-filling stage, respectively (Table 1). Similarly, the stomatal conductance was reduced by 44.2% while instantaneous water use efficiency was significantly increased as compared to the control at both stages. An increase in iWUE could be due to more reduction in E than P_n by water deficit. The ratio of intercellular to ambient CO_2 concentration (Ci:Ca) and the internal CO_2 concentration were not affected by water deficit induced at anthesis and grain-filling stages (Table 2). Variability for net photosynthesis rate, stomatal and mesophyll conductances and instantaneous water use efficiency was observed among genotypes both at anthesis and grain-filling stage (Table 2).

No significant correlations were found between net photosynthesis rate and stomatal conductance and photosynthesis and transpiration rates (at both stages under stress and well-watered conditions. Similarly, no overall correlations were found between P_n and g_s and P_n and Ci:Ca both under stress and well-watered conditions. However, strong correlations were noted between net photosynthesis rate and mesophyll conductance under both stress (r = 0.603, P<0.001) and well-watered (r = 0.902, P<0.001) conditions.

Table 1. Mean value of photosynthetically active radiation (PAR), net photosynthetic rate (P_n), transpiration rate (E), stomatal conductance (g_s) and instantaneous water use efficiency (iWUE) of durum wheat genotypes grown under water deficit and well-watered conditions during anthesis and grain-filling stages.

Growth stage	***PAR*** *(µmol m^{-2} s^{-1})*	***P_n*** *(µ mol m^{-2} s^{-1})*		***E*** *(mmol m^{-2} s^{-1})*		***gs*** *(mmol m^{-2}s^{-1})*		***iWUE*** *(µ mol /mmol)*	
		S	**C**	**S**	**C**	**S**	**C**	**S**	**C**
Anthesis	1994.8	8.1	11.6	0.42	0.88	109.1	195.5	28.8	15.8
Grain-filling	2084.8	4.3	10.5	0.15	0.53	43.9	115.8	33.5	23.5
Mean	**2039.8**	**6.2**	**11.1**	**0.29**	**0.71**	**76.5**	**155.7**	**31.2**	**19.7**

S-Stress, C-Control

Relative water content, specific leaf area and ash content

The mean values of the RWC across genotypes under well watered and water deficit treatments were 87.6% and 55.7%, respectively. Water deficit caused a 36.7% reduction of the RWC of the leaves but genotypes were varied in maintaining their RWC under both water deficit and well-watered conditions. Specific leaf area (SLA) was significantly influenced by water regimes and genotypes but not by their interaction (Table 2). The SLA of all the genotypes was increased under water deficit by an average value of 12.6% relative to the well-watered treatment. Mean ash accumulated (on dry mass basis) in the kernels in the M1 treatment was higher by 70.1% compared to the well-watered treatment. However, the mean values of the grain ash content in the M2 and M3 treatments were lower than the values in the C (data not shown). Kernel ash content was negatively and significantly correlated with grain yield and WUE under the prolonged water deficit induced at tillering and anthesis stages.

Table 2. Analysis of variance showing the mean squares relative water content, specific leaf area and leaf gas exchange characteristics of durum wheat genotypes grown under water deficit and well-watered conditions measured anthesis and grain-filling stages.

Variables	***Mean squares*** Water deficit (M) (*df* = 1)	Genotypes (G) (*df* =17)	M x G (*df* =17)	Error (*df* =68)	*CV (%)*
RWC	2.74627***	0.0151***	0.0085***	0.0025	7.0
SLA	17449.81***	3909.75***	704.42NS	832.33	12.9
P_n	317.8***	8.17***	8.34***	2.57	16.4
E	5.717***	0.09NS	0.103NS	0.092	46.4
g_s	201716.4***	2413.6***	2197.6	746.5	17.9
m_{gs}	0.0065***	0.0003**	0.0004**	0.00013	26.8
Ci	860.6NS	2390.4NS	1766.3NS	2481.8	21.4
Ci:Ca	0.016NS	0.0188NS	0.013	0.02	20.3
iWUE	4601.3***	393.9***	556.5***	117.9	48.6
Grain- filling stage					
P_n	1014.3***	5.22**	6.37***	2.24	20.1
E	3.79***	0.055**	0.047**	0.022	44.1
g_s	137316.7***	552.7NS	736.1NS	497.6	28.0
m_{gs}	0.021**	0.00016NS	0.0003*	0.00012	31.3
Ci	411.7NS	2028.9NS	2488.0NS	1575.0	18.2
Ci:Ca	0.0045NS	0.0158NS	0.019NS	0.013	18.4
iWUE	1510.5*	293.8NS	455.7NS	329.0	58.6

NS, *, ** and *** means non- significant and significant at 5%, 1% and 0.1% level of probability, respectively.

Chlorophyll fluorescence parameters

Water deficit treatment induced at tillering stage increased the Fo value by 10.9% and lowered the Fv, Fm and Fv/Fm values by 12.3%, 6.2% and 6.4%, respectively as compared to the control treatment (Table 3). Similarly, at grain filling stage, water deficit treatment had significantly reduced the value of Fv, Fm and Fv/Fm. However, water stress at anthesis had no significant effect on all fluorescence parameters except on Fo (Table 4) in which the value of Fo was increased under water deficit condition. Deferential sensitivity of chlorophyll fluorescence parameters to water deficit was also observed among the tested genotypes measured at tillering, anthesis and grain-filling stages (Table 4).

Leaf posture and rolling

The numbers of genotypes with erect, semi-erect and droopy-leaved were 6 (33.3%), 2 (11.1%) and 10 (55.6%), respectively. Of these, 50% of the genotypes did not exhibit leaf rolling and only 22 % and 27% showed intermediate and a strong leaf rolling. Based on orthogonal contrast analysis, both leaf posture and rolling had significant effect on yield and some other yield attributes. Erect-leaved genotypes exhibited 9.4% and 9.1% more grain yield and harvest index, respectively than the droopy genotypes (Table 5). However, the two groups were not significantly different in the number of kernels per spike. Kernel weight and aboveground biomass were 8.8 and 12.0% higher in droopy genotypes than in erect genotypes. Conversely, erect-leaved genotypes had 4.7, 7.3 and 6.3% greater number of kernels per spikelet, WUE_G and grain filling rate than droopy genotypes, respectively.

Table 3. Mean values of chlorophyll fluorescence parameters of 18 durum wheat genotypes grown under water deficit and well-watered conditions at three growth stages**.**

Growth	*Fo*		*Fv*		*Fm*		*Fv/Fm*	
	Stress	**Control**	**Stress**	**Control**	**Stress**	**Control**	**Stress**	**Control**
Tillering	810.8	730.8	2271.1	2590.3	3105.8	3310.3	0.73	0.78
Anthesis	703.9	689.7	2477.1	2423.7	3126.5	3108.9	0.77	0.78
Grain Filling	939.0	940.3	1828.2	2273.3	2807.3	3211.5	0.66	0.73
Mean	**817.9**	**786.9**	**2192.1**	**2429.1**	**3013.2**	**3210.2**	**0.72**	**0.76**

*Fo = initial Fluorescence, Fv = Variable fluorescence, Fm = Maximum fluorescence and Fv/Fm = the ratio of variable to maximum fluorescence (Optimum quantum yield).

Genotypes that exhibited strong leaf rolling had 21.1% more grain yield than genotypes exhibited no leaf rolling (Table 5). Similarly, those genotypes showing strong leave rolling had more number of kernels per spike (10.2%), grain filling rate (17.7%) and WUE_G (16.5%). However, biomass yield, kernel weight and HI were not significantly different between genotypes that showed strong leaf rolling and no rolling. Positive significant relationships were found between leaf rolling and WUE_G ($r = 0.37^*$), and leaf rolling and grain yield ($r = 0.33^*$). However, the relationships with kernel ash were negative and non-significant ($r = -0.15^{ns}$). Generally, leaf rolling exhibited a significant positive effect on grain yield and WUE under stress condition.

Table 4. Analysis of variance showing the mean squares for chlorophyll fluorescence parameters of durum wheat genotypes grown under water deficit and well-watered conditions measured at tillering, anthesis and grain-filling stages.

	Mean squares				
Variables	Water deficit (M) (*df* = 1)	Genotypes (G) (*df* =17)	M x G (*df* =17)	Error (*df* =67)	*CV (%)*
		Tillering stage			
Fo	22751.59***	20280.06***	19548.51***	2523.86	6.5
Fv	2602847.1***	261043.34***	73915.15NS	82096.02	11.8
Fm	1038783.03***	220017.15***	82577.49NS	65943.75	8.0
Fv/Fm	0.0609***	0.00419***	0.00301***	0.000549	3.10
		Antheis Stage			
Fo	5431.66*	6590.52***	5959.04***	1534.67	5.6
Fv	76860.27NS	268792.69***	70811.79NS	45378.22	8.7
Fm	123178.77NS	207916.02***	46393.53NS	59124.51	7.7
Fv/Fm	0.00028NS	0.0034***	0.00184***	0.00033	2.4
		Grain-filling stage			
Fo	28.1NS	19204.04**	17090.0*	6829.86	8.8
Fv	2915589.27***	314881.75*	179519.8NS	139619.37	18.0
Fm	2658279.9***	346278.71***	211094.81*	108036.4	10.8
Fv/Fm	0.451***	0.10544*	0.00387NS	0.00289	7.9

NS, *, ** and *** means non- significant and significant at 5%, 1% and 0.1% level of probability, respectively.

Correlation studies

The relationship between leaf gas exchange parameters and yield and aboveground biomass at anthesis and grain-filling stages under water deficit is given in Table 6. No significant correlations were found between P_n and grain yield and between P_n and biomass yield at both stages under water stressed condition (Table 6). Similarly, there was no relationship between grain yield and the internal to ambient CO_2 concentration ratio at both anthesis and grain-filling stages. However, significant positive relationship was found between Ci:Ca and biomass at grain-filling stage.

The correlation analysis between chlorophyll fluorescence parameters and yield and biomass yield at three growth stages under water deficit conditions is given in Table 7. Fo was positively correlated with yield and aboveground biomass at all stages but correlation was significant at tillering stage only. The correlation between grain yield and Fv/Fm was negative at tillering stage while it was positive at other stages.

Table 5. Effect of leaf posture and rolling on grain yield and yield component of durum wheat genotypes grown under water deficit condition during anthesis**.**

Leaf posture	^{a}GY	Biomass	HI	KSPKL	KWT	WUE_G	KSPK	GFR
Leaf posture								
Erect	1.92	4.09	0.44	2.34	3.66	0.82	32.97	40.32
Semi-erect	1.52	4.22	0.39	2.15	3.82	0.75	29.30	35.05
Droopy	1.74	4.45	0.40	2.23	4.10	0.76	32.56	37.80
Leaf rolling								
Strong	1.99	1.99	0.40	2.30	4.10	0.85	33.31	43.00
Intermediate	1.96	2.41	0.39	2.60	3.38	0.85	35.10	39.20
No leaf rolling	1.57	2.00	0.39	2.10	4.10	0.71	29.90	35.40

^{a}GY= grain yield (g $plant^{-1}$), Biomass = aboveground biomass yield (g $plant^{-1}$), HI = harvest index, KSPKL= numbers of kernels per spikelet, KWT= 100-kernel weight, WUE_G= water use efficiency, KSPK = numbers of kernels per spike and GFR = grain filling rate.

The relationship between grain yield and yield components with peduncle length and excursion in the M1 water regime is given in Table 8. Peduncle length and excursion were positively and significantly correlated with grain yield per plant. The relationships between these morphological traits and biomass yield and as well as with HI were positive and significant. Similarly, the correlation between peduncle excursion and kernel weight was positive and strong. A significant positive correlation was also observed between number kernels per spike and both peduncle length and peduncle excursion (Table 8).

A significant and positive correlation could be established between peduncle length and WUE and between peduncle excursion and WUE (Table 8). The relationships between peduncle length and grain ash content was significantly negative. Similarly, negative significant correlations were noted between peduncle excursion and grain ash content. Correlation between peduncle length and drought susceptibility index (S) was significantly negative (Table 8).

Table 6. Relationship between leaf gas exchange parameters, grain yield and aboveground biomass of durum wheat genotypes grown under water stressed condition at anthesis and grain-filling stages.

Character[a]	***Anthesis stage***		**Grain-filling stage**	
	Grain yield	Aboveground biomass	Grain yield	Aboveground biomass
P_n	-0.315	-0.443	0.178	0.087
E	0.050	0.010	0.199	0.195
iWUE	-0.196	-0.068	0.053	-0.198
g_s	-0.028	-0.112	-0.380	0.082
m_{gs}	-0.378	-0.337	-0.014	-0.204
Ci:Ca	0.020	0.029	0.080	0.484*

*P < 0.05, [a] P_n = Net photosynthetic rate, E = Transpiration rate, g_s = Stomatal conductance, m_{gs}= Mesophyll conductance, iWUE = Instantaneous water use efficiency Ci:Ca = Internal to atmospheric carbon dioxide ratio.

Table 7. Correlation coefficients of the relationship between chlorophyll fluorescence parameters, yield and biomass yield of durum wheat genotypes grown under water deficit conditions at three growth stages.

Character[a]	**Tillering stage**		**Anthesis stage**		**Grain-filling stage**	
	GY	Biomass	GY	Biomass	GY	Biomass
Fo	0.539*	0.431	0.300	0.144	0.308	0.020
Fv	-0.097	0.193	0.369	0.107	0.033	0.194
Fm	-0.010	-0.125	0.319	-0.101	0.135	0.148
Fv/Fm	-0.385	-0.439	0.048	-0.052	0.250	0.688**

*P < 0.05 and ** P < 0.01 [a] GY = Grain yield per plant (g/plant), Biomass = Aboveground biomass per plant.

Discussion

The results showed that net photosynthesis and transpiration rate was severely reduced under water deficit condition. These results are in agreement with Condon *et al.* (2002). The P_n decrease could be explained by reduction in stomatal conductance, which reduced CO_2 diffusion into the leaves. In the present study, however, the internal CO_2 concentration was remained stable under water deficit condition and it was similar to that observed in well-watered condition. Thus, reduced stomatal conductance was not supposed to be a major cause for the reduced P_n so that the effect of water deficit on photosynthesis may be due to enzyme inactivation because of high leaf temperature and low leaf water potential (non-stomatal limitation). The instantaneous water use efficiency significantly increased under water deficit as compared to the control at both stages. An increase in iWUE could be due to more reduction in E than P_n by water deficit. An increase iWUE under water deficit condition was also reported by Abbad *et al.* (2004).

Table 8. Correlation coefficients of the relationship between peduncle length, peduncle excursion grain yield and yield components of durum wheat genotypes grown under water deficit condition from tillering to physiological maturity.

Characters	***Peduncle length***	***Peduncle excursion***
Plant height (PLH)	0.64**	0.57**
Grain yield (GY)	0.58**	0.66**
Biomass yield (BY)	0.65**	0.70***
Harvest index (HI)	0.56**	0.37
Kernel weight (KWT)	0.40	0.77***
Kernel number per spike (KSPK)	0.57**	0.71***
Kernel number per spikelet (KSPKL)	0.26	0.50*
Water use efficiency (WUE_G)	0.70**	0.68**
Grain-filling rate (GFR)	0.51*	0.67**
Kernel ash content (G_aM_a)	-0.67**	-0.75***
Drought susceptibility index (S)	-0.57**	-0.43

*P < 0.05; ** P < 0.01, *** P < 0.001.

No significant correlations were found between net photosynthesis rate and stomatal conductance and photosynthesis rate and transpiration rate (at both stages under stress and well-watered conditions. Both P_n and E are depending on g_s were proportionally affected by water deficit as a result there was no significant correlation observed between P_n and g_s. Simane (1993) also reported similar results under moisture deficit conditions. Gutièrrez-Rodriguez *et al.* (2000) and Monneveux *et al.* (2006) reported a positive correlation between P_n and Ci:Ca is expected if CO_2 supply is the dominant factor causing differences in P_n. In the present study, however, no overall correlations were found between P_n and g_s and P_n and Ci:Ca both under stress and well-watered conditions. However, strong correlations were noted between net photosynthesis rate and apparent mesophyll conductance under both stress ($r = 0.603$; $P < 0.001$) and well-watered ($r = 0.902$; $P<0.001$) conditions. Thus, it suggested that mesophyll conductance was found to be the dominant factor that control P_n in the studied genotypes. In previous studies, mesophyll conductance was found to be the dominant factor for the expression of genotypic differences under irrigated (Fischer *et al.*, 1998; Koc *et al.*, 2003) and drought conditions (Siddique *et al.*, 1999).

The RWC values in control and water deficit treatments were comparable with those reported by Strauss and Agenbag (2000) and Abbad *et al.* (2004). Water deficit caused a 36.7% reduction of the RWC of the leaves but genotypes were varied in maintaining their RWC under both water deficit and well-watered conditions. During drought stress, the water balance of a plant is disrupted and as a result of which the RWC and water potential of leaves decreased (Bajjii *et al.*, 2001). 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).

The SLA the genotypes increased under water deficit relative to the well-watered treatment. The observed increase in SLA under water deficit condition is in agreement with the previous reports on durum wheat (Rascio *et al.*, 1990; Araus *et al.*, 1997a), barley (Araus *et al.*, 1997b) and cowpea (Anyia and Herzog, 2004). Studies have shown that drought stress can affect the growth of plant organs differently (Spollen *et al.*, 1993), which may result in alteration of the morphological features of the plant (French and Turner, 1991). The current increase in SLA under water deficit condition may be due to the loss of weight than the decrease in leaf area under water deficit. Araus *et al.* (1997b) also indicate that an increase in SLA under water deficit condition probably reflects adaptation to drought conditions.

Chlorophyll fluorescence analysis is a sensitive indicator of the tolerance of the photosynthetic apparatus to environmental stress (Maxwell and Johnson, 2000). Chlorophyll fluorescence parameters in this study were sensitive to water deficit at tillering and grain- filling stages. The value of the minimal fluorescence yield (Fo) was increased under water deficit as compared to the control at tillering and anthesis stages. On the other hand, the value of the variable fluorescence (Fv), the difference between Fo and Fm, and the maximal fluorescence yield (Fm), were reduced due to water deficit at tillering and grain-filling stages. Similarly, the Fv/Fm ratio, which characterizes the maximum yield of the primary photochemical reaction in dark-adapted leaves and frequently used as a measure of the maximal photochemical efficiency of PSII (Krause and Weis, 1991), was reduced under water deficit condition. The patterns of changes in fluorescence parameters observed in this study are supported by the pattern of change reported by many authors under drought conditions (Long *et al.*, 1994; Aruas *et al.*, 1998; Zlatev and Yordanov, 2004). An increase in Fo is a characteristic of PSII inactivation whereas a decline in Fv under stress may indicate the increase in nonphotochemical quenching process at or close to reaction centers (Baker and Horton, 1987). Similarly, the increase in Fo and the decrease in Fm under water deficit with concomitant decrease in Fv/Fm, indicate the occurrence of chronic photoinhibition due to photoinactivation of PSII centers, most probably associated with the degradation of D1 protein (He *et al*., 1995; Giardi *et al*., 1996).

Leaf posture has been proposed as a trait that could increase crop yield potential by improving radiation use efficiency in high radiation environments (Reynolds *et al*., 1999). In our present study, it was observed that genotypes that exhibited erect leaf posture had more grain yield, HI, kernel number per spikelet and water use efficiency but lower kernel weight and biomass yield than prostrate leaved genotypes. Innes and Blackwell (1983) also reported a 4% yield advantage in wheat isolines in the Unit Kingdom. Similarly, in a comparison of two barley cultivars contrasting in this trait, net photosynthesis measured at different heights within the canopy was more evenly distributed in the more erect leaf cultivar, and leaf sheath photosynthesis was greater than in the curved leaf canopy (Angus *et al*., 1972). Araus *et al*. (1993) reported that erect-type lines had 5-16% greater number of grains per square meter. Monneveux *et al*. (2004) in their experiment also found that erect leaf cultivars had lower grain weight than droopy-type. The physiological basis of the trait was studied in near isogenic CIMMYT lines of wheat and showed that more erect leaf posture was associated with higher grain number and higher stomatal conductance based on carbon isotope discrimination measurements of the mature grain (Araus *et al*., 1993).

Leaf rolling is also an important trait for shading radiant energy and is likely result in cooler leaf temperature, less transpiration and lower respiratory losses (Richards *et al*., 2002). Genotypes that exhibited strong leaf rolling had more grain yield, number of kernels per spike, grain filling rate and water use efficiency than genotypes exhibited no leaf rolling. This result indicated that leaf rolling exhibited a significant positive effect on grain yield and WUE under stress condition. This is because; leaf rolling decreases stomata closure and represent an important drought-avoidance mechanism under drought stress (O'Toole *et al*., 1979).

In the present study, it was observed that leaf gas exchange parameters were not strongly correlated with grain yield and biomass yield under water deficit condition at both stages. There are a number of factors that affecting dry matter accumulation besides gas exchange (Boyer, 1996). For example, biomass production of a plant is not only determined by photosynthesis but also by respiratory losses at night (Lambers *et al*., 1998). Besides, spot measurement of leaf photosynthesis may vary with leaf age, position, leaf surface, light intensity, and general plant and development stage (Richards, 2000). Variations in daily time course of weather parameters such as light intensity, temperature, relative humidity, etc. also affect leaf gas exchange (Simane, 1993). It could also be due to differences in single leaf photosynthesis and canopy photosynthesis. The lack of significant correlation between P_n and grain yield under water stress in the present study suggests that selection for higher rates of leaf photosynthesis has not improved yield most probably because the source is less limiting than the sink. Moreover, yield is by nature a very integrative trait. Therefore, any trait consistently related to yield should also be integrative, either in time or in level of organization or both (Araus, 1996; Araus *et al*., 2001).

No significant correlations were found between chlorophyll fluorescence parameters and grain yield under moisture stress condition. However, initial fluorescence was positively correlated with yield and aboveground biomass at all stages but correlation was significant at tillering stage only (Table 7). Araus *et al*.

(1998) also reported positive correlation between Fo and grain yield under water stressed condition. The correlation between grain yield and Fv/Fm was negative at tillering stage while it was positive at other stages. Contrary to our findings, Araus *et al.* (1998) reported a positive relationship between grain yield and Fv/Fm, between grain yield and Fm and between grain yield and Fv.

In the present study, peduncle length and excursion were positively and significantly correlated with grain yield per plant. This positive relationship between grain yield and morphological traits under water deficit condition indicate that low growth rate of plants is one of the limiting factors of yield under water deficit conditions (Simane *et al.*, 1993, Villegas *et al.*, 2001). Therefore, genotypes with greater growth rate under such condition would provide the highest grain yield. Favorable conditions during growth may permit an expansion of the last internodes as well as a higher yield (Gupta *et al.*, 2001). Carbohydrates are also remobilized from the peduncle and flag leaf to the grain during grain-filling period (Zamski and Grunberger, 1995).

The significant and positive correlation observed between peduncle length, peduncle excursion and yield and negative correlation of peduncle length and excursion with drought susceptibility index found in the present study suggest that peduncle length and excursion could be good indicators of grain yield for breeding purpose in areas where water is limiting for an extended period of the growing season in durum wheat. This result is in conformity with previous reports that showed peduncle length as an indirect selection criterion in wheat under drought conditions (Kaya *et al.*, 2002).

Conclusion

Water deficit had paramount effects on chlorophyll fluorescence and leaf gas exchange parameters. Photosynthesis rate decreased with decrease in stomatal conductance, but a weak relationship between them implied that non-stomatal limitation to photosynthesis might have been in operation. The results also indicated that apparent mesophyll conductance rather than stomatal conductance has an effect on genotypic variation in net photosynthesis rate both under stress and well-watered conditions. Chlorophyll fluorescence parameters were weakly associated with grain yield and aboveground biomass yield under water deficit condition. Significant correlation was observed only between Fo and grain yield at tillering stage. Similarly, leaf gas exchange parameters were weakly associated with grain yield and aboveground biomass yield under water deficit conditions. The present study showed that leaf gas exchange and chlorophyll fluorescence parameters were not proper criteria for screening the drought tolerant wheat genotypes under water-limited environments. Peduncle length and excursion were positively correlated with grain yield under water deficit condition. Negative and significant relationship was found between peduncle length and drought susceptibility index suggests the peduncle length as an indirect selection criterion in wheat under drought conditions. Leaf posture and rolling had a profound effect on grain yield and other attributes. The genetic variability found for these morphological traits among durum wheat genotypes studied also suggest opportunity for selection superior genotype in water-limited environments.

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