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STOMATAL AND CHLOROPHYLL LIMITATIONS OF WHEAT CULTIVARS SUBJECTED TO WATER STRESS AT BOOTING AND ANTHESIS STAGES

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Abstract

The stomatal and chlorophyll effects were determined on yield and its contributing traits of wheat cultivars (Damani, Hashim-8, Gomal-8, DN-73, Zam-04 and Dera-98) under stress conditions at booting and after anthesis stages during 2009 at the University of Reading, UK. Significant water stress effects were observed on chlorophyll fluorescence, chlorophyll content, stomatal conductance, leaf area, relative water content (RWC %) and grain yield per plant. All the cultivars behaved independently and significantly different for physiological and yield traits. Cultivars Hashim-8, Zam-04 and Damani were observed as best candidate genotypes for rain-fed regions, and minimum effect and percent reduction was recorded at their booting and after anthesis stages for all traits at stress conditions. These rain-fed cultivars were also persistent at higher level of stomatal conductance and RWC % even under water stress condition, which reflects their adaptability under drought environment.

Introduction

Rain-fed farming fields share about 60-70% of the arable land in South Asian countries (Singh & Dhillon, 2004). In Pakistan, total cultivated area is 21.25 million hectares (5.35 million hectares is rain-fed). Wheat is grown on more than 30% of the total cultivated area that produce food for more than 35% of the world population. In Khyber Pakhtunkhwa province, more than 67% of the area where wheat is grown is rain-fed (FBS, 2010-2011). Agricultural performance is very low in dry land farming areas. However, it is possible to raise it through the adoption of approved dry farming technology.

Drought negative effects on plant growth and development causes up to 50% decrease in wheat productivity (Wang *et al.,* 2003; Khakwani *et al.,* 2012). Plant response to drought is a complex physical-chemical process, in which many biological macro and micro molecules are involved, such as nucleic acids, proteins, carbohydrates, lipids, hormones, ions, free radicals and mineral elements (Ingram & Bartels, 1996). The drought effects on crops yield depend on its severity and the stage of plant growth during which it occurs (Asgharipour & Heidari, 2011). Seed germination is the first stage of growth that is sensitive to water deficit. Therefore, seed germination, vigor and coleoptile length is rudiment for the success of stand establishment of crop plants. The rate and degree of seedling establishment are extremely important factors to determine both yield and time of maturity (Rauf *et al.,* 2007; Noorka *et al.,* 2013).

There are three components of successful rain-fed agriculture: retaining precipitation in soil, reducing evaporation losses of water, and sowing of crops that have drought tolerance characteristics and fit the rainfall pattern (Anonymous, 2007; Khan *et al.,* 2013). Farmers in rain-fed areas usually adopt conventional methods and grow traditional wheat cultivars suitable for their area. Wheat cultivars grown in these regions are mostly low yielding and susceptible to pests and diseases but are well acclimatized to the local environment and thrive best under adverse climatic conditions i.e., drought etc., whereas, the approved irrigated wheat cultivars do not tolerate such biotic and abiotic stresses.

Plants cope with drought through stress avoidance and tolerance strategies that vary with genotype (Khan *et al.,* 2012). Plants under drought stress conditions struggle to revise their metabolic and structural capabilities mediated by modified gene expression, which assists to improve their potential under stress environment (Bohnert & Sheveleva, 1998). Stimuli are generated in the leaf or elsewhere (roots) to make physiological and biochemical alterations in plants to survive under adverse environment (Pereira & Chaves, 1993). However, some acclamatory physiological changes also occurred in plants under stress environment such as modification in root shoot ratio or temporary storage of reserves in stem (Rodrigues *et al.,* 1995). Water scarcity closes the stomata and reduces carbon entry; therefore, it has been assumed that plants grown under drought conditions are under carbon starvation, which affects their growth (Muller *et al.,* 2011). Due to carbon limitations, the photosynthesis is highly affected under water stress conditions and it is recognized that sucrose and other sugars regulate the expression of many genes involved in this process.

It is well known that emission of chlorophyll *a* fluorescence provides an indicator of the primary photochemistry of photosynthesis, which is therefore used for detecting plant tolerance to water stress. Fluorescence may also provide information on the carbon reduction cycle such as leaves affecting from drought stress, the slow phase of fluorescence induction is altered (Ogren & Oquist, 1985; Toivonen & Vidaver, 1988; Ogren, 1990).

Their findings revealed that, beside stomatal closure, the primary effect of drought stress is impairment of the carbon assimilation. Therefore, it was assumed that membrane stability was increased in dehydrated leaf tissues (Havaux, 1992). The above findings raise the possibility of using fluorescence as an indicator for drought stress. Plant stomata are the gateway between plant and atmosphere and play a vital role in plant responses to environmental conditions (Nilson & Assmann, 2007). Similarly, measurements of stomatal conductance, dimension and density, leaf or soil water potential provide meaningful quantitative data and are necessary in a detailed physiological analysis of drought response characteristics (Ahmad *et al*., 2006; Woo *et al.,* 2008; Baloch *et al*., 2012). The ability of a cultivar to keep its stomata open despite internal water stress has been considered a form of drought resistance trait (Seropian & Planchon, 1984; Johnson *et al.,* 1987). In light of above review, an experiment was designed to determine the response of six wheat cultivars (three each rain-fed and irrigated) to water stress at booting and anthesis stages of growth and development.

Materials and Methods

Five bread wheat cultivars viz. Hashim-8 (ICW91), Gomal-8 (CM85836), DN-73 (CMSS96T03253T), Zam-04 (CRG732), Dera-98 (CM76688) and one local cultivar Damani were sown in pots during 2009 in a glasshouse under ambient environment at the University of Reading, UK. The pots (4 L size) were filled with "John Innes No. II growing media". At emergence, only three seedlings per pot were left growing while others were thinned out. Plants were exposed to two different environments i.e., T_1 (control, 100% field capacity) and T_2 (20 days water stress was given at booting stage and 20 days water stress after anthesis). All the treatments were replicated four times.

After 20 days stress at booting stage, the following parameters were recorded viz; chlorophyll content was recorded using chlorophyll meter and reading was taken on the second expanded leaf from the top of two plants in each pot at final tillering stage. Chlorophyll fluorescence (Fv/Fm = variable fluorescence/maximum fluorescence) was recorded by "Handy PEA (Hansa Tech., Industries Ltd, England)". The reading was taken on the upper most fully expanded leaf from the top of two plants in each pot at final tillering stage. Stomatal conductance was recorded by "Delta-T porometer AP4 (Delta-T Devices Ltd., Burwell, Cambs., UK)" on the second expanded leaf from the top of two plants in each pot at final tillering stage. Thermal couples were stamped on leaves to record leaf temperature after every five seconds using Data logger, thereafter an average temperature was estimated. These parameters were also recorded at the end of 20 days stress after anthesis.

Leaf area (LA) was measured in cm^2 using an automatic "leaf area meter (Delta-T Devices Ltd., Burwell, Cambridgeshire, UK)". Relative water content (RWC %) was recorded at booting stage according to Schonfeld *et al.,* (1988), where fresh weight from three youngest fully expanded flag leaves were determined within two hours after excision. Turgid weight was obtained after soaking the leaves for 16 to 18 h in distilled water. After soaking, leaves were quickly and carefully blotted dry with tissue paper prior to determine of turgid weight. Dry weight was obtained after drying the leaves sample in oven for 72 hours at 70°C. Relative water content was calculated with following equation:

$$RWC = \frac{Fresh\ weight - dry\ weight}{Turgid\ weight - dry\ weight} \times 100$$

Total grain yield per plant (g) was recorded at harvest from main spike and tillers using Sartorius analytical balance. A completely randomized design (CRD) was applied for ANOVA using the Genstat version II (Lawes Agricultural Trust, Rothamsted Experimental Station, UK) and also to estimate correlation coefficient between various attributes.

Results and Discussion

Photosynthesis is one of the main physiological processes affected by water stress and the emission of chlorophyll fluorescence provides an indicator of the primary photochemistry of photosynthesis. In present study, chlorophyll fluorescence of rain-fed cultivars (Damani, Hashim-8 and Zam-04) was reduced to 15-16% when plants received 20 days water stress at booting and 12-15% (at 20 days water stress after anthesis). However, in irrigated cultivars the said values reduced to 24 (Dera-98), 25 (DN-73) and 30% (Gomal-8) at 20 days water stress at booting while 24 (DN-73 and Dera-98) and 32% (Gomal-8) chlorophyll fluorescence reduction was observed in said cultivars at to 20 days water stress after anthesis (Table 1). However, rain-fed cultivars retained significantly ($p \leq 0.05$) maximum chlorophyll fluorescence as compared to irrigated ones under stressed conditions. Sayar *et al.,* (2008) observed that chlorophyll fluorescence extinction measurement seems to be the most reliable test enabling the discrimination of wheat cultivars according to their drought tolerance and all wheat tolerant cultivars in group 5 showed an average of 16% decrease in chlorophyll fluorescence which was significantly lower than values owned by cultivars in other groups having drought susceptible cultivars. Similar results were obtained in drought susceptible cultivar of bean (Dobrudjanski ran) where a significant higher decrease in chlorophyll fluorescence was recorded, however, drought tolerant cultivar Prelom showed a slight tendency to decrease (Zlatev & Yordanov, 2004). It is also reported that decrease of chlorophyll fluorescence under drought stress seems to indicate the occurrence of chronic photo-inhibition due to photo-inactivation of photosystem II centers, possibly attributable to D1 protein damage which usually limit photosynthetic activity (Zlatev, 2004).

Table 1. Physiological response of six wheat cultivars received 20 days drought stress at booting stage and after anthesis stage.

Treatments	Wheat cultivars	Physiological traits recorded at booting stage				Physiological traits recorded at anthesis stage			
		Chlorophyll fluorescence (ratio)	Chlorophyll content (units)	Stomatal conductance (mmol $m^{-2}s^{-1}$)	Leaf temperature (°C)	Chlorophyll fluorescence (ratio)	Chlorophyll content (units)	Stomatal conductance (mmol $m^{-2}s^{-1}$)	Leaf temperature (°C)
T_1 (Control)	Damani	0.81±0.01	14.08±0.54	215.13±22.03	8.53±0.30	0.78±0.01	12.13±0.52	74.88±4.60	14.50±0.58
T_1 (Control)	Hashim-8	0.80±0.01	14.49±0.31	260.00±37.31	8.73±0.22	0.79±0.01	12.25±0.83	73.35±6.22	15.00±0.29
T_1 (Control)	Gomal-8	0.80±0.01	20.11±1.05	267.13±48.86	8.15±1.18	0.82±0.00	18.50±0.96	82.75±5.02	15.00±0.87
T_1 (Control)	DN-73	0.80±0.01	20.54±1.29	267.88±50.65	7.14±0.40	0.79±0.00	18.50±0.94	82.63±5.16	13.75±1.01
T_1 (Control)	Zam-04	0.80±0.00	14.20±1.16	220.38±18.64	7.03±0.97	0.81±0.00	13.00±0.61	74.25±4.83	14.75±0.14
T_1 (Control)	Dera-98	0.79±0.01	20.69±0.45	240.50±38.17	6.88±0.65	0.79±0.00	18.00±1.15	81.50±3.75	14.50±0.00
T_2 (Stress)	Damani	0.68±0.01	13.25±0.63	183.89±30.59	11.85±0.26	0.68±0.01	11.65±0.71	63.48±8.36	16.50±0.65
T_2 (Stress)	Hashim-8	0.68±0.01	13.86±0.83	229.13±24.63	11.63±0.71	0.68±0.01	11.88±0.97	64.50±2.10	16.25±0.72
T_2 (Stress)	Gomal-8	0.56±0.01	16.80±0.58	142.40±04.14	12.15±0.89	0.56±0.01	14.88±0.72	55.75±5.22	16.00±1.15
T_2 (Stress)	DN-73	0.60±0.00	17.16±0.23	138.88±13.30	13.30±0.12	0.60±0.00	15.25±1.11	53.50±5.33	15.50±0.61
T_2 (Stress)	Zam-04	0.68±0.01	13.15±0.50	192.13±30.40	11.60±1.10	0.68±0.01	12.25±1.11	64.00±1.73	15.50±0.61
T_2 (Stress)	Dera-98	0.60±0.00	15.63±1.09	151.94±29.72	10.70±0.81	0.60±0.00	13.75±0.48	60.00±5.20	15.00±0.41
SED	Treatments	0.005 *	0.46 *	13.99 *	0.42 *	0.004 *	0.48 **	6.08 **	0.40 *
	Cultivars	0.009 *	0.79 *	24.22 *	0.73 ^{NS}	0.007 *	0.84 *	10.50 *	0.68 ^{NS}
	Interaction	0.012 *	1.12 **	34.26 ^{NS}	1.04 ^{NS}	0.009 *	1.18 ^{NS}	14.89 ^{NS}	0.97 ^{NS}
% Reduction in Stress Treatment									
	Damani	15	6	15		12	4	15	
	Hashim-8	15	4	12		14	3	12	
	Gomal-8	30	16	47		32	20	33	
	DN-73	25	16	48		24	18	35	
	Zam-04	16	7	13		16	6	14	
	Dera-98	24	24	37		24	24	26	

Values showing * and ** stand for significant at 0.05 and 0.01 probability level, respectively, whereas ^{NS} represents a non-significant value. SED stands for standard error of difference between varietal means

Similarly, a minimal decrease (4%) in chlorophyll content was observed in Hashim-8 and was having non-significant difference to its counterparts Damani (6%) and Zam-04 (7%) at 20 days water stress at booting stage (Table 1). However, maximum decrease in chlorophyll content was recorded in irrigated cultivars under similar stressed condition i.e., Dera-98 (24%) and DN-73 and Gomal-8 (16%). Almost similar trend was noticed when same cultivars were given 20 days water stress after anthesis i.e., 3, 4 and 6% reduction in chlorophyll content of rain-fed cultivars Hashim-8, Damani & Zam-04, respectively and 18, 20 and 24% reduction in irrigated cultivars DN-73, Gomal-8 and Dera-98, respectively. All the rain-fed cultivars (Damani, Hashim-8 and Zam-04) gain significantly ($p \leq 0.05$) maximum chlorophyll content as compared to irrigated cultivars (Gomal-8, DN-73 and Dera-98). Christopher *et al.,* (2004) observed that chlorophyll contents were similar for both drought susceptible and resistant cultivars until grain filling. However, drought susceptible cultivar leaves lost chlorophyll contents earlier than those of drought resistant lines. This showed that irrigated cultivars (drought susceptible) leaves senesced earlier than those of rain-fed ones (drought resistant). This implies that differences in the rate of carbon acquisition before the onset of leaves senescence were not responsible for grain yield of rain-fed cultivars. This also suggests that ability of rain-fed cultivars to maintain leaf chlorophyll content, and most probably to continue carbon acquisition longer during grain filling as also observed in sorghum ‘stay green’ line, is likely to be more important in contributing to the increased yield under drought environment.

Plants growing in water stress conditions showed alteration in cell carbon metabolism which is possibly mediated with low CO_2 availability due to stomatal closure (Meyer & Genty, 1999; Lawlor, 2002). Results revealed significant ($p \leq 0.05$) differences between water stress given at booting and anthesis stages and wheat cultivars regarding stomatal conductance (Table 1). Under optimum condition (non-stressed), the average stomatal conductance was quite higher which was decreased up to 12 (Hashim-8), 13 (Zam-04) and 15% (Damani) in rain-fed cultivars when subjected to 20 days water stress at booting. Whereas in irrigated cultivars i.e. Dera-98, Gomal-8 and DN-73 it was reduced to 37, 47 and 48%, respectively. Similar response in stomatal conductance was observed in rain-fed cultivars (with 20 days water stress at anthesis) with reduction of 12, 14 and 15% in Hashim-8, Zam-04 and Damani, respectively, and 26, 33 and 35% stomatal conductance reduction in irrigated cultivars Dera-98, Gomal-8 and DN-73, respectively. There is a strong link between stomatal conductance and photosynthesis in which leaf dehydration can lead to turgid loss of guard cells causing passive stomatal closure which reduce stomatal conductance and consequently the supply of CO_2 to fixation site is reduced (Ahmadi & Siosemardeh, 2005). However, the ability of stomata to remain open in rain-fed cultivars was closely linked to their greater capacity for osmotic adjustment as compared with irrigated cultivars. Similar results were reported by El-Hafid *et al.,* (1998).

However, some studies indicated altered relationships between RWC % and photosynthetic rate and changes in metabolism (Flexas & Medrano. 2002; Ahmadi & Siosemardeh, 2005). Findings of one of the studies (Flexas & Medrano, 2002) showed that there was a good correspondence between the onset of drought-induced inhibition of different photosynthetic sub-processes and stomatal conductance. The contents of ribulose bisphosphate (RuBP) and adenosine triphosphate (ATP) decreased at early stage during drought at still relatively high stomatal conductance. This suggests that RuBP regeneration and ATP synthesis are impaired. Decreased photochemistry and Rubisco activity typically occur at lower stomatal conductance, whereas permanent photo-inhibition is only occasional. This study suggested that stomatal closure was the earliest response to drought and the dominant limitation to photosynthesis at mild to moderate drought. However, in parallel, progressive down-regulation or inhibition of metabolic processes leads to decreased RuBP content, which becomes the dominant limitation at severe drought (almost complete stomatal closure), and thereby inhibits photosynthetic CO_2 assimilation. It is also believed that stomatal responses are often more closely linked to soil moisture content than to leaf water status. This suggested that stomata are responding to chemical signals (e.g. ABA) produced by dehydrating roots, whilst leaf water status is kept constant (Davies & Zhang, 1991; Chaves *et al.,* 2002). Leaf temperature was increased to 3-4°C in all cultivars at 20 days water stress at booting stage, however, in water stress during anthesis stage the increase was 1°C, which indicated that drought stress significantly decreased the leaf water potential which had pronounced effect on photosynthetic rate. This decrease in water content in leaves increased the leaf temperature that might have occurred due to increased respiration and decreased transpiration resulting from stomatal closure. The said results are in line with findings of Siddique *et al.* (2000) who reported that exposure of wheat cultivars (Kanchan, Sonalika, Kalyansona and C306) to drought led to noticeable decrease in leaf water potential and RWC % with a concurrent increase in leaf temperature.

On average, 15% leaf area was decreased in rain-fed cultivars (Damani, Hashim-8 and Zam-04) as compared to 35% decline in irrigated cultivars (Gomal-8, DN-73 and Dera-98) when subjected to 20 days water stress at booting and anthesis stages (Table 2). The differences were significant ($p \leq 0.05$) between treatments and cultivars for both variables. Results were in corroboration with Xu & Zhou, (2008) who reported that leaf area was significantly declined in plants grown under stress environment due to limited availability of assimilates. However, a possible reason for minimum reduction in leaf area of rain-fed cultivars under stress environment could be that accumulated solutes were used as substances for rapid recovery growth when stress is relieved. Similar results were reported by Christopher *et al.,* (2004) where differences between drought resistant and susceptible cultivars under stressed condition were due to partitioning of resources during development. The drought susceptible genotypes had more thin leaves that reduced total dry weight. Hence, present findings clearly showed that water scarcity affects stomatal behavior which imbalance the photosynthesis that results into minimum assimilates production.

Table 2. Response of six wheat cultivars received 20 days drought stress at booting stage and after anthesis stage regarding yield and its components.

Treatments	Wheat cultivars	Leaf area (cm^2)	RWC (%)	Yield per plant (g)
T_1 (Control)	Damani	26.53 ± 1.03	94.50 ± 1.44	2.64 ± 0.14
T_1 (Control)	Hashim-8	25.10 ± 0.57	96.00 ± 0.91	3.78 ± 0.26
T_1 (Control)	Gomal-8	31.22 ± 0.57	93.41 ± 1.69	2.87 ± 0.29
T_1 (Control)	DN-73	41.65 ± 0.68	93.86 ± 2.30	2.85 ± 0.12
T_1 (Control)	Zam-04	26.62 ± 0.88	92.67 ± 2.99	3.37 ± 0.13
T_1 (Control)	Dera-98	36.83 ± 1.62	93.17 ± 0.89	2.70 ± 0.09
T_2 (Stress)	Damani	22.20 ± 0.30	86.56 ± 2.30	2.43 ± 0.09
T_2 (Stress)	Hashim-8	21.63 ± 1.67	88.50 ± 2.60	3.56 ± 0.27
T_2 (Stress)	Gomal-8	20.82 ± 0.34	74.92 ± 1.60	1.81 ± 0.20
T_2 (Stress)	DN-73	26.82 ± 0.88	72.80 ± 1.66	1.89 ± 0.13
T_2 (Stress)	Zam-04	22.34 ± 1.06	85.26 ± 2.78	3.03 ± 0.11
T_2 (Stress)	Dera-98	23.58 ± 0.14	74.58 ± 2.36	1.87 ± 0.10
SED	Treatments	0.53 *	1.19 *	0.09 *
	Cultivars	0.92 *	2.06 *	0.15 *
	Interaction	1.31 *	2.92 *	0.22 *
% Reduction in stress treatment				
	Damani	16	8	8
	Hashim-8	14	8	6
	Gomal-8	33	20	37
	DN-73	36	22	33
	Zam-04	16	8	10
	Dera-98	36	20	31

*, ** Significant at $p \leq 0.05$ and $p \leq 0.01$, NS = Non-significant. SED = Standard error of means difference

The RWC % was reduced 8% in rain-fed cultivars (Damani, Hashim-8 and Zam-04), whereas the said reduction was 20-22% in irrigated genotypes (Gomal-8, DN-73 and Dera-98) with 20 days water stress at booting and anthesis stages (Table 2). Minimal reduction in RWC % in rain-fed cultivars may be attributed to differences in the ability to absorb more water from the soil and or the ability to control water loss through the stomata. Moreover, retaining sufficient RWC % in leaves by rain-fed cultivars has a significant effect on photosynthesis, which suggests that because of osmotic adjustment the rain-fed cultivars may avoid non-stomatal limitations to photosynthesis. It may also be due to differences in the ability of the tested cultivars to accumulate and adjust osmotically to maintain tissue turgid and other physiological activities (Sinclair & Ludlow, 1985). Varietals differences in RWC % may also be a result of their varied genetic ability to absorb water in the existing rooting zone and or extending rooting depth to increase water reserve for crops (Schonfeld *et al.,* 1988; Siddique *et al.,* 2000). At the cellular level, plants attempts to alleviate the damaging effects of stress by altering their metabolism to cope with stress (Korir *et al.,* 2006). On the other hand, a significant decline regarding grain yield per plant (31% in Dera-98, 33% in DN-73 and 37% in Gomal-8) was observed in irrigated cultivars with 20 days water stress at booting and anthesis stages. However, in rain-fed cultivars the grain yield was declined 6 (Hasim-8), 8 (Damani) and 10% (Zam-04) under similar stress environment which indicated that these cultivars have full potential to be grown in drought environments. As observed for yield, 20 days water stress at booting and anthesis stages caused a significant reduction in stomatal and chlorophyll traits that subsequently reduced photosynthesis hence yield was declined across cultivars. It appears to be a possible physiological mechanism by which drought can affect growth and productivity of crops. However, rain-fed cultivars thrived well under stress condition because of their minimum reduction in the above-mentioned traits. Similar results have been reported by Ahmadi & Siosemardeh (2005), Ratnayaka & Kincaid (2005) and Razzaq *et al*., (2013).

Correlation coefficients, in general were positive and significantly high across prominent parameters at booting (Table 3) and anthesis (Table 4) stages. The yield showed significantly positive association between chlorophyll fluorescence, stomatal conductance and RWC %. However, yield has non-significant association with chlorophyll content, leaf temperature and leaf area. Similarly, RWC % showed significantly positive correlation with chlorophyll fluorescence, stomatal conductance, and leaf area. The relationship of RWC % was significantly negative with leaf temperature and was non-significant with chlorophyll content. Leaf area has significantly positive association with chlorophyll fluorescence, chlorophyll content and stomatal conductance and significant negative relationship with leaf temperature. Similarly, leaf temperature has significantly negative correlation with chlorophyll fluorescence, chlorophyll content and stomatal conductance. Significant positive relationship was observed between stomatal conductance and chlorophyll fluorescence and chlorophyll content. However, chlorophyll fluorescence was non-significantly correlated with chlorophyll content. The significant and positive correlation between stomatal/chlorophyll parameters with yield indicated that under stressed condition, if some physiological traits (stomatal/chlorophyll) are below optimum it can significantly affects net assimilates production which will eventually reduce the yield (Zlatev, 2004; Ahmadi & Siosemardeh, 2005). Similar results were reposted by Attarbashi *et al.,* (2002), Subhani & Chowdhry (2000) and Munir *et al.,* (2007) which showed that most of the physiological traits significantly affected the grain yield in wheat crop.

Table 3. Correlation between physiological traits recorded at booting stage and yield.

Variables	Chlorophyll fluorescence	Chlorophyll content	Stomatal conductance	Leaf Temperature	Leaf area	RWC
Chlorophyll content	0.22 ^{NS}					
Stomatal conductance	0.89 **	0.34 *				
Leaf temperature	-0.88 **	-0.45 *	-0.78 **			
Leaf Area	0.60 **	0.83 **	0.60 **	-0.72 **		
RWC	0.96 **	0.11 ^{NS}	0.93 **	-0.80 **	0.47 *	
Yield	0.68 **	-0.23 ^{NS}	0.77 **	-0.46 *	0.09 ^{NS}	0.79**

*, ** Significant at $p \leq 0.05$ and $p \leq 0.01$, NS = Non-significant

Table 4. Correlation between physiological traits recorded after anthesis stage and yield.

Variables	Chlorophyll fluorescence	Chlorophyll Content	Stomatal conductance	Leaf Temperature	Leaf area	RWC
Chlorophyll content	0.25 ^{NS}					
Stomatal conductance	0.94 **	0.50 *				
Leaf temperature	-0.62 **	-0.54 *	-0.72 **			
Leaf area	0.60 **	0.82 **	0.76 **	-0.81 **		
RWC	0.95 **	0.10 ^{NS}	0.89 **	-0.48 *	0.47 *	
Yield	0.69 **	-0.23 ^{NS}	0.53 *	-0.15 ^{NS}	0.09 ^{NS}	0.79**

*, ** Significant at $p \leq 0.05$ and $p \leq 0.01$, NS = Non-significant

Conclusion

Wheat cultivars yield was significantly affected due to reduction in chlorophyll, stomatal and leaf water status parameters with drought conditions at booting and anthesis stages of development. The chlorophyll fluorescence, chlorophyll content and stomatal conductance were significantly reduced in response to water status, which may be closely associated to photosynthesis and water use efficiency hence reduced wheat yield with 20 days water stress at booting and anthesis stages. However, stomatal and non-stomatal inhibition to photosynthesis under stress conditions varied in drought susceptible (irrigated) and tolerant (rain-fed) wheat cultivars. Minimum reduction in chlorophyll fluorescence and chlorophyll content, stomatal conductance and RWC % could be some adaptive strategies in drought resistant cultivars which appear to be involved in drought tolerance. Therefore, three wheat cultivars, Damani, Hashim-8 and Zam-04 appeared as drought tolerant cultivars for rain-fed regions. Although, cultivar Damani is already under cultivation in local rain-fed area of Dera Ismail Khan, Pakistan, however, present results showed its yield was highly reduced in stress conditions. Therefore, Hashim-8 and Zam-04 can be recommended as the most suitable candidates to replace low yielding local cultivars and can be introduced in other rain-fed regions as well where drought prevails at booting and anthesis stages.

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