

## Effect of Terminal Drought Stress on Morpho-physiological Traits of Wheat Genotypes

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**Abstract.** Development of wheat varieties with low moisture requirements and their ability to withstand moisture stress may cope-up well with the on-coming peril of drought conditions. Ten wheat genotypes including two new strains, PBGST-3, Hero, Bhittai, Marvi, Inqlab, Sarsabz, Abadgar, Kiran, Khirman and PBGST-4 were sown in split plot design with factorial arrangement in four replications at Experimental Field, Department of Plant Breeding & Genetics, Sindh Agriculture University, Pakistan during 2012-13. The results revealed that water stress caused significant reductions in all morpho-physiological traits. The genotypes differed significantly for all the yield and physiological traits. The interaction of treatments × genotypes were also significant for all the traits except plant height, productive tillers/plant, grains/spike and harvest index, were non-significant which indicated that cultivars responded variably over the stress treatments suggesting that breeders can select the promising genotypes for both stress and non-stress environments. Among the genotypes evaluated Bhittai, Kiran-95, PBGST-3 and Sarsabz showed good performance as minimum reductions occurred under terminal stress conditions for all the traits studied. Hence, above mentioned genotypes were considered as drought tolerant group. The high positive correlations of physiological traits like chlorophyll content and relative water content with almost all yield traits indicated that these physiological traits could serve as reliable criteria for breeding drought tolerance in wheat. The negative correlations of electrolyte leakage with several important yield traits indicated that though this physiological trait has adverse effect on yield attributes, yet it could reliably be used to distinguish between drought tolerant and susceptible wheat genotypes.

**Keywords:** drought stress, yield attributes, physiological traits, correlations, wheat genotypes, electrolyte leakage

### Introduction

Although breeders are continuing to improve the yield potential of wheat, however, progress to achieve increasing wheat yields in drought environments has become more difficult (Jones, 2007). In defining a strategy for wheat breeding under drought tolerance, Rajaram *et al.* (1996) suggested that simultaneous evaluation of germplasm should be carried-out both under near optimum conditions (to utilize high heritability and identify genotypes with high yield potential) and under stress conditions (to preserve alleles for drought tolerance). In wheat, yield is reduced mostly when drought stress occurs during heading or flowering and soft dough stages. Drought stress during maturity resulted in about 10% decrease in yield, while moderate stress during the early vegetative period had essentially no effect on yield (Jatoui *et al.*, 2012). Munjal and Dhanda (2016) noted that mean performance of wheat genotypes for grain yield under irrigated conditions was significantly

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higher than under drought stress conditions. Therefore, physiological and biochemical approaches have a great importance in order to understand the complex responses of plants to water deficiency which could help to develop new varieties rapidly. Development of cultivars with high yield is the main goal in water limited environments but success has been modest due to the varying nature of drought and the complexity of genetic control of plant responses (Mirbahar *et al.*, 2009). Various quantitative traits (including morphological and physiological characteristics) have been proposed for the selection of tolerant genotypes to drought stress (Hammad *et al.*, 2014). A wide range of putative selection criteria that could be used to increase drought tolerance in plants is available, however, very few examples of success obtained using physiological traits in breeding programmes. A physiological approach would be the most attractive way to develop new wheat varieties, but breeding for specific and sub-optimal environments involve a deeper understanding of yield determining process (Araus

*et al.*, 2008). Total chlorophyll content and the chlorophyll a/b ratio were found to reduce under water stress conditions. A decrease in this index was faster in drought sensitive than in drought tolerant genotypes (El-Tayeb, 2006). Rong-Hua *et al.* (2006) concluded that chlorophyll content with SPAD units could be considered as a reliable indicator in screening barley genotypes for drought tolerance. Water deficiency was found to reduce the relative water content (RWC) in plant leaves. A high RWC and low excised leaf water loss (RWL) have been suggested as important indicators of water status (Arjenaki *et al.*, 2012).

Drought is a serious problem in many parts of the world (Moayedi *et al.*, 2011) where wheat, barley and other small-grained cereals are part of the staple diets. Opportunities for marker-assisted selection are also considered. Incorporating specific drought resistance traits in breeding programmes should facilitate more rapid improvement in the drought resistance of wheat and other small-grained cereals (Quarrie *et al.*, 1999).

Drought at grain filling stage reduces the cell size and number and results in shriveled grains with small size and reduced weight and early maturity (Gomaa *et al.*, 2014). It has been found that under the drought stress conditions, those genotypes that show the highest harvest index and highest yield stability are drought tolerant (Rathore, 2005). The main objectives of present study therefore were: (i) to determine the mean performance of wheat genotypes for water stress tolerance, (ii) to identify drought tolerance indicators based on morpho-physiological traits and (iii) to determine correlations between various morpho-physiological traits under terminal water stress.

## Materials and Methods

The field experiment was conducted at Experimental Field of the Department of Plant Breeding and Genetics, Sindh Agriculture University, Tandojam, Sindh, Pakistan so as to screen drought tolerance wheat genotypes during 2012-13 cropping season. The experiment was carried-out in split plot design with two treatments (non-stress and stress at anthesis) in four replications. Ten genotypes viz. PBGST-3, Hero, Bhittai, Marvi, Inqilab, Sarsabz, Abadgar, Kiran, Khirman and PBGST-4 were sown through hand drill. The water regimes were considered as the main factors while wheat genotypes as sub-factor. The irrigation regimes with no stress treatment received frequent irrigations without any water stress (a total of

6 irrigations were applied), while in water stress treatment, stress was imposed at anthesis by with-holding water for 40 days from initiation of anthesis till start of grain formation.

The essential cultural operations were adopted uniformly in all the plots throughout the growing period. Before first irrigation, seedlings were thinned to ensure uniform and reduced plant competition for optimum plant growth and development. All the agronomic practices were done at proper time. Fertilizer at the rate of 125-75 kg N&P/ha, respectively, was applied in the form of Urea and DAP. Full dose of phosphorus with 1/3<sup>rd</sup> of nitrogen was applied at the time of land preparation while remaining 2/3<sup>rd</sup> nitrogen were split in three equal doses and applied with first, third and fifth irrigations. Other inputs like herbicides were applied as and when required. All the required cultural practices including dry hoeing, weeding etc. were adopted uniformly in all plots throughout the growing period. Data were collected from ten randomly tagged index plants from each genotype per replication for yield traits like plant height (cm), productive tillers/plant, grains/spike, seed index (1000 grain wt. in g), grain yield (kg/ha) and harvest index (%). The relative water content (RWC%) was determined with formula developed by Schonfeld *et al.* (1988):  $RWC\% = (\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight}) \times 100$ , chlorophyll content was measured by SPAD meter (SPAD-500 Plus) as relative greenness in arbitrary units and electrolyte leakage(%) was assayed by estimating the ions leaching from the cell wall with the procedure developed by Sairam *et al.* (1998). Plant material (0.3 g) was taken in 10 mL of de-ionized water in two sets. One set was subjected to room temperature (approx. 25 °C) for 4 h and its conductivity (C1) was recorded using a conductivity instrument (LC116, Mettler-Toledo Instruments Co., Ltd, Shanghai, China). The other set was kept in a boiling water bath (100 °C) for 10 min and its conductivity was also recorded (C2). Electrolyte leakage was calculated as:

$$\text{Electrolyte leakage} = [1 - (C1/C2)] \times 100.$$

**Statistical analysis.** Analysis of variance was carried out according to procedures developed by Gomez and Gomez (1984) whereas, phenotypic correlations were determined according to Raghavrao (1983) by using the following formula:

$$r = \sqrt{\frac{[\sum xy - (\sum x)(\sum y)/n]}{\sum x^2 - (\sum x)^2/n \times \sum y^2 - (\sum y)^2/n}}$$

## Results and Discussion

**Analysis of variance.** Mean squares from analysis of variance (Table 1) revealed that water stress caused significant declines in plant height, productive tillers/plant, grains/spike, seed index, harvest index, grain yield (kg/ha), relative water content (RWC %), chlorophyll content (relative greenness) and electrolyte leakage (EL %). Significant differences were also observed among the cultivars for all the yield and physiological traits studied that could help wheat breeders to select the drought tolerant varieties on the basis of one or more morpho-physiological attributes. The mean squares due to treatment  $\times$  genotype interactions were also significant for all these traits except that plant height, productive tillers/plant, grains/spike and harvest index were non-significant. The significance of treatment  $\times$  genotype interactions indicated that varieties performed variably over the stress treatments. These interactions could help wheat breeders to select the promising varieties based on one or more reliable drought tolerant indicators and put them in a breeding programme to develop new drought tolerant breeding material. Similarly, several researchers like Allahverdiyev *et al.* (2015); Baloch *et al.* (2012) and Jatoi *et al.* (2012) reported significant difference in response of wheat varieties to terminal water stress conditions.

**Mean performance of wheat cultivars under terminal water stress. Plant height (cm).** Optimum plant height is considered as an important trait for avoiding lodging, thus maximizes harvest index. On an average, water stress caused -5.06 cm reduction in plant height yet, minimum reduction was observed in Sarsabz (-3.00 cm) while maximum in Kiran (-7.37cm) followed by Hero

(-6.47) and PBGST-03 (-6.18 cm) (Fig. 1a). The lowest decrease in plant height of later group of varieties indicted their tolerance however, such statement may not hold true where terminal drought is expected having no effect on plant height when it is already attended before stress was imposed. Similar results were noted by Jatoi *et al.* (2012); Khakwani *et al.* (2011) and Mirbahar *et al.* (2009); who observed that water stress significantly reduced the plant height.

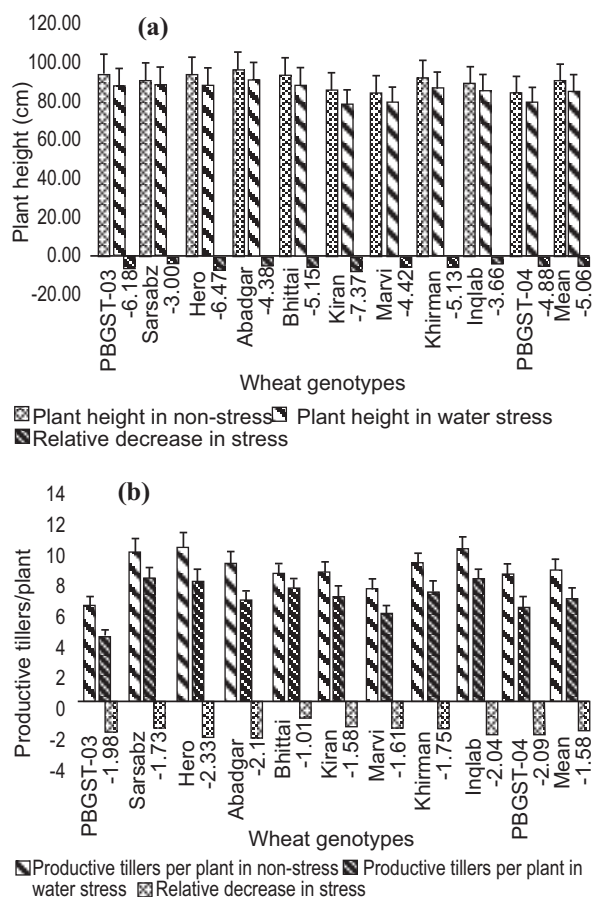
**Productive tillers/plant.** On an average, water stress caused a decline of -1.85 tillers per plant (Fig. 1b). The minimum relative decreases were recorded in Bhittai, Kiran, Sarsabz and Khirman being stress tolerant genotypes. While the prominent reductions were observed in cultivars Abadgar, Hero and Inqlab. The minimum and maximum reductions due to stress in above cultivars for tillers/plant characterised first group as drought tolerant and second as drought susceptible ones. Present results are in accordance with those recorded by Jatoi *et al.* (2011) and Baloch *et al.* (2012)

**Grains/spike.** The range of seeds set by single spikes was counted as 57.73-84.39 in normal water conditions, while in stress, the range was 51.55-78.91 grains/spike. On an average, water stress caused -5.98 seeds decline in grains/spike. When comparing the cultivars, highest number of grains/spike were set by Bhittai, Hero and PBGST-4 in water stress at anthesis, respectively (Fig. 2a). Usually, water stress at terminal stage causes infertility which results into lower number of grains/spike. Similar results were suggested by Allahverdiyev *et al.* (2015) and Elhafild *et al.* (1998) who demonstrated that drought stress results in reduced pollination and reduces the number of grains/spike.

**Table 1.** Mean squares from analysis of variance for various morpho-physiological traits of wheat genotypes grown under water stress conditions

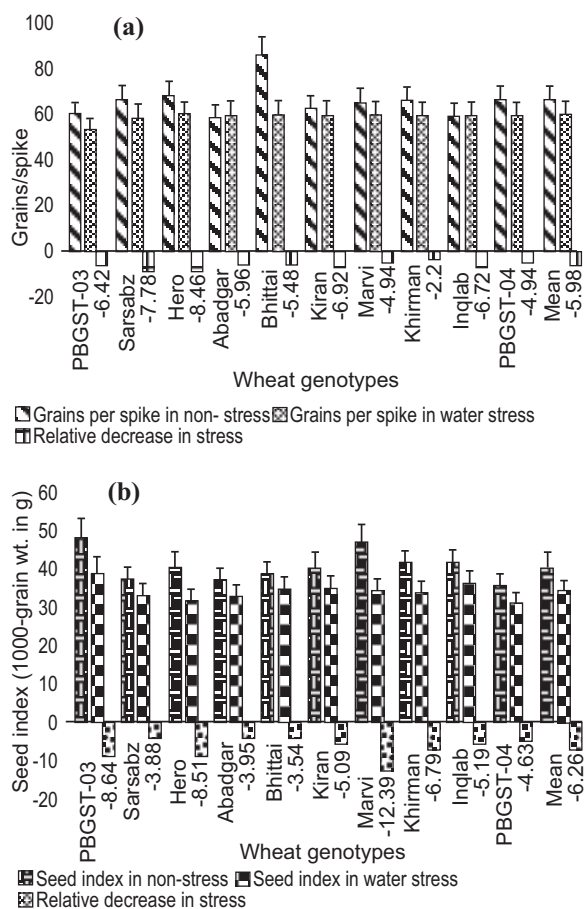
Yield traits	Replication D F = 3	Treatment (T) D F = 1	Error (a) D F = 3	Genotypes (G) D F = 9	T $\times$ G D F = 9	Error (b) D F = 54
Plant height	28.50	513.08**	3.34	140.04**	3.49ns	6.07
Prod. Tillers/plant	0.62	68.62**	0.66	11.50**	0.33 ns	0.36
Grains/spike	3.64	715.33**	9.33	489.39**	6.15ns	7.44
Seed index	18.49	783.82**	1.40	73.60**	16.02**	3.44
Harvest index	4.56	555.30**	3.82	35.20**	1.87 ns	9.32
Grain yield (kg/ha)	499	8156206**	1487	1305922**	138968**	3395
<b>Physiological traits</b>						
Relative water content	1.00	26938.90**	1.40	144.70**	59.10**	1.80
Chlorophyll content	4.32	1496.80**	5.90	100.38**	3.64*	3.80
Electrolyte leakage	67.75	8181.01**	6.88	98.67**	51.40**	7.05

\*\*,\* = significant at 1 and 5% probability levels; DF = degrees of freedom; ns = non significant.



**Fig. 1(a-b).** Mean performance for plant height (a) and number of productive tillers plant-1 (b) of wheat genotypes grown under non-stress and water stress at anthesis.

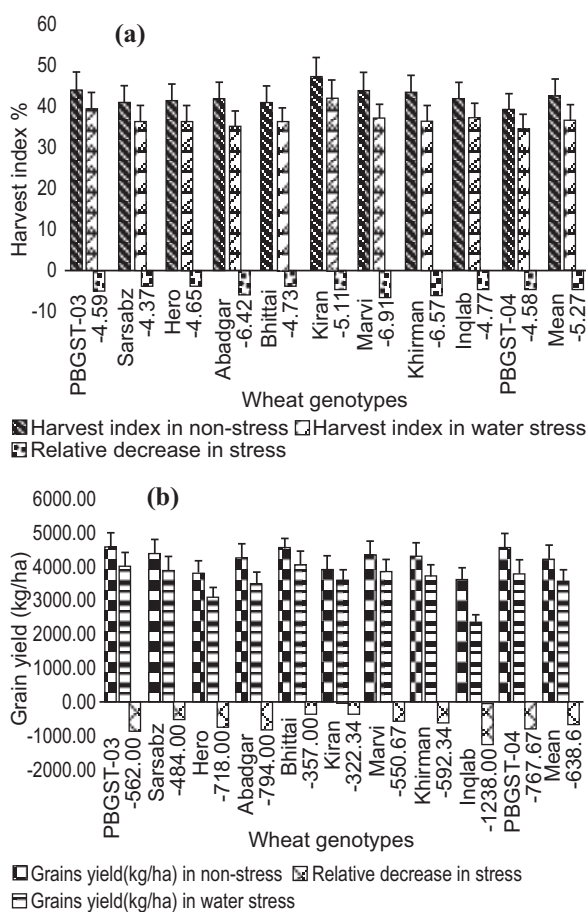
**Seed index (1000-grain wt. in g).** The average seed index in non-stress was 40.04 g while in stress conditions was 33.78g, thus on an average, water stress caused -6.26 g reduction in thousand grain weight (Fig. 2b). The little declines in seed index due to terminal stress however were recorded in cvs. Bhittai, Sarsabz, Abadgar and PBGST-4, whereas, sharper reductions were noted in cultivars Marvi, PBGST-03 and Hero. Based on these results, the first group of cultivars was considered as drought tolerant and second group as drought susceptible. During grain formation, water stress reduces transport of assimilates to the grains resulting smaller seeds, consequently lower seed index. Plaut *et al.* (2004) reported that 1,000 kernel weight and weight of kernels per spike were more severely decreased by water deficit i.e., the rate of dry matter accumulation and number of kernels were considerably decreased due to water deficit. Jatoi *et al.* (2012) also recorded similar results.



**Fig. 2(a-b).** Mean performance for grains/spike (a) and seed index (b) of wheat genotypes grown under non-stress and water stress at anthesis.

**Harvest index (%).** One useful approach to increase wheat productivity is to split yield into biomass at maturity and harvest index (HI). Most yield potential progress in wheat has been associated with increased HI. It is often stated that progress in HI is exhausted because values are approaching the limits of 60%, hence the focus should be on biomass rather than on HI. In present study, the average HI due to water stress dropped by -5.27%, however, this decline was smaller in some of the cultivars such as Sarsabz, PBGST-4 and PBGST-3 while sharper reductions occurred in Marvi, Khirman and Abadgar as shown in Fig. 3a. Thus, these two groups of cultivars could be considered as highly drought tolerant and highly susceptible ones, respectively, yet the remaining cultivars fall in moderately tolerant. These results are in conformity with those of Jatoi *et al.* (2011), who reported that the average HI dropped due to water stress however, this decline was smaller in tolerant cultivars.

**Grain yield (kg/ha).** Grain yield (kg/ha) is the ultimate result of all physiological and agronomical responses of varieties to drought stress conditions. The average decline due to terminal water stress was recorded as -638.60 kg/ha (Fig. 3b). The higher grain yield in kg/ha was produced by PBGST-03, PBGST-04, Bhattai and Sarsabz in water stress imposed at anthesis. Thus, these cultivars sustained the water stress by showing drought tolerance against other cultivars under evaluation. Drought during grain filling could be limiting the rate and duration of filling processes, causing small grain size, earlier physiological maturity, reduce number of grains, low grain weight and grain yield of wheat (Gupta *et al.*, 2001). Allahverdiyev *et al.* (2015) reported that drought led to decrease in yield and yield components of wheat genotypes. Munjal and Dhanda (2016) also reported that about 50% reduction was observed in grain yield under stress condition.



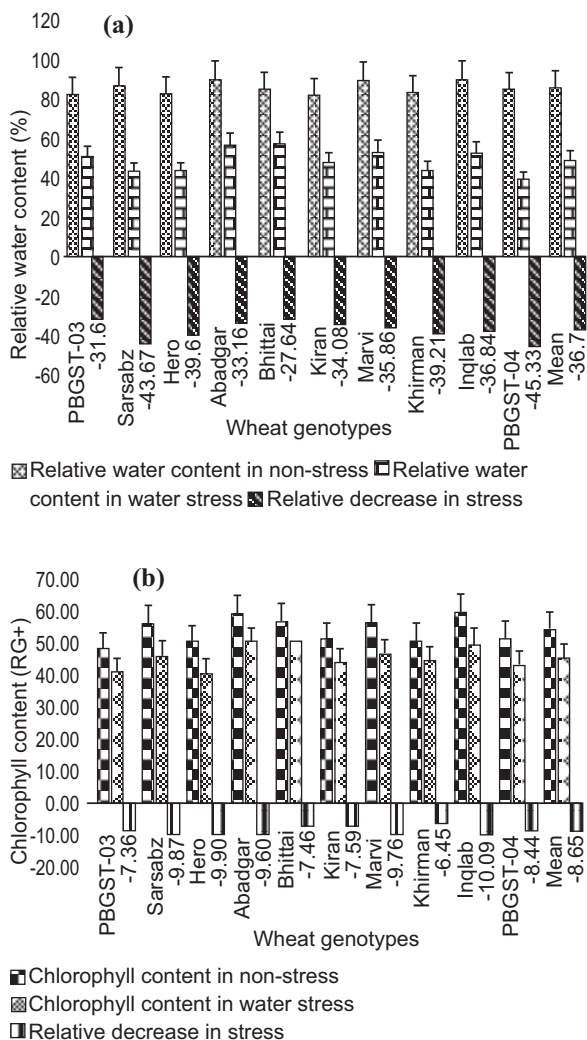
**Fig 3(a-b).** Mean performance for a) harvest index and (b) grain yield kg/ha of wheat genotypes grown under non-stress and water stress at anthesis.

**Relative water content (%).** Relative water content (RWC %) is very essential criteria of water stress in wheat leaves. The average reduction of -36.70% in RWC % was noticed due to water stress (Fig. 4a). The top three cultivars having higher RWC% in stress conditions were Bhattai, Abadgar and Marvi, while, the lowest RWC% was observed in PBGST-04, Hero, Sarsabz and Khirman. These results indicated that first group of cultivars was drought tolerant and second being drought susceptible, yet the remaining cultivars were moderately drought tolerant. These results indicated that first group of cultivars was drought tolerant and second being drought susceptible, yet the remaining cultivars were moderately drought tolerant. These findings were similar to those noted by Gunes *et al.*, (2008) who reported that water deficient was found to reduce the relative water content (RWC%) in plant leaves.

A high RWC% and low excised leaf water loss (RWL) have been suggested as important indicators for selection of drought tolerant genotypes. Schonfeld *et al.* (1988) also observed a decline in the amount of RWC% of wheat due to drought stress and reported the highest RWC% in the tolerant genotype.

**Chlorophyll content (relative greenness, RG).** Leaf chlorophyll increases the photosynthetic activity, hence contributes toward more grain yield. The chlorophyll content decreased in water stress treatment with an average of -8.65 RG (Fig. 4b). The maximum chlorophyll content was recorded in Inqlab followed by Abadgar and Marvi in non-stress whereas, in stress cultivars Abadgar gave highest chlorophyll content (49.26 RG) followed by Inqlab and Bhattai (48.88 RG). High chlorophyll content is a desirable characteristic because it indicates a lower degree of photo-inhibition of photosynthetic apparatus, therefore, reducing carbohydrate losses for grain growth (Farquhar *et al.*, 1989). These findings are in conformity with those obtained by Iturbe *et al.* (1998) who reported that water stress condition caused reduction in chlorophyll content. Decrease in the chlorophyll content under drought stress was also observed by Sayar *et al.* (2008) in wheat.

**Electrolyte leakage (%).** Cell membranes are one of the first targets of many plant stresses and it is generally accepted that the maintenance of their integrity and stability under water stress conditions is a major component of drought tolerance in plants. The degree of cell membrane injury induced by water stress may be easily estimated through measurements of electrolyte leakage from the cells (Bajji *et al.*, 2001). Less electrolyte leakage



**Fig 4(a-b).** Mean performance for a) relative water content and b) chlorophyll content of wheat genotypes grown under non-stress and water stress at anthesis.

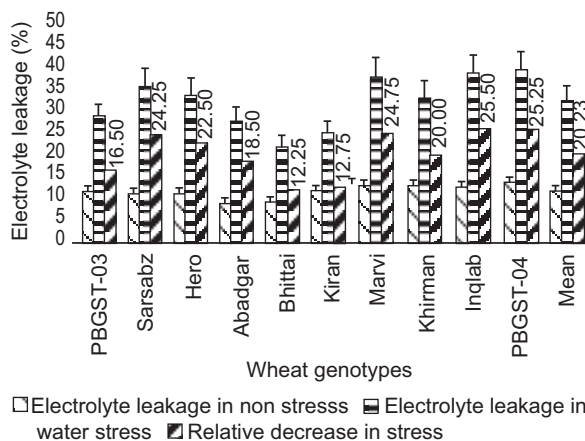
(EL %) is an important indicator of water stress tolerance in leaves under drought conditions. In non-stress, the EL% varied from 9.25-13.75% whereas in stress, the range was 22.00 to 39.00%. However, on an average, cell membrane leakage of 20.23% was noticed due to water stress (Fig. 5). The top three cultivars with lower percent of membrane leakage in stress conditions were, Bhittai, Kiran and Abadgar, while the highest leakage percentage was marked in PBGST-04, Inqlab, Marvi and Sarsabz. These results indicated that first group of cultivars was drought tolerant and second being drought susceptible, yet the remaining cultivars were moderately drought tolerant. Similar to our results (Bajji *et al.* 2001) also noted that injury index of drought sensitive cultivar

Kabir-1 exhibited highest values as compared with the drought resistant cultivars which gave lower injury percentage. Sayar *et al.* (2008) noted that electrolyte leakage reached at 21 and 11% after 2 h in drought susceptible and tolerant wheat cultivars.

**Correlations between yield and physiological traits.**

Though most of the yield traits were significantly correlated with each other, yet the correlation coefficient (r) was at lower side to most part (Table 2). The plant height, productive tillers/plant were significantly but moderately associated with all the yield traits under study. The high correlation ( $r = 0.45^{**}$ ) however was recorded between productive tiller/plant and grain yield/plant. Among the yield traits, the maximum correlations nevertheless was obtained between grains/spike and grain yield/plant ( $r = 0.63^{**}$ ) and seed index with harvest index ( $r = 0.62^{**}$ ). The most valuable correlations nonetheless were noted among grains/spike, seed index, harvest index and seed yield/plant and kg/ha. In drought stress and non-stress conditions, spike length had positive and significant correlation with number of grains/spike. Azadi *et al.* (2009) also reported positive and significant correlation between spike length with number of spikelets/spike, spike weight and number of grains/spike in wheat under drought stress condition. Golparvar *et al.* (2006) investigated some bread wheat cultivars in two conditions of drought stress and non-stress and they observed positive and significant correlation between spike length with number of grains/spike.

The correlation coefficients between yield and physiological traits were also recorded (Table 2) which



**Fig 5.** Mean performance for electrolyte leakage of wheat genotypes grown under non-stress and water stress at anthesis.

indicated that traits like plant height, tillers/plant, grains/spike, seed index and harvest index were negatively but significantly associated with electrolyte leakage. Other physiological traits like relative water content was significantly and positively associated with tillers/plant ( $r = 0.56^{**}$ ), grains/spike ( $r = 0.35^{**}$ ), grain yield/plant ( $r = 0.56^{**}$ ), seed index ( $r = 0.66^{**}$ ) and harvest index ( $r = 0.60^{**}$ ). The chlorophyll content also exhibited fairly good association with yield traits like tillers/plant ( $r = 0.58^{**}$ ), grains/spike ( $r = 0.31^{**}$ ), grain yield/plant ( $r = 0.56^{**}$ ), seed index ( $r = 0.40^{**}$ ), and harvest index ( $r = 0.35^{**}$ ). Similar to present findings, Allahverdiyev *et al.* (2015) reported that chlorophyll content was positively and significantly correlated with plant height, spike/m<sup>2</sup> and grain yield. Likewise, plant height, seed weight, spikelets/spike, grains/spike were positively and significantly correlated with most physiological parameters. Therefore, these traits may deem a good criterion for selection.

The correlations between physiological traits were also determined (Table 2) and the results revealed significantly positive correlations between relative water content and chlorophyll content ( $r = 0.83^{**}$ ) while relative water content and chlorophyll content both were highly but negatively associated with electrolyte leakage ( $r = -0.91^{**}$  and  $r = -0.73^{**}$ ), respectively. These results, by and large, suggested that physiological traits which exhibited high correlations with yield traits in drought condition may be used as selection criteria to select drought tolerant wheat genotypes. Hence, there is a greater scope of using physiological traits along with yield traits in selection for improving yield productivity in water shortage condition.

## Conclusion

Ten popular wheat genotypes were evaluated for drought tolerance by imposing water stress at anthesis stage. The results revealed that water stress caused significant decline in all morpho-yield and physiological traits studied. The genotypes Bhitai, Kiran, PBGST-03 and Sarsabz which recorded good performance by giving minimum reductions in majority of the traits under stress were regarded as drought tolerant among ten cultivars that were evaluated. The high positive correlations of physiological traits with almost all yield traits indicated that these physiological traits could serve as reliable criteria for breeding for drought tolerant wheat cultivars.

**Table 2.** Correlation coefficient (r) between yield and physiological traits of wheat genotypes grown over non-stress and water stress conditions

No.	Correlation between yield traits	Correlation (r)
1	Plant height vs productive tillers/plant	0.39**
2	Plant height vs grains/spike	0.21*
3	Plant height vs grain yield/plant	0.16ns
4	Plant height vs seed index	0.33**
5	Plant height vs harvest index	0.22*
6	Plant height vs grain/yield (kg/ha)	0.26**
7	Productive tillers/plant vs grains/spike	0.29**
8	Productive tillers/plant vs grain/yield plant	0.45**
9	Productive tillers/plant vs seed index	0.15ns
10	Productive tillers/plant vs harvest index	0.24**
11	Productive tillers/plant vs grain/yield (kg/ha)	0.05ns
12	Grains/spike vs grain/yield plant	0.63**
13	Grains/spike vs seed index	0.09ns
14	Grains/spike vs Harvest index	0.10ns
15	Grains/spike vs grain yield kg/ha	0.47**
16	Grain yield/plant vs seed index	0.31**
17	Grain yield/plant vs harvest index	0.37**
18	Grain yield/plant vs grain yield (kg/ha)	0.36**
19	Seed index vs harvest index	0.62**
20	Seed index vs grain yield (kg/ha)	0.42**
21	Harvest index vs grain yield (kg/ha)	0.35**
<b>Correlation between yield and physiological traits</b>		
22	Plant height vs relative water content	0.49**
23	Plant height vs electrolyte leakage	-0.54**
24	Plant height vs chlorophyll content	0.44**
25	No. of tillers/plant vs relative water content	0.56**
26	No. of tillers/plant vs electrolyte leakage	-0.50**
27	No. of tillers/plant vs chlorophyll content	0.58**
28	Grains/spike vs relative water content	0.35**
29	Grains/spike vs electrolyte leakage	-0.41**
30	Grains/spike vs chlorophyll content	0.31**
31	Grain yield/plant vs relative water content	0.56**
32	Grain yield/plant vs electrolyte leakage	-0.57**
33	Grain yield/plant vs chlorophyll content	0.56**
34	Seed index vs relative water content	0.66**
35	Seed index vs electrolyte leakage	-0.62**
36	Seed index vs chlorophyll content	0.40**
37	Harvest index vs relative water content	0.60**
38	Harvest index vs electrolyte leakage	-0.61**
39	Harvest index vs chlorophyll content	0.35**
<b>Correlation between physiological traits</b>		
40	Relative water content vs chlorophyll content	0.83**
41	Relative water content vs electrolyte leakage	-0.91**
42	Chlorophyll content vs electrolyte leakage	-0.73**

\*\* = significant at  $P < 0.01$  and  $P < 0.05$ ; ns = non significant.

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