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GENETIC VARIABILITY OF DIFFERENT WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES/CULTIVARS UNDER INDUCED WATER STRESS

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Abstract

Drought significantly depresses yield of many crop plants including wheat in the world. Identification of wheat genotypes that can tolerate limited water condition is vital to boost the wheat production. Under drought, cell membrane stability (CMS) and its relationship with different agronomic traits has been thoroughly described as an important screening trait to breed for drought tolerant wheat genotypes. In Pakistan, we first time extensively screened a large collection of wheat gene pool using the CMS as a screening tool. In the present study, 50 wheat cultivars/genotypes were screened for CMS by exposing the flag leaf discs with instant drought using PEG (6000). Significant differences were found among the wheat genotypes for CMS, number of tillers and 100 seed weight. Interaction between water regimes and genotypes was also significant which showed the genetic variation among the 50 cultivars/genotypes. Correlation between CMS and number of tillers was significant, while it was non significant with 100 seed weight. The association between the number of tillers and 100 seed weight was non significant. Among these parameters, CMS was found the most reliable screening parameter for characterization of cultivars/genotypes for drought. This study will be helpful in warranting wheat genome mapping studies.

Key words: Bread wheat; *Triticum aestivum* L.; Cell membrane stability; Drought; Pakistan.

Introduction

Wheat (*Triticum aestivum* L.) is the world's most widely adapted crop, supplying one-third of the world population with more than half of their calories and nearly half of their protein. Wheat is mainly grown on rainfed land, and about 37 % of the area of developing countries consists of semiarid environments in which available moisture constitutes a primary constraint on wheat production. By 2020, world demand for wheat is expected to be 40 percent higher than that of its level in the later half of the 1990s (Rosegrant, 1997). Wheat is a staple food crop of Pakistan. Drought is one of the major factors detrimental to wheat production in Pakistan.

Selection of wheat genotypes/cultivars with better adaptation to water stress should increase the productivity in rainfed areas (Rajaram, 2001). Genetic improvement in drought tolerance requires identification of relevant drought resistance mechanism and the development of suitable methodology for their measurement in a large breeding population (Blum, 1979). It can be accomplished by selecting for grain yield under field condition in the breeding programme. (Sammons *et al.*, 1978) but it require full season. Thus, certain physiological or biochemical selection criteria should be identified for rapid assessment of tolerance to water stress. Several physiological characteristics have been reported as being reliable indicators for the selection of genotypes/cultivars for drought

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tolerance (Ashraf *et al.*, 1999), e.g., photochemical activity of photosystem II (PS-II) calculated as F_v/F_m and chlorophyll in canola (Kauser *et al.*, 2006), and cell membrane stability (CMS) (Dedio, 1975). CMS reflects the capability of wheat/plant cell tissues to hold electrolytes under drought condition by retaining the cell membrane structure intact (Sullivan, 1971). The genetic variation in CMS has been quantified by conductivity meter in different crop plants including wheat (Shanahan *et al.*, 1990)

The CMS has been extensively used as selection criterion for different abiotic stresses including drought and high temperature in wheat (Blum *et al.*, 2001; Bajji *et al.*, 2002; Rahman *et al.*, 2006a), rice (Tripathy *et al.*, 2000), mustard (Hashem *et al.*, 1998), cotton (Ullah *et al.*, 2006; Rahman *et al.*, 2006b) and sorghum (Premachandra *et al.*, 1992). Several associations were established between CMS and different agronomic traits by inducing in vitro with polyethylene glycol (PEG-6000) (Dhanda *et al.*, 2004). To the extent of our knowledge, the CMS assay has not been conducted on wheat at length in Pakistan.

The objectives of the present study were to determine the extent of genotypic differences for CMS in different wheat cultivars/genotypes developed at different wheat research institutes of Pakistan and its relationship with different agronomic traits. The information generated from this study will add to the existing database regarding the utilization of this physiological feature for selecting drought tolerant wheat genotypes.

Materials and Methods

Plant material: The plant material used in the study consisted of 50 wheat cultivars/varieties/ genotypes, which have been developed/bred in different ecological zone of Pakistan (Table 4). These were collected from wheat breeding program, National Agricultural Research Council (NARC), Islamabad.

Experimental design: All the genotypes were planted in the field of the National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan during the normal wheat growing season (2004-2005) under two irrigation regimes, well watered (normal) and water limited (drought) environments. This Institute is situated 31^o--26'N latitude, 73^o-06'E and at an elevation of 184.5 meters from the sea level. Daily rainfall during each growing season was recorded at experimental site. The experimental design was a triruplicated split-plot with water regimes assigned in main plot and cultivars in sub-plots. The plant-to-plant distance was maintained at 7.5 cm by hand thinning 10 days after germination, while rows were spaced at 30 cm apart. Appropriate control measures were taken to eradicate weeds.

Measurement of productivity traits: At maturity, ten plants of each wheat genotype in each replication were selected to count the number of tillers. Seed from each plant was also harvested. Hundred seed weight was measured on analytical balance.

Measurement of physiological attributes: For estimating cell membrane stability (CMS), artificial desiccation was induced by polyethylene glycol (PEG-6000) method as proposed by Sullivan (1971). A bulked sample of fully expanded five flag leaves from randomly selected five plants was collected at noon from the normal plots. Samples were rinsed with deionized water to remove surface contamination and carefully blotted dry.

Two groups of containing twenty 1.0 cm^2 leaf discs were made from the bulked leaves sample. One group was exposed to 30% polyethylene glycol (PEG-6000) in 20 ml screw-cap vials and the second group was submerged in 10 ml deionized water in the vials (control sample). These vials were kept at room temperature in dark for 24 hours.

Subsequently, conductance of the sample solutions was measured with a conductivity meter (Model, 145 A+, Thermo Electron USA). The vials with samples were then autoclaved for 15 minutes and conductance of the sample solutions measured a second time to obtain an estimate of total electrolyte concentration. All measurements were recorded at 25 ^{0}C by keeping vials submerged in a water bath and vials were shaken vigorously to mix contents. CMS was the mean percentage (5 observations/plot) of relative cell injury (Blum & Ebercon, 1981) with the formula,

$$CMS\% = \{(1-(T_1/T_2))/ (1-(C_1/C_2))\} \times 100$$

Where T_1= Stress sample conductance before autoclaving; T_2= Stress sample conductance after autoclaving; C_1= control sample conductance before autoclaving; C_2= control sample conductance after autoclaving

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The mean values for genotypes were used to compute the coefficients of variation (Burton & Devane, 1953) and also to calculate the simple correlation (Miller *et al.,* 1958) between number of tillers, 100 seed weight and CMS.

Statistical analysis: Analysis of variance (ANOVA), appropriate for the specified experimental design, was performed with MSTAT-C software to evaluate the genetic difference among the wheat genotypes. Statistical significance was assumed at 5 and 1% levels of probability. Differences among means were tested by least significant difference (LSD) test at 5% probability level.

Results and Discussion

Significant variations for CMS occurred among the 50 cultivars/genotypes of wheat (Table.1). Mean values for CMS ranged from 21.58% for B-Silver to 98.6 % for Dirk. Lower value of CMS shows the susceptibility under drought condition while the genotypes with high CMS value shows drought tolerance. The genotypes exhibiting values lower than 50% are highly susceptible to drought condition. Most of the wheat genotypes fell in the range of 71-80%. However, very few genotypes were in the range of 51-60% (Figure.1). Mostly, the wheat genotypes exhibiting higher CMS values have been bred for rainfed areas. Hence, CMS value can be used to select the drought tolerant genotypes in the wheat-breeding program (Dhanda *et al.,* 2004; Rahman *et al.,* 2006[a]).

Analysis of variation indicated considerable amount of genetic variability for 100 seed weight and number of tillers under the well watered (W_1) and limited water (W_2) conditions (Table. 2). The magnitude of mean square values were higher in the W_1 regime than the W_2 regime. Reduction in the expression or variability of plant attributes under W_2 regime has been reported (Richards, 1989) Mean square values due to genotype–water regime interactions were significant for all the traits. This suggests that the choice of water regime and genotypes was appropriate (Dhanda *et al.,* 2004) which may have certain implications for breeding drought tolerant wheat cultivars.

Table 1. Analyses of variance for Cell membrane stability (CMS) of 50 wheat cultivars/genotypes.

		Mean squares
SOV	**d.f**	**CMS**
Replication	2	5.749^{ns}
CMS	49	1030.825***
Error	98	2.859
CV %		2.38

*P<0.05, **P<0.01 and *** P<0.001. n.s.-Non-significant

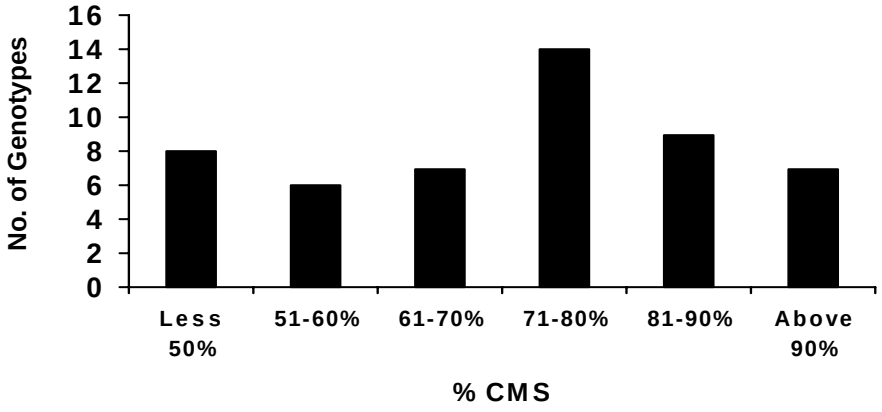

Fig. 1. Genetic variability of CMS exhibited by wheat genotypes/cultivars

Table 2. Analyses of variance for number of Tillers and 100 seed weight of 50 wheat cultivars/genotypes under two water regimes.

		Mean squares	
SOV	**d.f**	**100 Seed Weight**	**No. of Tillers**
Replication	2	0.025^{ns}	0.343^{ns}
Water Regime	1	10.043***	184.083***
Error A	2	0.016	0.863
Genotype	49	0.587***	2.882***
Water Regime x Genotype	49	0.166***	1.689*
Residual	196	0.013	0.658
CV %		2.34	20.99

*P<0.05, **P<0.01 and *** P<0.001. n.s.-Non-significant

Table 3. Phenotypic correlation among 100 seed weight, number of tillers and cell membrane stability (CMS) in 50 wheat cultivars.

	100 seed Weight	No. of Tillers	CMS
100 seed weight	1		
No. of Tillers	-0.1132^{ns}	1	
CMS	-0.18516^{ns}	0.4849**	1

Mean square values for replication of tillers and 100 seed weight were non significantly different. The interactions of the genotypes with water regime were highly significant for the 100 seed weight while it was significant for the number of tillers. It may be suggested that the choice of the environments (W_1 and W_2) and genotypes was appropriate. Thus the variation in genotypes over the environments could provide scope for breeding drought tolerant cultivars (Dhanda *et al.,* 2004).

Phenotypic correlations were computed among number of tillers, 100 seed weight and CMS (Table-3). Correlation between number of tiller and CMS was also significant. It shows that the genotypes with high CMS value produce high number of tillers and thereby perform better in drought conditions (Rahman *et al.,* 2006[a]). As far as correlation for 100 seed weight with number of tillers and CMS is concerned, it was non significant.

The cell membrane stability is a measurement of resistance induced in plants exposed to desiccation created artificially by the PEG (Sullivan, 1971). The factor(s) mainly responsible for desiccation tolerance of the leaf tissue are osmotic potential and water potential. Reduction in both results in depletion of water and ultimately the dehydration of the cell coupled with electrolyte leakage (Bartel & Nelson, 1994). Sullivan *et al.,* (1971) were the first to apply this test as a measure of CMS for selection work. The method involves the conductometric measurements of rate of solute leakage, such as electrolytes, amino acids, saccharides, organic acids, hormones, phenolics, and fluorescent materials from leaf-tissue segments after exposure to desiccation (Leopold *et al.,* 1981). The leakage from the water-stressed tissue sample is taken as an index of injury or the proportion of dead cells in the tissue (Blum, 1979).

The post desiccation electrolyte leakage is inversely proportional to CMS and has often been used successfully to screen for drought tolerance. The genotypic difference revealed by the CMS provides good relations with yield and the genotypes and can be screened on the basis of the CMS (Ashraf *et al.,* 1999). This is most useful and efficient method to screen germplasm for drought tolerance and many workers have used this for screening purposes (Shanahan *et al.,* 1990) like in Sorghum (Sullivan & Ross, 1979), wheat (Blum & Ebrecon, 1981) and maize (Premachandra *et al.,* 1989).

The traits; CMS, number of tillers and 100 seed weight showed considerable variability; however, screening under water limited conditions, CMS can be used as a preliminary selection tool in wheat breeding programme. The present study will prove the first step towards molecular markers studies. To understand drought tolerance, there is a need to initiate physico-molecular approaches to address adaptation of crop plants to under drought including escape and avoidance. The present information generated will also supplement the international data on CMS.

Table 4. 100 seed weight, number of tillers and cell membrane stability (CMS) in 50 wheat cultivars.

Cultivars	100 seed Weight	No. of Tillers	CMS
B-SILVER	**4.370**	**4.0**	21.589
BAKHTAWAR	**4.433**	**6.0**	**88.464**
BHWP-79	3.433	3.7	33.259
BHWP-94	3.810	3.7	37.851
C-271	**4.180**	2.3	53.792
C-591	3.217	**5.0**	**92.478**
CHENAB-2000	**4.263**	**4.7**	61.447
DIRK	2.903	**6.7**	**98.610**
DRAWAR-95	**3.927**	4.0	**76.445**
FBD-83	**4.033**	**5.9**	**94.710**
FPD-83	3.400	2.3	**91.206**
GA-2002	3.553	**5.0**	**83.496**
KAGHAN-	**4.293**	**6.0**	**94.024**
KHYBER-79	3.317	3.7	64.786
KOHISTAN-97	**4.473**	3.0	68.085
KOHSAR-95	3.720	**5.6**	**88.871**
LU-26	3.870	3.0	**79.535**
MARGALLA-99	3.200	3.0	**89.533**
MARVI-2000	**4.337**	1.3	54.537
MARWAT-JOL	3.863	**5.0**	49.616
MEHRAN-88	**4.120**	**5.7**	**81.061**
MH-97	**4.117**	**4.8**	**79.232**
M-PAK	**4.397**	3.0	69.530
NOWSHERA-96	**4.390**	4.1	**73.298**
NR-150	3.907	**5.0**	**89.651**
NR-180	**4.163**	**4.3**	**74.377**
NR-204	**4.057**	**4.7**	40.382
NR-212	**3.987**	3.7	35.491
NR-214	3.870	3.7	39.565
NR-228	**4.623**	3.7	**76.352**
NR-228	**4.007**	3.0	**79.246**
NR-235	**4.620**	**4.0**	**72.124**
NURI-70	**4.030**	**4.0**	53.322
PAK-81	3.730	**5.8**	**86.685**
PARI-73	3.800	3.300	**74.607**
PIRSABAR-91	3.887	2.300	**88.214**
POTHWR-93	3.743	3.000	**95.548**
PUNJAB-81	3.597	3.000	58.860
PUNJAB-85	**3.927**	2.000	50.073
RAWAL-87	**4.337**	**4.000**	**76.963**
ROHTAS-90	3.143	**3.700**	**72.075**
SALEEM-2000	3.570	2.700	67.595
SANDAL	**3.930**	3.000	**76.373**
SARSABZ	**4.117**	2.700	67.232
SHAHKAR-5	3.480	3.300	**83.421**
SHALIMAR-88	**4.420**	2.700	**74.146**
SIND-81	**4.027**	2.000	**92.214**
SONURA	**4.113**	**3.700**	**76.626**
SULTAJ-86	3.360	2.700	67.933
TANDJAM-83	**4.163**	2.700	59.333

Bold figures were representing cultivars showing 100 seed weight, number of tillers and CMS above average whereas non-bold figures that showing below average.

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