EXPRESSION OF VARIOUS PHYSIOLOGICAL TRAITS IN BREAD WHEAT UNDER DROUGHT STRESS

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ABSTRACT

Wheat, the world's third important cereal, is cultivated in Pakistan both in irrigated and rainfed areas. One of the major constraints of wheat production in rainfed area is drought that needs to be addressed. Although many genotypes were released that were tolerant to drought stress by improving various physiological traits but there is a need to lessen the effects of drought to boost wheat productivity. The present study was designed to evaluate different genotypes by analyzing various physiological traits such as relative water content, cell membrane stability, proline content and chlorophyll content to check their association with drought stress in different wheat genotypes. The research material consisted of sixteen different wheat genotypes. The experiment was planted during winter 2013-14 following RCBD with three replications. Drought stress was induced by withholding water during heading and anthesis stages. Data of various physiological traits were recorded; statistically analyzed and interpreted. Significant variation was observed for studied traits which revealed that selection can be performed to improve the genotypes. High heritability, observed for relative water content, proline content and cell membrane stability, indicated that selection would be effective for these traits as they are heritable to next generation. Correlation analysis revealed that cell membrane stability and proline content were negatively correlated with relative water content while proline content was positively correlated with cell membrane stability. Yield was only positively correlated with chlorophyll content. Among tested wheat genotypes, Maxi-Pak was found to be potential variety that can be used in future wheat breeding programme for rainfed conditions.

Key words: Wheat, cell membrane stability, proline content, chlorophyll content, drought.

1. INTRODUCTION

Triticum aestivum L., a cereal grain, is the third most produced cereal after maize and rice. Improvement in wheat yield came through dwarfing genes which was first used by Japanese wheat breeders and it was major cause of green revolution. Dwarf varieties prevent losses that occur from lodging. Wheat is grown as a food crop for human but 10 % is saved for seed and industry purpose. Earlier selection was done for improving those characters which were associated with domesticated wheat improvement. Wheat is a complex crop as regards the selection and breeding.

Water stress is worldwide issue which predicts the sustainable agricultural production [1]. As a major crop of Pakistan, wheat is planted in October-November and harvested in April to May. It is grown on rainfed as well as irrigated land. The lack of adequate soil moisture occurs at early growth stages commonly in rainfed areas leading to water stress, due to infrequent rains and non availability of irrigation facilities in rainfed area. In rainfed areas, the crop is grown on stored soil moisture received from the monsoon rains and during growth period very scanty rainfall occurs, whereas during late growing stages the rains are intermittent and variable in quantity resulting in terminal drought stress; as a consequence the final yield is adversely affected. Drought leads to stomata closure and reduction of water content, turgor loss. Sometime it leads to death of plant by disturbance of metabolism [2]. Drought affects the physiological traits of wheat crop. Efforts have been made to improve the yield of wheat crop under rainfed condition by improving the traits which are affected by drought. Main problem faced by wheat growers in rainfed region is water stress. Water stress not only affects the morphological traits but also affects the physiological traits; severity of stress depends on cultivar, growth stage, duration and intensity of stress. All the stages respond differently to water stress, some stages can cope with the stress by maintaining its water potential or turgor pressure or efficient utilization of water. Water stress can reduce the biomass, tillering ability, grain size etc. Photosynthesis is the process that is affected by drought first; various factors that contribute to photosynthesis include chlorophyll content, relative water content and various pigments. Drought causes leaf senescence in various wheat genotypes thus causing the chlorophyll degradation. Proline is an amino acid, which accumulates during various stresses as an osmo-regulatory protein, genotypes that accumulate more proline shows tolerance towards stress by maintaining the plant water potential. The present study was conducted to evaluate response of different wheat genotypes for various physiological traits under drought stress.

2. MATERIALS AND METHODS

The present study was conducted in the field area of the Department of Plant Breeding and Genetics, PMAS Arid Agriculture University Rawalpindi. A set of sixteen different genotypes of wheat (Table 1), maintained in the department, were planted in the rain-shelter (60 x 15 feet), covered with polythene sheet during growing season 2013-14, following RCBD with three replications. Seed of each genotype was sown with the help of dibbler making two rows of length 4 feet with row to row distance 9 inches. Drought stress was induced by withholding the irrigation during the growth season. Whereas, the control plants were irrigated normally during the stress period, and all plants were left to grow until grain maturation with normal irrigation.

Data for physiological traits like relative water content (RWC), proline content (PC), cell membrane stability (CMS) and chlorophyll content (CC) were recorded. Relative water content was measured following Schonfield *et al*. (1988) [3]. Fresh leaves were detached from each genotype and cut into smaller pieces of about 2 inches lengths and placed separately into zipper bags to avoid excessive transpiration losses. Fresh weight (FW) was immediately recorded and then samples were soaked for 16 hours in distilled water. Turgid weights (TW) of the samples were recorded after blotting with tissue paper to remove extra water. Leave samples were then dried for 72 hrs at 70 °C in hot air oven and dry weight (DW) was recorded. Finally RWC were calculated using following formula:

$$\boldsymbol{RWC\%} = \left[\frac{\boldsymbol{FW - DW}}{\boldsymbol{TW - DW}}\right] \times \mathbf{100}$$

Proline content was determined by the method of Bates *et al.* (1973) [4]. Fresh leaves of each genotype were used to take samples weighing 0.25g; the samples were grinded in 5ml sulfosalicyclic acid (3 %, w/v) and poured in falcon tubes. These tubes were then centrifuged at 14000rpm for 10min to filtrate the homogenate. Afterwards, acetic acid and acidic ninhydrin reagent (2 ml) was added into the filtrate. After mixing the solution it was incubated for 1 hour at 100 °C and then transferred to ice bath to stop reaction. When falcon tubes attained the room temperature then toluene (4 ml) was added, vortexed and absorbance was recorded at suitable wavelength on spectrophotometer while toluene was used as blank sample.

For cell membrane stability, samples from leaves of each genotype were collected and washed with distilled water to remove any contaminations. These samples were then put into two groups' test tubes, one control and other treated. In treated samples, 10ml of 30% polyethylene glycol (PEG-6000) was poured and in control tubes double distilled water was added. These tubes were incubated at 10°C in cold chamber for 24 hours. First reading of electrical conductivity was taken after incubation and then the tubes were covered with aluminum foil and autoclaved at 121°C with 15 lbs pressure for 15 minutes. The electrical conductivity was measured once again and CMS was determined by following formula:

$$\boldsymbol{CMS\%} = \left[\frac{\mathbf{1} - \left(\frac{\boldsymbol{T1}}{\boldsymbol{T2}}\right)}{\mathbf{1} - \left(\frac{\boldsymbol{C1}}{\boldsymbol{C2}}\right)}\right] \times \mathbf{100}$$

Where;

T1= Conductance of stress samples before autoclaving

T2= Conductance of stress samples after autoclaving

C1= Conductance of control samples before autoclaving

C2= Conductance of control samples after autoclaving

Chlorophyll content was estimated on a sunny day by using Chlorophyll Meter (SPAD-502). Three readings were recorded from the middle section of leaves of each genotype, and their averages were recorded

Analysis of variance was worked out following Steel *et al.* (1997) [5] to determine differences among different physiological traits under drought stress in wheat. Genotypic and phenotypic variance and Broad sense heritability were estimated according Johnson *et al*. (1955) [6]. Genotypic and phenotypic co-efficient of variation were estimated according to Burton (1952) [7] whereas, correlation coefficient was worked out according to Panse and Sukhatme (1967) [8].

3. RESULTS AND DISCUSSION

3.1 Relative water content

Highly significant variation was present among the genotypes sown in the rain shelter for relative water content (RWC) as depicted in Table 2. Genotype "WC-20" had maximum (81.92 %) RWC and minimum value (64.88 %) for this trait was observed in genotype "WC-24" (Table 3). According to Schonfeld *et al*. (1988) [3], genotypes having higher RWC are more tolerant to water scarce conditions compared to genotypes having lower RWC.

The values for genotype variance (GV) and phenotype variance (PV) were 18.92 and 29.62, respectively. The difference between these values showed that environmental influence was present. Little difference between genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) was depicted from the table which showed that phenotype was representative of the genotype and environmental influence is less on the trait. Since the broad sense heritability was high for this trait, hence this also means that greater proportion of variability was due to genetic factor (Table 5).

3.2 Proline Content

Analysis of variance depicted that proline content was highly significant for the genotypes under study which revealed that selection can be done for this trait (Table 2). Range for this trait was 0.02 mg/g-0.15 mg/g with mean value of 0.09 mg/g. Maxi-pak showed minimum proline content while Chenab-70 showed maximum proline content (Table 3). Genotypes having maximum proline content would be able to survive under drought stress conditions as it protects the membranes from damage under stress and can be used for the development of varieties for rainfed areas.

The value for GV was not different from the PV value as depicted from the Table 5. The GCV value was 46.77 % and PCV value was 53.75%, which showed that environmental effect was present for this trait. High heritability value (75 %) predicted that trait is under less influence of environment (Table 4.2), these results were similar to Naroui Rad *et al.* (2013) [9] who reported 97 % of heritability for this trait. Selection for this trait would be helpful in future for improvement of drought tolerant varieties for rainfed area.

Table 1: List of different wheat genotypes studied under rainfed conditions

Sr. No.	Names of Genotypes	Sr. No.	Names of Genotypes
1	99FJ-03	9	Zarlashta
2	Marvi-2000	10	Punjab-96
3	WC-13	11	Shafaq
4	WC-24	12	Maxi-pak
5	WC-19	13	WC-20
6	Faisalabad-85	14	Chenab-70
7	Kaghan	15	AUR-0809
8	Bahawalpur	16	Chakwal-50

Table 2: Mean squares of physiological traits of different wheat genotypes under rainfed conditions

SoV	Df	RWC	PC	CMS	CC
Varieties	15	67.47	0.006	429.752	3.339
Replication	2	47.016	0	0.641	2.914
Error	30	10.699	0.001	11.014	1.486
F.RATIO (Varieties)	-	6.306 **	10.363 **	39.017 **	2.246 *
F.RATIO (Replications)	-	4.395 *	.68 ^{ns}	.058 ^{ns}	1.96 ^{ns}

** = highly significant, * =significant, ns = non-significant, RWC = Relative water content, PC = Proline content, CMS = Cell membrane stability, CC = Chlorophyll content,

Table 3: Mean values of physiological traits for different wheat genotypes under rainfed conditions

Genotypes	RWC	PC	CMS	CC	Yield
99FJ-03	70.76cd	0.12ab	62.42f	38e	1720ab
Marvi-2000	80.71a	0.04de	73.12df	39.67bcde	1750ab
WC-13	67.72de	0.05de	63.51f	40.19abcd	1780ab
WC-24	64.88e	0.07d	50.17g	41.11abc	2090ab
WC-19	69.52cde	0.07cd	79.62bc	41.29abc	1610b
Faisalabad-85	70.36cd	0.11bc	64.91f	39.91bcde	1770ab
Kaghan	70.98cd	0.12ab	72e	39.28cde	1450b
Bahawalpur	70.83cd	0.05de	54.38g	40.55abcd	1660b
Zarlashta	77.79ab	0.07cd	55.67g	40.98abcd	1350b
Punjab-96	71.31cd	0.15a	78.57cd	40.22abcd	2140ab
Shafaq	69.2cde	0.03de	83.6abc	41.09abc	1570b
Maxi-pak	77.45ab	0.02e	85.62a	42.14a	2310ab
WC-20	81.92a	0.12ab	84.72ab	40.91abcd	2040ab
Chenab-70	68.89cde	0.15a	55.56g	41.52ab	2290ab
AUR-0809	71.92cd	0.12ab	80bc	40.81abcd	2400ab
Chakwal-50	73.49bc	0.13ab	62.32f	39de	2770a
Average	72.36	0.09	69.14	40.42	1918.75
LSD 0.05	5.45	0.04	5.53	2.03	1070

RWC = Relative water content, PC = Proline content, CMS = Cell membrane stability, CC = Chlorophyll content.

3.3 Cell Membrane Stability

ANOVA table showed that variation between genotypes was highly significant and the genotypes under study were not uniform for this trait suggesting selection can be done for this trait for the improvement (Table 2). Range for this trait was 50.17-85.62. Genotype WC-24 showed minimum value while Maxi-pak showed maximum value for this trait (Table 3). Genotypes that would be able to maintain their stability under drought stress shows more tolerance towards drought. Similar results were also discussed in Bayoumi *et al.,* (2008) [10].

The GV and PV values for CMS were 139.57 and 150.59 respectively, which show that environment effect was present for this trait that led to variation in expression of this trait. The GCV and PCV values showed little difference between them. Under environmental conditions different genotypes behave differently, those genotypes which showed stable cell membrane stability can be used for further breeding programs aimed at improving drought tolerance. High broad sense heritability was observed for this trait, which showed that selection would be effective (Table 5).

3.4 Chlorophyll Content

Analysis of variance showed that this trait had significant variation among the genotype and selection could be done to obtain desirable results (Table 2). Range for this trait was 38-42.14 with the mean of 40.42. 99FJ-03 and Maxi-pak were the genotypes with minimum and maximum value for chlorophyll content respectively (Table 3). Similar findings were also observed by (Keyvan, 2010) [11].

The GV and PV values for this trait were 0.61 and 2.10 respectively. While the GCV value was 1.94 % and PCV value was

3.58 %, these values depict that more variation in this trait was due to environment. Selection for this trait should be done with great care for development of drought tolerant cultivars. Broad sense heritability for this trait was low (29 %) and low heritability estimates for trait revealed that character has low genetic potential (Table 5).

3.5 Yield per hectare

Grain yield is the ultimate goal of breeder under stress condition. Analysis of variance showed non-significant differences for the yield per hectare, indicating less or no variation was present among the genotypes under study (Table 2). Range for this trait was 1350-2770 kg/hec. The genotype Zarlashta showed minimum yield under this experiment while Chakwal-50 showed maximum yield which means that this genotype performed better under stress condition and can be cultivated under rainfed conditions (Table 3).

GV and PV values were 14485.5 and 426343.8 respectively. While the GCV value was 6.27 % and PCV value was 34.04 %, which showed that genotypes was not true representative of genetic factor, environmental variation played its role and caused variation for this trait (Table 5). Rashidi (2011) [12] and Kotal *et al.* (2011) [13] also revealed the influence of environment for this trait. Low heritability was present for this trait. Yield of a plant greatly depends on the condition of experiment if they were favorable more yield can be obtained.

3.6 Correlation

Simple correlation coefficients were carried out between traits of genotypes under study with the help of Statistix 8.1 software and shown in table 4. It revealed that CMS and PC were negatively correlated with RWC while PC was positively correlated with CMS. Yield per Hectare was found to be significant and positively correlated with Chlorophyll content only. Proline is an amino acid which accumulates under drought stress and protects the membranes for ion leakage and maintains the stability of the membrane as under stress condition ion leakage increases which disrupt the membrane and ultimately become more prone to abiotic stress like drought. These results were similar to Ganbalani *et al*. (2009) [12].

Table 4: Correlation coefficients between various physiological traits under rainfed conditions

	RWC	**CC**	**CMS**	**PC**	**Yield**
RWC	1				
CC	-0.12 ^{NS}	1			
CMS	-0.89**	0.060 ^{NS}	1		
PC	-0.87**	0.063 ^{NS}	0.991**	1	
Yield	-0.190 ^{NS}	0.820*	0.164 ^{NS}	0.164 ^{NS}	1

** = highly significant, NS = Non-Significant, - = negatively correlated, RWC = Relative water content, PC = Proline content, CMS = Cell membrane stability, CC = Chlorophyll content

Table 5: Components of variation for physiological traits of different wheat genotypes under rainfed conditions

	RWC	PC	CMS	CC	Yield
GV	18.924	0.002	139.579	0.617	14485.5
PV	29.623	0.002	150.594	2.104	426343.8
GCoV	6.012	46.778	17.088	1.944	6.273
PCoV	7.522	53.753	17.75	3.589	34.03
CoH	0.639	0.757	0.927	0.293	0.034

GV= Genotypic variance, PV= Phenotypic Variance, GCoV= Genotype Coeffecient of variation, PCoV= Phenotype Coeffecient of variation, CoH= Coeffecient of Heritability.

4 CONCLUSION

Genotypes possessed significant variation for studied traits suggesting that selection can be performed to improve the genotypes for physiological traits. Traits that help to reduce the drought stress and maintain drought tolerance under rainfed condition like relative water content, proline content and cell membrane stability can be used in further wheat breeding programs.

As different wheat genotypes were evaluated for physiological traits under drought stress, one can improve and develop drought tolerant cultivars by improving the traits like proline content, relative water content and cell membrane stability that would help to overcome the drought stress under rainfed condition to obtain maximum yield. Among tested wheat genotypes, Maxi-Pak was found to be potential variety for higher relative water content, cell membrane stability and chlorophyll content. Hence it can be used in future wheat breeding programme for rainfed conditions.

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