GENETIC DIVERSITY IN SPRING WHEAT GENOTYPES UNDER DROUGHT STRESS IN BANGLADESH

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ABSTRACT

Thirty local and exotic wheat genotypes were evaluated in alpha lattice design with three replications at the Regional Wheat Research Centre (RWRC), Bangladesh Agricultural Research Institute (BARI), Rajshahi during Rabi season of 2010-11. The main objective of this study was to identify the extent of genetic diversity for traits related to drought tolerance mechanism. Genotypes were grouped into five different clusters. Cluster V comprised with the maximum number of genotypes (eleven) followed by cluster III with six and IV with five genotypes. Inter-cluster distance was maximum between clusters I and II (15.03) indicated wide genetic diversity between the genotypes of these two clusters followed by the distance between cluster I and III (14.20). The highest inter genotypic distance (1.5566) was estimated between the genotypes G4 and G18 and the lowest (0.2240) between G13 and G14. Among the characters, early ground coverage, initial plant m^2 , days to heading, peduncle length and chlorophyll content of flag leaf at 14 days after anthesis contributed the maximum for divergence in the genotypes investigated. It revealed that these parameters contributed more to grain yield under drought stress. Cluster I had the highest grain yield ha⁻¹, spikes m⁻², TDM and LAI at 86 DAS, CT_{cf} peduncle length and plant height. Cluster II exibitted the lowest days to heading, days to maturity, plant height, CT_{cf} , chlorophyll content at 14 days after anthesis, LAI, TDM and spikes $m²$. Crosses between I and II, I and III, I and IV, III and V and II and V have greater chance to generate more heterotic F_1 s. Considering magnitude of genetic distance, contribution of different traits towards total divergence, magnitude of cluster means for different traits and performance the genotypes; G30, G6 and G28 of cluster I, G18 and G7 of cluster II and G25, G27 , G19, G2 and G1 of cluster V could be utilized as parents for future hybridization programme to improve drought-tolerant high yielding wheat crop.

Keywords: Multivariate analysis, genetic diversity, spring wheat, drought stress

Abbreviations:

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CIMMYT: International Maize and Wheat Improvement Center BARI: Bangladesh Agricultural Research Institute RWC: Relative water content RWRC: Regional Wheat Research Centre

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INTRODUCTION

Wheat (*Triticum sp*) is one of the largest cereal crop of the world belongs to the *Graminae* (*Poaceae*) family. It is also the second most important cereal crop next to rice in terms of area and usefulness in Bangladesh. The area and production of wheat was gradually increased after liberation to the cropping season of 1999-2000 in Bangladesh but area has been decreasing due to unfavorable weather conditions specially drought and heat and competition with winter crops viz. maize, Boro rice and potato (BBS, 20011). In the coming period leading up to 2020, the demand of wheat for human consumption in developing countries is expected to grow at 1.6% per annum. The global average of wheat yield will have to be increased for the coming 25 years from 2.6 to 3.5 tons ha⁻¹ to meet up human consumption (Ortiz *et al.* 2008). This increased yield is very much essential to protect global food security, hence, it requires a continuous supply of improved germplasm with best regards to climate change particularly for drought. The demand of wheat is increasing day by day in this century because it needs less inputs and minimum irrigation but has high food value and high yield potential. Drought is the most common environmental stress affecting about 32% of 99 million hectares under wheat cultivation in developing countries and at least 60 million hectares under wheat cultivation in developed countries (Shamsi *et al*. 2011). Therefore, most of the countries of the world are facing the problem of drought. It has been estimated that average yield loss of 17 to 70% in grain yield is due to drought stress in wheat (Ahmadizadeh *et al*. 2011). Drought creates great challenges to the plant breeder, physiologist and agronomist around the world. In Bangladesh, scientists are primarily working to develop wheat genotypes suitable for irrigated areas whereas developments of genotypes for rainfed or dry environment are given less importance. The Wheat Research Centre (WRC) of Bangladesh Agricultural Research Institute (BARI) has so far developed 28 high yielding varieties but most of them are suitable for irrigated condition. Potentiality may exist among these cultivars and in other advanced lines for further genetic manipulation for dry environments. But efforts to identify drought tolerance characters among the existing varieties/lines to incorporate the tolerance characters into the newly developed varieties have so far not been made at least systematically in Bangladesh. New varieties need to be developed that can withstand adverse climatic condition, particularly the soil moisture stress in order to produce satisfactory yield per unit area.

Genetic diversity plays an important role in plant breeding either to exploit heterosis or to generate productive recombinants. The importance of genetic diversity for selecting plants for recombination breeding in an autogamous crop such as wheat to recover transgressive segregants has also been repeatedly emphasized (Murthy and Anand, 1966; Jatasara and Paroda, 1983). Reduction in the genetic variability makes the crops increasingly vulnerable to diseases and adverse climatic changes. Precise information on the nature and degree of genetic diversity present in wheat genotypes collected from their principal areas of cultivation would help to select parents for evolving superior varieties. In the present study, 30 wheat genotypes were tested for assessing the diversity considering yield as one of the important selection criterion. The multivariate analysis has been established by several investigators for measuring the degree of divergence and for ascertaining the relative contribution of different characters towards the total divergence (Natarajan *et al.* 1988; Golakia and Makne, 1992). D 2 statistics, cluster and factor analysis have been proved to be useful in selecting accessions for hybridization. Mahalanobis's (1949) \overrightarrow{D}^2 analysis has been successfully used in measuring the diversity in several crops. Therefore, this study would help to determine the extent of genetic diversity for drought tolerant traits that inherently present

in the evaluated breeding materials through classification them into different groups. Results of this study would also help in designing future improvement program for the development of drought tolerant varieties by selection desirable parental lines based on multivariate analysis.

MARERIALS AND METHODS

The experimental material was consisted of thirty diverse genotypes (Table 1) of spring wheat (*Triticum aestivum* L.) including a local check variety Shatabdi (BARI Wheat-21), chosen on the basis of their differences in yields and the performance of several physiological traits under drought stress conditions. This study was conducted at the experimental field of the Regional Wheat Research Centre (RWRC), Bangladesh Agricultural Research Institute (BARI), Rajshahi during 2010-11 cropping season. The experiment was laid out in Alpha Lattice design with three replications and recommended production package of RWRC was followed to ensure a good crop. The genotypes were grown in rainfed plots.

The moisture content of the experimental field soil was determined by gravimetric method. The following formulas were used to calculate soil moisture content and water at field capacity.

% Soil moisture (weight basis):
$$
\frac{\text{Weight of soil moisture}}{\text{Weight of oven dry soil}} \times 100
$$

Soil moisture contents were 22%, 20.16%, 18.42% and 18.00% after 50, 62, 74 and 86 days after sowing (DAS), respectively. The water at field capacity of the experimental field soil was 38%. At the time of crown root initiation (CRI) stage (17-21DAS) 38.4 mm rain fall was recorded. No supplementary irrigation was provided as the experiment was conducted under rain-fed condition. The crop was fertilized with NPKS and B @ 100, 28, 40, 20 and 2.5 kg ha-1 respectively to ensure proper growth and development. The elements N, P, K, S and B were applied in the form of Urea, Triple Super Phosphate, Muriate of Potash, Gypsum and Boric acid respectively.

Data on seventeen morpho-physiological traits were recorded. Early ground coverage, days to heading (DTH), days to maturity (DTM), plant height, peduncle length were recorded. Canopy temperature was measured using a handheld infrared thermometer (Mikron M90 series, Santa Clara, CA, USA). Relative water content (RWC) of leaf was calculated using following formula (Schonfeld *et al.* 1988). RWC (%) = [(Fresh weight – Dry weight) / (Turgid weight–Dry weight)] \times 100. Chlorophyll content was measured by a Minolta SPAD meter at 14-days after anthesis and expressed in SPAD unit. Leaf area index (LAI) was measured according to the formulas described by hunt (1978), $[LAI = Total leaf area of the plant / Area of the$ ground surface covered by the plant] using a Green leaf area meter, Model-GA-5, Made in Japan. Dry weight of all vegetative and reproductive organs including grain was taken after drying at 70˚C for 72-hours in an oven except root weight in total dry matter calculation. Spikes per square meter, spikelets per spike and grain per spike were measured at physiological maturity. Thousand grain weight and grain yield (t ha-1) was measured after harvesting the crop. Genetic diversity were studied following Mahalanobis (1936) generalized distance (D²) extended by Rao (1952). Based on the D² values, the studied genotypes were grouped into clusters according to the Tocher's method (Rao, 1952).The methods of Singh and Chowdhary (1985) were used for calculating the intra and inter cluster distances. Statistical analyses were performed by computer software Genstat 5.13 edition.

Genotypes	CROSS/PEDIGREE	SOURCE
G 01	Shatabdi (BARI Gom-21)	WRC, BARI
G 02	W15.92/4/PASTOR//HXL7573/2* BAU/3/WBLL1	CIMMYT
	PTSS02B00I02T-0TOPY-0B-0Y-0B-11Y-0M-0SY	
G 03	MILAN//PRL/2*PASTORI4/CROC_1/AE.SQUARROSA (213)//PGO/3/	CIMMYT
	CMSA02M00435T-040M-040P0Y-040ZTM-040SY-040M-18ZTY-04M-0Y	
G 04	CROC_1/AE.SQUARROSA (213)//PGO/3/CMH81.38/2*KAUZ/4/	CIMMYT
	CMSA02Y00059S-040P0Y-040ZTM-040SY-040M-5ZTY-0I M-0Y	
G 05	MTRWA92.161/PRINIA/5/SERI*3//RL6O1O/4*YR/3/PASTOR/4/	CIMMYT
	CM SA02M00279S-040ZTM-040ZTY-040ZTM-040SY-2ZTM-03Y-0B	
G 06	POTCH 93/4/MILAN/KAUZ//PRINIA/3/BAV92/5/MILAN/KAUZ//	CIMMYT
	CM SA05M00619T-050Y-040ZTM-040ZTY-26ZYM-03Y-08	
G 07	ACHTAR*3//KANZ/KS85-8-5/4/MILAN/KAUZ//PRINIA/3/BAW92/5/	CIMMYT
	CMSA05M00661T-050Y-040ZTM-040ZTY-33ZTM-02Y-0B	
G 08	QG 78.5//2*INQALAB 91*2/TUKURU	CIMMYT
	CMSA05M00713T-050Y-040ZTM-040ZTY-13ZTM-02Y-0B	
G 09	QG 78.5//2*INQALAB 91*2/TUKURU	CIMMYT
	CMSA05M00713T-050Y-040ZTM-040ZTY-33ZYM-01Y-0B	
G 10	QG 78.5//2*INQALAB 91*2/fIJKURU	CIMMYT
	CMSA05M00713T-050Y-040ZTM-040ZTY-33ZTM-02Y-0B	
G 11	HUANIL//2*WBLL1*2/KUKUNA	CIMMYT
	CMSA05M00752T-050Y-040ZTM-040ZTY-28ZTM-01Y-0B	
G 12	SERI*3//RL6O1O/4*YR/3/PASTOR/4/BAV92/5/WBLL1*2/TUKURU/	CIMMYT
	CMSA05M00790T-050Y-040ZTM-040ZTY-22ZYM-02Y-0B	
G 13	SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92/5/WBLL1*2/TUKURU/.	CIMMYT
	CMSA05M00790T-050Y-040ZTM-040ZTY-30ZTM-01Y-0B	
G 14	NSM*4/14-2/FRTL/2*PIFED/3/VORB	CIMMYT
	CMSA05M00650T-050Y-040ZTM-040ZTY-10ZTM-03Y-0B	
G 15	NSM*4/ 14-2/FRTL/2*PIFED/3/VORB	CIMMYT
	CM SA05M00650T-050Y-040ZTM-040ZTY-20ZTM-01Y-0B	
G 16	NSM*4/ 14-2//FRTL/ 2*PIFED/3/VORB	CIMMYT
	CMSA05M00650T-050Y-040ZTM-040ZTY-21ZTM-02Y-0B	
G 17	BABAX/LR42//BABAX/3/BABAX/LR42//BABAX/4/	CIMMYT
G 18	CM SA05M00772T-050Y-040ZTM-040ZTY-14ZTM-02Y-0B ONIX/4/MILAN/KAUZ//PRINIA/3/BAV92	
	CMSA05Y00338S-040ZTP0Y-040ZTM-040SY- I 2ZTM-03Y-0B	CIMMYT
G 19	CN079//PF70354/MUS/3/PASTOR/4/BAV92/5/FRET2/KUKUNA//	CIMMYT
	CMSA05Y01011T-040M-040ZTM-040SY-2ZTM-03Y-0B	
G 20	CN079//PF70354/MUS/3/PASTOR/4/BAV92/5/FRET2/KUKUNA//	CIMMYT
	CMSA05Y01011T-040M-040ZTPOY-040ZTM-040SY-4ZTM-03Y-0B	
G 21	CN079/ /PF70354/MUS/3/PASTOR/4/BAV92/5/FRET2/KUKUNA//	CIMMYT
	CMSA05Y0 1011T-040M-040ZTPOY-040ZTM-040SY-5ZTM-01Y-0B	
G 22	CN079//PF70354/MUS/3/PASTOR/4/BAV92/5/FRET2/KUKUNA//	CIMMYT
	CMSA05Y01011T-040M-040ZTP0Y-040ZTM-040SY-14ZTM-02Y-0B	
G 23	SOKOLL//PBW343*2/KUKUNA/3/ATTILA/PASTOR	CIMMYT
	CMSA05Y0 1188T-040M-040ZTPOY-040ZTM-040SY- I 7ZTM-02Y-0B	
G 24	GK ARON/AG SECO 7846//2180/4/2*MILAN/KAUZ//PRINIA/3/	CIMMYT
	CMSA05Y00954T-040M-040ZTP0Y-040ZTM-040SY-9ZTM-04Y-0B	
G 25	GK ARON/AG SECO 7846//2180/4/2*MILAN/KAUZ//PRXNIA/3/	CIMMYT
	CMSA05Y00954T-040M-040ZTP0Y-040ZTM-040SY-I2ZTM-01Y-0B	
G 26	GK ARON/AG SECO 7846//2180/4/2*MILAN/KAUZ//PRINIA/3/	CIMMYT
	CMSA05Y00954T-040M-040ZTP0Y-040ZTM-040SY-I5ZTM-01Y-0B	
G 27	MILAN/KAUZ//PRINIA/3/BAV92/4/WBLL1*2/KUKUNA	CIMMYT
	CMSA04M00040S-040ZTB-040ZTY-040ZTM-040SY-2ZTM-3Y-0B	
G 28	BOW/VEE/5/ND/VG9144//KAL/BB/3/YACO/4/CHIL/6/CASKOR/3/	CIMMYT
	CMSA04M01201T-050Y-040ZTP0M-040ZTY-040ZTM-040SY-2ZTM-01Y-0B	
G 29	BOW/VEE/5/ND/VG9144//KAL/BB/3/YACO/4/CHIL/6/CASKOR/3/	CIMMYT
	CMSA04M01201T-050Y-040ZTP0M-040ZTY-040ZTM-040SY-6ZTM-01Y-0B	
G 30	BOW/VEE/5/ND/VG9144//KAL/BB/3/YACO/4/CHIL/6/CASKOR/3/	CIMMYT
	CMSA04M01201T-050Y-040ZTPOM-040ZTY-040ZTM-040SY-6ZTM-03Y-0B	

Table 1. Pedigree of spring wheat genotypes used in the experiment

RESULTS AND DISCUSSION

From the principal component analysis, the eigen values are often used to determine how many factors to retain. The first 6 axes accounted 80.02% of the total variation among the 17 traits describing 30 wheat genotypes while the first two accounted 44.70% (Table 2) which suggested that these components were highly responsible for genetic divergence in the present materials. Principal component analysis reflects the importance of the largest contributor to the total variation (Sharma, 1998).

Principal component axes	Eigen	Percent contribution	Cumulative percentages
	values		
I	4.4648	26.26	26.26
П	3.1346	18.44	44.70
Ш	2.0567	12.10	56.80
IV	1.8319	10.78	67.58
V	1.0813	6.36	73.94
VI	1.0339	6.08	80.02
VII	0.8152	4.80	84.82
VIII	0.7281	4.28	89.10
IX	0.4872	2.87	91.97
X	0.3585	2.11	94.08
XI	0.2693	1.58	95.66
XII	0.2064	1.21	96.87
XIII	0.1933	1.14	98.01
XIV	0.1507	0.89	98.90
XV	0.1068	0.63	99.53
XVI	0.0492	0.29	99.82
XVII	0.032	0.19	100

Table 2. Eigen values (latent roots) and percent contribution of traits towards divergence in 30 spring wheat genotypes.

The genotypes were significantly different from each other for grain yield and different morpho-physiological traits. Using Mahalanobis' D^2 statistics and Tocher's method, the genotypes were grouped into five clusters (Table 3). Cluster I and II had same number of genotypes i.e. four and they were the smallest cluster. The cluster V was the largest cluster with eleven genotypes. The cluster III and V contained six and five genotypes, respectively. Pattern of distribution of genotypes among various clusters reflected the considerable genetic variability present in the genotypes and this wider genetic variability may be due to the adaptation of these genotypes to specific environmental conditions. Ferdous *et al.* (2011) carried out an experiment with 24 genotypes of bread wheat and got similar results with five clusters. Similar results were also obtained by Arega *et al*. (2007) on exotic and indigenous durum wheat genotypes in the northeast Ethiopia.

Table 3. Distribution of 30 spring wheat genotypes among the five clusters based on Mahalanobis' D^2 - values

Cluste	Number of	$\%$ of total	Genotypes
	genotypes	entries	
		13.33	G 4,G 6, G 28, G 30
		13.33	G 7, G 9, G 10, G 18
Ш	o	20.00	G 3, G 8, G 13, G 14, G 15, G 17
IV		16.67	G 11, G 12, G 16, G 22, G 23
		36.67	G1, G2, G5, G19, G20, G21, G24, G25, G26, G27, G29

Genotypes grouped in the same cluster (intra-cluster) are expected to be genetically related than genotypes grouped in different clusters (inter-clusters). The $D²$ analysis yielded intra (bold) and inter cluster distances (Table 4). The highest intracluster distance was observed for cluster II (0.633) and the lowest for cluster IV (0.529). The maximum inter-cluster distance was observed between the clusters I and II (15.03) followed by the distance between cluster I and III (14.20) and between clusters I and IV (13.02). The maximum value of inter-cluster distance indicated that the genotypes belonging to cluster I were far diverged from those of cluster II. Similarly the higher inter-cluster values between cluster I and III, clusters I and IV indicated that the genotypes belonging to each pair of clusters were far diversed. However, the distance between cluster II and IV was the minimum (3.10) followed by the distance between cluster III and IV (3.35) indicating genotypes within these pair of clusters were less diversed. These relationships were also reflected in the scatter diagram (Figure 1). Parents for hybridization could be selected on the basis of large inter-clusters distance for isolating useful recombinants in the segregating generations (Ghaderi *et al*. 1984; Diers *et al*. 1996). Thus, diverse lines from different clusters should be chosen for crossing in wheat breeding program. The average inter-cluster distances were higher than the average intra-cluster distances, which indicated the presence of wide genetic diversity among the genotypes of different clusters than those of the same cluster. Similar results were found by Choudhury *et al*. (2006) in wheat.

Table 4. Average intra (bold) and inter cluster distances (D^2) for 30 spring wheat genotypes

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Cluster number	I	$\overline{\mathbf{u}}$	$\overline{\rm III}$	\overline{IV}	\mathbf{V}
\overline{I}	0.589				
$\rm II$	15.030	0.633			
$\rm III$	14.200	5.880	0.579		
${\rm IV}$	13.025	3.105	3.355	0.529	
$\mathbf V$	6.993	8.399	9.549	7.164	0.579
	400 300 200 100 7 15 0 8 14 -100 Ш $13\,$ -200 -300 -400	18 10 ٠ \mathbf{I} 9 24 ۵ ٠ 16 3 12 ٠ $\boldsymbol{\mathsf{IV}}$ $\bf{22}$ $17\,$ \rightarrow 23	26 27 21 $\mathbf v$ $2 + 25$ 19 \star^5 20 $1 + 1$ ٠ 29 ó 28 ٠ I 4	$30\,$ 6	
	-600 -400	-200 $\pmb{0}$	200 400	600	

Figure 1. Scatter distribution of 30 spring wheat genotypes based on their principal component scores superimposed with clustering

Mean values of the traits for each cluster are presented in Table 5. The results revealed that the clusters remarkably distinguished for most of the traits which is indicative of proper clustering. This cluster I had the highest grain yield ha⁻¹, spikes $m⁻²$, TDM and LAI at 86 DAS, chlorophyll content, peduncle length and plant height. This cluster had the lowest intra-cluster mean for CT_{gf} . This result indicates that it has ability to maintain cooler canopy at the late grain growth stage. In addition to that this cluster also had the $2nd$ lowest early ground coverage, relative water content and TGW. In drought stress conditions, the cultivars that have more relative water content (RWC) is more resistant to drought stress and their yield is stabile (Shamsi, 2010).

Cluster III ranked the lowest for grain yield $ha⁻¹$ included six genotypes. This cluster had the lowest initial plants $m²$ and RWC. It also had the 2nd lowest early ground coverage, peduncle length and LAI. This cluster had the highest intra-cluster mean for CT_{vg} and CT_{gf} days to heading, days to maturity and plant height. The genotype in this cluster was unable to maintain cooler canopy at the vegetative growth stage and grain filling stage, long duration and low RWC might have played the key role for all negative attributes. Cluster II showed the $2nd$ highest days to heading and RWC but exibitted the lowest days to maturity, EGC, CT_{gf} , Chlorophyll content at 14-days after anthesis, LAI, TDM, spikes $m²$, spikelets spike⁻¹ and grains spike⁻¹. For relative water content cluster IV consisting of five genotypes ranked the highest. This cluster had the lowest CT_{ve} , the $2nd$ lowest peduncle length, TDM, spike per $m⁻²$ and Chlorophyll content at 14-days after anthesis. The cluster had eleven genotypes ranked the highest for TGW and initial plants population. This cluster had the lowest intra-cluster mean for grains spike⁻¹and peduncle length but this cluster had the $2nd$ highest grain yield ha⁻¹, days to heading and chlorophyll content under drought stress condition.

 The trait contributing maximum to the divergence are used to give greater emphasis for deciding on the cluster for the purpose of further selection and in the choice of parents for hybridization (Jagadev and Samal, 1991). Contributions of traits towards divergence obtained from Canonical Variate Analysis (CVA) are presented in Table 6. Negative values for traits in any of the vectors indicated lower contribution of the trait in divergence while positive values indicated higher contribution. However, plant height, canopy temperature at vegetative and anthesis stage, relative water content, leaf area

index and total dry mater at 86-days after sowing, spikes per $m²$ and grains per spike had negative values in both the vectors indicated that these seven traits had the lowest contribution to the total divergence. In vector I (the major axis of differentiation) the important traits responsible for genetic divergence were spikelets spike⁻¹ and 1000-grains weight. On the other hand in vector II (the second axis of differentiation) grain yield and maturity days were important traits having positive values. CVA revealed that early ground coverage, initial plant $m²$, days to heading, peduncle length and chlorophyll content of flag leaf at 21-days after anthesis had positive values in both vectors. Such results indicated that these five traits had the highest contribution towards the divergence among the thirty spring wheat genotypes. The result suggests that the divergence in the present materials due to these five traits would offer a good scope for improvement in drought tolerance through selection of parents. Spike length and grains spike⁻¹ were found to have the highest contribution towards total divergence as observed by Choudhury *et al*. (2006) while studying genetic diversity in 37 spring wheat genotypes with seven traits. Zaman *et al*. (2010) studied genetic diversity and reported days to maturity and1000 grain weight were found to have contribution towards total divergence.

The results of the present study indicate the presence of genetic diversity among the tested spring wheat genotypes. Parents from divergent clusters (G-30, G-6, G-28 from cluster I, G-18, G-17 from cluster II, G-25, G-27, G-19, G-2 and G-1) can be used for hybridization in order to isolate useful recombinants in the segregating generations. This information might be used in the genetics and breeding program for improvement of drought-tolerant high yielding wheat crop.

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