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GENETIC DIVERSITY FOR HEAT TOLERANCE TRAITS IN SPRING WHEAT (*Triticum aestivum* L.)

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ABSTRACT

The present investigation was undertaken with the objective to identify the extent of genetic diversity for traits related to heat tolerance among 25 currently available spring wheat genotypes under late sowing condition during the cropping season 2009-2010. All genotypes were distributed into five clusters showing considerable genetic divergence for most of the heat tolerant traits under study. The role of grain filling rate and biomass production in both the vectors had the highest contribution to genetic divergence. The positive values of both the vectors for flag leaf senescence, ground coverage, spikes number, 1000-grain weight, grain yield (g m^{-2}) and harvest index indicating high contribution of these traits towards the divergence among 25 genotypes of wheat. Three pair of clusters, viz. I & II, I & III and I & V can be considered for obtaining more heterotic progeny as the genetic distance between these clusters were larger. Considering yield performances, cluster distance and cluster mean the genotype G-22 from cluster I and genotype G-14 from cluster IV may be considered better parents for further breeding of heat tolerance as they showed maximum divergence and high degree of tolerance to heat under late sowing condition. Moreover, the genotype G-22 could be exploited for direct release as a heat tolerant variety after testing under wider range of environments.

Key words: Wheat (*Triticum aestivum* L.); SPAD value; physiological maturity; heat tolerance; heritable traits; heterotic progeny.

INTRODUCTION

Genetic diversity is the extent to which the heritable traits differ within a group of plants (Hintum and Van, 1995). High level of out-crossing within the populations, human selection, introgression and hybridization enhanced variability and diversity. Determination of genetic diversity is useful for plant breeding and hence production of more efficient plant species under different conditions (Khodadadi *et al*., 2011). Genetic diversity assessment helps to choose the parents from diversified sources for hybridization. Therefore, appropriate selection of the parents is essential to be used in crossing nurseries to enhance the genetic recombination for potential yield increase (Islam, 2004). The advanced materials are good sources of diverse germplasm. Wheat Research Centre of Bangladesh Agricultural Research Institute now has a wide range of

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spring wheat germplasm collection from different sources. It is important to evaluate the extent of diversity present in this collected germplasm and identify useful variations associated with heat tolerance. Genetic diversity for heat tolerance traits in cultivated spring wheat is well established (Midmore *et al*., 1984; Rawson, 1986; Wardlaw *et al*., 1989; Al-Khatib and Paulsen, 1990; Reynolds *et al*., 1994). Many yield related traits were identified as contributing to heat tolerance in wheat. The most responsive yield components are grains per spike, spikelets per spike, 1000-grain weight, duration and rate of grain filling, biomass, harvest index, test weight of grain etc. (Shpilar and Blum, 1986). Few other morpho-physiological traits such as canopy temperature (Reynolds *et al*., 1994; Amani *et al*., 1996 and Barma *et al*., 1997) leaf chlorophyll retention (Rees *et al*., 1993 and Reynolds *et al.*, 1994), early seedling vigor (Mann, 1994) were also demonstrated to be associated with heat tolerance. The multivariate analysis has been established by several investigators for measuring the degree of divergence and for ascertaining the relative contribution of different characters to the total divergence (Natarajan *et al.,* 1988; Sindhu *et al*., 1989 and Golakia and Makne, 1992). D^2 cluster and factor analysis have been proved to be useful in selecting accessions for hybridization. Mahalanobis's (1936) D^2 analysis has been successfully used in measuring the diversity in several crops. Divergence analysis is a useful tool in quantifying the degree of divergence between biological population of geographical level and to access in assessing relative contribution of different components to the total divergence both at intra and inter cluster levels (Jatasra and Paroda, 1978). Precise information on the nature and degree of genetic divergence helps the plant breeder in choosing the diverse parents for purposeful hybridization (Arunachalam, 1981 and Samsuddin, 1985). This study will help to determine the extent of genetic diversity for heat tolerant traits and to identify divergent spring wheat genotypes for hybridization program expecting to provide superior segregates.

MATERIALS AND METHODS

The present study was conducted at the experimental field of the Regional Wheat Research Centre, Bangladesh Agricultural Research Institute, Gazipur during the cropping season 2009-2010. The experiment covered the period from last week of December, 2009 to first week of April, 2010 and considered as late sowing condition (high temperature during later growth stages). The experimental site was situated between 23°46´N latitude and 90°23´E longitude with elevation of 8 m. above sea level. The climate of this place is characterized by wet summer and dry winter. For conducting the present study, the advanced materials were collected from the ongoing breeding program of Wheat Research Center, BARI, Dinajpur. Twenty four spring wheat genotypes along with one released popular variety "Shatabdi" were planted in RCBD with three replications. Seeds of each genotype were sown in unit plot size of 2.5m long with 6 rows in lines 20cm apart. Standard agronomic practices were adopted for the experiment. At maturity, the central 0.8 m^2 areas of each of the plot were harvested for recording spike number, grain yield (g m^{-2}) and biomass (g m^{-2}). Data were collected on the different phenological and physiological characters *viz.,* days to anthesis, flag leaf senescence (day), stay green period (day), grain filling duration (day), physiological maturity (day), canopy temperature (^{0}C), chlorophyll content (spad unit), ground coverage (scale) and grain filling rate (g m^{-2} d^{-1}).

A hand held infra-red thermometer was used to measure canopy temperature for individual genotype. The canopy temperature was measured 2 times at 3 days interval at vegetative and grain filling stage during noon period under bright sun and less wind. Mean of 2 data was used for statistical analysis. Chlorophyll content of leaves was

measured in 5 fully expended sunlit flag leaves in vivo by a Minolta spad meter at anthesis and 21 days after anthesis and expressed in spad unit. Ground coverage was recorded visually at 35 days after sowing. Usually 0-10 scale was used for the measurement of this trait. Stay green period was measured by subtracting flag leaf senescence days to the physiological maturity days.

Multivariate analysis was done by computer using GENSTAT 5.13 and Microsoft Excel 2000 software through four techniques viz, principal component analysis, principal coordinate analysis, cluster analysis and canonical vector analysis. Cluster analysis was performed by D^2 analysis (originally outlined by Mahalanobis, 1936 and extended by Rao, 1952), which divides the genotypes based on the data set into more or less homogeneous groups. Intra-cluster and inter-cluster distance, cluster mean and contribution of each character to the divergence were estimated as suggested by Singh and Chaudhary (1985).

RESULTS AND DISCUSSION

The variations among the genotypes of wheat advanced lines were studied on multivariate scale using Mahalanobis (1936) D^2 statistics. It appears that there were significant variations among all the genotypes. The covariance matrix gave non hierarchical clustering among 25 wheat genotypes and grouped them into five clusters (Table 1). Cluster IV contain the largest number (8) of genotypes followed by cluster III (6). The cluster II and V each included five genotypes. The cluster I had only one genotype. The genotypes accumulated in the same cluster are not sharply diversified.

Table 1. Distribution of 25 spring wheat genotypes in five different clusters

Cluster	Number of genotypes	Percent of total entries	Genotypes
I	1	4	G-22
II	5	20	G-9, G-10, G-13, G-19, G-23
III	6	24	G-3, G-7, G-8, G-16, G-18, G-20
IV	8	32	G-1, G-2, G-5, G-6, G-12, G-14, G-17, G-24
V	5	20	G-4, G-11, G-15, G-21, G-25

Canonical variate analysis

Average intra and inter cluster distances of five clusters are presented in Table 2. It was observed that the cluster I had high distance from the rest. It was suggested that the genotype in the cluster I was distinctly different from others. The highest distance was obtained between the cluster I and V (32.71) and it was followed by the distance between the clusters I and III (27.46), I and II (27.43). The higher inter-cluster values indicated that the genotypes belonging to each pair of clusters was far diverse, accordingly the higher heterosis in progeny could be observed. Gupta *et al*. (2002) constructed four clusters from 24 advanced lines of bread wheat and reported that the clusters I and III were highly divergent. The distances between the clusters I and II, I and III had almost the same values. Moreover, the cluster IV also showed almost the same distance from the cluster II and III, respectively. It was suggested that the genotypes belong to the cluster II and III would not result significant variation when crosses were made between the genotypes of cluster I and IV, respectively. The distance between the clusters II and III was minimum (3.50) followed by the distance between the clusters III and V, II and V suggesting genotypes belonging to these clusters were less diverged. The

genotypes within a cluster tend to diverse less from each other possibly due to similarity of parentage. Intermediate distances were found in between the clusters IV and the rest. The intra-cluster distance was the highest in cluster III (0.480) followed by the cluster IV (0.425). The lowest intra- cluster distance was in cluster I (0.00) having one genotype. Nimbalkar *et al.* (2002) studied genetic divergence of 24 cultivars and observed the highest and the lowest intra cluster distances in the clusters III and I, respectively. The intra-cluster distances were less than the inter-cluster distances, suggesting wider genetic diversity among the genotypes between the groups. Chowdhury *et al.* (2006) obtained larger inter-cluster distances than the intra-cluster distances in a multivariate analysis of wheat.

Table 2. Average intra (bold) and inter cluster distances (D^2) for 25 wheat genotypes

Cluster	I	II	III	IV	V
I	**0.00**				
II	27.43	**0.372**			
III	27.46	3.50	**0.480**		
IV	15.06	12.38	12.58	**0.425**	
V	32.71	6.60	5.25	17.82	**0.399**

Principal coordinate analysis

The results obtained from principal coordinate analysis showed that the highest inter genotypic distances were made with G-14 (Table 3). The genotype G-14 made the top four highest genotypic distances with the genotypes G-11, G-16, G-18 and G-7, respectively. The lowest distance was found in between the genotypes G-15 and G-21 as well as between G-1 and G-6. The difference between the highest and the lowest inter-genotypic distance indicated the prevalence of variability among the 25 genotypes of wheat studied.

Table 3. Five highest and five lowest inter genotypic distances among the 25 genotypes of wheat

	Inter genotypic distance				
Sl. No.	Genotypic combination	Highest distance	Sl. No.	Genotypic combination	Lowest distance
1.	G-11 and G-14	0.8825	6.	G-15 and G-21	0.2047
2.	G-14 and G-16	0.8449	7.	G-1 and G-6	0.2056
3.	G-14 and G-18	0.8389	8.	G-3 and G-25	0.2308
4.	G-7 and G-14	0.8218	9.	G-6 and G-19	0.2355
5.	G-16 and G-24	0.8198	10.	G-8 and G-25	0.2408

Principal component analysis

Principal component analysis was carried out with 25 genotypes of wheat. In PCA, the first axis accounted for 21.72% variation among the genotypes followed by 17.05% of the second axis. The first six axes accounted 80.99% of the total variation among the 17 traits describing 25 wheat genotypes while the former two accounted 38.77%. Scores obtained for the first two components were plotted against two main axes and then superimposed with clustering (Fig 1). The positions of the genotypes in the

scatter diagram were apparently distributed into 5 groups, which indicated that there exists considerable diversity among the genotypes.

Contribution of characters towards the divergence of genotypes

The values of vector I and vector II obtained from PCA are presented in table 4. From the positive absolute value of the two vectors, it revealed that grain filling rate and biomass had the greatest contribution to genetic divergence. The values of vector I and vector II revealed that both these vectors had positive values for flag leaf senescence, ground coverage, spikes number m^{-2}, 1000-grains weight, grain yield and harvest index indicating high contribution of these traits towards the divergence among 25 genotypes of wheat. Therefore, the divergence in the present materials due to these traits will offer a good scope for improvement heat tolerance through selection of parents. The positive absolute values of vector I and negative absolute values of vector II for anthesis and canopy temperature both at vegetative and grain filling stage indicated the responsibility of primary differentiation. On the contrary, the negative absolute values of vector I and positive absolute values of vector II for the characters physiological maturity, stay green period, grain filling period and chlorophyll content of flag leaf both at anthesis and 21 days after anthesis indicated the responsibility of secondary differentiation. However, negative values in both vectors for the grains number $spike^{-1}$ had lower contribution towards the divergence. Suri and Sharma (1999) reported that grain yield and tiller per plant were major contributors towards genetic divergence with moderate contribution from 1000-grain weight and harvest index. Elias and Shamsuddin (2000) grouped 16 genotypes of wheat into six clusters and reported that grain yield m^{-2} contributed maximum to the total divergence followed by 100-grain weight.

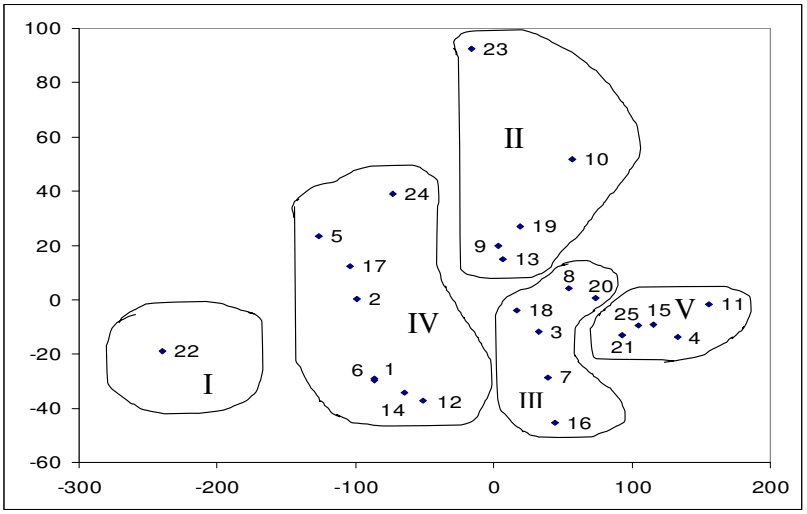

Fig 1. Scatter distribution of 25 wheat genotypes based on their principal component scores superimposed with clustering (1…25= Serial no. of the wheat genotypes).

Class mean analysis

Mean values of cluster for different phenological, physiological and primary yield contributing traits related to heat tolerance are presented in Table 5. Distribution pattern of all the genotypes into various clusters showed the presence of considerable genetic divergence among the genotypes for most of the traits studied. Exceptionally,

mean values for harvest index was same for all the clusters. Hasan (2012) obtained same class mean values of all the 7 clusters for harvest index and plant waxiness when studied in a multivariate analysis of 168 spring wheat genotypes. Genetic divergence among wheat genotypes through cluster analysis was also reported by Singh and Dwivedi (2002) and Ali *et al.* (2008). It appears that the average values of the genotype for stay green period, grain filling rate, chlorophyll content both at anthesis and 21 days after anthesis, biomass, spikes number m^{-2}, grains number $spike^{-1}$ and grain yield belongs to the cluster I were greater than the total mean of all the genotypes. The average of almost all the traits of genotypes in cluster IV were found higher than the mean of all genotypes except stay green period, chlorophyll content at 21 days after anthesis, grains number $spike^{-1}$ and harvest index.

Table 4. Contribution of characters towards divergence in spring wheat

Sl. No.	Trait	Vector I	Vector II	Sl. No.	Trait	Vector I	Vector II
1.	Anthesis	0.1735	-0.1292	10.	CHL_A	-0.2912	0.2556
2.	FLS	0.0141	0.2053	11.	CHL_{21}	-0.3529	0.2330
3.	PM	-0.1373	0.3175	12.	Biomass m^{-2}	0.2411	0.4263
4.	Stay green	-0.2381	0.1797	13.	Spikes m^{-2}	0.2721	0.2415
5.	GFD	-0.2795	0.4084	14.	Grains $spike^{-1}$	-0.0136	-0.1899
6.	GFR	0.4728	0.1298	15.	1000-grains wt.	0.0066	0.2308
7.	GC_{35}	0.1159	0.1525	16.	Grain yield m^{-2}	0.3601	0.3828
8.	C T_{veg}	0.1211	-0.0370	17.	Harvest Index	0.2661	0.0054
9.	C T_{gf}	0.1494	-0.0653				

FLS: Flag leaf senescence days; PM: Physiological maturity days; GFD: Grain filling duration (day); GFR: Grain filling rate (g m^{-2} d^{-1}); GC_{35}: Ground coverage at 35 days (scale); CT_{veg}: Canopy temperature at vegetative stage (^{0}C); CT_{gf}: Canopy temperature at grain filling stage (^{0}C); CHL_A: Flag leaf chlorophyll content at Anthesis period (SPAD); CHL_{21}: Flag leaf chlorophyll content at 21 days after Anthesis (SPAD).

In cluster II, mean of spikes number m^{-2} and 1000-grain weight were more than the total average and for other traits were less than the total average. Mean of grains number $spike^{-1}$ of the cluster III was more than the total average and rest of the traits were approximately less than or equal to the total average. These results concur with the findings of Khodadadi *et al*. (2011). Average values of all the traits belong to the genotypes in cluster V were approximately equal or less than the mean of all the genotypes. Cluster I with one genotype (G-22) was able to lead for the traits grain filling rate, chlorophyll content of flag leaf, canopy temperature, biomass, spikes number m^{-2} and grain yield in respect of cluster means of 17 characters. The cluster I included early genotype for anthesis and maturity period with the highest grain filling rate. Bruckner and Frohberg (1987) suggested that high grain filling rate and short grain filling duration appeared to contribute to increased stress tolerance. The high chlorophyll content of flag leaf both at anthesis and 21 days after anthesis belongs to the genotype of cluster I indicates high opportunity for the fixation of photosynthates to developing grains under late sowing condition. The genotype of cluster I showed low canopy temperature both at vegetative and grain filling stage suggesting that the genotype can keep their canopy cool under heat stress condition.

Considering the performances of the genotypes in five clusters it is observed that the genotypes included in the cluster IV and V took longer period for days to anthesis in late planting condition suggesting that the genotypes had the ability for anthesis even

under heat stress condition. The genotypes of cluster IV took maximum time for leaf senescence and their maturity delayed under late sowing condition. These genotypes had the green foliage for longer period under heat stress condition. Similarly this cluster included the genotypes which had high ground coverage at mid vegetative stage and produced high biomass under heat stress condition indicates their high opportunity for photosynthetic activity under late sowing condition. The genotypes belongs to the cluster I had produced the highest number of spikes m^{-2} followed by the cluster II and IV indicates that these genotypes had the ability to produce higher number of fertile spikes under heat stress.

Table 5. The average of traits for each cluster (above number) and the difference between each cluster from the total mean (within parentheses)

Sl. No.	Trait	Cluster				
		I	II	III	IV	V
1.	Anthesis (day)	64.0 (-1.42)	65.3 (-0.12)	65.0 (-0.42)	65.7 (0.28)	65.9 (0.48)
2.	Flag leaf senescence (day)	86.5 (-2.16)	88.6 (-0.06)	88.2 (-0.46)	89.4 (0.74)	88.4 (-0.26)
3.	Physiological maturity (day)	91.5 (-1.82)	92.8 (-0.52)	93.7 (0.38)	93.9 (0.58)	92.9 (-0.42)
4.	Stay green period (day)	5.0 (0.34)	4.2 (-0.46)	5.4 (0.74)	4.4 (-0.26)	4.5 (-0.16)
5.	Grain filling duration (day)	27.5 (-0.4)	27.5 (-0.4)	28.7 (0.8)	28.2 (0.3)	27.0 (-0.9)
6.	Grain filling rate (g m^{-2} d^{-1})	17.9 (3.58)	14.2 (-0.12)	13.2 (-1.12)	15.5 (1.18)	13.2 (-1.12)
7.	GC_{35} (scale)	5.3 (-0.134)	5.3 (-0.134)	5.4 (-0.034)	5.7 (0.266)	5.3 (-0.134)
8.	C T_{veg} (^oC)	23.0 (-1.19)	23.7 (-0.49)	24.2 (0.01)	24.5 (0.31)	24.4 (0.21)
9.	C T_{gf} (^oC)	30.6 (-1.45)	31.9 (-0.15)	31.6 (-0.45)	32.5 (0.45)	32.3 (0.25)
10.	CHL_A (spad)	50.2 (1.67)	47.6 (-0.93)	48.6 (0.07)	48.9 (0.37)	48.5 (-0.03)
11.	CHL_{21} (spad)	46.5 (1.89)	43.6 (-1.01)	44.4 (-0.21)	44.6 (-0.01)	45.5 (0.89)
12.	Biomass m^{-2} (g)	1150.0 (216.8)	910.0 (-23.2)	900.4 (-32.8)	1010.6 (77.4)	828.5 (-104.7)
13.	Spikes number m^{-2}	385.0 (44.4)	377.0 (36.4)	316.7 (-23.9)	355.9 (15.3)	299.5 (-41.1)
14.	Grains number $spike^{-1}$	52.3 (2.67)	48.3 (-1.33)	53.3 (3.67)	47.3 (-2.33)	49.7 (0.07)
15.	1000-grains weight (g)	37.4 (-1.14)	39.7 (1.16)	37.1 (-1.44)	39.9 (1.36)	37.1 (-1.44)
16.	Grain yield m^{-2} (g)	492.5 (94.1)	391.0 (-7.4)	377.5 (-20.9)	434.4 (36.0)	354.5 (-43.9)
17.	Harvest Index (ratio)	0.4 (-0.026)	0.4 (-0.026)	0.4 (-0.026)	0.4 (-0.026)	0.4 (-0.026)

GC_{35}: Ground coverage at 35 days; CT_{veg}: Canopy temperature at vegetative stage; CT_{gf}: Canopy temperature at grain filling stage; CHL_A: Flag leaf chlorophyll content at anthesis period; CHL_{21}: Flag leaf chlorophyll content at 21 days after anthesis

On the other hand, the cluster II and IV produced the lowest number of grains spike^{-1}. Accordingly, it can be expressed that because those genotypes has high number of spikes m^{-2} thus number of grains spike^{-1} was reduced (Khodadadi *et al.*, 2011). The genotypes in the cluster III got maximum time to stay green and took longer period for maturity under heat stress condition. As a result the genotypes in this cluster got the highest period for grain filling under late sowing. The genotypes belong to the cluster III, I and V had produced high number of grains spike^{-1} with below average 1000-grain weight. On the contrary, the genotypes of cluster IV and II had produced high 1000-grain weight with minimum number of grains spike^{-1}. This indicates a competitive demand of both sinks (grain number and size) for photosynthates from a common source, which expressed through a compensating balance between two traits under stress condition.

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