

Diversity and association analysis in bread wheat (*Triticum aestivum* L. em. Thell) under terminal heat stress condition

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In India, wheat is grown in a wide range of environments covering from northern hills to southern hills and Sourashtra to Assam. Out of six agro climatic zones of wheat cultivation, four experience high temperature stress. Considering the emerging challenges, improvement of wheat germplasm for heat tolerance emerges as one of the priority area in wheat improvement (Ortiz *et al.* 2008). Grain yield is a complex character and its maximum expression under a specific stress conditions needs a particular plant type. Association analysis are useful in providing the information on association between different characters under targeted environment for improving the plant type as whole rather than individual character. Besides, use of genetic diverse genotypes is essential in recombination breeding for generating wide range of variability for selection. Therefore, the present study was undertaken to assess level of genetic diversity and inter relationship among grain yield and its components as well as some physiological characters under heat stress environment.

The experimental material comprised of 35 diverse genotypes of wheat representing a range of phenotypic variation in terms of yield and yield components and zone of adaptations (Suppl Table 1). These 35 genotypes were grown during rabi season 2011-12 at Experimental

Farm, Division of Genetics, IARI, New Delhi. The material was sown using Randomized Block Design replicated thrice under very late sown (10th January) conditions to expose the genotypes to heat stress. Each genotype was sown in a six-row plot having a gross area of 5 m x 1.38 m with a row spacing of 23 cms with the help of self propelled Norwegian seed drill. Field was irrigated at regular intervals depending upon the rainfall and recommended standard cultural and agronomic practices were followed to raise the healthy crop. Observations were recorded on 17 quantitative characters viz., days to 50% flowering, days to maturity, effective tillers m⁻², grain number /spike, grain weight /spike, spike length, 1000 grain weight, grain yield, biological yield, harvest index, hardness index, canopy temperature depression (CTD), membrane stability index (MSI), chlorophyll a, chlorophyll b, total chlorophyll content and carotenoid content.

The canopy temperature depression (CTD) was measured at anthesis stage of the unirrigated crop using a portable infrared thermometer (Model AG-42, Teletemp Corporation, Fullerton, CA) with a view of 2.50. Membrane stability index (MSI) was estimated at anthesis stage as per Sairam *et al.* (1997). Photosynthetic pigment content was estimated at anthesis stage by extracting 0.05 g of the leaf material

in 10 ml dimethylsulfoxide (DMSO) following procedure given by Hiscox and Israelstam (1979) and Lichtenthaler and Welburn (1983). Mean values over the replication were used for statistical analysis. Variance analysis was carried out as per Panse and Sukhatame (1967); phenotypic and genotypic correlation and path coefficient analysis were carried out as suggested by Al Jibouri *et al.* (1958) and Dewey and Lu (1959) respectively. Using Mahalanobis D² statistic (Mahalanobis, 1936) diversity analysis was carried out to assess genetic divergence. The genotypes were grouped based on minimum generalized distance using Tocher's Method as suggested by Rao (1952). Analysis of variance revealed highly significant mean sum of square of treatment for all the traits under study suggesting the presence of substantial amount of genetic variability in the material under study (Table 1).

The phenotypic and genotypic correlation coefficient is presented in Suppl Table 2. The perusal of phenotypic and genotypic correlations revealed that they had same sign but the magnitude of genotypic correlations was found to be higher than that of phenotypic correlations, indicating that the elimination of environmental effects led to strengthen the genetic association between the pairs of characters. Grain yield recorded highly significant and positive correlation with biological

yield (0.754 **) and harvest index (0.507**). These relationships suggested that selection for high biological yield along with high harvest index are desirable under heat stressed irrigated condition in increasing the yield. The results are in agreement with the finding of Rahman *et al.* (1997) and Singh *et al.* (2007). Biological yield had positively significant correlation with effective tillers m⁻² (0.415**) and harvest index (0.343*). The harvest index showed positively significant correlation with photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoid) content and spike length. Thus, these characters were identified as important traits in enhancing the grain yield under stress condition and can effectively be used as selection criteria. Both days to 50 % flowering and maturity exhibited positive and significant correlation with chlorophyll a (0.351*, 0.429**), chlorophyll b (0.375*, 0.393*) and total chlorophyll content (0.364 *, 0.424 **) contents. Days to flowering also showed positive correlation with days to maturity (0.851**) and MSI (0.354*) while days to maturity showed positive correlation with carotenoid content (0.481 **) and 1000 grain weight (0.490**). Chlorophyll a showed positively significant relation with chlorophyll b (0.966**), total chlorophyll (0.999**) and carotenoid content (0.993**). Grain number /spike

Table 1. Analysis of variance for different characters of wheat under heat stress conditions

SN	Characters	Rep [2]	Treatment [34]	Error[68]	CD	CV (%)
1	Days to 50 % flowering	4.906	7.755**	0.444	1.09	1.034
2	Days to maturity	1.006	14.353**	4.029	3.28	1.907
3	MSI (%)	57.89	193.873**	61.373	12.79	15.47
4	CTD	2.464	2.835**	0.685	1.35	25.56
5	Chlorophyll a	0.352	1.398**	0.17	0.673	14.78
6	Chlorophyll b	0.052	0.093**	0.011	0.171	17.01
7	Total chlorophyll	0.674	2.184**	0.263	0.209	15.03
8	Carotenoid	0.00001	0.00006**	0.000006	0.0013	23.16
9	Effective tillers m ⁻²	25.5	9077.03**	2049.49	73.9	14.49
10	Spike length (cm)	0.018	1.789**	0.098	0.51	3.439
11	Grain weight /spike (g)	0.166	0.187**	0.27	0.267	8.73
12	Grain number /spike	35.008	156.847**	20.431	7.38	9.06
13	1000 grain weight (g)	34.406	49.357**	8.289	4.7	7.54
14	Biological yield (g/m ⁻²)	8640	110597.18**	3931.294	102.37	6.558
15	Grain yield (g/m ⁻²)	1260	236583.853**	2171.764	76.09	12.67
16	Harvest index	0.117	114.757**	24.859	8.14	12.87
17	Hardness index	1.406	87.035**	0.328	0.933	2.198

** Significant at 1% level of probability, Figure with parenthesis indicate the degree of freedom

found to be positively correlated with spike length (0.349*) and grain weight /spike (0.776**). 1000 grain weight exhibited significantly negative correlation with number of traits like photosynthetic pigments, grain number /spike (-0.596**). These findings are in conformity with the findings of other researchers. Hanchinal *et al.* (1994) reported that spike m⁻² under very hot environment may serve as valuable selection criteria. Choudhary *et al.* (1996) concluded that kernel weight and tillers number per plant were highly correlated with heat tolerance. Association of CTD, leaf chlorophyll content and 1000 grain weight with heat tolerance was reported by Hode *et al.* (1999). Semeena *et al.* (2001) reported strong correlation with harvest index under heat stress environment. Munjal and Dhanda (2004) reported positive correlation of grain yield with 1000 grain weight under heat stress conditions. Reynolds *et al.* (2007) concluded that under heat leaf chlorophyll content and canopy temperature were well correlated with yield.

These association among the characters suggested that under heat stress conditions presence of more photosynthetic pigment content had effective relationship with higher harvest index and 1000 grain weight. Therefore, under heat stress conditions selection for high biological yield along with high harvest index, more photosynthetic pigments are desirable under heat stressed irrigated condition in increasing the yield. The path coefficient analysis (Suppl Table 3) carried out at genotypic level revealed that total chlorophyll content (99.860), had maximum direct effect on grain yield followed by harvest index (3.124), 1000 grain weight (2.37), days to flowering (1.475), CTD (1.345) and grain number /spike (1.214). Of these, harvest index only showed positive correlation indicating strong and true relationship between the two traits. On the contrary biological yield which showed the maximum direct correlation but it had direct effect of lower magnitude due to negative contribution via total chlorophyll content.

All other traits like total chlorophyll content, carotenoid content, 1000 grain weight, days to flowering, CTD and grain number /spike exhibited high positive direct effect but have non-significant

positive correlations with grain yield suggesting that they are influenced by other characters. Interestingly, different photosynthetic pigments like chlorophyll a, chlorophyll b, total chlorophyll content and carotenoid content largely influenced these characters negatively in general. Besides, negative direct effect due to days to maturity, chlorophyll a and chlorophyll b, grain weight /spike and effective tillers m⁻² on grain yield while they contributed positively through carotenoid content. The present investigation, thus, revealed that harvest index, effective tillers m⁻², 1000 grain weight and photosynthetic pigments mainly chlorophyll b and carotenoids emerged out the major contributing traits towards grain yield under heat stressed environment. Plant breeders also argue that selection for yield components is more effective than yield per se (Graffius, 1959). Therefore, for improving the grain yield of heat stresses conditions, wheat breeder should aim for selecting genotypes with higher harvest index, more numbers of spike /plant, bold grains and higher amount of photosynthetic pigments.

Divergence analysis for 35 genotypes was carried out following Mahalanobis D² statistics procedure. The D² values which determine the statistical distance among various genotypes reflecting their genetic diversity was estimated for each pair of genotypes under study. Direct group constellation was formed using total D² values for each character combination by non hierarchical Euclidean cluster analysis. The 35 bread wheat genotypes were grouped into five multi genotypic clusters (Suppl Table 4). Around 28.5 percent of total genotypes (10 genotypes) were found in each of two clusters *viz.*, cluster III and IV while cluster II consist of the minimum three genotypes *i.e.*, 8.5 percent of total genotypes was grouped in cluster II. Both clusters I and V consisted of equal number of six genotypes (about 17 percent of total genotypes in each). No two clusters taken together had the similar pattern for all the traits indicating thereby wide diversity in the experimental material for majority of traits. The clustering pattern of genotypes followed random nature *i.e.*, the tendency of genotypes from different centres to be grouped in one cluster and genotypes evolved at one centre to find placed in different clusters.

This distribution pattern of genotypes into clusters also suggests lack of relationship between pedigree of genotype and genetic divergence. Walia and Garg (1996), Patil and Bhavsar (1997), Gartan and Mittal (2003), Sharma and Suri (2005) and Yousaf *et al.* (2008) also reported lack of relationship between geographic distribution/pedigree and genetic divergence.

Inter (D^2) and intra cluster distances ($=\sqrt{D^2}$) values were calculated for each pair of cluster (Suppl Table 5) revealed that maximum intra cluster distance (3.286) was found in cluster III followed by cluster I (3.125) while minimum intra cluster distance was observed in cluster II (2.521). The highest inter cluster distance was noted between cluster III and IV (5.446) followed by cluster III and V (5.141) whereas minimum inter cluster distance was observed between cluster I and IV (3.379). The perusal of inter and intra cluster distances suggested that maximum intra cluster distance values were lower than lowest inter cluster distances. Thus, the genotypes included within a cluster tended to less diverse from one another as compared to genotypes grouped in separate clusters. Therefore, genotypes belonging to the clusters separated by high statistical distance could be used in hybridization programme for obtaining a wide spectrum of variation among the sergeants. The cluster mean for each of the 17 characters was calculated by averaging the total mean values of each member belonging to that cluster and presented in Suppl Table 6. The cluster means for different characters were reflections of genetic differences prevalent among the clusters. The different clusters showed differences with each other for one or more traits. The genotypes belong to Cluster I showed desirable cluster mean value for biological and grain yields, hardness index and for early flowering and less maturity period and lower values of photosynthetic pigments. Maximum cluster mean values for MSI, effective tillers m⁻² and spike length were found in cluster II but recorded lowest biological and grain yield. High content of chlorophyll a, chlorophyll b, total chlorophyll, grain number /spike and harvest index were exhibited by genotypes grouped in cluster III. Entries grouped in cluster IV showed high value for grain weight /spike but exhibiting lowest number of effective tillers m⁻². High cluster mean value for 1000

grain weight and CTD were recorded by the cluster V with minimum harvest index, grain number /spike and spike length. Therefore, the genetically diverse genotypes included in different clusters may be selected for effective crossing programme. Considering the inter cluster distance, cluster mean and per se performance of the genotypes MP 4010, HD 3190, CL 34108, HD 2967, CL 3198, HD 2932, CL 31908, HD 2864 and DBW 17 may be chosen for effective recombination breeding which may result into wide spectrum of variability to operate section in segregating population.

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