

GENETIC DIVERSITY STUDIES AMONG DIFFERENT MORPHO-PHYSIOLOGICAL TRAITS IN SPRING WHEAT (*TRITICUM AESTIVUM* L.) IN RELATION TO HEAT TOLERANCE

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ABSTRACT

The present investigation in fifty wheat (*Triticum aestivum* L.) genotypes was undertaken to study the genetic variability and selection response for heat tolerance through measuring canopy temperature depression and chlorophyll content as selection criterion at various crop growth stages. Wide genetic diversity was observed among the accessions which were grouped into eight clusters by Tocher's method based on D² values. The intra-cluster distances were lower in all cases reflecting homogeneity in genotypes within the clusters. The cluster V contained the maximum numbers of genotypes (11) and cluster III had the minimum number of genotypes (2). The highest intra-cluster distance was observed in cluster III (228.132) that represented maximum variability within the cluster and Cluster VII (68.365) had the lowest intra-cluster distance. The maximum inter cluster distance was noticed between the clusters II and VIII (1154.262) and the lowest between clusters VI and VII (165.073). Cluster with large distances were considered more diverged than those with small distances. The major contributing character towards genetic divergence was found to be plant height (24.82%) followed by grains per spike (21.39%), chlorophyll content (16.57 and 19.10) and spikelets per spike (6.12).

Abbreviations: CTD-canopy temperature depression, PCA-principle component analysis, CVA- conical vector analysis, PCO- principle coordinate analysis.

INTRODUCTION

Wheat is the second most important cereal in India next to rice, where wheat is being grown in a relatively less cool than temperate climate and short winter. Of the cultivated wheat area, half is located in less developed countries where there have been steady increases in productivity since green revolution, associated with genetic improvements in yield potential (Reynolds and Borlaug, 2006). High temperature stress in the recent years has been major factor affecting the wheat productivity especially, in the arid and semi arid regions of the world. The earth temperatures are constantly increasing. An important goal in wheat breeding is the development of new lines with tolerance to high temperature stress (Reynolds et al. 2007, Ortiz et al., 2008). CTD and chlorophyll content measurement are two important criteria for heat stress tolerance. CTD has been used as a selection criterion for tolerance to drought and high temperature stress in wheat breeding. CTD is directly or indirectly affected by a number of physiological processes therefore is a good indicator of a genotype's fitness in a given environment (Reynolds et al., 1997). Significant reduction in chlorophyll content is due to different biotic and abiotic stresses in wheat varieties. Significant correlation between chlorophyll content and grain yield under heat and drought stresses can contribute to decrease of drought intensity damage due to reduction of chlorophyll content (Mohammadi et al., 2009). Genetic

diversity is the extent of genetic variation existed among selected cultivars, breeding lines or species.

With the development of advanced biometrical techniques such as multivariate analysis based on the Mahalanobis (1936) statistics, quantification of divergence among the biological population and assessing the relative contribution of different components to the total divergence at intra and inter-cluster levels have now become possible. Such a study also permits to select the genetically diverse parents to obtain the desirable recombinant in the segregating generations upon crossing. Among the various methods identified/developed to study the genetic divergence in the genotypes/accessions, the Mahalanobis D² (Mahalanobis, 1936) is reliable and most frequently used. It is a very useful technique of measuring genetic divergence (Meena and Bahadur, 2013; Sharma and Devi, 2013; 2014; Srivastava et al., 2014). Inclusion of more diverse parents (within a limit) is believed to increase the changes for obtaining stronger heterosis and gives broad spectrum of variability in segregating generations (Joshi and Dhawan, 1966; Ananda and Murty, 1968). In addition, crossing in moderately diverse parents also showed maximum heterosis (Chauhan and Singh, 1982).

The knowledge and degree of nature of divergence is useful in selection of the desirable parents for breeding program. To recommend few promising lines for heat stress tolerance in wheat (*Triticum aestivum* L.) was the main objective of the communication.

MATERIALS AND METHODS

Plant materials

The genotypes of wheat (*Triticum aestivum* L.) were taken from the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences. It consisted of 50 different genotypes from CIMMYT trails (Genotype 1-Genotype-50, including one check HUW-234) (Table 1).

Location

The study was carried out in Varanasi at the Agriculture Research farm of Institute of Agricultural Sciences, Banaras Hindu University during two consecutive growing years (2010-11 and 2011-12). Varanasi lies in the North eastern part of the country and enjoys sub-tropical climate. The Agriculture Research Farm is situated in South- Eastern part of Varanasi city at 25°15' North latitude and 83°03' East longitude at an elevation of 75.7m above the mean sea level.

Observations recorded

The observations of various morpho-physiological traits such as germination %, Days to 50% heading, chlorophyll content, canopy temperature depression, days to 50% anthesis, days to 50% maturity, plant height, spike length, number of spikelets per spike, number of grains per spike, 1000 grain weight, and plot yield were taken.

Agro-climatic and weather conditions

Varanasi is subjected to extremes of weather conditions. Temperature ranged during crop season was from 16.5°C to 41.6°C during day and from 7.2°C to 27.9°C during night with relative humidity 96% to 17%.

Methods

The experiments were designed in a randomized block design

with three replications. The Minolta SPAD-502, a hand held chlorophyll meter was used for rapid estimation of the chlorophyll level in plant leaves. Canopy temperature was measured and recorded on per plot basis using a hand held infrared thermometer-based equipment (Sixth Sense LT-300). CTD was calculated as per Bahar *et al.*, 2008. CTD = Air temperature (°C) - Canopy temperature (°C).

Statistical analysis

Mean values were subjected to analysis of variance to test the significance for each character as per methodology advocated by Panse and Sukhatme (1967). Mahalanobis D2 analysis The data collected were subjected to multivariate analysis utilizing Mahalanobis D2 statistic as suggested by Mahalanobis (1936) and Rao (1952) using statistical software WINDOSTAT 9.1 developed by INDOSTAT services Ltd. Hyderabad, India. Accessions were grouped into various clusters following Tocher's method as suggested by Rao (1952). The necessity of principal component analysis (PCA), principal coordinate analysis (PCO), non-hierarchical clustering and conical root analysis or canonical vector analysis (CVA) for measuring the degree of divergence has been established by several investigators in rice and other crops (Selvakumar *et al.*, 1989; De *et al.*, 1988; Pathan *et al.*, 1993).

RESULTS AND DISCUSSION

The analysis of variance (ANOVA) for all the characters was studied showed in Table 2. Among the genotypes almost all characters showed highly significant variation indicating wide range of variability present among the genotypes.

Cluster analysis

By application of non-hierarchical clustering using covariance

Table 1: List of genotypes of *Triticum aestivum* L. with their pedigree

No.	Genotype name/Pedigree	No.	Genotype name/Pedigree
1	HUW-234	26	ALTAR84//AE.SQUARROSA(219)//2*SERI/4/....
2	DHARWAR DRY	27	ALTAR84//AE.SQUARROSA(219)//2*SERI/4/....
3	CHAM 6	28	SOKOLL//SUNCO/2*PASTOR
4	SOKOLL/PASTOR/HXL7573/2*BAU	29	SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/4/PARUS/PASTOR
5	PASTOR/HXL7573/2*BAU/3/SOKOLL/WBLL1	30	OR 9437534/SOKOLL//SOKOLL
6	NS-732/HER/3/PRL/SARA/TST/VEE#5/4/FRET2	31	MRC//KAUZ//SKAUZ/3/SUNSTATE/5/VEE/LIRA//BOW/3/BCN..
7	CROC_1/AE.SQUARROSA(213)//PGO/10/ATT...	32	MRC//KAUZ//SKAUZ/3/SUNSTATE/5/VEE/LIRA//BOW/3/BCN..
8	MILAN//PRL/2*PASTOR/4/CROC_1/AE.SQUA...	33	W97.234//SW89.5181//KAUZ/5/VEE/LIRA//BOW/3/BCN/4/KAUZ
9	CROC_1/AE.SQUARROSA(213)//PGO/3/CMH81..	34	MTRWA92.161//PRINIA/5/SERI*3//RL6010/4*YR/3/PASTOR....
10	CROC_1/AE.SQUARROSA(213)//PGO/3/CMH81..	35	MTRWA92.161//PRINIA/5/SERI*3//RL6010/4*YR/3/PASTOR....
11	CHEN/AEGILOPS SQUARROSA(TAUS)//BCN..	36	MTRWA92.161//PRINIA/5/SERI*3//RL6010/4*YR/3/PASTOR....
12	CHEN/AEGILOPS SQUARROSA(TAUS)//BCN..	37	MTRWA92.161//PRINIA/5/SERI*3//RL6010/4*YR/3/PASTOR....
13	FILIN/2*PASTOR//PRL/2*PASTOR	38	PSN/BOW//MILAN/3/2*BERKUT
14	ATTILA*2/9/KT/BAGE/FN/U/3/BZA/4/TRM/5/....	39	PSN/BOW//MILAN/3/2*BERKUT
15	QT9155//JUN/BOMB/3/FILIN/2*PASTOR	40	SLVS/PASTOR/3/PASTOR//MUNIA/ALTAR 84
16	SHAM4//VEE#5/NAC/3/ATTILA*2/M10.....	41	672.2/P66.D.270//AE.SQUARROSA(320)/3/CUNNINGHAM/4....
17	CHRZ//BOW/CROW/3/WBLL1/4/CROC_1/AE.SQ...	42	BERKUT/EXCALIBUR
18	CHRZ//BOW/CROW/3/WBLL1/4/CROC_1/AE.SQ...	43	672.2/P66.D.270//AE.SQUARROSA(320)/3/CUNNINGHAM/4....
19	KLDR/PEWIT1//MILAN/DUCULA	44	BARKUT/HTG
20	WBLL1//ATTILA*2/M10(MUTATEDC-306)/3/QT...	45	BARKUT/HTG
21	HSB1313/2*WBLL1	46	SOKOLL//PRL/2*PASTOR
22	FILIN/IRENA/5/CNDO/R143//ENTE/MEXI_2/3/AE..	47	SOKOLL/92.00/E7.32.5
23	SOKOLL/EXCALIBUR	48	SOKOLL/EXCALIBUR
24	SUNCO/2*PASTOR//EXCALIBUR	49	SOKOLL/EXCALIBUR
25.	CROC_1/AE.SQUARROSA(205)//KAUZ/3/LANG	50	KRICHAUFF/FINSI/3/URES PRL//BAV/92/4/ALTAR84/AE.SQ..

Table 2: Analysis of variance (ANOVA) for all the characters under study in fifty lines of wheat

Mean sum of squares											
Source	Degrees of freedom	Germination percent	Days to heading	Days to anthesis	Plant height	Spike length	Spikelet per spike	Days to maturity	Grains per spike	1000 grain weight	Yield per plot
Replication	1	0.25	3.24	0.04	2.41	0.00	0.99	0.09	8.60*	0.08	18769.00*
Treatment	49	28.78**	4.56***	6.47***	1.84***	1.68***	5.02***	13.72***	53.98***	47.03***	8890.33**
Error	49	14.54	1.61	1.59	0.71	0.19	0.42	1.72	1.61	9.26	4247.92

Mean sum of squares											
Source	Degree of freedom	Chlorophyll content-1	Chlorophyll content-2	Chlorophyll content-3	Canopy temperature-1	Canopy temperature-2	Canopy temperature-3	CTD-1	CTD-2	CTD-3	
Replication	1	0.04	0.86	0.19	2.41	3.06	0.03	1.57	5.52*	0.03	
Treatment	49	18.74***	20.10***	19.79***	1.84***	2.49***	2.39*	1.59***	2.82***	2.39	
Error	49	0.76	0.36	0.46	0.71	0.87	1.42	0.61	1.07	1.83	

* Significant at Pd* 0.05 level of significance ** Significant at Pd* 0.01 level of significance; *** Significance at Pd*0.001 level of significance

Table 3: Grouping of wheat genotypes into eight clusters (Ward)

Clusters	Cluster Members(genotypes)	Number
1	6 20 21 31 36	5.000
2	2 12 23 32 34	5.000
3	13 16	2.000
4	3 7 14 17 19 24 27 37 39 43	10.000
5	9 10 11 15 16 25 28 30 33 35 50	11.000
6	1 8 26 29 38 44	6.000
7	22 41 42	3.000
8	4 5 40 45 46 47 48 49	8.000

matrix, the fifty genotypes were grouped into eight different clusters shown in Table 3. In line with this, M. Ferdouset al. (2011) grouped wheat genotypes into 5 clusters. Ribadia et al., (2007) also studied the genetic divergence among 50 exotic genotypes of wheat by employing Mahalanobis’s D2 analysis based on 10 characters. The genotypes were grouped into six clusters. Cluster V contained the maximum number of eleven genotypes followed by cluster IV, VII and VI having ten, eight and six genotypes, respectively. Cluster III contained the least number of two genotypes. Canonical root analysis was done to compute the intra and inter cluster distance (D²) values are presented in Table 4. Results indicated that the inter cluster distances were larger than intra cluster distances in most of the cases suggesting wider genetic diversity among the genotypes of different groups. Singh et al. (1987) also reported about the cluster by using D²-statistics. The maximum inter cluster distance was observed between clusters II and VIII (1154.262) followed by I and VIII (1003.560), II and V (889.028), II and IV (857.856). The maximum inter cluster distance indicated the genotypes in these clusters were far diverse than those of other clusters. The minimum inter-cluster distance was observed between clusters VI and VII (165.073) (Table 4) indicating a close relationship among the genotypes of these cluster. The highest intra-cluster distance was found in cluster III (228.132) followed by cluster II (172.491) and IV (169.530). The lowest intra-cluster distance was noticed for cluster VII (68.365). These results revealed that the genotypes in cluster III were distantly related; on the other hand the genotypes in cluster VI were closely related. These results confirmed the clustering pattern of the genotypes according to principal component analysis. It is expected in our results that the crosses between the genotypes of cluster II and VIII, I

and VIII and II and V would exhibit high heterosis and produce new combination with desirable traits. The genotypes of distant cluster could be used for further hybridization program.

Cluster means for the characters

The mean performances of nineteen characters in six clusters are shown in Table 5. Most of the characters showed distinct difference among the clusters. In cluster I, it contained the highest values for the character germination percent and canopy temperature depression and the lowest values for the characters canopy temperature, chlorophyll content and plant height. The highest values indicated that heat tolerant genotypes of *Triticum aestivum* L. could be found from this cluster. Cluster II produced the highest cluster mean for the characters spikelets per spike and grain per spike and the lowest mean values observed from the character days to 50% heading and days to maturity. So, the short duration and optimum yielding genotypes could be found from this cluster. Cluster V had the lowest cluster mean for the character spike length, spikelets per spike, grains per spike, plot yield and CTD at all the three stages and the highest cluster mean value for the character days to maturity and canopy temperature at vegetative and early reproductive stages, so this cluster could neither provide us high yielding nor short duration genotypes and also they are susceptible to heat stress. Cluster VII had the highest cluster

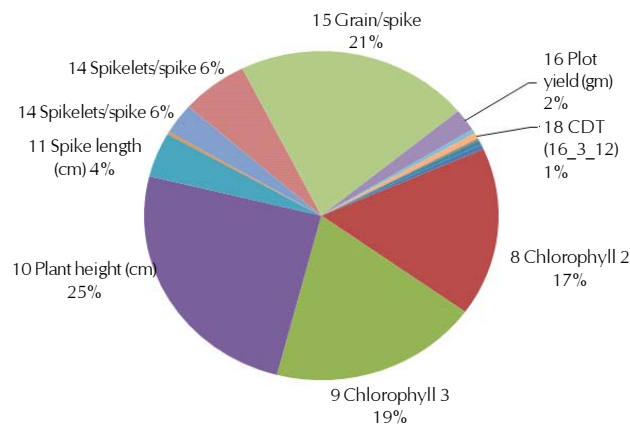


Figure 1: Contribution of various traits towards divergence in 50 *Triticum aestivum* L. genotypes

Table 4: Average Intra and Inter-cluster D²-Values (Ward's) among eight clusters of 50 wheat genotypes.

	1 Cluster	2 Cluster	3 Cluster	4 Cluster	5 Cluster	6 Cluster	7 Cluster	8 Cluster
1 Cluster	164.094	376.604	332.59	562.312	584.162	449.007	795.739	1003.560
2 Cluster		172.491	333.446	857.856	889.028	499.423	715.600	1154.262
3 Cluster			228.132	562.624	400.383	332.132	539.120	856.454
4 Cluster				169.53	359.716	216.487	319.296	256.753
5 Cluster					80.639	309.930	525.084	576.419
6 Cluster						96.927	165.073	293.821
7 Cluster							68.365	223.293
8 Cluster								159.962

Table 5: Cluster mean values (ward's) for different traits

	Germination percent	Days to heading	Days to anthesis	Plant height (cm)	Spike length (cm)	Spikelet/spike	Days to maturity	grains/spike	1000 grain wt.(g)	Yield per plot
1 Cluster	95.000	76.400	80.000	94.200	9.700	17.429	111.400	42.495	40.865	698.800
2 Cluster	93.000	76.100	80.200	103.100	9.400	17.897	110.200	47.762	39.354	707.800
3 Cluster	90.938	77.125	80.500	101.688	9.156	16.455	111.875	36.746	41.138	665.250
4 Cluster	93.333	77.083	80.417	94.333	8.917	16.579	112.667	39.246	36.067	683.000
5 Cluster	91.250	79.250	84.000	98.750	8.000	14.080	117.500	29.245	42.285	621.500
6 Cluster	93.636	76.545	80.545	100.727	9.045	16.617	115.500	40.935	39.533	695.727
7 Cluster	90.625	76.125	79.625	104.875	9.875	18.080	115.000	40.330	43.270	799.750
8 Cluster	90.556	76.667	80.444	99.611	9.389	17.126	114.667	40.478	39.767	656.333

	Chlorophyll content 1	Chlorophyll content 2	Chlorophyll content 3	Canopy temperature1	Canopy temperature2	Canopy temperature3	CTD 1	CTD 2	CTD 3
1 Cluster	40.040	39.040	37.820	19.310	24.060	28.830	6.590	7.540	4.170
2 Cluster	40.520	39.510	38.080	18.475	24.340	29.326	7.125	6.660	3.674
3 Cluster	41.525	40.869	38.906	19.200	24.128	29.319	6.587	6.872	3.681
4 Cluster	45.333	44.450	43.100	19.558	23.772	29.142	6.042	7.228	3.858
5 Cluster	43.675	43.200	41.350	20.050	24.862	29.800	5.550	6.137	3.200
6 Cluster	44.955	44.155	42.605	18.880	24.280	29.304	6.857	6.720	3.696
7 Cluster	47.663	46.675	44.850	17.719	24.113	29.006	7.881	6.888	3.994
8 Cluster	47.939	47.583	46.056	19.108	24.450	29.381	6.492	6.717	3.619

Table 6: Relative contributions of the ten characters to the total divergence in wheat

Character	Vector I	Vector II
1. Germination %	0.07961	0.17095
2. Days to 50% heading	0.00296	-0.46779
3. Days to 75% anthesis	0.02618	-0.45630
4. Canopy temperature-1	0.05404	-0.04677
5. Canopy temperature-2	0.03656	-0.20810
6. Canopy temperature-3	0.00741	0.27326
7. Chlorophyll content-1	-0.39335	-0.03805
8. Chlorophyll content-2	-0.40155	0.01197
9. Chlorophyll content-3	-0.40296	0.02832
10. Plant height	0.26230	-0.09996
11. Spike length	0.06237	0.10944
12. 1000 kernel weight	0.06053	-0.22715
13. Days to maturity	0.12046	-0.28174
14. Spikelets/spike	0.10547	0.31833
15. Grains/spike	0.06370	0.20355
16. Plot yield	0.37376	-0.08267
17. CTD-1	0.28885	0.17320
18. CTD-2	0.21537	0.26839
19. CTD-3	-0.36709	0.12885

mean values for the characters plant height, spike length, 1000 kernel weight, spikelets per spike, plot yield and CTD at vegetative stage and had lowest cluster values for the character days to 50% anthesis and canopy temperature at vegetative stage. So, this cluster could provide as high yielding as well as

heat tolerant genotype.

Contribution of the characters towards divergence of the genotype

Contribution of characters towards divergence of the genotypes can be studied through Figure 2 and Table 6. The major contributing character towards genetic divergence was found to be plant height (24.82%) followed by grains per spike (21.39%), chlorophyll content (16.57 and 19.10) and spikelets per spike (6.12) Goel *et al.* (2005). Plant height contributes highest following grains per spike, chlorophyll content at early and late reproductive stages. Earlier researchers also reported significant variation among characters like CTD (Binod Kumar *et al.*, 2013). CTD is an efficient parameter for stress diagnostic and selection of heat stress adapted genotypes. It works on the principle that surface temperature of the canopy is related to the amount of transpiration resulting in evaporative cooling. According to the conical root analysis the characters germination percent, canopy temperature at late reproductive stage, spike length, spikelets per spike, grains per spike and CTD at reproductive stage had positive signs in both the vectors which indicated that these were the important component character having higher contribution to the genetic divergence among genotypes studied (Table 6). It is interesting that the greater divergence in the present materials due to these two characters will offer a good scope for improvement of yield with high heat tolerance thorough rational selection of parents for producing heterotic wheat hybrids.

Selection of parents for future breeding program

Genotypes contained in clusters exhibiting high cluster mean for specific traits may be selected for breeding programme. The crossed involving parents belonging to maximum divergent clusters were expected to manifest maximum heterosis and also wide genetic variability. A higher heterosis could be produced from the crosses between genetically distant parents (Ghaderi *et al.*, 1984). Keeping this in view, it appears that crosses between genotypes belonging to cluster II and VIII, I and VIII and II and VI would give high manifestation of heterosis as well as wide spectrum of genetic variation in F₂ generation. Genotypes included in cluster II was important for earliness in maturity, cluster VII for maximum plant height, spike length, 1000 kernel weight, spikelets per spike, plot yield and canopy temperature depression at all the three crop stages. Cluster VIII is important for chlorophyll content. Considering cluster distance, inter-genotypic distance, cluster mean and other agronomic performances, genotypes of cluster I, II, VII and VIII may be selected for further hybridization program.

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