Original Research Article

Evaluate the genetic divergence and Principal component analysis in Bread wheat (*Triticumaestivum*L.)

Abstract

The current investigation was carried out on somebread wheat (*Triticumaestivum*L.) genotypesthroughoutspring season to evaluate their heat tolerancevia Cluster Analysis and principal component analysis (PCA). The experiment was accomplished in an augmented block design with 60 genotypes and three replications. Evaluations werecarried out on 26 quantitative traits.Cluster analysis showed five clusters, cluster I with 56 genotypes and clusters II, III, IV and V with only one genotype each.The clusters II, III, IV and V have only one genotype each, so their intra-cluster distances were zero. The intra-cluster distance for cluster I was57.879.The maximum and minimum inter cluster distance was found between cluster II andIII (267.377) and between cluster I andII (86.469), respectively. Cluster I showed theearliest(76.689 days)average forearly maturity(n° of days to 50%flowering)andcluster III showed the maximum (27.664) average for grain yield(grain yield per plant). PCA indicated that the five principal components (PC1 to PC5) accounted for65.61%of the total variance. PC1 accounted for 11.51% of the total variance and showed positive factor loading for almost traits. Harvest index, grain yield per plant, flag leaf width and leaf rolling showed the highest factor loadings for PC1.As a result of the foregoing dataand analysis, it is possible to conclude that there is great potential for effective geneticimprovement for grain yield and correlated traits in the present wheat genotypes.(Genetic improvement through:Hybridizationby crossing genotypes from cluster I to the further clusters? What about crossing genotypes within the cluster I?And what about recurrent selection in cluster I in order to get new genotypes that show higherheterosis with further clusters? I think it is important to emphasise these aspects).

Key word: Wheat; genetic divergence; cluster analysis; morphological traits.

INTRODUCTION

Wheat (Triticum*aestivum*L., 2n=42) is the most important cereal in the world.Wheat belongs to the family Poaceae (Gramineae) and tribe Triticeae containing more than 15 genera and 300 species including wheat and barley.T. *aestivum* is a segmental allohexaploid (2n = 6x = 42, AABBDD) originated in the Fertile Crescent area of South-Western Asia its geographical centre of origin and spreaded globally for cultivation and consumption. It is an allohexaploid (AABBDD, 2n = 6x = 42) that arose by hybridization between a cultivated tetraploid wheat *T. turgidum* (AABB, 2n = 4x = 28) and the wild goatgrass *Aegilopstauschii* (DD, 2n = 2x = 14).

The nutritional composition of the wheat grain varies somewhat with differences in climate and soil. On an average, the kernel contains 10-12 per cent water, 65-70 per cent carbohydrates, 9-12 per cent protein, 1-2 per cent fat, 1-1.8 per cent minerals, and 2-2.2 per cent crude fibres. Thiamine, riboflavin, niacin, and small amounts of vitamin A are present, but the milling processes removes most of those nutrients with the bran and germ.

The yield and productivity of wheat are seriously threatened by high temperatures. In India, wheat cultivation suffers significant injuries each year due to high temperature stress (Kumar et al.2013). As wheat is a crop that grows in the winter, it needs an extended period of low temperatures to attain its highest grainyield. According to Wahid *et al.* (2007) and Sareen*et al.* (2015), high temperature stress is the outcome of a temperature increase that lasts longer than a threshold and has the potential to permanently harm plant growth and physiological development.

In the hybridization programme, D2 statistics analysis is used to select genetically dissimilar parents. P.C. Mahalanobis introduced the concept of D2 statistics in 1936. Rao utilised this approach to estimate genetic divergence in plant breeding. It is used to assess the degree of divergence and identify the relative contribution of each component characteristic to overall divergence. Genetic divergence is important in plant breeding because hybrids from divergentlines exhibit more heterosisthan closely related parents. Principal Component Analysis (PCA) is a statistical technique that transforms a set of correlated variables into a smaller set of uncorrelated variables, called principal components. These components retain most of the variance of the original data, allowing for a simplified analysis without losing important information.This experiment was undertaken in order toassessgenetic divergence among wheat genotypes using cluster analysis and PCAto select desirable parents in hybridization programmes.

MATERIAL AND METHODS

The current investigation wasperformed at three different locations; namely:Crop research farm, Nawabganj (UP); Crop research farm, Araul(UP); and Crop research farm, Daleep Nagar

(UP),during crop season of spring in2021-2022 and 2022-2023years, under normal (non-stressed) and late sown (heat-stressed) conditions. The field experiments comprised 60 germplasm of bread wheat. Furthermore, the experimental trials were conducted in 12 environments (E1 to E12), which included six non-stressed (NS) and six heat-stressed (HS) environments. The field experiments were laid out in Augmented Block Design (ABD). Each experimental plot consisted of three rows of 2m length by 20 cm between rows covering an area of 2 m x 0.20m x 3 (1.2 m^2) under irrigated condition. The 26 physio-morphological and grain yield related traitsevaluated were:Days to 50% flowering (D50H); Number of spikelets per spike(NSPS); Plant height (PH,cm); Physiological maturity (PM, days); Spike bearing tillers per plant(SBTPP); Plant biomass (PB, g); Peduncle length (PL, cm); Number of grains per spike(NGPS); Flag leaf length (FLL, cm); Number of grains per plant(NGPP); Flag leaf width (FLW, cm); Grain length(GL, mm); Flag leaf area (FLA, cm2); Grain width(GW, mm); Chlorophyll content(CC), L/W ratio of grains(??);Canopy temperature depression (CTD, ^{0}C); Grain yield /plant(GYPP, g); Plant waxiness (PW, 0-10); Harvest index (HI, %); Leaf rolling (LR, 0-10 scale); 1000 grain weight(??, g); Grain filling period (GFP, days); Protein content (PC, %); Spike length (SL, cm);and Gluten content (GC; %).~~The F test (ANOVA) for genotypes wassignificant for all traits, indicating the presence of genetic variability among the genotypes.~~(This is a result, not a method. Let´s put it in the beginning of the Results and Discussion)

List 1 :Details of bread wheat accessions used in the study. (I think this list is not necessary).

Sr. No.	Genotypes	Sr. No.	Genotypes
1.	K-1711	31.	K-2105
2.	K-1903	32.	K-2109
3.	K-1805	33.	K-0307
4.	K-1907	34.	K-0607
5.	K-1910	35.	K-1803
6.	K-2003	36.	K-1317
7.	K-0306	37.	PBW-852
8.	K-0402	38.	DBW-173
9.	K-2107	39.	HD-3388
10.	K-2121	40.	HD-2359
11.	K-8962	41.	K-9644
12.	K-9351	42.	K-2101
13.	K-9465	43.	KRL-213
14.	K-8027	44.	KRL-19
15.	K-2103	45.	PBW-826
16.	K-1006	46.	DBW-187
17.	K-1616	47.	HD-3392

18.	K-1905	48.	HD-2967
19.	K-1809	49.	DBW-107
20.	K-1908	50	DBW-222
21.	K-2001	51.	PBW-833
22.	K-2007	52.	HD-3399
23.	K-9107	53.	PBW-835
24.	K-9162	54.	KRL-210
25.	K-9533	55.	KRL-1-4
26.	K-2108	56.	K-2010
27.	K-9423	57.	KRL-283
28.	K-8434	58.	DBW-350
29.	K-7903	59.	HD-3086
30.	K-2104	60.	WH-1142

RESULT AND DISCUSSION

Genetic Divergence:

~~The non-hierarchical Euclidean cluster analysis was employed to study the genetic divergence existing among 60 bread wheat germplasm collection based on twenty-six characters.~~(This is not discussion, this is method)

The F test (ANOVA) for genotypes was significant for all traits, indicating the presence of genetic variability among the genotypes.

Supported by the pseudo-F-test, the 60 genotypes were grouped into 5 non-overlapping clusters. The distribution of 60 bread wheat genotypes in 5 clusters is presented in Table 1.

~~The clustering pattern of the sixty genotypes were grouped into five different non-overlapping cluster.~~(Already written before)

Cluster analysis showed five clusters, cluster I with 56 genotypes and clusters II, III, IV and V with only one genotype each.This indicatesdivergencebetween cluster I and the subsequent four clusters as well as among these four clusters.The clusterstypically comprised genotypes of different origins, indicatinga low correlation between genetic and geographic divergence. However, genotypes from the same origin or geographical area were also shown to be clustered together. Grouping genotypes fromdifferent origin or geographic region in same cluster were frequently observed.The estimates of intra and intercluster distance represented by D^2 values are given in Table 2.As the clusters II, III, IV and V have only one genotype each, their intra-cluster distances were zero. The intracluster distance for cluster I was57.879. The maximum inter cluster distance was found between cluster IIandIII (267.377) andthe minimum was foundbetweencluster I andII (86.469) (Table 2). The higher inter- cluster distance indicated greater genetic divergence between the genotypes of those clusters, while lower inter-cluster values between the clusters suggested that the genotypes of the clusters were not much genetically diverse from each other.

These results are in close conformation with the findings of Rahman*et al.* (2013) and Kumar *et al.* (2016),Pandey*et al.* (2021)and Abdelghany*et al.*(2023). (Have they donesomeinvestigation with the same genotypes that you used in this work?)

A lookon table 3 showed that cluster means for the different traits indicated considerable differences between the clusters. The entire cluster from cluster I to cluster V had average mean performance for most of the characters.(I think there is a miss interpretation here. The cluster means differences among clustersfor each trait are small, of course. And the cluster means differences among traits for each cluster are big, of course too.)

Cluster I showed earliest mean value for day to 50 per cent flowering (76.689 day), cluster II showed earliest mean value for the grain filling period (29.536 day), cluster II showed maximum mean value for flag leave length (23.579), cluster III showed maximum mean value for flag leave width (1.988), cluster II showed maximum mean value for flag leave area (46.832) cluster IV showed maximum mean value for plant height (101.590), cluster III showed maximum mean value peduncle length (36.941), cluster V showed maximum mean value for spike bearing tiller per plant (12.415), cluster III showed maximum mean value for chlorophyll content (30.244), cluster I maximum mean value for canopy temperature depression (7.864), cluster III showed maximum mean value for plant waxiness (5.694), clusterII showed maximum mean value for leave rolling (5.596), cluster I showed maximum mean value for spike length (10.768), cluster I showed maximum mean value for no. of spikelet per spike (20.413), cluster III showed maximum mean value for physiological maturity (117.984), cluster I showed maximum mean value for plant biomass (51.717), cluster V showed maximum mean value for no. grain per spike (46.519), cluster III showed maximum mean value for no. of grain per plant (489.685), cluster I showed maximum mean value for grain length (6.778), cluster V showed maximum mean value for grain width (2.869), cluster I showed maximum mean value for length/width ratio (2.439), cluster I showed maximum mean value for testweight (39.125), cluster III showed maximum mean value for grain yield per plant (27.664), cluster III showed maximum mean value for harvest index (55.976), cluster IV showed maximum mean value for protein content (12.203), cluster III showed maximum mean value for gluten content (7.263). These results are in close conformation with the findings of Khalid *et al*. (2022), Abdelghany*et al*. (2023) and Khalid *et al.* (2023). (This paragraph is totally unnecessary. It is only a reiteration of what is previously showed on Table 3. Besides this, as I mentioned before, of course the cluster means differences among clusters for each trait are expected to be almost the same. Likewise, the cluster means differences among traits for each cluster are expected to be large since the differences among traits are also themselves large).

PRINCIPAL COMPONENT ANALYSIS

In the present study,thefirst eight PC accounted for 65.62% (Table 4).(This is what is shown at Table 4, isn´t it?). Besides that, one of the premises of PCA is that the first two PC must account for at least 80% of the total variance.)~~were transformed to five independent principal components by getting loaded on common principal factors, indicating that these five components contributed maximum towards variation of the data *set* the first five principal components having eigen values altogether explained 65.61 per cent of the total variation and were retained for further studies. The relative contribution to the variation by different principal components was proportional to their eigen values and it decreased progressively.~~

PC1 accounted for 11.51 % of the total variance and almost all studied characters showed positive loading in this principal component(Table 5). The factor loading of principal components showed that, PC1 accounted maximum variability for characters like harvest index, grain yield per plant, flag leaf width, leaf rolling, protein content, chlorophyll content, grain L/W ratio, days of 50% heading, grain length, gluten content and grain yield per plant. These results agreed in Khodadadi*et al.* (2011),Rymuza*et al.* (2012),Sareen*et al.* (2014), Hamam*et al.* (2015), Adilova*et al.* (2020), Farheen*et al.* (2021), Bhatti*et al.* (2022), Khalid *et al.* (2023) and Kumar *et al.* (2024).(Have they done some investigation with the same genotypes that you used in this work?)

~~Principal Component Analysis (PCA) is a valuable technique which is used to classify the relationships among the traits in a complete multi-trait system and helps in identification of data pattern by reducing the number of dimensions. PCA accomplishes this reduction by identifying directions, called principal components (PCs), along which the variation in the data is maximal. By using a few components, each sample can be represented by relatively few numbers instead of by values for thousands of traits~~(This is not results, neither discussion. It was already shown in the introduction).In the present investigation, PCA was performed for twenty-six yield and yield component traits in wheat lines. The principal components with eigenvalues more than 1 and which explained at least 5 per cent of the variation in the data should be considered(Again: one of the premises of PCA is that the first two PC must account for at least 80% of the total variance.). Eigenvalue measures the amount of variation explained by a particular factor out of the total variation. The factor loading of a principal component is the correlation between the scores determined by this principal component and the values or means of each original variable. The eigenvalues from PCA determines the number of factors to be retained which accounts for most of the variability in the original data set. The principal components with higher eigenvalues and traits which had high factor loading were considered as best representative of system attributes. The sum of all eigenvalues is always equal to the number of traits. (Oh my God! What was sorted out here? What a mess, what a chaos).

In our study, first eight principal components had eigenvalue greater than one and they cumulatively explained 65.61 per cent of the total variation present in the original data set. So, these eight principal components were considered important for further explanation. The first principal component explained 11.51 per cent while, the second, third, fourth, fifth, sixth, seventh and eighth principal component exhibited 10.03 per cent, 9.65 per cent, 8.81 per cent, 7.81 per cent, 6.48 per cent, 5.99 per cent and 5.30 per cent variability, respectively among the lines for the traits under study (Table 4). The first principal component accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible. (What is in this paragraph is a repetition of what is already in Table 4).

Scree plot explains the percentage of variation associated with each principal component and is obtained by drawing a graph between principal component numbers (X-axis) and percentage of variation explained (Y-axis). The Principal Component 1 showed 11.51 per cent variability with eigenvalue 2.99 which then declined gradually. From the graph, the maximum variation was observed in Principal Component 1. (Is there a screen plot in this manuscript?).

The result of the PCA explained the genetic divergence of wheat lines. Eigenvalues assess the importance and role of each component to total variation, while the factor loading indicates the scale of contribution of every origin trait with which each principal component is associated. Within each principal component, only highly loaded factors or traits were retained for further explanation. Component matrix revealed that Principal Component 1 showed high positive loading for harvest index (0.435), grain yield per plant (0.293), flag leaf width (0.214), leaf rolling (0.195), protein content (0.174) and chlorophyll content (0.159). Principal Component 2 enabled high positive loading for grain L/W ratio (0.499), days of 50% heading (0.351), grain length (0.245), gluten content (0.268) and grain yield per plant (0.231). The prominent traits contributing maximum variability and desegregating in different principal components have the tendency to remain together which may be kept into consideration during utilization of these characters in crop improvement programme as a donor for the associated traits.(Likewise, this is a repetition of what is in Table 5).

Table: 1 Cluster analysis:(The title of a table must be complete, such that the reader does not need to refer to the text to understand it.)

Clusters	Size	Genotypes
I	**56**	DBW-350, PBW-833, HD-3399, KRL-213, KRL-1-4, DBW-173, K-0402, WH-1142, KRL-19, K-2104, K-2108, KRL-283, K-1908, HD-2359, K-8962, K-9533, K-1907, DBW-107, PBW-826, K-1711, K-2109, K-0607, K-0306, K-1809, PBW-852, K-2101, K-1805, PBW-835, K-1317, K-2105, KRL-210, DBW-187, K-1910, K-2103, HD-3086, K-2010, K-9644, K-9351, HD-3388, HD-2967, K-1905, K-2007, DBW-222, K-9162, K-1616, HD-3392, K-2107, K-2001, K-1006, K-1903, K-7903, K-1803, K-9423, K-8027, K-0307, and K-9107
II	**1**	K-2003
III	**1**	K-2121
IV	**1**	K-8434
V	**1**	K-9465

Table: 2 Average inter and intra cluster distance(The title of a table must be complete, such that the reader does not need to refer to the text to understand it.)

	cluster 1	cluster 2	cluster 3	cluster 4	cluster 5
cluster I	**57.879**	86.469	142.980	88.334	86.769
cluster II		**0.000**	267.377	101.141	172.747
cluster III			**0.000**	201.540	112.853
cluster IV				**0.000**	137.586
cluster V					**0.000**

Table: 3 Average cluster mean for 26 traits(The title of a table must be complete, such that the reader does not need to refer to the text to understand it.)

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
D50H	76.689	76.931	77.027	76.774	76.856
GFP	29.685	29.536	29.573	29.560	29.555
FLL	23.033	23.579	23.249	22.882	23.009
FLW	1.981	1.964	1.988	1.950	1.973
FLA	45.790	46.832	46.788	45.154	45.999
PH	100.009	100.288	100.975	101.590	99.925
PL	36.309	35.957	36.941	36.451	35.704
SBTPP	12.305	12.272	12.212	12.294	12.415
CC	29.556	29.317	30.244	29.534	29.935
CTD	7.864	7.783	7.557	7.756	7.581
PW	5.446	5.631	5.694	5.626	5.726
LR	5.425	5.596	5.583	5.335	5.375
SL	10.768	10.726	10.483	10.662	10.661
NSPS	20.413	20.202	20.098	20	20.057
PM	117.199	117.559	117.984	117.841	117.705
PB	51.717	50.657	50.987	51.604	50.077

NGPS	44.391	44.793	44.343	43.901	46.519
NGPP	425.896	441.773	489.685	455.669	467.843
GL	6.778	6.746	6.710	6.740	6.716
GW	2.813	2.835	2.849	2.844	2.869
GLWR	2.439	2.411	2.383	2.398	2.365
TW	39.125	38.555	38.568	38.863	38.413
GYPP	27.006	27.364	27.664	27.401	27.142
HI	53.767	55.777	55.976	54.923	55.722
PC	11.998	12.083	12.175	12.203	12.226
GC	7.255	7.252	7.263	7.257	7.247

Table: 4 Principal Component analysis for yield and yield related traits(The title of a table must be complete, such that the reader does not need to refer to the text to understand it.)

	Eigenvalue	**Variance percent**	**Cumulative variance percent**
PC1	2.993	11.513	11.513
PC2	2.608	10.033	21.547
PC3	2.509	9.653	31.200
PC4	2.291	8.814	40.015
PC5	2.032	7.818	47.833
PC6	1.687	6.489	54.323
PC7	1.557	5.992	60.315
PC8	1.378	5.302	65.618
PC9	1.199	4.611	70.229
PC10	1.101	4.237	74.467
PC11	1.001	3.853	78.320
PC12	0.811	3.119	81.439
PC13	0.809	3.113	84.553
PC14	0.673	2.588	87.142
PC15	0.612	2.354	89.497
PC16	0.565	2.173	91.670

PC17	0.499	1.920	93.591
PC18	0.397	1.529	95.120
PC19	0.357	1.376	96.496
PC20	0.292	1.126	97.623
PC21	0.274	1.057	98.680
PC22	0.197	0.759	99.440
PC23	0.116	0.446	99.886
PC24	0.014	0.055	99.942
PC25	0.011	0.041	99.984
PC26	0.004	0.015	100.000

Table: 5 Factor loadings of principal components(The title of a table must be complete, such that the reader does not need to refer to the text to understand it.)

	PC1	**PC2**	**PC3**	**PC4**	**PC5**
D50H	0.000	0.351	0.000	0.128	0.000
GFP	0.123	0.000	0.000	0.152	-0.144
FLL	0.313	0.000	0.393	0.000	0.000
FLW	0.214	0.000	0.129	-0.278	0.184
FLA	0.371	0.000	0.380	-0.181	0.117
PH	-0.265	0.000	-0.146	-0.349	0.000
PL	-0.107	-0.119	0.000	-0.527	0.000
SBTPP	0.000	0.000	0.117	0.000	-0.103
CC	0.159	-0.242	0.000	0.000	0.000
CTD	-0.109	-0.185	0.286	0.000	-0.288
PW	0.000	0.000	-0.135	0.000	-0.211
LR	0.195	0.000	0.000	0.161	-0.207
SL	-0.141	0.000	0.247	0.274	0.000
NSPS	0.000	0.000	0.415	0.171	0.270
PM	0.000	0.122	-0.309	0.238	0.000
PB	-0.312	0.126	0.187	0.213	0.241
NGPS	0.195	0.000	-0.161	0.000	0.307
NGPP	0.167	-0.128	-0.216	0.000	0.281
GL	0.000	0.245	0.169	0.000	-0.367
GW	0.000	-0.447	0.000	0.249	0.000
GLWR	0.000	0.499	0.000	-0.224	-0.174
TW	-0.190	0.104	0.000	-0.214	0.228
GYPP	0.293	0.231	-0.119	0.141	0.000
HI	0.435	0.000	-0.210	-0.111	-0.235
PC	0.174	0.166	0.000	0.000	0.123
GC	0.000	0.268	0.000	0.000	0.340

CONCLUSION:

Based on the above result of genetic divergence with the help of principle component analysis it could be concluded for all traitsCluster I had highest number of genotypes (56). The minimum intra cluster distance (0.00)was found for II to V and maximum was found for cluster I (57.879).The maximum inter-cluster distance was found between cluster II to III (267.377).The minimum inter- cluster D^2 value found in case of cluster I to II (86.469). Cluster I showed earliest mean value for day to 50 per cent flowering (76.689 day) and most important character grain yield per plant cluster III showed maximum mean value for (27.664). Principal component analysis (PCA) indicated that the five principal components (PC1 to PC5) showed 65.61 per cent of the total variability(*These are not conclusions. These are results, just results*.). Thus, this finding indicated that these traits could utilize in various breeding as well as improvement programmes. The information may further help the breeder in formulation appropriate strategy aimed at getting higher yield and character improvement in wheat.(*Even if it were written correctly, it would still be an empty conclusion. It could almost be valid even if no analysis had been conducted*.)

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