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Genetic Divergence Analysis of late Sown Wheat (*Triticum aestivum* L.) Accessions using Principal Component Analysis and Euclidean distance Method

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ABSTRACT: The current study was conducted using 60 bread wheat genotypes under late sown environment in randomized block design. The analysis of variance revealed that considerable variation existing among the genotypes for all the traits under study. The highest estimates of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were observed for grain growth rate at 28 (GGR28) days after anthesis *i.e.*, 22.31 and 27.76 respectively. The highest value for heritability (broad sense) was observed for seedling dry weight (SDW) (98.57 %) whereas grain yield per plot (GY/P) had moderately high heritability (62.09 %). Based on D² Statistics cluster analysis, 60 genotypes were grouped into seven clusters. The maximum inter-cluster distance was recorded between cluster I&V (10.38) and cluster I&VII (9.72). Genetic diversity analysis facilitates plant breeders to choose divergent lines which they utilize in hybridization breeding programmes. Genotypes selected from these clusters may be used in hybridization programmes, as divergent parents are likely to produce heterotic F₁s and most promising segregants. Based on Principal component analysis (PCA) first seven components with >1 eigen values contributed 73.6 % variation to the total variation. Maximum value explained by the PC1 (22.2 %) and PC2 (13.0 %).

Keywords: Wheat, Genetic variability, Genetic diversity, Cluster analysis and Principal component analysis.

INTRODUCTION

Bread wheat (Triticum aestivum L.) is the widely grown staple cereal crop after paddy. Earlier, it has been cultivated only as a food crop but, nowadays there is sudden increase in demand for wheat driven by increase in China's domestic corn prices, which is causing more wheat to be used in animal feed (Shilpa Samant, 2020). Globally, wheat production in 2020-21 reaches upto 796.6 million metric tons (FAOSTAT, 2021). As per 4th Advance estimates production for major crops during 2020-2021, area under wheat cultivation were 34.6 million hectares and production was 109.52 million tonne (Anonymous, 2021). For breeders it is a tough grind to increase the production of wheat to stand up with the global food and nutritional security standards. Especially, for late sown wheat this task becomes particularly challenging when late sown wheat undergoes forced maturation and test weight reduction, due to the prevailing high temperature and desiccating winds during the month of April (Singh and Dhaliwal 2000). This can only be achieved if, adequate genetic diversity is present among the gene pool for developing climate resilient, high yielding and stress tolerance varieties and hybrids.

Crop improvement in any breeding programme relies on the amount of genetic variability and diversity present in the gene pool. Genetic variability is a measure of variability present among genotypes in experimental material. Its presence is essential to adapt to change in environmental conditions by the population. The extent of genetic diversity among the selected genotypes may be estimated by genetic divergence studies (Mondal, 1997). D-square statistics (D²) developed by Mahalanobis (1936), has been used to evaluate the net divergent genotypes present in different clusters. Genetic distances are measures of the average genetic divergence between cultivars or populations. If variation is present in the tactical gene pool, the materials can be utilized directly to develop competitive varieties (Pfeiffer and McClafferty, 2007). Principal Component Analysis (PCA) reveals the major contributor to the total variations at each distinct point. It is a multivariate technique which reduces data with large number of correlated variables substantially into new set of variables. Correlation matrices are better for estimating PCA because it does not require standardization.

Keeping in view, an effort has been made to evaluate a set of 60 bread wheat genotypes with an aim, to analyze the genetic variability, heritability, and genetic advance for yield and its component traits and diversity among biological population by D^2 statistics and principal component analysis.

MATERIALS AND METHODS

Accessions used in the present investigation comprises of 60 bread wheat genotypes, sown in Randomized Block Design (RBD) under late sown conditions with three replications at experimental area of Wheat & Barley section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar. The prescribed bundle of practices were followed.

A. Morpho-physiological trait

The observations were recorded on five randomly selected plants from every genotype in each replication for the accompanying morpho-physiological traits namely, Days to 50% heading (DH), days to anthesis (DA), canopy temperature at heading (CTH, °C), canopy temperature after anthesis (CTA, °C), grain growth rate (14, 21, 28 days after anthesis) (GGR14, GGR21 and GGR28 respectively; mg grain⁻¹day⁻¹), plant height (PH, cm), peduncle length (PL; cm), peduncle extrusion (PE; cm), number of effective tillers per meter (ET/M), spike length (SL; cm), spikelet per spike (S/S), no. of grains per spike(G/S), grain yield per plot(GY/P, g), biological yield per plot(BY/P, gm) and harvest index (HI, %).

B. Seed vigour parameters

Standard germination (SG; %), density (SD, g cc⁻¹), 1000 grain weight (TGW; gm), seedling length (SL; cm), seedling dry weight (SDW; mg), seedling vigour index-I and II (SVI and SV II respectively). "Between the papers" (BP) method was used for estimating standard germination. The seedling vigour indices were calculated as per the method given by Abdul-Baki and Anderson, (1973).

C. Statistical analysis

The ANOVA (Fisher, 1925), heritability, phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV), Wards minimum method and Squared Euclidean Distance for clustering the genotypes and principal component analysis (PCA) is estimated by using correlation matrices to measure the diversity.

RESULTS AND DISCUSSION

A. Genetic Variability analysis

The mean sum of square values presented in ANOVA Table 1 was observed as highly significant for all the traits considered, elucidating satisfactory variability present among the genotypes. Similar results were obtained by Phougat *et al.* (2017); Fikre *et al.* (2015). The presence of variability among the experimental material might be due to diverse sources of collected breeding material as well as environmental effects on phenotypes.

The heritability, genetic advance, genotypic coefficient of variance (GCV) and phenotypic coefficient of variance (PCV) are presented in Fig. 1 and 2. The high GCV and PCV values were obtained in GGR28 and seedling vigour index II, moderate PCV and GCV values were accounted for GY/P, GGR21, SD, SL, SDW and SV I. Moderate to high value of GCV and PCV obtained in this study fall in line with those observed by Kaddem *et al.* (2014); Kumar *et al.* (2013); Nukasani *et al.* (2013).

Whereas lowest value for GCV and PCV were reported for DH, DA, CTH, CTA, PL and SG indicated very less variability for such traits among the genotypes. Similar findings were reported by Pooja *et al.* (2018); Rani *et al.* (2018). High difference between GCV and PCV value were reported in traits such as GGR21, GGR28, BY/P and GY/P indicating environment effect was prominent in such traits as compared to others.

 Table 1: Analysis of variance for morpho-physiological traits and seed vigour parameters of 60 wheat genotypes.

Source of	Degree	of	Mean sum of squares											
variation (SV)	freedom (D.F.)	n	DH	DA	GGR14	GGR21	GGR28	СТН	СТА	РН	ET/M	PL	PE	SL
Replications	2		1.67	0.51	0.03	0.02	0.05	0.47	0.24	1.27	10.05	0.96	0.42	0.12
Genotypes	59		6.22* *	4.68	0.17**	0.28**	0.12**	0.70**	0.60**	66.73**	183.1**	10.09**	5.74**	3.10 [*]
Error	118		0.49	0.19	0.01	0.06	0.01	0.19	0.06	1.63	12.88	0.56	0.28	0.07
SV	DE						Me	an sum of s	quares					
51	D.r.	S/S	6 6	G/S	BY/P	GY/P	TGW	HI	SG	SD	SL	SDW	SV I	SV II
Replications	2	0.3	7 0	.44	277599.02	79638.45	5 0.12	21.21	0.32	0.00	0.16	0.28	0.00	0.44
Genotypes	59	6.44 **	4 57.	.54**	1377336.32**	354382.53	3** 25.27 ^{**}	37.56**	78.06**	0.13**	22.21**	13.65**	0.07^{**}	711.6 2 ^{**}
Error	118	0.33	3 1	.00	696166.33	59910.15	5 1.97	14.10	1.90	0.00	0.18	0.12	0.00	4.43

**Significant at p= 0.01

The proportion of variability inherited from parents to offspring is manifested by heritability (Lush, 1949). High estimates for heritability *i.e.*, > 80 per cent was reported by days to anthesis, plant height, effective tillers per meter, peduncle length, peduncle extrusion, spike length, spikelet per spike, number of grains per spike and all the six seed vigour parameters.

Similar results were reported by Singh *et al.* (2017) (SG, SL, SDW, SV I and II), Kumar *et al.* (2013) (PH and BY/P) and Deshewa and Kyosev (2015) (ET/M). For improvement of these traits selection can be practiced as they are governed by additive genes. Traits

such as CTH, BY/P and HI showed low heritability indicating high environmental influence and selection may not be effective for their improvement.

Estimating heritability with genetic advance reveals the type of gene action involved in expression of the traits. Thus, the traits showing high heritability with high genetic advance indicates additive gene action and direct selection for such traits are effective. In current study direct selection may be effective for G/S, S/S, SD, SDW, SL, SV I and II. Similar results were reported by Singh *et al.*, 2017 (SDW and SV I and II).



Fig. 1. Graph showing PCV, GCV, Heritability and Genetic Advance for morpho-physiological traits.



Fig. 2. Graph showing PCV, GCV, Heritability and Genetic Advance for seed vigour parameters.

B. Genetic Divergence Analysis

Sixty bread wheat genotypes were assembled into seven clusters using D^2 - statistics, genotypes within cluster were less genetically diverse than those among two distinct clusters (Table 2). The distribution pattern showed that group II and VI have the maximum

number of genotypes (14), trailed by cluster III, IV and V (10, 9 and 5 respectively), whereas, cluster I and VII has minimum number (4 each). Findings of present study are supported by Arya *et al.* (2017); Dutamo *et al.* (2015): Mishra *et al.* (2015); Fikre *et al.* (2015).

Table 2: Clustering pattern of 60 wheat genotypes on the basis of D² cluster analysis.

Cluster	No.	Genotypes
Cluster I	04	P12574, P12576, P13559, P13656
Cluster II	14	P13646, P13652, P13590, P13673, P13639, P13674, P13676, P13663, P13587, P13597, P13659, P13679, P13679, P13648, P13660
Cluster III	10	P13582, P13377, P12717, P13650, P13666, P13653, P13618, P13637, P13657, P13658
Cluster IV	09	P13653, P13655, P13682, HD3059, WH1021, WH1124, DBW90, P12592, P13294
Cluster V	05	P13565, P13588, P13583, P13644, P13260
Cluster VI	14	P13584, P13624, P13585, P13620, P13634, P13622, P13623, P13350, P13589, P13645, P13638, P13635, P13635, P13636, P12645
Cluster VII	04	P13633, P13642, P13640, P13641

The intra- and inter-cluster average distances has been presented in Table 3 and Fig. 3. The diagonal representing the intra-cluster distance ranged from 5.43 (II) to 6.39(V) and above diagonal represents intercluster distance ranged from 6.50 (III & II) to 10.38 (V & I) indicating considerable genetic diversity between different the clusters. A maximum difference among the genotypes within the same cluster was reported in cluster V (6.39) followed by cluster I (6.16) and cluster VII (5.60), this indicates genotypes present in these cluster with high degree of divergence might produce more desirable breeding material (Dobariya *et al.*, 2006) whereas minimum intra-cluster distance was reported by cluster II (5.43) indicating homogenous nature of genotypes in this cluster, hence selection will not be effective. Maximum genetic divergence was reported between cluster I & V (10.38) followed by between cluster I & VII (9.72), suggesting the genotypes from clusters possessing maximum genetic distance when involved in hybridization programme, produces more heterotic F1's and most promising segregants in the segregating generations. Similar findings were obtained by Phougat *et al.* (2017); Sharma *et al.* (2017).

Table 3: Estimates of average intra (diagonal) and inter (above diagonal) cluster distance values for 60 wheat genotypes.

Cluster	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII
Cluster I	6.17	7.41	8.17	7.32	10.39	9.22	9.72
Cluster II		5.44	6.50	6.36	7.57	6.81	7.89
Cluster III			5.59	6.18	7.87	6.54	7.20
Cluster IV				5.60	7.62	6.69	7.38
Cluster V					6.40	7.22	8.47
Cluster VI						5.52	6.72
Cluster VII							5.60

Kiran et al.,



Fig. 3. Cluster diagram for inter and intra cluster distances between wheat genotypes.

The cluster mean analysis has been presented in Table 4. Cluster VII comprised of early genotypes by having least number of DH and DA (69.83 and 76.08, respectively) and reported highest cluster mean GY/P (3532.9 g), SL (11.60 cm), PE (17.15 cm), ET/M, GGR14 (3.58 mg g⁻¹ day⁻¹) and SG (94.83%). Thus, genotypes may complete their life cycle before the onset of heat stress due to late sown conditions. Cluster III consist of genotypes with highest BY/P (8193 g), low CTH and CTA (26.35 & 25.17 °C respectively and third highest mean GY/P (3347.3 g), indicating these genotypes can tolerate high temperature stress condition by lowering their canopy temperature and maintains

yield. Cluster IV genotypes had high values for G/S (54.93) and short stature plants (94.97cm) and Cluster V consists of genotypes with maximum values for GGR21 (2.17 mg g⁻¹ day⁻¹), TGW (39.50 g), HI (44.28 %), SD (1.80 g cc⁻¹), SDW (11.29 mg) and SV II (1064.19), thus the genotypes from these clusters also have desirable traits which can be utilized in hybridizing programme to get the ultimate aim of plant breeder *i.e.*, grain yield. Similar results were reported by Dutamo *et al.* (2016); Imran *et al.* (2016); Kabir *et al.* (2015). Therefore, genotypes from cluster VII could be exploited for the direct release as a variety after testing under wide range of environment.

 Table 4: Cluster Mean values of 7 clusters for morpho-physiological traits & seed vigour parameters in wheat.

Cluster	DH	DA	GGR14	GGR21	GGR28	СТН	СТА	PH	ET/M	PL	PE	SL
I	73.67	78.58	3.09	1.68	0.70	26.22	27.53	98.22	93.08	34.64	15.44	9.40
II	74.02	78.93	3.15	1.98	0.95	25.62	26.81	101.90	94.26	36.13	16.48	9.86
III	73.73	78.72	3.38	1.63	0.88	25.17	26.35	99.19	103.20	34.51	14.77	9.79
IV	73.18	77.97	3.27	1.65	0.77	25.78	27.13	94.97	96.74	34.54	15.42	10.13
v	73.40	78.27	3.14	2.17	0.90	25.21	26.78	99.04	92.13	35.41	16.16	10.14
VI	73.76	78.72	3.43	1.66	0.71	25.43	26.80	105.59	99.76	35.42	15.82	11.21
VII	69.83	76.08	3.58	1.81	0.58	25.33	26.60	103.77	105.00	35.10	17.15	11.60
Mean	73.43	78.12	3.30	1.78	0.81	25.51	26.81	100.91	97.87	35.23	15.83	10.31

Cluster	S/S	G/S	BY/P	TGW	HI	SG	SD	SL	SDW	SVI	SVII	GY/P
Ι	18.25	51.08	7417	34.03	39.71	78.17	1.34	17.44	5.58	1360.50	433.89	2944.2
Π	18.95	49.38	7537	36.92	37.46	92.41	1.50	19.32	7.56	1781.92	698.19	2783.1
III	19.40	52.43	8193	35.52	41.04	94.33	1.49	20.04	7.26	1891.90	685.71	3347.3
IV	19.33	54.93	6816	34.86	40.57	92.60	1.54	21.48	7.41	1994.90	686.10	3060.6
v	19.73	50.00	7233	39.50	44.28	94.27	1.80	23.40	11.29	2202.67	1064.19	3166.2
VI	20.29	51.76	7993	38.98	39.11	94.24	1.67	23.91	8.39	2254.71	788.90	31.95.2
VII	19.17	51.00	8176	37.39	40.14	94.83	1.51	22.02	7.80	2087.91	740.11	3532.9
Mean	19.43	51.54	7624	36.91	40.33	92.55	1.56	21.22	7.87	1969.83	731.13	3107.6

C. Principal component analysis

Principal component analysis simplifies the complex data by changing the number of related variables into smaller number of variables called principal components. The analysis had grouped the wheat variables into seven main components which showed eigen value >1 and accounted for 73.6% of total variation with yield. Importance should be given to the traits falling in these seven components because of their maximum contribution to the total variation (Table 6, Fig. 4). Eigen values represents the spreading of data on *al* 14(1): 1793-1798(2022) 1796

Kiran et al.,

Biological Forum – An International Journal 14(1): 1793-1798(2022)

the line which is an eigen vector. The maximum eigen value was 5.31 for PC1 while the minimum was 1.28 for PC7 (Table 5). Maximum per cent of variance explained by component 1(22.2) followed by component 2 (13.0) and component 3 (10.57) which means they had contributed more to the total variation. The high principal component score for a particular genotype in a particular component denotes high value

for the traits mentioned in that component for that specific genotype. The traits included in all the seven components with the genotypes having maximum values in that component are presented in Table 5. Similar finding regarding the traits present in seven main components have been obtained by Bhanupriya *et al.* (2014); Mobullah *et al.* (2013); Upadhyay *et al.* (2016).

Table 5: Per cent of variance, cumulative variance and Eigen values in the seven main components.

Components	Eigen value	% Variance	Cumulative variance
1	5.31	22.2	22.2
2	3.11	13.0	35.1
3	2.46	10.3	45.4
4	2.44	9.4	54.8
5	1.69	7.1	61.8
6	1.54	6.4	68.3
7	1.28	5.4	73.6

Table 6: Princi	pal components w	ith traits and	genotype with	maximum	values in eacl	i component.
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Component	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Traits	SV II, SV I, SDW, SD,SG	ET/M, BY/P, GY/P, GGR14	SS, GS, SL	DH, DA	PE, PL, TGW, PH	HI	СТН, СТА
Top three							
genotypes	P12574	P13260,	P13638,	P13294,	P13641,	P13648,	P13648,
having	P12576	P13588,	P13652,	P13589,	P13648,	P13653,	P12645,
maximum	P13656	P13639	P13674	P13622	P13660	P13584	P12717
value							



Fig. 4. Biplot of grain yield per plot and yield related traits on the first 2 principal components under late sown environment.

CONCLUSION AND FUTURE SCOPE

High heritability with high genetic advance estimated in current study showed that the direct selection for traits such as number of Grain per spike, spikelet per spike, seed density, seedling dry weight, seedling length, seedling vigour index I and II may be effective as they are governed by additive gene action. Based on cluster analysis, it can be concluded that wider genetic divergence found among the genotypes. Maximum inter-cluster distance was reported in cluster I and V (10.38) however minimum distance observed in cluster IV and III (6.18) indicating greater the distance between these genotypes revealed broad spectrum of genetic diversity, maximum heterosis can be obtained in crosses between these clusters for varietal improvement. The maximum intra-cluster distance was shown in cluster V (6.39) followed by cluster I (6.16) indicating maximum genetic diversity among the genotypes. Cluster with minimum intra-cluster distance was cluster II (5.43) and cluster VI (5.52), indicating less diversity and more homogeneity among the genotypes and hence selection will be less effective as compared to cluster I and V. From the seven principal components, the first two components, PC1 and PC2 with values 22.2% and 13.0% respectively, contributed more to the total variations. On the basis of PCA the promising genotypes namely, P13260, P13588, P13639, P13638, P13652, P13674, P13641, P13648, P13660,

P13648, P13653 and P13584 were identified for yield and quality attributing traits which showed the maximum values in the main components. Hence, genotypes selected from current study can be used for further investigations and in hybridizing programmes to produce more heterotic F1's and most promising segregants in the segregating generations.

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Conflict of Interest. None.

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