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Assessing Genetic Diversity in Bread Wheat (*Triticum aestivum* L.) using D^2 Statistical Analysis

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ABSTRACT

Background: Genetic diversity is one of the potent components used to develop improved cultivars with a broad genetic base and wider adaptability. Genetic diversity between two parents of diverse origin generally displays greater heterosis and yields transgressive segregants. Therefore, knowledge of genetic diversity is the prerequisite for the genetic improvement of crop plants including wheat. D^2 statistical analysis is one of the potent biometrical techniques used for the assessment of genetic diversity.

Methods: The essence of the current experiment involved 40 providential bread wheat lines that were arranged and laid down in a randomized block design (RBD) with three replications at the experimental research farm of Suresh Gyan Vihar University, Jagatpura, Jaipur, during the *rabi* crop season (2020-21). The bread wheat lines of the same geographical range were assessed to determine the genetic diversity using D^2 statistical analysis and heritability estimate in apropos to ten quantitative/qualitative characters.

Result: Based on D^2 analysis, the 40 promising wheat strains were lined up into ten different clusters to estimate the average distance at intra and inter-cluster levels. The uttermost intra-cluster distance was recorded in cluster X, whereas, the appreciative inter-cluster distance pertained among cluster-VI and cluster-X pursued by cluster-IX and cluster-X. Entrenched to the mean or average performance, most of the bread wheat lines were spotted to disclose appreciable distinctions concerning to their cluster means. It was perceived that the maximum percent contribution towards total divergence was exhibited by grain yield plant^{-1}, followed by spike length, effective tiller plant^{-1}, 1000 grain weight and plant height. Moreover, the number of effective tillers plant^{-1}, spike length, harvest index ,1000 grain weight, number of grains spike^{-1} and protein content were quite substantial since these traits exhibit high heritability estimate together with high genetic advance as percent of mean.

Key words: Bread wheat, D^2 analysis, Genetic diversity, Heritability, Intra/inter clusters.

INTRODUCTON

Genetic diversity is the sum total of genetic variation prevailing within or among the crop population or species. Genetic diversity is one of the potent components used to develop improved cultivars with a broad genetic base and wider adaptability. Moreover, the existence of genetic diversity inside and among the crop species helps to meet the current and future breeding requirements as it allows the breeders to identify worthier lines whichever promptly could be released as a new variety or used as a parent in the hybridization programmes. Genetic diversity between two parents of diverse origin generally displays greater heterosis and yields transgressive segregants than those between closely related strains. Therefore, knowledge of genetic diversity is the prerequisite for the genetic improvement of crop plants including wheat (Tejasvi *et al.*, 2017; Girnam *et al.*, 2018). Though, it has been asserted that many of the improved cultivars of bread wheat currently under cultivation have one or more parents in common. For example, almost all the semi-dwarf wheat varieties have *Rht1, Rht2,* or both these genes for reduced height; these genes have been derived from a single wheat variety *viz.*, Noirin-10. This has led to the narrowing down of the genetic base so as the diversity as well of the wheat crop. Thus, triggered genetic vulnerability *i.e.* susceptibility of bread wheat to various biotic and abiotic stresses (Singh, 2015). Consequently, it is crucial to avoid genetic vulnerability by using diverse and unrelated parents in the breeding programmes. In that way, the genetic diversity of any crop population including wheat can be broadened which will encourage breeders to flourish new varieties for traits of specific interests like quality improvement and tolerance to biotic and abiotic stresses. One of the potent biometrical techniques used for the assessment of genetic diversity present within a population is the D^2 statistic (Singh and Narayanam, 2015). It measures divergence in two folds *viz*., intracluster and intercluster. Thus, it assists the plant breeder

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in the choice of genetically divergent parents for manipulation in hybridization programmes. In addition, D^2 statistics measure the degree of diversification and determine the relative proportion of each component character to the total divergence. The genotypes together grouped in a single cluster are less divergent than the ones, which are placed in different clusters (Santosh *et al*., 2019). On the other hand, heritability associated with genetic advance as per cent of mean is one of the effective selection parameters that ushers the plant breeder to perform selection and ascertain the traits showing inheritance that are purely genetical rather than environmental. On that account, a trial was accomplished with the purpose of evaluating 40 promising bread wheat lines for the study of genetic diversity using D^2 statistics aiming to identify elite divergent parents and their possible efficacy for the genetic improvement of bread wheat and also to find out the magnitude of heritability and genetic advanc as percent of mean in ten quantitative/qualitative characters of bread wheat.

MATERIALS AND METHODS

The on-going field investigation was organized during the *rabi* crop season (November) of 2020-21 at the experimental research farm, School of Agriculture, Suresh Gyan Vihar University (SGVU), Jagatpura, Jaipur. Geographically, the study area is situated at 26°55′N latitude, 75°46′E longitude and lies on an elevation of 431 m above sea level with an average annual rainfall of 546.2 mm. The soil of the experimental farm was classified as sandy loamy with a pH level of 6.5 to 7.12. Throughout the investigation, the daily temperature ranges from 29.2°C to 31°C with average humidity extending from 40 to 47% (November to March). The farm of SGVU is turned to many field experimental studies, hence, soil management, cultural practices (irrigation and fertilizer application) and the way growing different crops form a vast diversity within the field premises.

The entities for the current research comprise 40 promising wheat lines that were acquainted from IARI, New Delhi and Durgapura Research Station, Jaipur, Rajasthan. All these 40 propitious lines showed differential genetical behaviour in terms of the contrasting attributes they possess. Therefore, tapping genetic diversity among or within all the 40 wheat strains and assembling the same in one place were examined and screened throughout this current study.

The current trial was laid down in a randomized block design (RBD) with three replications. The plot size of 2 m width and 1.5 m length (2×1.5 m = 3 m^2) was chosen with a plant geometry of 25×5 cm. Thus, each plot consisted of 8 rows and each row comprised approximately 30 plants as per 25×5 cm row-to-row and plant-to-plant distance. The distance between the adjoining blocks and plots was held at 1.5 m and 0.5 m, respectively. All the recommended cultural practices *i.e.* one deep ploughing followed by three harrowing's were given at the time of field bed preparation. Pre-sowing seed treatment with thiram @ 2 g/kg seed was applied to control seed-borne diseases like loose smut. Fertilizers (NPK) were applied as per the recommended dosage *i.e.*, 100 N: 50 P and 40 K ha^{-1}. The crop was raised with a seed rate of 100 kg/ha. Apart from that five irrigations at different stages of crop growth were given to grow a healthy wheat crop.

To maintain homogeneity and avoid any sampling error, the central 4 rows of each plot were selected as representative/working samples for documentation of data sampling and observations. Plants that were raised on each side of 2 rows acts as border effects were left and not used for data collection**.** The observations were set down on five competitive randomly selected plants from each plot as well as from each replication for the characters under study *viz*., number of effective tillers $plant^{-1}$, spike length, number of grains $spike^{-1}$, plant height, 1000 grain weight and grain yield $plant^{-1}$ except for days to 50% flowering, days to maturity, harvest index and protein content. Both days to 50% flowering and days to maturity were recorded by counting the number of days starting from sowing up to when 50% of the plants in a plot bear flowers and when the whole plot attains maturity as well. The grain protein content was estimated by the method given by Lowry *et. al* (1951) whereas, harvest index is the ratio of economic yield to total biological yield and it was calculated according to Abrar *et. al.* (2011)

The genetic diversity was estimated using Mahalanobis D^2 statistics to workout divergence at two levels *viz*., intracluster and intercluster levels (Mahalanobis, 1928). Therefore, the bread wheat lines were grouped into 10 clusters based on the D^2 value and by using the Tocher's method suggested by Rao (1952). D^2 statistics were estimated by working on the following formula:

$$D^2\, ij = (\bar{X}i - \bar{X}j)\ cov^{-1}\ (\bar{X}i - \bar{X}j)$$

The per cent contribution of characters towards genetic divergence was estimated according to Nedarajan and Gunasekaran (2008) whereas heritability in a broad sense (h^2b) and genetic advance as percent of the mean was estimated according to the formula suggested by Comstock and Robinson (1952) and (Johnson *et al*., 1955a).

RESULTS AND DISCUSSION

Cluster pattern

As per the assumption from D^2 analysis, the 40 bread wheat lines under investigation were grouped into ten clusters (Table 1). The cluster size varies, each comprising one to nine wheat lines. Cluster III emanated with the largest enumerate of components that make up 9 bread wheat lines, specifying comprehensive genetic closeness among them. Clusters II and IV pursued it, constituting 8 lines each, whereas clusters I and X had 6 and 4 entries. The remaining clusters, *i.e*., V, VI, VII, VIII and IX, comprise 1 bread wheat strain each, respectively. The framework of the grouping of wheat lines in discrete clusters and vice versa suggested that there was an inclination between geographical and genetic diversity as the wheat lines used in the present study belong to the same geographical region. These results conform with the observations of Verma *et al.* (2014), Singh *et al.* (2014), Vora *et al.* (2017), Santosh *et al*. (2019). The clustering arrangement may be exploited in picking the divergent parents by determining suitable cross combinations. It will foster intense feasible variability for several attributes. Thus, the lines with high intra and inter-cluster values can be utilized in the crop improvement programmes for further selection followed by hybridization.

Inter-cluster distances

The results (Table 2) specify that the maximum inter-cluster distances were perceived among VI and X clusters (66.654) followed by clusters IX and X (63.232), VI and IX (62.201), VIII and IX (61.677) and clusters VI and VIII (60.321). On the contrary, the low-lying inter-cluster distance was found between clusters II and III (14.302) followed by clusters I and VI (14.621), clusters II and V (15.321), clusters II and IV (16.111) and clusters I and VIII (16.321), propounded familiarity and the lowest level of genetic diversity among them. Cluster distance (Intra and Inter) is the foremost standard for the identification of diverse lines (parents) using the D^2 statistic (Rahman *et al.* 2015). The genotypes which possess the greatest inter-cluster distance are genetically more divergent and hybridization among them may result in the formation of transgressive segregants with more desirable features along with ample variability (Tewari *et al.,* 2015). Thus, the bread wheat lines fall under VI and X clusters (PBW 343 × CPAN 1796, PBW 343 × HS 420, PBW 343 × VL 892 and PBW 343 × RAJ 4393) will effectuate the possible hybridization among the parents showing broad genetic base/diversity and such crosses could hopefully ensure the blooming of desirable progenies in the F_2 and subsequent segregating generations.

Cluster means

The bread wheat line with respect to their various quantitative/qualitative characters, grouped under 10 clusters, discloses appreciable distinctions concerning their cluster means (Table 3). Based on the average or mean performance, genetically diverse lines with various desirable traits were spotted in each cluster. The findings of the present study designate that cluster I recorded earlier flowering (75.21 days) followed by cluster III (75.41 days), cluster VIII (76.54 days) and cluster IV (77.64 days). The same lines discussed above also mature earlier than the rest. Cluster-I matures in 113.88 days followed by cluster III (114.58 days), cluster VIII (114.87 days) and cluster IV (115.25 days). Such lines can escape the adverse effect of drought, terminal heat stress, disease/insect infestation and at the same time, they can give a better yield than those varieties which come under the adverse effect of terminal heat/drought, *etc*. (Singh, 2015). Thus, these lines can be used in hybridization programmes to transfer their earlier trait into other promising lines that lack such an important trait. The inflated mean

Table 1: Grouping of 40 genotypes of bread wheat in various clusters on the basis of D^2 statistic.

Cluster number	Number of lines in a cluster	Lines included in each cluster
I	6	DBW 14, DBW 69, HUW 468, UP 2554, UP2572, UP2594
II	8	HD 2687, HD2733, HD2781, HD 2824, HD 2932, HD 2967, PBW 502, PBW 550
III	9	HUW510, RAJ 3765, K0307,PBW 373, RAJ 4037, RAJ 3777, HD 2402, DBW 17, WH 1124
IV	8	WH 282, HD 2285, UP 2425, HW 2045, Sonalika, HI 1544. WR 544, LOK62
V	1	WH1080
VI	1	PBW 343
VII	1	RAJ 1482
VIII	1	LOK1
IX	1	GW366
X	4	CPAN 1796, HS 420, VL 892, RAJ 4393

Table 2: Average inter-cluster D^2 value, among 10 clusters for 40 bread wheat strains.

Cluster number	I	II	III	IV	V	VI	VII	VIII	IX	X
I	9.354	33.732	26.472	22.533	21.231	14.621	18.246	16.321	23.221	26.777
II		7.121	14.302	16.111	15.321	20.621	22.456	19.432	21.658	18.731
III			12.622	19.277	21.363	17.234	19.222	21.363	30.098	24.321
IV				15.237	17.322	19.211	23.222	18.421	19.541	21.432
V					0.00	56.767	51.543	55.679	58.543	55.444
VI						0.00	48.432	60.321	62.201	66.654
VII							0.00	58.343	55.762	56.123
VIII								0.00	61.677	58.658
IX									0.00	63.232
X										18.232

Table 3: Clusters means for 10 characters in bread wheat and percent contribution of different characters towards genetic divergene.

Characters/Number of clusters	I	II	III	IV	V	VI	VII	VIII	IX	X
Days to 50% flowering	75.21	82.33	75.41	77.64	78.67	83.33	79.54	76.54	78.71	80.61
Number of effective tiller plant^{-1}	8.32	9.22	7.23	7.34	6.70	10.12	7.43	8.44	7.54	7.86
Spike length (cm)	8.41	8.10	7.63	8.33	7.19	8.45	7.22	8.26	8.03	7.65
Number of grains spike^{-1}	40.66	39.87	37.54	40.23	36.41	40.71	36.81	40.09	39.66	38.15
Plant height (cm)	92.23	94.51	90.45	92.67	89.54	96.33	90.52	93.52	91.75	90.66
Days to maturity	113.21	119.33	114.67	115.45	117.33	121.45	117.65	114.58	116.85	118.65
Harvest index (%)	40.66	43.51	39.65	41.45	38.42	45.34	40.38	42.22	40.12	38.76
1000 grain weight (g)	39.67	41.54	37.64	38.85	37.44	42.75	38.71	41.25	40.66	39.08
Protein content (%)	10.22	11.26	11.33	10.61	9.48	12.22	10.11	11.32	10.56	9.28
Grains yield/plant^{1}	9.80	10.55	9.75	10.44	9.33	11.45	9.54	10.67	10.23	9.33

value for the number of effective tiller plant^{-1} was displayed by cluster VI (10.12) followed by cluster II (9.22) and cluster VIII (8.44), whereas the lowest was recorded in cluster V (6.70). The maximum average value for spike length (8.45 cm) was found in cluster VI followed by cluster I (8.41 cm) and cluster IV (8.33 cm). Cluster VI exhibited a greater cluster mean (40.71) for the number of grains spikes^{-1} which was at par with cluster I (40.66), cluster IV (40.23) and cluster VIII (40.09). It is perceived from the present study that maximum cluster means concerning plant height were observed by cluster VI (93.33 cm) followed by cluster II (95.51 cm). Whereas, the lowest plant height was recorded by cluster V (89.54 cm). The mean data of the harvest index indicates that among all the clusters, cluster VI recorded the highest mean value for the harvest index (45.34%) whereas the lowest was recorded by cluster V (38.42%). From the current research, it is comprehended that the highest cluster mean values for 1000 grain weight were recorded in cluster VI (42.75 g) and the lowest in cluster V (37.44 g). Cluster VI observed maximum mean values for protein content (12.22%) and a minimum was observed by cluster X (9.28). The greatest cluster means value for grain yield plant^{-1} was displayed by cluster VI (11.25 g) and the lowest by cluster V (9.13 g). Grain yield is not an independent character but a product of a number of the constellation of yield contributing characters such as tillers per plant^{-1}, which contributes to raising plant population per unit area (Nazim *et al.,* 2014); spike length, grain per spike^{-1} and 1000 grain weight which form the ‘sink’ (Roman *et al.,* 2013) and the harvest index which is the ratio of economic yield to total biological yield. The harvest index is considered an effective parameter to measure yield advancement and is considered directly related to yield (Abrar *et al.,* 2011). In cereals including wheat, plant height is one of the essential components that is directly linked with lodging resistance and plant canopy (Singh, 2015). After yield, grain quality especially protein is a very important character and is directly needed by human beings (Abrar and Ram, 2018). All the above-cited attributes that have been grouped into different clusters under the present study play a pivotal role in the identification of more desirable diverse genotypes/lines within or among the clusters. On that account, hybridization between bread wheat lines grouped in 10 distinct clusters with the highest cluster means values will result in the induction of enormous genetic variability in the F$_2$ and subsequent generations providing an opportunity to encourage the production of desirable transgressive segregants. Thus, ensued genetic improvement in grain yield and yield contributing character as well as protein content either directly or indirectly in wheat. Similar findings were also confirmed by Kumar *et al.* (2013); Kumar *et al.* (2014a); Vora *et al.* (2017); Tejasvi *et al.* (2017); Girnam *et al.* (2018); Santosh *et al.* (2019). They all reported high cluster means for various characters in wheat.

Contribution of the characters toward total divergence

The involvement/contribution of the characters in connection with the manifestation of the entire genetic divergence (Table 4) indicated that the greatest per cent of the contribution comes from grain yield plant^{-1} (23.17%) followed by spike length (20.75%), effective tiller plant^{-1} (19.42), 1000 grain weight (14.33%), plant height (14.21%), harvest index (10.44%) and the number of grains spike^{-1} (8.42%). These results conform with (Lal *et al*., 2009; Kolakar *et al*., 2014; Kumar *et al*., 2014a; Vora *et al*., 2017 and Girnam *et al*., 2018). The characters with a maximum percent of contribution concerning the assertion of genetic divergence shall be acquired as an indicator in the selection of diverse parents from each cluster and utilized the same in the hybridization programmes for the development of desirable segregants which ultimately may result in the selection and release of new elite cultivars from the segregating population.

Heritability

Heritability is one of the important biometrical techniques, a reliable and excellent indicator aids in determining how much percentage of a trait is transmitted from parent to their offspring. In the ongoing investigation apart from focalizing the genetic diversity, heritability in the broad sense (the ratio of genotypic variance to the total or phenotypic variance) was also evaluated in all the 10 attributes under study. The values of heritability as per Johnson *et al.,* (1955a) were classified as low (less than 30%, moderate (30-60%) and high (greater than 60%). It was seen that all the 10 characters considered under the current research showed more than a 60% heritability estimate thus observing a high heritability percentage (Table 5). Amongst the 10 attributes, the highest heritability percentage in a broad sense was heeded by spike length (85.41%) ensued by 1000 grain weight (84.88%), harvest index (81.72%), number grains spike^{-1} (80.22%), number of effective tillers plant^{-1} (79.26%), plant height (74.56%), protein content (72.63%), grain yield plant^{-1} (69.45%), days to maturity (67.59%) and days to 50% flowering (65.41%). The findings of Yadav *et al.* (2011); Abrar *et al.* (2018); Pavan *et al.* (2018); Abrar *et al.* (2020) and Patil *et al.* (2023) are consistent with our results. A high heritability percentage for the aforementioned components shows that a portion of phenotypic variance has been linked to genotypic variance and hence credible selection for these traits based on phenotypic expression could be made. Characters with high heritability are wealthier for a plant breeder than those with low heritability since the traits with low heritability are more subject to environmental change and selection of such traits would result in misleading the findings. In addition to heritability, one more important biometric tool is the genetic advance which refers to the enhancement in the average genotypic value of selected genotypes over the original population (parental population) before selection. Howerver, as per Johnson *et al.* (1955a), heritable estimate combined with genetic advance expressed as per cent of mean is more useful than heritability alone in forecasting the eventual effect of selection and according to Johanson *et al.* (1955a) the range of genetic advance as per cent of mean is classified as low (less than 10%), moderate (10-20%) and high (more than 20). The number of effective tillers plant^{-1} (64.37%), spike length (51.88%) harvest index (45.05%),1000 grain weight (41.03%), number of grains spike^{-1} (39.64%) and protein content (34.55%) were all considered to be highly encouraging because of recording high genetic advance as percent of mean along with high heritability estimates. Consequently, these traits may also exhibit the least genotype x environment interaction as they are the least affected by environmental factors. Similar findings were also corroborated by (Yadav *et al.,* 2011; Abrar *et al*., 2018; Pavan *et al.,* 2018; Abrar *et al.,* 2020 and Patil *et al.,* 2023).

Table 4: Per cent (%) contribution of characters towards genetic divergence.

Characters	Contribution (%)
Days to 50% flowering	0.09
Number of effective tiller plant^{-1}	19.42
Spike length (cm)	20.75
Number of grains spike^{-1}	8.42
Plant height (cm)	14.21
Days to maturity	1.04
Harvest index (%)	10.44
1000 grain weight (g)	14.33
Protein content (%)	4.51
Grains yield plant^{1}	23.17

Table 5: Estimated heritability (bs) and genetic advance as percent of mean of 10 bread wheat attributes.

Characters	Heritability (%) in broad sense	Genetic advance as per cent of mean (Genetic gain)
Days to 50% flowering	65.41	18.45
Number of effective tiller plant^{-1}	79.26	64.37
Spike length (cm)	85.41	51.88
Number of grains spike^{-1}	80.22	39.64
Plant height (cm)	74.56	19.96
Days to maturity	67.59	10.20
Harvest index (%)	81.72	45.05
1000 grain weight (g)	84.88	41.03
Protein content (%)	72.63	34.55
Grains yield plant^{1} (g)	69.45	14.69

CONCLUSION

Based on the intra/inter-cluster distance, cluster means of different attributes and percent contribution of the characters towards total divergence aids in the selection of genetically divergent parents that can be exploited in the hybridization programmes for the enhancement of wheat crop in terms of yield, resistance and quality parameters. Apart from that, number of effective tillers plant^{-1}, spike length, harvest index,1000 grain weight, number of grains spike^{-1} and protein content demonstraed high heritability measures along with high genetic advance as percent of mean, indicating little or no influence of the environment on the expression of such characters. Thus, selection of such traits will be more beneficial in enhancing the yield and quality of bread wheat.

Conflict of interest

All authors declared that there is no conflict of interest.

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