Identification of genetic variability and diversity in selected wheat (*Triticum aestivum* L.) germplasm under three different dates of sowing

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Abstract

Genetic diversity offers crucial platform for improving the plant characteristics. Its exploitation is necessary to tackle the environmental fluctuations and for the effective exploitation of genetic resources in breeding programmes. Although wheat is one of the most thoroughly studied crops in terms of genetic diversity but its yield potential can further be enhanced by studying and identifying the suitable germplasm and utilizing them in crosses. In this study twenty genotypes of wheat germplasm were undertaken for identification of genetic variability and diversity for adaptation in NEPZ in three different dates of sowing. Observations were recorded on thirteen traits. All genotypes were evaluated for genetic variability and diversity by using Analysis of variance and D^2–Statistics. Analysis of variance revealed a significant difference among the genotypes for all the traits under study except for Normalized Difference Vegetation Index. The highest estimates of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were observed for no. of fertile spikes per plant (13.85) yield per plot (33.16) respectively. High heritability coupled with high genetic advance was observed for days to heading (0.67, 13.08) followed by plant height and biomass. Based on D^2–Statistics, 20 genotypes were grouped into seven clusters following Tocher method in which cluster I has maximum number of genotypes. The highest inter-cluster distance was found between cluster II and IV (21.19) followed by IV & VII (17.09). This suggests that genotypes included in these clusters possess wide genetic diversity. Highest cluster mean was found for biomass (692.50) followed by yield per plot and days to heading. On the basis of divergence and cluster mean it may be concluded that maximum heterosis and better transgressive segregants could be obtained in crosses among genotypes of cluster II, IV and VII in breeding programme for varietal improvement in wheat.

Keywords: Normalized difference vegetation index; number of fertile spikes per plant; biomass; days to heading

Introduction

Wheat (*Triticum aestivum* L.) belongs to the genus *Triticum* of the Poaceae (Gramineae) family, allohexaploid (2n=6x=42, AABBDD) having haploid genome of 16Gb. Wheat, being the most versatile and leading food grain crop of the world, both in area and production. Globally, it is cultivated in an area of 214.79 million ha and production of 735.18 million tonnes with an average productivity 34.22 quintals per hectare (FAOSTAT, 2018). In India, wheat ranks second after paddy both in area and production, occupying 29.14 million ha acreage area with production of 102.19 million tones and the productivity of 35.07 quintals per hectare (IWBR, 2018-19). Wheat is the mainstay of global food security, providing better nutrition to the human beings and considered as staple food for about 40% of the world’s population. The demand for wheat in next 10 years is expected to grow rapidly particularly in developing world due to a simultaneous increase in population and income; which needs 15-20% more food production by 2025 (FAO Technical brief, 2010). Thus; it is a challenging task before the breeders to enhance the production level without increasing area under production. Hence, only alternative left is to increase the productivity by improving the traits using genetic diversity, as the crucial platform in varietal improvement and better management of crop production.

Genetic diversity is crucial for adaptability and survival of wheat species against multiple biotic and abiotic stresses. The potential to select a superior line increases with genetic diversity. Diversity studies also facilitate the conservation and management goals of a particular plant species. Depletion of genetic variability reduces plant adaptability. For the effective use of genetic diversity in plant breeding, knowledge of its extent and distribution is of prime importance.
Morphological traits can be utilized to characterize genetic diversity, and are often influenced by environmental factors. Plant breeder’s major concern is to develop improved high yielding varieties among the best available genotypes either directly or through improvement of various factors which contribute indirectly to high yield. The breeding methodology should be such that, which in essence could incorporate the favorable changes either through selection or through hybridization of superior genotypes. Regarding this, information on nature and magnitude of genetic variability is of immense value for starting any systematic breeding programme in wheat. The presence of ample genetic variability in the base material ensures better opportunities for evolving desired plant types (Saharwal and Lodhi, 1995) (18). The knowledge of genetic parameters viz., heritability and genetic advance among characters under selection are very helpful for predicting genetic gain under selection in breeding programme and in adopting efficient breeding strategies (Falconer and Mackay, 1996) (6). The higher genetic distance between parents, the higher heterosis in progeny can be achieved (Joshi and Dhawan, 1966) (12). Keeping in view, an effort has been made in the current study to evaluate a set of 20 wheat genotypes with an aim, to analyze the genetic variability, heritability, and genetic advance for yield and its component traits and genetic diversity among germplasm by D2-statistics.

Materials and Methods

The experiment was carried out at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi during Rabi seasons 2016-17. The experimental materials included twenty different genotypes for NEPZ viz; HD-1982, K-307, HUW-468, K-9107, HD-2967, PBW-343, C-306, DBW-39, HD-2285, UP-262, HUW-234, DBW-14, LOK-1, PBW-550, HUW-1014, PBW-373, HD-2643, HUW-2036, WR-544, DBW-16. Experiment was conducted in 25 November 2016, 16 December 2016 and 8 January 2017, in a 2 rows each of one meter length. The row to row and plant to plant distance was maintained 22 cm and 10 cm, respectively. The border rows were HD-2967, also planted to neutralize the border effect. The recommended agronomic practices were followed for better crop growth and competitive crop stand. Observations were recorded on 13 agro-morphological traits viz; Number of fertile tillers per plant, Normalized Difference Vegetation Index, days to heading, Number of fertile spikes per plant, Number of spikelets per spike, plant height, yield per plot, biomass, 1000 grain weight, Iron and zinc content were measured using X-ray absorption spectroscopy machine. Besides these, chlorophyll content at heading and at anthesis stages by Minolta SPAD-502 Chlorophyll meter as well as canopy temperature depression (CTD) was measured by using a hand held infrared thermometer based equipment (Sixth Sense LT-300) from vegetative stage to dough Stages. The observations were recorded on five randomly selected competitive plants from each genotype on each sowing date for all the above-mentioned traits except CTD and NDVI, where single reading itself gives the average of scores of leaves. The data were subjected to Analysis of variance using the model proposed by Panse and Sukhatme (1969) (16). Phenotypic and Genotypic coefficient of variation (PCV and GCV) were estimated using procedures as suggested by Searle (1961) (19). Heritability in broad sense (h2) and genetic advance as per cent of mean were determined using the formula given by Allard (1960) (1). Genetic divergence among twenty genotypes were estimated using Mahalanobis (1936) (13) D2 statistic. The Tcher’s method as described by Rao (1952) (17) was used for clustering the D2 values. INDOSTAT software was used for the statistical analysis of the recorded data for the twenty genotypes.

Results and Discussion

The analysis of variance for 13 characters is shown in Table 1. The results reveal high significant difference due to replications for all the traits under investigation among twenty genotypes. This suggests the presence of heritable variance among the genotypes that can be exploited in wheat hybridization programs. The presence of ample extent of variability might be due to diverse sources of germplasm collected as well as environmental effects on phenotypes.

Table 1: Analysis of variance (ANOVA) for all the traits under study in twenty lines of wheat

| DF | Mean | Square |
|----|------|--------|
|    | TPP  | NDVI   | SPAD  | CT   | DH   | NS   | SPS  | PH   | PY   | BM   | TGW  | Fe   | Zn   |
| Replication 2 | 238.2*** | 0.05*** | 562.87*** | 16.80*** | 143.82*** | 66.95*** | 66.61*** | 136.56*** | 252924.5*** | 1022580*** | 1318.43*** | 189.03*** | 1059.75*** |
| Treatment 19 | 5.70 | 0.003 | 53.78 | 2.44 | 114.16** | 5.83 | 7.33** | 105.66 | 4987.37 | 33568.66 | 39.79 | 16.77 | 39.65 |
| Error 38 | 4.13 | 0.002 | 43.07 | 1.50 | 15.92 | 13.49 | 4.21 | 42.36 | 4672.37 | 23981.29 | 24.11 | 11.10 | 26.61 |

Abbreviations: TPP- No. of tillers per plant, NDVI- Normalized difference vegetation index, SPAD-The Soil Plant Analysis Development chlorophyll meter, CT-Canopy temperature, DH- Days to heading, NS- No. of spike per plant, SPS- No. of spikelet per spike, PH- Plant height, PY- Plot yield, BM- Biomass per m2 per genotype, TGW- thousand grain
Weight, Fe- Iron content, Zn- Zinc content.

The estimates of genetic parameters viz; phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (broad sense), genetic advance and general mean are presented in Table 2. The magnitude of phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the traits under investigation indicating the vital role of environmental interaction in the expression of the characters. The findings were in agreement with previous study in wheat (Gollen et al., 2011) (8). The trait like Number of fertile spikes per plant exhibited highest values of genotypic coefficient of variation (13.95%) followed by biomass (9.69%) and days to heading (7.74%). This suggests that these traits are having ample genetic potential for selection among genotypes in breeding programs. The highest phenotypic coefficient of variation (PCV) was observed for yield per plot (33.16%) followed by biomass (28.25%) and Number of fertile spikes per plant (27.67%). Wide gap between GCV and PCV was observed for yield per plot (4.92%, 33.16%) followed by biomass (9.69%, 28.25%) and Number of fertile spikes per plant (13.85%, 27.65%) indicating that environmental effect was
prominent for these traits. However least difference was observed for days to heading (7.74%, 9.44%) followed by canopy temperature difference (2.84%, 6.83%) and plant height (6.43%, 11.16%) suggesting that these traits are less affected by environment and having good heritable portion of genetic diversity. While the remaining characters exhibited moderate to low gap between GCV and PCV. This suggests low variability for such characters among genotype.

Heritability and genetic advance are important selection parameters. Heritability measures the contribution of genetic variability to the phenotypic variability observed for quantitative traits and it is good index of the transmission of variability to the phenotypic variability observed for such characters among genotype. When heritability coupled with genetic advance is studied the relative contribution of each component trait to the total divergence. On the basis of $D^2$ values, the 20 genotypes were grouped into seven clusters following Tocher’s method (Fig: 1) in such a way that the genotypes within a cluster had a low $D^2$ values than those of in-between the clusters. The grouping of genotypes into different cluster by using Tocher’s method were also reported by Arega et al., (2007) [2]. Tsegaye et al., (2014), Khodadadi et al., (2011) [14] and Singh et al., (2014) [23]. The composition of clusters has been depicted in fig: 1 and Table 3. Cluster I has the maximum number genotypes (11) followed by cluster IV (3) and cluster II (2) while, cluster V, VI and VII had one genotype only.

Genetic divergence ($D^2$) Mahalanobis (1936) [15]

$D^2$ analysis is one of the potent techniques of measuring genetic divergence. $D^2$ statistics measures the forces of differentiation at intra and inter cluster levels and determines the relative contribution of each component trait to the total divergence. On the basis of $D^2$ values, the 20 genotypes were grouped into seven clusters following Tocher’s method (Fig: 1) in such a way that the genotypes within a cluster had a low $D^2$ values than those of in-between the clusters. The grouping of genotypes into different cluster by using Tocher’s method were also reported by Arega et al., (2007) [2], Tsegaye et al., (2014), Khodadadi et al., (2011) [14] and Singh et al., (2014) [23]. The composition of clusters has been depicted in fig: 1 and Table 3. Cluster I has the maximum number genotypes (11) followed by cluster IV (3) and cluster II (2) while, cluster V, VI and VII had one genotype only.

![Fig 1: Clustering by Tocher Method](http://www.phytojournal.com)

Table 2: Evaluated genetic parameters in 13 traits for 20 genotypes of Wheat under study

| Traits Components | TPP | NDVI | SPAD | CT | DH | NS | SPS | PH | PY | BM | TGW | FE | ZN |
|-------------------|-----|------|------|----|----|----|-----|----|----|----|-----|----|----|
| GCV (%)           | 6.70| 1.99 | 4.15 | 2.84| 7.74| 13.85| 6.21| 6.43| 4.92| 9.69| 6.46| 3.58| 5.38|
| PCV (%) (Broad Sense) | 19.93| 7.46| 14.99| 6.83| 9.44| 27.67| 13.95| 11.16| 33.16| 28.25| 15.29| 9.38| 14.38|
| Genetic Adv5%     | 0.01| 0.07| 0.08| 0.17| 0.67| 0.25| 0.20| 0.33| 0.02| 0.12| 0.18| 0.15| 0.14|
| Genetic Adv1%     | 0.64| 0.01| 1.38| 0.61| 12.39| 2.17| 1.20| 6.99| 4.01| 51.18| 2.55| 1.39| 2.061|
| Gen Adv (Mean5%)  | 5.94| 1.40| 3.03| 5.16| 16.76| 18.30| 7.30| 9.79| 1.92| 8.77| 7.19| 3.60| 5.32|
| Gen Adv (Mean1%)  | 10.82| 0.71| 45.56| 19.73| 73.93| 11.87| 16.43| 71.39| 208.43| 583.58| 35.41| 8.432| 38.70|

Johnson et al., (1955) have reported that a character showing high heritability will also exhibit high genetic advance is not necessary. It can be estimated with greater degree of accuracy when heritability coupled with genetic advance is studied (Dudley and Moll, 1969). Therefore, heritability estimates along with genetic advance are normally more helpful in understanding the type of gene action involved in the expression of various polygenic traits and predicting the gain under selection. High heritability coupled with high genetic advance as percent mean were revealed for days to heading followed by plant height indicating substantial contribution of additive gene action in the expression of the characters and selection may be effective. High heritability along with low genetic advance for Number of fertile spikes per plant was indication of non-additive gene action hence; selection for such trait would mislead the expected results.
Table 3: List of 20 wheat genotypes in different clusters

| Groups | Name of genotypes |
|--------|-------------------|
| Cluster 1 | C-306, HD-2643, HUW-234, DBW-39, UP-262, K-9107, PBW-343, HD-1982, HUW-468, PBW-373, PBW-550 |
| Cluster 2 | HUW-1014, DBW-16 |
| Cluster 3 | LOK-1 |
| Cluster 4 | K-307, HD-2967, DBW-14 |
| Cluster 5 | HD-2285 |
| Cluster 6 | HUW-2036 |
| Cluster 7 | WR-544 |

The inter-cluster distance was observed to be higher than in intra-cluster distance, suggesting greater degree of diversification among clusters (Table 4). The inter-cluster distance varied from 5.35 to 21.19. The highest inter-cluster distance was observed between cluster I and IV (21.19) followed by between cluster IV and VII (17.09). This suggested that the genotypes included in these clusters were having broad spectrum of genetic diversity and may be used in hybridization programme for wheat improvement for producing good transgressive segregants having ample potential for developing high yielding varieties. However, lowest inter-cluster distances was observed between cluster I and cluster IV, indicating close relationship between these clusters and would not provide rewarding results.

Table 4: Computed inter and intra (bold and diagonal) cluster distances for seven clusters

| Cluster Distances | Cluster 1 | Cluster 2 | Cluster 3 | Cluster 4 | Cluster 5 | Cluster 6 | Cluster 7 |
|-------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Cluster 1         | 3.74      | 9.03      | 6.13      | 7.55      | 7.04      | 7.04      | 8.39      |
| Cluster 2         | 9.03      | 2.13      | 6.59      | 21.19     | 6.98      | 10.17     | 6.11      |
| Cluster 3         | 6.13      | 6.59      | 0.00      | 12.03     | 5.78      | 10.60     | 8.82      |
| Cluster 4         | 7.55      | 21.19     | 12.03     | 5.59      | 15.19     | 9.98      | 17.09     |
| Cluster 5         | 7.04      | 6.98      | 5.78      | 15.19     | 0.00      | 13.64     | 8.69      |
| Cluster 6         | 5.35      | 10.17     | 10.60     | 9.98      | 13.64     | 0.00      | 9.07      |
| Cluster 7         | 8.39      | 6.11      | 8.82      | 17.09     | 8.69      | 9.07      | 0.00      |

The maximum intra-cluster distance was observed in cluster IV (5.59), followed by cluster I (3.74) and cluster II (2.13). It was noticed that genotypes within cluster with high degree of divergence would produce more desirable breeding materials for attaining the maximum genetic advance (Dobariya et al., 2006). While, the cluster III, V, VI and VII had only one genotype only so no intra cluster distance was computed. The cluster means analyzed for 13 characters under investigation are presented under Table 5, revealed that the Cluster II exhibited highest mean value for biomass (692.50) followed by yield per plot (241.83) and plant height (70.03). Cluster IV exhibited highest mean value for days to heading (82.22). It was revealed that all clusters exhibit highest mean value for biomass followed by yield per plot, days to heading and plant height and they are major contributors of genetic divergence. It can be suggested from present study that crossing among genotypes from different clusters exhibiting good mean performance may help us in developing better yielding varieties. Incorporation of more divergent parents in hybridization program offers us a golden opportunity to exploit ample genetic variability in segregating generations and enhances the chances of attaining maximum heterosis. On the basis of divergence and cluster mean it may be suggested that maximum heterosis and good transgressive segregants could be achieved in crosses between genotypes of cluster II, IV and VII in wheat varietal improvement. Thus, crosses between the genetically diverse genotypes of cluster II with genotypes HUW-1014 and DBW-16 and cluster IV with genotypes K-307, HD-2967 and DBW-14 and cluster VII with genotype WR-544 are expected to exhibit maximum heterosis and are also likely to produce new recombinants with desired characters.
Table 5: Cluster Means by Tocher Method for 13 traits under study

| Cluster | TPP  | NDVI  | SPAD | CT   | DH   | NS   | SP5 | PH   | PY   | BM   | TGW | FE  | ZN  |
|---------|------|-------|------|------|------|------|-----|------|------|------|-----|-----|-----|
| Cluster 1 | 11.48 | 0.71  | 45.51 | 19.66 | 75.48 | 12.45 | 16.52 | 72.42 | 218.12 | 608.79 | 35.54 | 39.15 | 38.98 |
| Cluster 2 | 9.72  | 0.73  | 46.65 | 19.03 | 62.83 | 7.83  | 16.67 | 70.03 | 241.83 | 692.50 | 32.60 | 37.47 | 34.77 |
| Cluster 3 | 9.67  | 0.68  | 46.97 | 19.40 | 70.00 | 11.33 | 14.33 | 69.44 | 160.00 | 553.33 | 40.03 | 39.73 | 38.80 |
| Cluster 4 | 9.85  | 0.69  | 46.17 | 19.76 | 82.22 | 10.89 | 16.11 | 68.03 | 160.72 | 458.89 | 34.80 | 36.04 | 38.14 |
| Cluster 5 | 12.00 | 0.76  | 42.53 | 18.87 | 68.33 | 11.33 | 14.67 | 64.00 | 237.67 | 566.67 | 42.21 | 36.20 | 39.07 |
| Cluster 6 | 9.22  | 0.68  | 45.63 | 22.13 | 73.33 | 11.33 | 18.67 | 73.44 | 190.17 | 536.67 | 31.15 | 41.67 | 39.13 |
| Cluster 7 | 10.22 | 0.69  | 43.70 | 20.70 | 64.33 | 10.67 | 17.67 | 80.22 | 215.67 | 556.67 | 34.37 | 37.30 | 44.33 |

**Conclusion**
Current study on 20 wheat germplasm lines provides ample information about the genetic diversity present in them and will support breeders in expanding the varietal improvement programs by exploiting theses broad spectrum of genetic variability in segregating generation.

**References**
1. Allard RW. Selection under self-fertilization. Principles of Plant Breeding. John Wiley & Sons, Inc, 1960, 55.
2. Arega AD, Manyong VM. The effect of education on agricultural productivity under traditional and improved technology in Northern Nigeria: an endogenous switching regression analysis. Empire Econ, 2007; 32(1):141-159.
3. Arvind K, Raj S. Genetic variability in relation to qualitative and quantitative traits in wheat (Triticum aestivum L.). Agrica. Journal, 2015; 3(2):23-28.
4. Arya VK, Singh J, Kumar L, Kumar R, Kumar P, Chand P. Genetic variability and diversity analysis for yield and its components in wheat (Triticum aestivum L.). Indian J Agric. Res. 2017; 51(2):128-134.
5. Dudley JW, Moll RH. Interpretation and use of estimates of heritability and genetic variances in plant breeding 1. Crop science. 1969; 9(3):257-262.
6. Falconer DS, Mackay TFC. Introduction to Quantitative Genetics. 4th ed. Longman, Essex, UK, 1996.
7. FAOSTAT. Food and Agriculture Organization (FAO) of the United Nations, Rome, Italy, 2014. Available at http://aostat3.fao.org accessed. Kay,
8. Gollen B, Yadav RK, Kumar P. Assessment of genetic parameters for spike traits and yield attributes in bread wheat genotypes following Line X Tester mating system. Environ. Ecol. 2011; 29(2):752-756.
9. Harlan. Genetic resources in wild relatives of crop. Crop Sci. 1976; 16:329-333.
10. Indian Institute of Wheat and Barley Research (IIWBR). AICRP-W&B, Progress Report, Crop Improvement, 2017.
11. Johnson HW, Robinson HF and Comstock RF. Estimates of genetic and environmental variability in soybean. Agron. J. 1955; 47:314-318.
12. Joshi AB, Dhawan NL. Genetic improvement of yield with special reference to self-fertilizing crops. Indian J Genet. 1966; 26A:101-113.
13. Kaul DK, Singh B. Evolution for drought Tolerance in elite genotypes of Bread Wheat (Triticum aestivum L.). Advances in Plant Science. 2011; 24(1):141-144.
14. Khodadadi M, Fotokian MH, Miransari M. Genetic diversity of wheat (Triticum aestivum L.) genotypes based on cluster and principal component analyses for breeding strategies. Aust. J. 2011; 5(1):17.
15. Mahalanobis PC. On the generalized distance in statistics. Proc. Nat. Inst. Sci. 1936; 2:49-55.
16. Panse VG, Sukhatme PV. Statistical methods for agricultural workers. Indian Council of Agricultural Research, New Delhi, 1969.
17. Rao CR. Advance statistical methods in biometrical Research Edition I. John Willey and Sons, New York, 1952.
18. Sabhrawal PS, Lodhi GP. Germplasm evaluation for different traits in wheat (Triticum aestivum L.). HAU, J of Agri. Res. 1995; 25(4):207-210.
19. Searle SR. Phenotypic, Genotypic and environmental correlations. Biometric. 1961; 47:474-480.
20. Shankararao BS, Mukherjee J, Pal AK. Estimation of variability for yield parameters in bread wheat (Triticum aestivum L.). J of Plant breeding. 2010; 1:764-768.
21. Sharma AK, Garg DK. Genetic variability in wheat (Triticum aestivum) across different environments. Ann. Agric. Sci. 2002; 23(3):497-499.
22. Singh D, Singh KN. Variability analysis for yield and yield attributes of wheat under salt affected conditions. Wheat/information service. 2010; 110:35-39.
23. Singh P, Singh AK, Sharma M, Salgotr SK. Genetic divergence study in improved bread wheat varieties (Triticum aestivum L.). Afr. J Agric. Res. 2014; 9(4):507-512.
24. Tsegaye D, Dessalgn T, Dessalegn Y, Share G. Analysis of genetic diversity in some durum wheat (T. Durum) genotypes grown in Ethiopia. Afr. J Biotechnol. 2012; 11(40):9606-9611.
25. Yadav R, Gaikwad KB, Bhattacharyya R. Breeding wheat for yield maximisation under conservation agriculture. Indian J Genet. 2017; 77(2):185-198.