



## Multivariate analysis in wheat germplasm captures variability for agro-morphological and physiological traits

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### ABSTRACT

The present study was carried out with an objective to investigate genetic diversity in set of diverse wheat genotypes during 2018–19 at the experimental farm of Indian Institute of Wheat and Barley Research, Karnal. Principal Component Analysis was carried out on this set of 440 genotypes to study variability for different agro-morphological and physiological traits. The coefficient of variation ranged from 7.20–38.30 for the measured traits. Yield per plot was positively related with tillers per m, spike length, grains per spike and 1000 grains weight. The first three principal components explained 45.66% of variation. Agglomerative clustering grouped the genotypes into six groups and had a cophenetic correlation coefficient of 0.319. Almost all the components explained some variation for each trait and hence can be further used in hybridization for creation of variability in the breeding programs to develop improved cultivars. This remarkable variation in the set can be accounted to the fact that these lines were a collection of germplasm from different wheat growing countries and also their specificity for different traits.

**Keywords:** Agro-morphological traits, Principal Component Analysis, Variability, Wheat

A record harvest of 107.18 mt during 2019–20 has strengthened Indian position as the second largest producer of wheat in the world (Singh 2020). Wheat accounts for the majority of carbohydrate, protein, sugar, fat, fibre and minerals in the diet. Wheat as a crop also faces constraints in its growth and development because of biotic and abiotic stresses causing economic losses in the form of reduced yields. Among the major biotic stresses, are the fungal diseases such as the wheat rusts: stem rust, leaf rust and stripe rust. Other important diseases in India include Karnal bunt, powdery mildew and spot blotch. The major abiotic stresses encountered are the terminal heat, drought and salinity stresses. Yield stagnation in several cereals including wheat has been reported in past (Brisson *et al.* 2010, Fischer and Edmeades 2010, Grassini *et al.* 2013). The genetic diversity in hexaploid wheat, was always low as very few events of inter specific hybridizations got selected during the process of domestication. Domestication has caused bottlenecks in many crop species. The second major bottleneck in reducing the genetic variability was the early plant breeding methods such as selection. The semi dwarf Green Revolution wheats

further significantly contributed in reducing the variation (Smale *et al.* 2002). Also the cultivation of mega-cultivars and their further utilization in the breeding programs as better agronomic backgrounds, have added to the problem (Heal *et al.* 2004, Trethowan *et al.* 2018).

For adaptability of wheat cultivars in particular and survival of wheat species, genetic diversity is crucial and can mitigate the threat of biotic and abiotic stresses (Fu and Somers 2009, van den Broeck *et al.* 2010). Considering the significance of genetic variability in plant breeding, various methodologies have been applied for estimation of genetic diversity (Govindaraj *et al.* 2015, Kumar *et al.* 2019). Most of the wheat breeding programs utilize diverse sources of germplasm to widen the genetic base by using materials from other breeding programs, landraces, mutation stocks, translocation lines and exotic species (Zohary *et al.* 1969).

### MATERIALS AND METHODS

A set of 440 bread wheat genotypes, were characterized for twelve different physiological and agro-morphological traits including yield and its components (2019–20). The genotypes were broadly grouped into three;

- a) Indian genotypes including released varieties advanced breeding lines and genetic stocks registered for specific traits.
- b) 183 Australian genotypes representing released varieties, donors for specific traits such as rust resistance and water use efficiency.

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- c) The third group belonged to the 76 germplasm lines received from CIMMYT, Mexico, and included advanced lines having high yield, disease resistance and different grain quality parameters.

Each genotype was sown in two rows of 1.0 m length and each row was 30 cm apart. The experiment was raised following the recommended fertilizer dosage and irrigation schedules. Data on following characters were recorded: Days to flowering (DF), plant height (PH), tillers per m (TPM), number of spikelets per spike (SPS), spike length (SL), grains per spike (GPS), 1000-grain weight (TGW), peduncle length (PL) and grain yield per plot (GPP). Among Physiological traits, canopy temperature (CT), chlorophyll content index (CCI), and normalized difference vegetation index (NDVI) were recorded. CCI was recorded with chlorophyll metre (SPAD-502 Plus). CT was measured during 12.00 and 14.00 hr using hand-held infrared thermometer HTC MT4 with uniform distance above the canopy.

The estimates of genetic variability and bi-plot procedures for phenotypic correlation coefficients among various agronomic and physiological traits were calculated by utilizing adjusted mean as per statistical software STAR Version 2.0.1 (IRRI 2014).

## RESULTS AND DISCUSSION

The evaluation of the wheat lines revealed huge diversity for various agro-morphological and physiological traits studied. The basic statistic data carried out showed significant variation for all the traits. Among the yield attributing traits, SPS ranged from 12 to 52 with an average of 23.6 spikelets per spike. GPS also ranged from 10 to 84 with a mean value of 41.9. The values for TGW had a range of 21 to 70 g and mean of 53.3 g. The YPP showed a lot of variation with its values ranging from 15 g to 620 g per plot with average yield of 321.4 g. The coefficient of variation among the measured traits varied from 7.20 (DF) to 38.30 (SPS). Among the physiological traits studied, the coefficient of variation was reported maximum for the CCI (16.43) followed by NDVI (13.28) and CT (8.76).

The covariance derived for different traits showed relationships between the variances for each with some having positive relation while others had negative relationship (Table 1). The DF had a positive relation with TPM, PH, SPS, CCI and NDVI. The TPM showed positive relation with DTF, PH, PEDL, SPS, YPP, CCI and NDVI. PH was related positively with DTF, TPM, PEDL, SL, GPS, TGW, CCI and NDVI. The PEDL was related positively with TPM, PH, SL, TGW, CT and NDVI. The SL was related positively with PH, PL, GPS, YPP, TGW and CT. SPS had positive relation with DF, TPM, CCI and NDVI. GPS were positively associated with PH, SL, YPP, TGW and CT. YPP was positively related with TPM, SL, GPS and TGW. TGW was positively related with PH, PL, SL, GPS, YPP and CT. The CCI had a positive relation with DF, TPM, PH, SPS and NDVI. The CT was positively related with PL, SL, GPS and TGW. The NDVI was positively related with DTF, TPM, PH, PL, SPS and CCI. Positive and some

negative correlations were identified among different traits in the set of germplasm (Table 1). High positive correlation was observed for DTF with TPM, CCI and NDVI. Similarly, TPM were positively correlated with SPS and NDVI. SL was positively correlated with GPS and TGW. Also positive correlation was recorded for yield per plot with GPS and TGW. NDVI and CCI were also positively correlated. Some of the traits however showed high negative correlations. TGW had a high negative correlation between DF, TPM and SPS. Similarly, high negative correlation was observed for SL with TPM and SPS.

The principal component analysis was carried out on the set of 440 wheat genotypes, based on the data generated on 12 different physiological and agro-morphological traits including yield (Table 2). The first 10 principal components (PCs) explained 93.45% of the variation among 440 genotypes. A scree plot was derived based on decreasing values of the eigen vectors against the PCs. The PC1 explained 20.39% variation whereas PC2 and PC3 accounted for 13.27 and 12.0% variation respectively. PC1 accounted for variance in traits such as DF, TPM, SL, SPS, 100TGW, CCI and NDVI. PC2 explained variation for PH and PL. PC3 has high eigen values for grains per spike and yield per plot, whereas maximum variation for canopy temperature was explained by PC6. Similarly, the other PCs and their contributions are given in Table 2. The yield per plot was explained by four PCs (3, 4, 11 and 12). First three PCs, which accounted for 45.66% variation and were used to draw biplot diagrams (Fig 1) to differentiate the genotypes based on different traits. Group one identifies genotypes showing variation for TPM, CCI, SPSS and NDVI. Group two identified genotypes with variation for yield, GPS, TGW and SL, whereas group three had genotypes having variation for PH and PEDL. The biplot between the PC1 and PC3 could divide the genotypes into four groups and the biplot between PC2 and PC3 grouped the genotypes into three based on the variation for different traits studied. All the traits studied and their variation present in 440 wheat genotypes was partitioned into different groups based on the biplot analysis.

Agglomerative cluster analysis was done based on the euclidean distance method which classified 440 genotypes into six major clusters as is shown in the dendrogram (Fig 2). All the 12 physiological and agro-morphological traits were used to derive the clusters and had a cophenetic correlation coefficient of 0.319. Among the six major clusters, first cluster had 182 genotypes and contributed to 41.4% of the genotypes. Second, third and fourth clusters had 23, 48 and 77 members and accounted for 5.2%, 10.9% and 17.5% of the genotypes respectively. Fifth cluster was second largest with 107 individuals and thus had 24.3% of the total genotypes. The sixth cluster had only three genotypes. The 57% dissimilarity level between the genotypes was reported which depicts high level of variation between the genotypes

The coefficient of variation (CV) in present study varied from 7.20% for DF to 38.30% for SPS. A high degree of

Table 1 Matrix of simple correlation coefficients (r) between different traits studied

Trait	Parameter	DF	TPM	PH	PEDL	SL	SPS	GPS	YPP	TGW	CCI	CT	NDVI
DF	r	1	0.228*	0.153*	-0.065	-0.123*	0.123*	-0.147*	-0.032	-0.278	0.377*	-0.097*	0.307*
	p-value		0	0.0013	0.1719	0.0097	0.0101	0.0019	0.5053	0	0	0.0422	0
TPM	r	1	1	0.076	0.027	-0.240*	0.291*	-0.158*	0.173*	-0.238*	0.191*	-0.044	0.266*
	p-value			0.1093	0.5782	0	0	0.0009	0.0003	0	0.0001	0.3561	0
PH	r	1	1	1	0.526*	0.061	-0.010	0.042	-0.070	0.105*	0.058	-0.018	0.081
	p-value				0	0.2039	0.8382	0.3793	0.1413	0.0275	0.224	0.7033	0.0891
PEDL	r	1	1	1	1	0.067	-0.007	-0.024	-0.107*	0.079	-0.089	0.032	0.019
	p-value					0.1623	0.8911	0.6142	0.0251	0.0984	0.0618	0.503	0.6849
SL	r	1	1	1	1	1	-0.213*	0.268*	0.025	0.221*	-0.084	0.112*	-0.147*
	p-value						0	0	0.5984	0	0.0793	0.0183	0.002
SPS	r	1	1	1	1	1	1	-0.045	-0.123*	-0.384*	0.032	-0.046	0.092
	p-value							0.3446	0.0099	0	0.5025	0.3311	0.0551
GPS	r	1	1	1	1	1	1	1	0.356*	0.150*	-0.038	0.031	-0.133*
	p-value								0	0.0016	0.4217	0.5116	0.0053
YPP (g)	r	1	1	1	1	1	1	1	1	0.308*	-0.016	-0.033	-0.001
	p-value									0	0.7322	0.4948	0.9828
TGW (g)	r	1	1	1	1	1	1	1	1	1	-0.186*	0.082	-0.171*
	p-value										0.0001	0.0841	0.0003
CCI	r	1	1	1	1	1	1	1	1	1	1	-0.067	0.259*
	p-value											0.1632	0
CT	r	1	1	1	1	1	1	1	1	1	1	1	-0.078
	p-value												0.102
NDVI	r	1	1	1	1	1	1	1	1	1	1	1	1
	p-value												

\* significant at 5 % level. DF = Days to flowering, TPM = tillers per m, PH = plant height, PEDL = peduncle length, SL = spike length, SPS = spikelets per spike, GPS, grains per spike, YPP = yield per plot, TGW = thousand grains weight, CCI = chlorophyll content index, CT = canopy temperature, NDVI = normalized difference vegetation index.

Table 2 Principal component loadings for various traits along with standard deviation, proportion of variance, cumulative variance and eigen values

Statistics	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
DF	0.3914	-0.1085	0.2346	-0.3267	0.1192	-0.0319	0.2052	-0.1834	0.6843	0.0216	0.1855	0.2813
TPM	0.3685	-0.0496	0.2481	0.4070	-0.0753	0.2713	-0.0344	-0.4914	-0.2553	0.1894	-0.3271	0.3235
PH (cm)	0.0334	-0.6918	0.0484	0.0543	0.0213	-0.0845	0.1823	0.0412	0.1467	-0.0950	-0.5057	-0.4315
PEDL (cm)	-0.0395	-0.6586	-0.1471	0.2006	-0.0390	-0.143	-0.0200	0.0721	-0.1776	0.2074	0.5962	0.2560
SL (cm)	-0.3218	-0.1421	0.0934	-0.2757	0.4620	-0.0847	-0.4167	-0.6032	-0.0928	-0.0757	0.0736	-0.1167
SPS	0.3198	0.0715	-0.1559	0.5071	0.3879	-0.1248	-0.0446	0.0067	0.0647	-0.6341	0.1799	-0.0674
GPS	-0.2714	0.0054	0.4147	0.1941	0.5001	-0.2672	0.0112	0.4067	0.0084	0.1757	-0.2222	0.3864
YPP (g)	-0.1523	0.1054	0.6520	0.3228	-0.1478	0.0942	0.0710	-0.0563	0.1231	0.0885	0.3742	-0.4842
TGW (g)	-0.4179	-0.1385	0.2324	-0.0458	-0.3790	0.1714	0.0466	-0.0420	0.0380	-0.6432	-0.0559	0.3934
CCI	0.3101	-0.0406	0.3192	-0.4263	0.1682	0.0058	0.3750	0.1057	-0.6115	-0.2105	0.1273	-0.0638
CT	-0.1330	-0.0293	-0.1160	-0.0185	0.3946	0.8716	0.1142	0.1615	0.1000	0.0249	0.0093	-0.0487
NDVI	0.3423	-0.1240	0.2450	-0.1591	-0.1208	0.1609	-0.7658	0.3827	0.0105	-0.0803	-0.0303	-0.0349
Standard deviation	1.5642	1.2620	1.1999	1.0734	1.0146	0.9874	0.8564	0.8200	0.7901	0.7403	0.6633	0.5884
Proportion of Variance	0.2039	0.1327	0.1200	0.0960	0.0858	0.0813	0.0611	0.0560	0.0520	0.0457	0.0367	0.0289
Cumulative Proportion	0.2039	0.3366	0.4566	0.5526	0.6384	0.7196	0.7808	0.8368	0.8888	0.9345	0.9711	1.0000
Eigen values	2.4467	1.5927	1.4397	1.1523	1.0293	0.9750	0.7334	0.6724	0.6243	0.5481	0.4399	0.3462

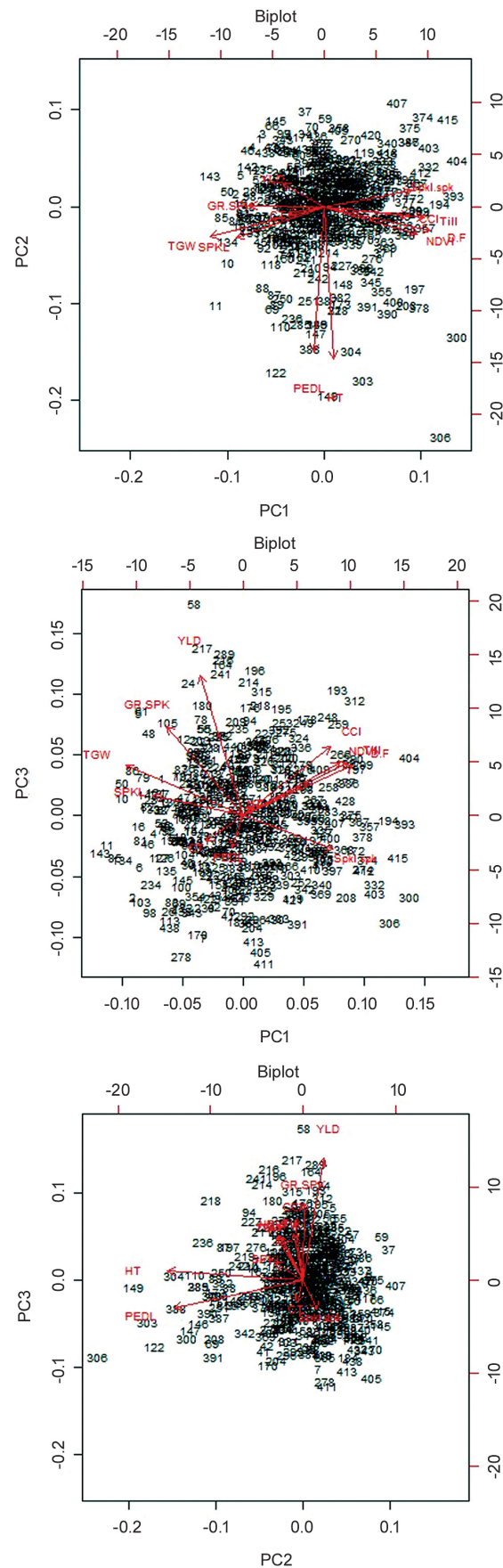


Fig1 Biplot diagrams of the first three principal components eigen vectors of 440 wheat fans.

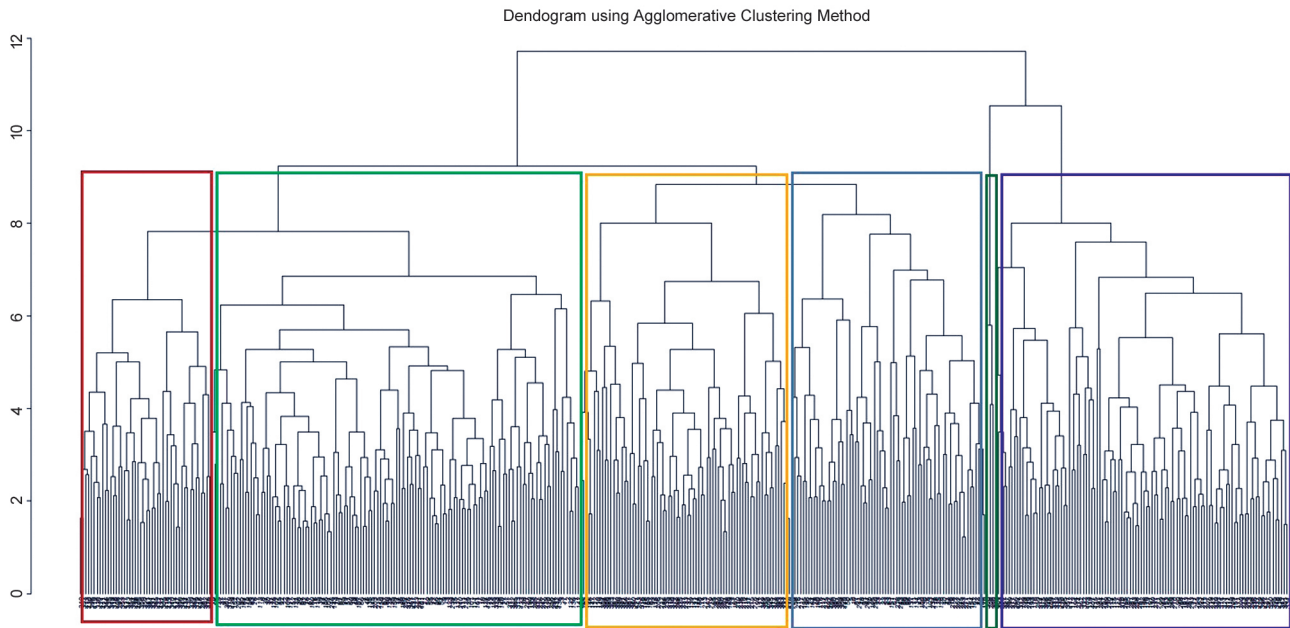


Fig 2 Cluster diagram of the principal components eigen vectors of 440 wheat genotypes by PCA.

variance for different traits such as TPM (25.64%), SL (19.50%), GPS (23.91%), YPP (26.71%) and TGW (17.37%) implies that, other than the environmental factors, this set exhibits a lot of variability for different yield components. Comparatively, low coefficient of variance was observed for DF (7.20%) and PH (13.48%). Thus plant height becomes an important trait as far as wheat breeding is considered. In context of the target area for which this germplasm will be used, the set covers the range of variability that is desired. For the three physiological traits studied, CCI (16.43%) had the highest CV followed by NDVI (13.28) and CT (8.76%). Variability on similar pattern was also reported in wheat for different economically important traits (Bityutskii *et al.* 2017, Cakmak *et al.* 2004, Kumar *et al.* 2015, Kumar *et al.* 2019, Mishra *et al.* 2020). The genetic variation for various traits, particularly yield and its components narrowed down after the advent of green revolution.

Both positive and negative trends were observed between the traits studied for correlation (Table 1). YPP was positively related with its component traits tillers per m, grains per spike and 1000 grains weight. However, it was negatively correlated with peduncle length and spikelets per spike. Among other yield components, TPM and the SPS were observed to be positively related. The agro-morphological traits such as DF had high positive relationship with TPM and CCI, whereas it had a slightly negative association with the overall yield. Among the physiological traits, only CCI seemed to be positively associated with DF and TPM. Earlier studies have also found similar results of correlation for various traits of economic importance in wheat (Cakmak *et al.* 2004, Peleg *et al.* 2009, Chatzav *et al.* 2010, Badakhshan *et al.* 2013, Amiri *et al.* 2015).

The PCA carried out showed that most of the traits studied explained the variability in the set of germplasm

indicating the importance of each trait that contributes to the overall variability. PCA allows data exploration to define correlations between different traits by reducing dimensionality and also investigates number of components needed to explain maximum variation (Samec *et al.* 2016). The biplot between PC1 and PC2 compares the genotypes in three groups each group explaining variation due to different traits (Fig 1). All the studied traits and their variation present in 440 wheat genotypes was partitioned into different groups based on the biplot analysis. PCA revealed that genetic diversity for both agro-morphological and physiological traits is available in the present set of germplasm. Agglomerative cluster analysis was done based on the euclidean distance method which classified 440 genotypes into six major clusters. All the 12 physiological and agro-morphological traits were used to derive the clusters and had a cophenetic correlation coefficient of 0.319. The dissimilarity level up to 57% between the genotypes observed, which depicts high level of variation between the genotypes. Many authors have reported similar results in wheat (Sajjad *et al.* 2011, Ashraf *et al.* 2012, Dutamo *et al.* 2015). Almost all the components explained some variation for each trait and hence can be further used in hybridization for creation of variability in the breeding programs to develop improved cultivars. This remarkable variation in the set can be accounted to the fact that these lines were a collection of germplasm from different wheat growing countries and also their specificity for different traits. More insights into this set can be achieved by molecular profiling of the genotypes and correlating with the information generated in the present study.

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