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RESEARCH ARTICLE

Assessment of Genetic Diversity in Bread Wheat (*Triticum aestivum* L.) Germplasm Grown under Rainfed Conditions of Kashmir

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Assessment of genetic diversity and germplasm improvement is fundamental for sustainable production of different crops including wheat. 200 accessions of wheat germplasm were evaluated during two successive *rabi* seasons of 2016-17 and 2017-18 along with two national (GW-322 and PBW-343) and one local (HS-240) checks at ICAR-NBPGR RS Srinagar. Principal component and cluster analysis were carried out involving 7 quantitative traits of plant height (cms), flag leaf length (cms), flag leaf width (cms), days to 75% spike emergence, days to 80% maturity, grain yield/plant (g) and 100-seed weight (g). Moderate positive correlations were observed between flag leaf length and days to 80% maturity as well as between these traits and plant height. Days to 80% maturity showed stronger positive correlation with 75% spike emergence which in turn was also strongly correlated with flag leaf length. The results of PCA revealed that the first 4 principal components contributed 76.1% of the total variability with first two components accounting for more than half (51.1%). The divergence analysis based on Euclidian method indicated the presence of adequate genetic diversity with four clusters showing distinct characteristics. 25 promising genotypes identified for each trait, could be useful to plan crosses and study the extent of heterosis.

Key Words: Cluster analysis, Genetic diversity, Principal components, Promising genotypes, *Triticum aestivum*

Introduction

Triticum aestivum L. known as common or bread wheat represents 90% of the total world wheat production while other 10% is contributed by *Triticum turgidum* subsp. *durum* which is known as durum wheat (Ateş Sonmezoğlu *et al*., 2012). In fact, bread wheat is a very diverse and widely adaptable cereal crop (Levandi *et al*., 2014) and is one of the most important and staple food crops in the world standing next to rice, both in area and production occupying 17% of crop acreage world over, feeding about 40% of world population and providing 20% of the total food calories and protein in human nutrition (Gupta *et al.,* 2005). India is second largest producer of wheat after China and with 99.70 million tonnes during 2017-18 contributed 12.91% of total global production (Ramdas *et al.,* 2019). In India, the major wheat producing states are Uttar Pradesh, Punjab, Haryana, Madhya Pradesh, Rajasthan, Bihar, Maharashtra and Gujarat. These states contribute about 87.5% of total wheat production in the country (Kumar *et al.,* 2014). In the union territory of Jammu and Kashmir, wheat is cultivated as *rabi* crop, however in Gurez and Tulail areas and also in adjacent union territory of Ladakh it is cultivated as summer crop. In Jammu province along with maize, it is the staple food crop while in Kashmir rice is the staple food crop and wheat is cultivated in limited areas predominantly as rainfed crop especially in remote hilly areas and in the foothills of the valley. In Ladakh, wheat is cultivated less commonly than naked barley, the latter being staple food crop of that region. Few decades back wheat was more commonly cultivated in Kashmir, however owing to the popularization of horticultural as well as fodder crops especially oats, there has been a drastic decline in wheat cultivation in the region. Absence of cold tolerant and short duration varieties may be among other reasons for loss of interest in this crop among local farmers. Nevertheless there has always been a good scope for wheat cultivation especially under rainfed conditions in dry areas of the region. The demand for wheat is expected to grow faster than any other major agricultural crop. With a limited scope for increasing the crop acreage the only option is to increase existing average yield as the production target in the country has been fixed at 140 million tonnes by

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the year 2050 (ICAR-IIWBR Vision Document, 2015). Exploring genetic diversity among wheat genotypes besides evaluation and utilization of this genetic diversity is fundamental to the sustainable production of the crop. It is very well documented that morphological and agronomic characterization provides a simple and direct way of determining genetic variations among genotypes while at the same time assessing their performance under normal growing conditions. The present study was therefore undertaken to assess the magnitude of genetic diversity and characters contributing towards total genetic diversity in some bread wheat genotypes under rainfed conditions of Kashmir for effective selection in future breeding programmes. In the study, cluster and principal component analyses has been used for genetic diversity analysis.

Materials and Methods

The study was carried out under rainfed conditions at the Experimental Farm of ICAR-NBPGR Regional Station Srinagar, Jammu and Kashmir (33°59′ N latitude, 74°47′ E longitude, 1639 m above sea level) during two successive *rabi* seasons of 2016/2017 and 2017/2018. The experimental site is situated on dry *Karewa* land having soil pH of 6.23 and average normal monthly rainfall 52 mm. Two hundred accessions of *Triticum aestivum* L. germplasm along with two national (GW-322, PBW-343) and one local (HS-240) check varieties were used in the present study. These 200 accessions are given in supplementary list. The germplasm was supplied by Evaluation Division of ICAR-National Bureau of Plant Genetic Resources, New Delhi. Some of the genotypes used in the study were earlier collected by the station from far-flung, remote and hilly areas of Kashmir and Ladakh. The experiments were laid out in augmented block design with bed width of 2 m and row to row and plant to plant distance of 25 cm and 10 cm respectively. Recommended agronomic practices and protective measures were followed to grow a healthy crop. Fertilizers @120:60:40 kg NPK/ha were applied for better crop production. Weeding was done by hand whenever necessary. Data on plant height (cm), flag leaf length (cm), flag leaf width (cm), days to 75% spike emergence, days to 80% maturity and grain yield/plant (g) were recorded on five randomly selected plants of each germplasm line ignoring those on peripheries. 100 seed weight (g) was also recorded in every genotype. These quantitative traits were then analyzed by cluster and principal component analysis. Principal components analysis (PCA) was carried out on the correlation matrix calculating the mean data of the accessions. The genotypes in each resultant cluster were also analyzed for basic statistics. Correlations between different traits were determined by Pearson's correlation. Statistical tests were carried out by the Statistical Package for Social Sciences (version 21) and OpenStat softwares.

Results and Discussion

Basic statistics on pooled data for quantitative traits showed a considerable variability in 200 accessions of wheat under study (Table 1). Very high variance was observed for plant height. Medium to high variance was observed for flag leaf length, days to 75% spike emergence and grain yield/plant. Small genetic variance was observed for flag leaf width, days to 80% maturity and 100-seed weight. In Kashmir, wheat is sown as *rabi* crop and its growth is arrested during winter months remaining under snow cover thus crop duration is significantly increased. If the winter is prolonged over the months of February and March and temperature is significantly reduced, the crop duration is further increased. Even in our present study during the year 2016/17, the days to 75% spike emergence and days to 80% maturity ranged from 136-137 days and 195-200 days respectively, while during the year 2017/18 these parameters ranged from 116-150 days and 180-185 days respectively. The reason for early spike emergence and maturity during later year of 2017/18 was comparatively drier winter with almost negligible snowfall and rainfall during the months of February and March. After the winter is over and temperature increases fast, the spike emergence and ultimately maturity ensues. Most of the wheat accessions used in the present study are reported to be early maturing types in other parts of the country. However, we have not been able to identify some significant early maturity lines under local Kashmir conditions.

Simple correlation analysis: In order to assess relationship between different traits, simple correlation coefficients between grain yield and its components has been computed and is presented in Table 2. While positive correlations of different magnitude were observed between different traits studied, the seed yield/plant showed weak negative correlation with plant height and flag leaf length. Stronger positive correlations were observed between days to 80% maturity and days to 75% spike emergence and between days to 75% spike emergence and flag leaf length.

Table 1. Basic statistics for seven quantitative traits of 200 accessions of wheat evaluated during 2016/17 and 2017/18

Trait	Mean ± SD	Minimum value	Maximum value	Variance
Plant height (cms)	96.9 ± 11.4	68.0	134.3	130.1
Flag leaf length (cms)	22.9 ± 2.4	16.8	31.4	5.7
Flag leaf width (cms)	1.49 ± 0.19	1.10	2.15	0.04
Days to 75% spike emergence	141.9 ± 4.6	131.5	152.0	21.7
Days to 80% maturity	190.7 ± 1.0	188.0	192.5	1.0
Grain yield/plant (g)	20.499 ± 5.533	5.933	45.820	30.6
100-Seed weight (g)	3.695 ± 0.533	2.250	5.350	0.3

Table 2. The Pearson correlation coefficient matrix for agro-morphological traits in 200 accessions of wheat evaluated during 2016/17 and 2017/18

Trait	PH	FLL	FLW	DSE	DMT	SY	SW
PH	1.0000						
FLL	**0.3031	1.0000					
FLW	**0.1473	**0.2813	1.0000				
DSE	**0.2165	**0.4138	**0.1589	1.0000			
DMT	**0.3020	**0.3680	**0.1484	**0.6039	1.0000		
SY	-0.0368	-0.0937	**0.1375	0.0621	0.0542	1.0000	
SW	0.0519	0.0411	**0.1378	**0.1520	0.0672	**0.2567	1.0000

PH=Plant height, FLL=Flag leaf length. FLW=Flag leaf width, DSE=Days to 75% spike emergence, DMT=Days to 80% maturity, SY=Grain yield/plant and SW=100-Seed weight
** Significant at 1% level of probability

Principal component and Cluster analysis: The quantitative trait of yield is a stated goal for plant breeders which is under the control of many genes, thus selection based on yield *per se* may not be successful for its improvement. Selection of yield contributing traits having relatively high heritability thus becomes important in overall improvement in yield potential. However, selection based on simple correlation coefficients between different traits may not be too helpful. Thus, multivariate statistical analysis of the agro-morphological data obtained on 200 wheat germplasm accessions during the course of present investigation has been done for deeper understanding of relationship between the traits. The correlation coefficient analysis has been found to be useful in the identification of characters that are positively correlated with yield (Maqbool *et al.*, 2010; Bode *et al.*, 2012) while the evaluation of phenotypic variability by multivariate analysis gives the possibility to include a large number of accessions and to identify the most suitable resources for special traits (Goel *et al.*, 2015). The identified accessions for special traits can then be effectively used in wheat breeding programmes**.** Therefore, extent of genetic diversity in wheat accessions in present study has been measured by cluster analysis and principal component analysis (PCA) for their effective evaluation and utilization. The cluster analysis is an appropriate method for assessing family relationships (Mellingers, 1972) and the merit of using PCA over cluster analysis is that each germplasm line can be assigned to one group only (Mohammadi, 2002).

Results from the principal component analysis for the two year combined data showed that four components of PC1, PC 2, PC 3 and PC 4 accounted for 76.1% of the total variation in agro-morphological traits studied contributing 32.4%, 18.6%, 13.5% and 11.5% of the total variation respectively (Table 3). Eigen values given in the table show that relative discriminating power of the principal components was high for PC 1 (2.271) followed by PC 2 (1.304), PC 3 (0.942) and PC 4(0.806). The first four components explaining 76.1% of total variation were taken under study. The number of components was also determined with the help of the highest slope in Scree plot (Fig. 1). The Scree plot explains the percentage of variance associated with each principal component. The most effective traits in the first component were flag leaf length, days to 75% spike emergence and days to 80% maturity (Table 4). Major effective traits in the second component were grain yield/plant and 100-seed weight. In the third component, flag leaf width was the main effective trait while plant height and 100-seed weight were the most effective traits governing fourth

Table 3. Eigen values and percentage of total variance for PCA in 200 accessions of wheat evaluated during 2016/17 and 2017/18

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
Eigen value	2.271	1.304	0.942	0.806	0.748	0.554	0.374
Percent variance	32.4	18.6	13.5	11.5	10.7	7.9	5.3
Cumulative percent variance	32.4	51.1	64.5	76.1	86.7	94.7	100.0

Table 4. Vector loadings for yield contributing traits in 200 accessions of wheat evaluated during 2016/17 and 2017/18

Trait	Communalities	Vector 1	Vector 2	Vector 3	Vector 4	Vector 5	Vector 6	Vector 7
PH	0.989	-0.361	-0.176	0.230	0.788	-0.370	-0.068	-0.153
FLL	0.672	-0.469	-0.213	0.252	-0.129	0.232	0.758	0.162
FLW	0.911	-0.295	0.251	0.746	-0.359	-0.043	-0.401	-0.036
DSE	0.805	-0.518	-0.021	-0.396	-0.225	0.102	-0.113	-0.708
DMT	0.778	-0.513	-0.091	-0.383	-0.119	-0.159	-0.328	0.659
SY	0.925	-0.067	0.693	-0.138	-0.085	-0.594	0.369	-0.011
SW	0.993	-0.164	0.609	-0.067	0.402	0.647	-0.062	0.116

PH=Plant height, FLL=Flag leaf length. FLW=Flag leaf width, DSE=Days to 75% spike emergence, DMT=Days to 80% maturity, SY=Grain yield/plant and SW=100-Seed weight

component. The first PC thus, was more related to vegetative growth, second PC solely to yield and yield contributing traits whereas third and fourth PC contrasts variables that again related to vegetative growth. All the studied traits thus seem to effectively govern the variation in these four components. Traits loading more on first two principal components with Eigen values greater than 1 and contributing more than half of the variance were used to cluster 200 wheat genotypes into closely related groups. It has been observed that the first principal component accounts for maximum variability in the data with respect to succeeding components (Leilah and Al-Khateeb, 2005). According to Chahal and Gosal (2002), characters with largest absolute values closer to unity within the first component influence the clustering more than those with lower absolute values closer to zero. Besides, the first two principal components were used to cluster genotypes into groups since they contributed more than half of the total variation as also suggested by different authors (Ajmal *et al*., 2013; Poudel *et al.*, 2017). According to Badu-Apraku *et al*. (2006), the traits that loaded more on PC1 and PC2 showed the strong discriminatory power in separating genotypes. Clustering was done through Ward's method. The critical examination of the dendrogram generated after the cluster analysis and PCA, revealed four clusters (Fig. 2). The mean value ± SD of all the traits studied of these four clusters are presented in Table 5. Seventeen (17)

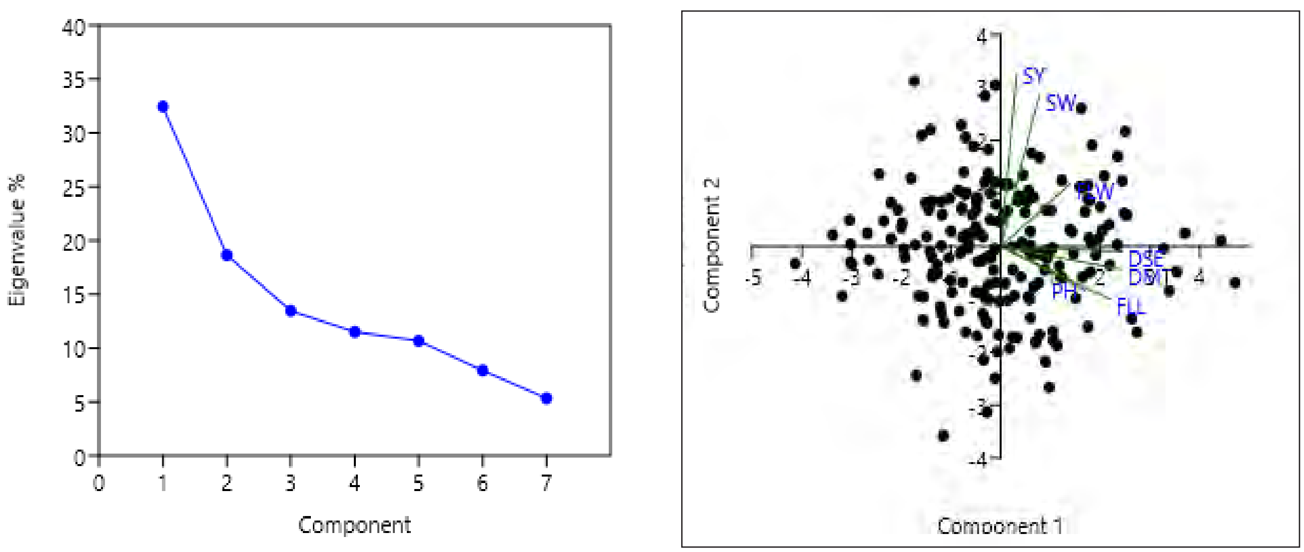

Fig. 1. Scree plot and Biplot of 200 wheat accessions based on first and second components

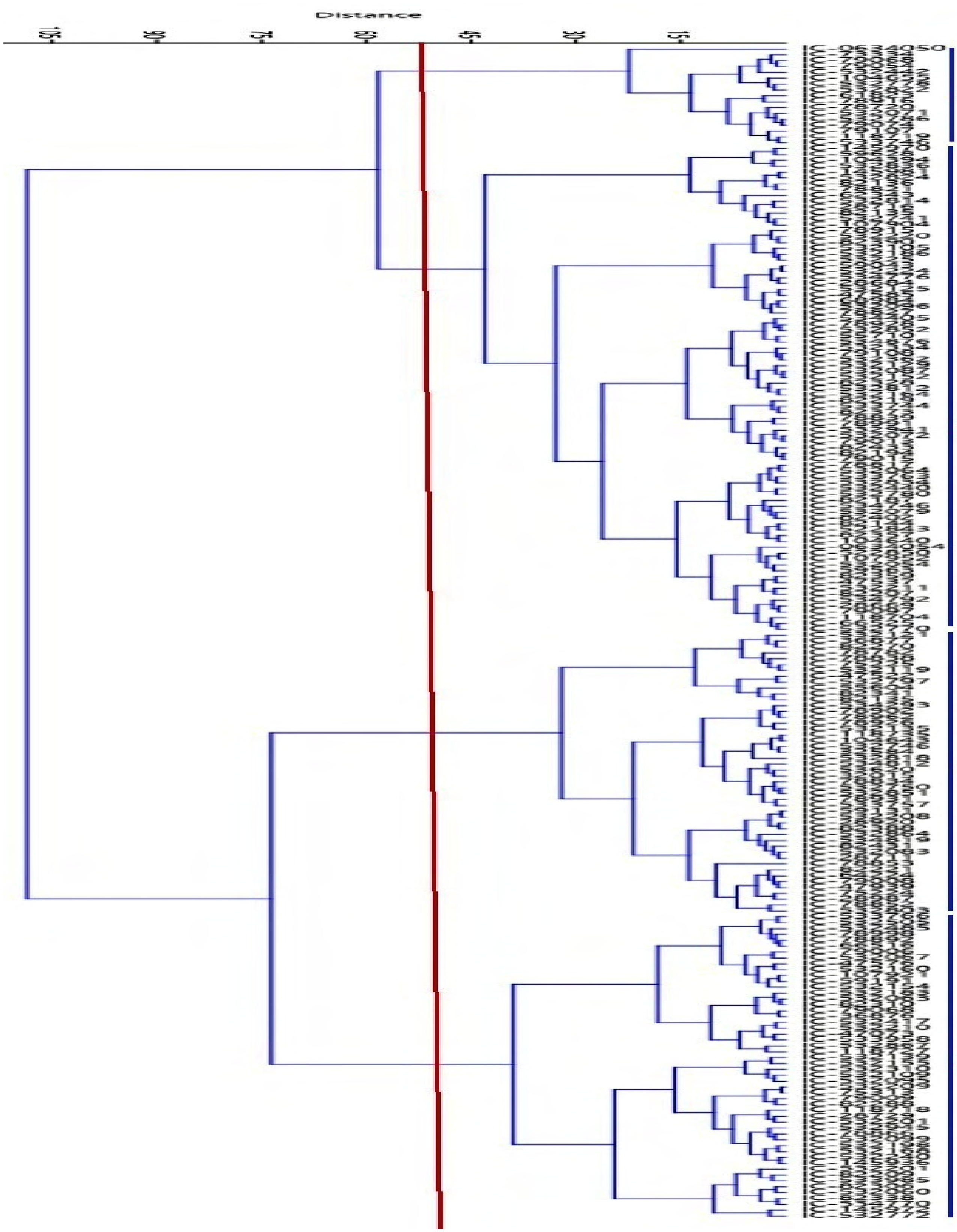

Fig. 2. Dendrogram of 200 wheat accessions based on agro-morphological traits using hierarchical cluster analysis (Ward's method)

Table 5. Mean and standard deviation for four clusters based on seven quantitative characters of 200 accessions of wheat evaluated during 2016/17 and 2017/18

Trait	Cluster 1 (17 accessions)	Cluster 2 (83 accessions)	Cluster 3 (48 accessions)	Cluster 4 (52 accessions)
PH	92.2 ± 9.6	96.2 ± 11.0	96.4 ± 9.5	100.8 ± 13.1
FLL	22.3 ± 1.4	22.2 ± 2.2	23.3 ± 2.3	23.9 ± 2.5
FLW	1.48 ± 0.16	1.49 ± 0.19	1.46 ± 0.19	1.54 ± 0.22
DSE	140.9 ± 2.5	139.1 ± 3.1	140.6 ± 2.8	148.2 ± 2.0
DMT	190.4 ± 0.9	190.4 ± 1.0	190.6 ± 0.9	191.5 ± 0.6
SY	30.180 ± 4.711	21.830 ± 2.686	13.900 ± 2.803	21.350 ± 4.280
SW	3.960 ± 0.450	3.690 ± 0.510	3.510 ± 0.510	3.800 ± 0.570

PH=Plant height, FLL=Flag leaf length. FLW=Flag leaf width, DSE=Days to 75% spike emergence, DMT=Days to 80% maturity, SY=Grain yield/plant and SW=100-Seed weight

Table 6. Promising accessions identified for each trait as a result of evaluation of 200 accessions of wheat (These accessions have performed equally well and were among top 25 accessions during both the years of 2016/17 and 2017/18)

Trait	Promising accessions identified	Best performing check mean value
Plant height (cms)	IC-79009, IC-532310, IC-533985, IC-532152, IC-36877, IC-59043, IC-532201, IC-532095, IC-532183	>PBW-343 (109.1 cms)
Flag leaf length (cm)	IC-532310, IC-78853, IC-532183, IC-79068, IC-36876, IC-532095, IC-78915, IC-82341, IC-532063, IC-78782	>GW-322 (25.25 cms)
Flag leaf width (cm)	IC-68984, IC-78915, IC-532063, IC-532183, IC-64224, IC-532083, IC-532864, IC-75224	>GW-322 (1.55 cms)
Days to 75% spike emergence	IC-62343, IC-145981, IC-82135, IC-532741, IC-532814, IC-28716, IC-532824, IC-78821, IC-107904, IC-532803, IC-82136, IC-532818, IC-82144	<PBW-343 (142.0)
Days to 80% maturity	IC-145970, IC-28716, IC-533757, IC-532071	<GW-322 (190.5)
Grain yield/plant (g)	IC-0634050, IC-78916, IC-61823, IC-78720	>GW-322 (26.604 g)
100-seed weight (g)	IC-532210, IC-533985, IC-118704, IC-82288, IC-532139, IC-118719, IC-75224, IC-532099, IC-78926, IC-61823, IC-53225, IC-532164, IC-79068, IC-534792, IC-532160	>GW-322 (3.562 g)

genotypes in cluster 1 represent only 8.5% of the total genotypes have highest grain yield/plant (g) and 100-seed weight (g). Cluster 2 constituted the biggest cluster with 83 genotypes representing 41.5% of the total genotypes characterized by comparatively lesser days taken for 75% spike emergence. Cluster 3 with 48 genotypes representing 24% of the total genotypes showed least values for grain yield/plant (g) and 100-seed weight (g). Finally cluster 4 with 52 genotypes representing 26% of the total genotypes have highest value of plant height (cms), flag leaf length (cms) and flag leaf width (g). Thus, four distinct clusters were observed in the present study as a result of the multivariate analysis. Although the cluster analysis groups genotypes on the basis of greater morphological similarity, the clusters did not necessarily include all genotypes from same origin (Zubair *et al*., 2007). Same is true of our study also, the accessions collected by our station from Jammu and Kashmir grouped in different clusters. The study has thus indicated the presence of appreciable variability in the agro-morphological traits within these 200 accessions of wheat. The accessions that ranked among the first 25 accessions for the studied traits during both the years of 2016/17 and 2017/18 and which were significantly superior to the check varieties have been identified as promising accessions (Table 7). For example, maximum grain yield/plant (g) has been recorded in accession IC-0634050 originally collected from a remote hilly area in Kashmir. This accession grouped as accession first in cluster 1. There is an ample scope and in fact need for exploitation of the accessions identified in the present study for utilization in wheat breeding programmes in the region.

Conclusion

The study has recorded tremendous amount of variability occurring in two hundred *Triticum aestivum* germplasm accessions studied here. PCA and cluster analysis has grouped these accessions in distinct groups. Selection of accessions from the first two principal components

Supplementary list of wheat germplasm accessions studied

S No.	IC No.	S No.	IC No.	S No.	IC No.	S No.	IC No.	S No.	IC No.
1	IC-532071	41	IC-28920	81	IC-75224	121	IC-532109	161	IC-532505
2	IC-82144	42	IC-82136	82	IC-104643	122	IC-532095	162	IC-118727
3	IC-534776	43	IC-532746	83	IC-532183	123	IC-532310	163	IC-104655
4	IC-532786	44	IC-32584	84	IC-82270	124	IC-78827	164	IC-82209
5	IC-61823	45	IC-532220	85	IC-532778	125	IC-532867	165	IC-533744
6	IC-532741	46	IC-47522	86	IC-78839	126	IC-534770	166	IC-533958
7	IC-53620	47	IC-532135	87	IC-532770	127	IC-533766	167	IC-82187
8	IC-532164	48	IC-78915	88	IC-59043	128	IC-82288	168	IC-78866
9	IC-532334	49	IC-532125	89	IC-118704	129	IC-79015	169	IC-145936
10	IC-79108	50	IC-534293	90	IC-533757	130	IC-532772	170	IC-82431
11	IC-78892	51	IC-532836	91	IC-532818	131	IC-79074	171	IC-59563
12	IC-78928	52	IC-55710	92	IC-532945	132	IC-47537	172	IC-28684
13	IC-532209	53	IC-532362	93	IC-78902	133	IC-532154	173	IC-532208
14	IC-79068	54	IC-532750	94	IC-82338	134	IC-534876	174	IC-107952
15	IC-532201	55	IC-78931	95	IC-47073	135	IC-532139	175	IC-78998
16	IC-62343	56	IC-532186	96	IC-145972	136	IC-107904	176	IC-78991
17	IC-532485	57	IC-532087	97	IC-533763	137	IC-532803	177	IC-79013
18	IC-68984	58	IC-532083	98	IC-47576	138	IC-32015	178	IC-47585
19	IC-78817	59	IC-78852	99	IC-78916	139	IC-79068	179	IC-532060
20	IC-78853	60	IC-532152	100	IC-82390	140	IC-532210	180	IC-79054
21	IC-533985	61	IC-532417	101	IC-82194	141	IC-532442	181	IC-82310
22	IC-145981	62	IC-53225	102	IC-82306	142	IC-82388	182	IC-532099
23	IC-532814	63	IC-28711	103	IC-534792	143	IC-532160	183	IC-532105
24	IC-28669	64	IC-82135	104	IC-522843	144	IC-532072	184	IC-82207
25	IC-66525	65	IC-78838	105	IC-82359	145	IC-107921	185	IC-82402
26	IC-64224	66	IC-532440	106	IC-532797	146	IC-59191	186	IC-78720
27	IC-79009	67	IC-66516	107	IC-73334	147	IC-79006	187	IC-79107
28	IC-145970	68	IC-532064	108	IC-522850	148	IC-532121	188	IC-82219
29	IC-28716	69	IC-532063	109	IC-82357	149	IC-532240	189	IC-118719
30	IC-104394	70	IC-82328	110	IC-78834	150	IC-78962	190	IC-532224
31	IC-534746	71	IC-78782	111	IC-532812	151	IC-532155	191	IC-532834
32	IC-55604	72	IC-82135	112	IC-532852	152	IC-532119	192	IC-79008
33	IC-532824	73	IC-532054	113	IC-534405	153	IC-36876	193	IC-82373
34	IC-79097	74	IC-75225	114	IC-532079	154	IC-104640	194	IC-533746
35	IC-59628	75	IC-28987	115	IC-59131	155	IC-82221	195	IC-532851
36	IC-532941	76	IC-47939	116	IC-28729	156	IC-532822	196	IC-118718
37	IC-82341	77	IC-534812	117	IC-532198	157	IC-532067	197	IC-78926
38	IC-534819	78	IC-118735	118	IC-532168	158	IC-82433	198	IC-532211
39	IC-532129	79	IC-36877	119	IC-75313	159	IC-78821	199	IC-0634050
40	IC-532864	80	IC-532780	120	IC-532096	160	IC-532207	200	IC-0634054

as well as from identified promising genotypes for each trait can be utilized for specific breeding purposes as possible parental lines. While we have not found any particular accession/s best for all the traits studied here, some accessions performed equally well for some of these traits as follows.

1. IC-532310, IC-532095 and IC-532183 are the genotypes with maximum plant height and flag leaf length, later accession i.e. IC-532183 is best for flag leaf width as well.
2. IC-533985 is the genotype with highest plant height as well as seed weight.
3. IC-78915, IC-532063, IC-532183 are best for both flag leaf length and flag leaf width.
4. IC-79068 is the genotype with greater flag leaf length and seed weight.
5. IC-75224 is the genotype with greater flag leaf width and seed weight.
6. IC-28716 has been found to be earliest genotypes

for both the traits of days to 75% spike emergence as well as days to 80% maturity.

7. IC-61823 is the genotype with maximum grain yield/plant as well as 100-seed weight.

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