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Multivariate Analysis to Screen Heat Tolerant Lines of Bread Wheat based on Morphological and Quality Traits

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ABSTRACT: Sixty advance lines of wheat with four standard checks (WH 1021, WH 1124, HD 3059 and DBW 90) were evaluated during *Rabi* 2020-21 at the research area of Wheat and Barley section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar using multivariate approaches to sort fifteen quantitative and eight qualitative traits into respective categories that describe the share of components to the total variation in the study. The cluster analysis revealed that advance lines and checks were classified into seven clusters, with crosses between members of cluster III and cluster IV producing a preferable combination of morphological and quality traits. The results of PCA depicted that the first seven out of twenty three principal components accounted for 70.66 % of the total variance in the study. The biplot illustrated that traits *viz.*, grain yield per plot, biological yield per plot, flag leaf length, grain weight per spike and number of spikelets per spike had longer lengths on both axes, depicting an ample contribution to both PC1 and PC2. The observed patterns indicated the presence of extensive diversity that may be considerate in choosing parents, promising enough to produce heterotic stress tolerant combinations and to determine their implicit role in genetic enhancement.

Keywords: Multivariate approaches, Cluster analysis, PCA, Biplot, Genetic diversity.

INTRODUCTION

Wheat (*Triticum aestivum* L em.Thell) is a significant cereal crop produced worldwide and utilized for both human and livestock nutrition. It has an unprecedented spectrum of agriculture practises, some of which date back to the Neolithic revolution, commenced 10,000 years ago. It is grown on roughly one-sixth of the world's agrarian land and is an essential sustenance for tens of millions of people. The allohexaploid genomic structure of wheat, which may confer some degree of flexibility, is partially responsible for its propensity to thrive under a variety of environmental predicaments (Dubcovsky and Dvorak 2007). Ninety five per cent of the farmed wheat is hexaploid bread wheat, which is specifically suited for use in contemporary utilitarian baking. Despite having a recent genealogy, bread wheat has enough genetic diversity to assist its growth in a variety of conditions. As a result of recent crop improvement efforts, the elite germplasm pool has a stipulated genetic diversity, which makes it difficult to create new strains for stress tolerance and adaptation (Rao and Hodgkin 2002; Zhang *et al*., 2005).

However, a range of abiotic as well as biotic stresses are still pervasive and have a substantial negative impact on yield, with wheat being particularly vulnerable to heat stress (Gupta *et al*., 2013). According to Mondal and Khajuria (2001), heat stress affects plants in a variety of ways such as modifying their growth patterns as well as reducing grain emergence and production. The adverse consequences of climatic patterns on crop yield are posing a menace to global food security, and it is anticipated that rising temperatures will have a greater influence on perpetuating wheat production (Braun *et al*., 2010). Therefore, genetic diversity must be assessed in order to cope up with the effects of the climate crisis in the foreseeable future.

Multivariate analysis has opened a new domain of crop advancement. Principal component analysis (PCA) and cluster analysis are two vital strategies for exploring genetic diversity that differ from one another profoundly. Each germplasm line can be assigned to just one group when using PCA as opposed to cluster analysis (Mohammadi, 2002), however, cluster analysis is a useful method for analysing kinship (Mellingers, 1972). The grail of this study was to identify wheat advance lines with competent genetic variation for heat tolerance.

MATERIALS AND METHODS

Sixty advance lines of hexaploid bread wheat along with four standard checks (WH 1124, WH 1021, HD 3059 and DBW 90) were screened for genetic diversity based on fifteen yield ascribing morphological traits and eight quality traits. The field experiment was carried out in *Rabi* 2020-21 with three replicated levels in a randomized block design (RBD) at the research area of the Wheat and Barley section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar (29.09°N parallel and 75.43°E meridian, respectively and at 215.2 m above MSL (mean sea level) in the sub-tropical region of North Western Plains Zone (NWPZ) of India.

Data were recorded from five plants selected at random from each entry of three replications for days to heading, days to maturity, plant height, number of spikelets per spike, spike length, peduncle length, flag leaf length, main spike weight, number of grains per spike, grain weight per spike, 1000 grain weight, number of effective tillers per metre, grain yield per plot, biological yield per plot, harvest index, grain appearance score, hectolitre weight, sedimentation value, wet gluten, dry gluten, total gluten, crude protein, and total soluble sugars.

Statistical analysis: The mean data were put through cluster analysis and principal component analysis using R software (version R4.2.1) based on Ward's minimum variance method (1963) and Kaiser's (1958) approach of using principal components, respectively.

RESULTS AND DISCUSSION

Cluster analysis based on Ward's minimum variance method: The minimal variance clustering technique developed by Ward (1963) in the first instance, was used to determine the genetic divergence among different wheat advance lines. As shown in Table 1 and Fig. 1, this hierarchical clustering divided the advance lines and checks into seven clusters, revealing the presence of noteworthy genetic diversity between them, as also reported by Mastafa *et al*. (2019). In this study, cluster I was found to be the dominant comprising 19 advance lines, followed by cluster V (14), cluster II and IV (9 each), cluster III (7), cluster VI (4) and cluster VII (2). Cluster II (WH 1021 and WH 1124), cluster IV (HD 3059), and cluster VI (DBW 90) were the precise places of the checks. The heat map illustrated in Figure 2 shows the dissimilarity pattern among individual genotypes, with the blue line designating the point at which the identical genotypes appear on both axes. The transition from blue to red indicated more diversity present among the corresponding genotypes.

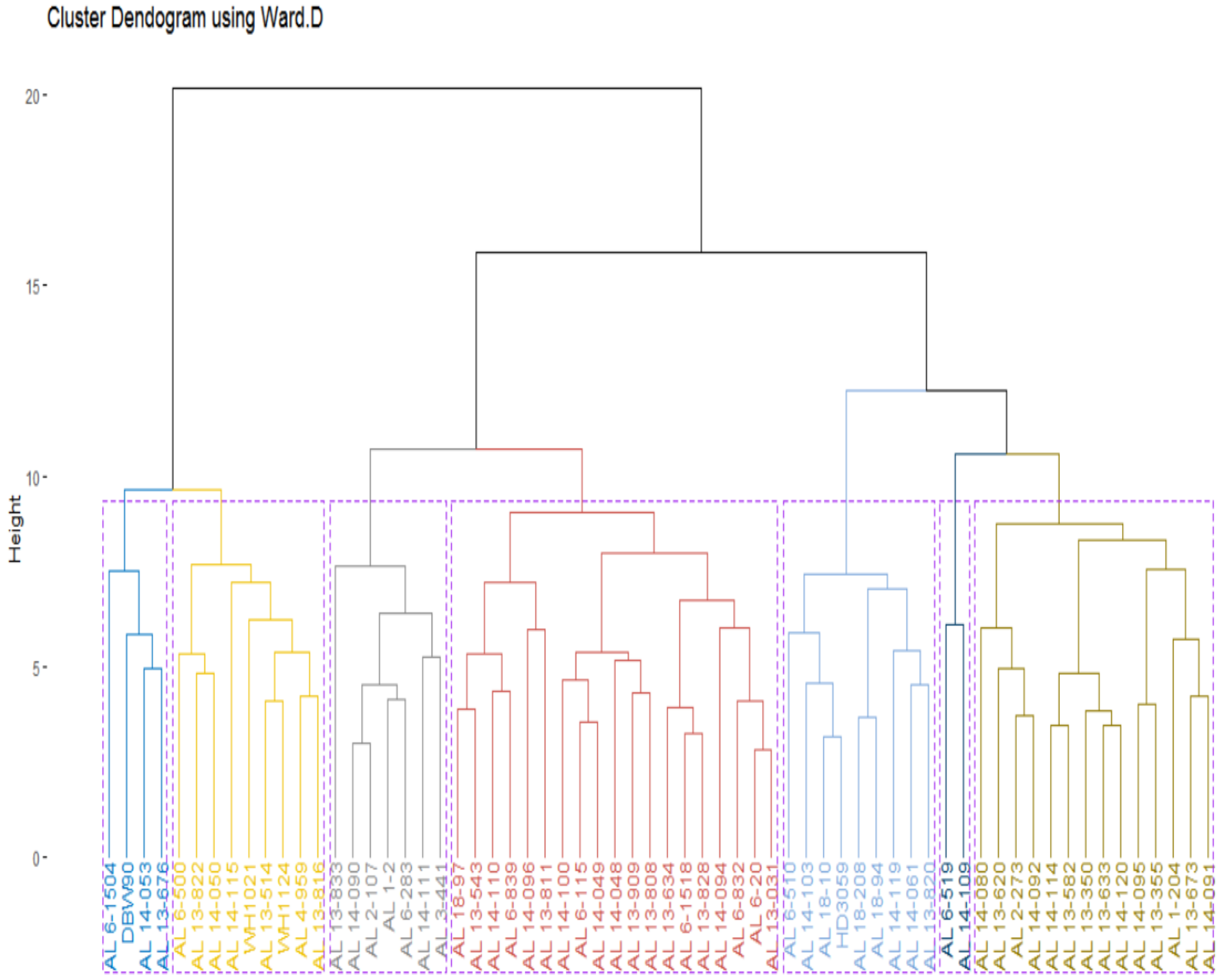

Fig. 1. Dendrogram representing clustering pattern of advance lines of bread wheat and checks based on Euclidean distance under late sown conditions.

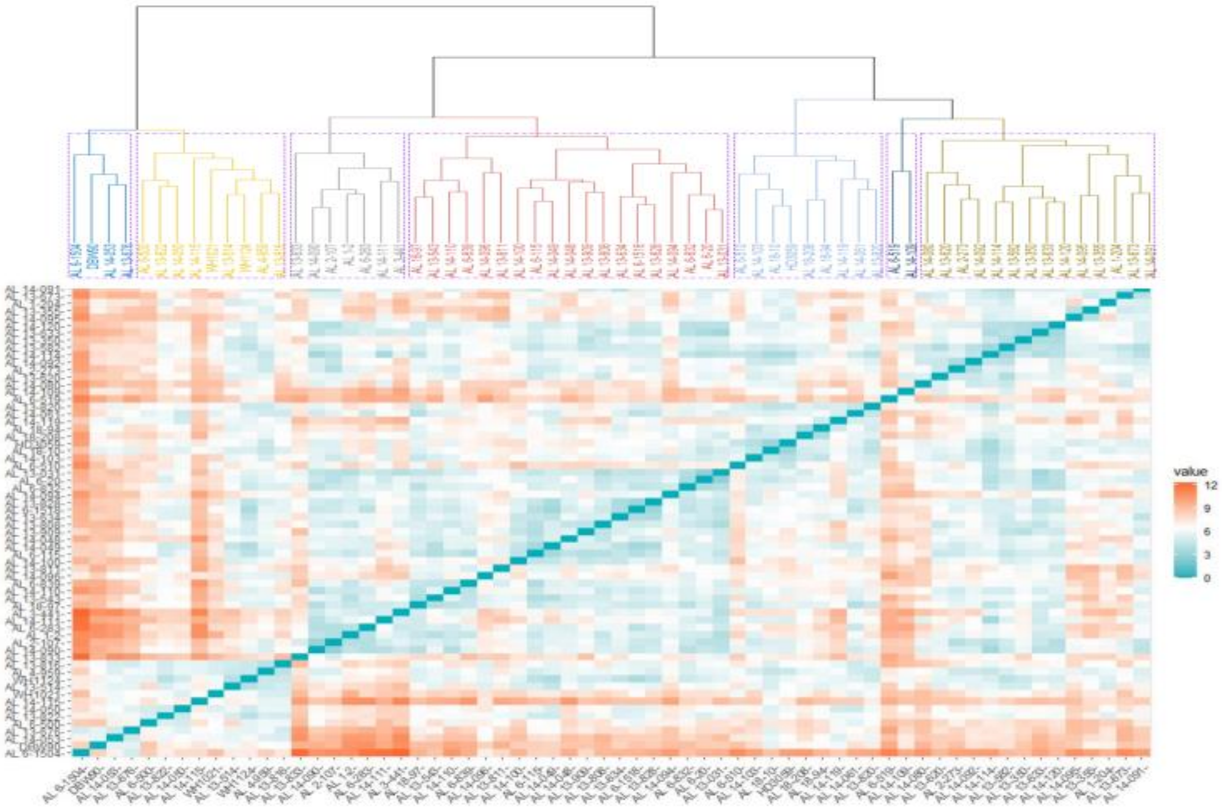

Fig. 2. Dissimilarity heat map for advance lines and checks of bread wheat under late sown conditions based on cluster analysis.

Table 1: Clustering pattern of the advance lines and checks of bread wheat based on Euclidean distance under late sown conditions.

Clusters	Intra cluster distance	Number of genotypes	Cluster members
I	32.89	19	AL 14-110, AL 14-094, AL 6-832, AL 18-97, AL 13-909, AL 14-048, AL 14-096, AL 13-543, AL 6-20, AL 13-808, AL 13-031, AL 13-811, AL 6-839, AL 14-100, AL 6-115, AL 6-1518, AL 13-634, AL 13-828, AL 14-049
II	32.36	9	AL 6-500, AL 13-822, AL 14-115, AL 4-959, AL 14-050, AL 13-816, AL 13-514, **WH 1021, WH 1124**
III	27.51	7	AL 14-111, AL 3-441, AL 14-090, AL 1-2, AL 6-283, AL 2-107, AL 13-833
IV	30.67	9	AL 18-208, AL 14-103, AL 18-10, AL 14-061, AL 14-119, AL 18-94, AL 13-820, AL 6-510, **HD 3059**
V	26.72	14	AL 14-114, AL 14-095, AL 14-080, AL 13-582, AL 13-673, AL 13-633, AL 14-120, AL 2-273, AL 14-092, AL 13-620, AL 1-204, AL 14-091, AL 13-355, AL 13-350
VI	27.95	4	AL 14-053, AL 6-1504, AL 13-676, **DBW 90**
VII	19.77	2	AL 6-519, AL 14-109

Average intra and inter cluster distances: The intra cluster distance varied from 19.77 to 32.89, while the inter cluster distance varied from 27.02 to 42.60, in accordance with Table 2. The largest corresponding values were found for cluster I (32.89) and between clusters I and IV (42.60), suggesting that lines from these clusters could be employed as parents in breeding programmes to produce more heterotic pairings. Cluster VII (19.77) displayed the smallest average intra cluster distance, indicating that the genotypes in this cluster are less diverse, as also observed by Arya *et al*. (2017); Tanveer *et al*. (2021); Reddy *et al*. (2022).

Table 2: Average intra (diagonal) and inter cluster (off-diagonal) distances among seven clusters of wheat advance lines under late sown conditions.

Clusters	I	II	III	IV	V	VI	VII
I	**32.89**						
II	39.36	**32.36**					
III	34.38	36.77	**27.51**				
IV	42.60	37.16	36.97	**30.47**			
V	42.52	35.09	39.17	40.87	**26.72**		
VI	39.22	37.80	39.92	30.58	31.32	**27.95**	
VII	35.49	32.88	36.49	31.63	27.02	29.05	**19.77**

Cluster mean analysis: Table 3 displays the average values of the examined traits in each cluster, from which it could be interpreted that yield contributing and quality traits can be integrated using the advance lines from cluster III and IV. For nearly all the quality traits, cluster I displayed the lowest characteristics. Cluster II showed a high sedimentation value (48.26) together with a short spike length (9.85). Maximum plant height (96.86), effective tillers per metre (97.6), spikelets per spike (1.94), flag leaf length (25.28), main spike weight (3.22), grain weight per spike (2.84), 1000 grain weight (35.57), biological yield (9752.38), harvest index (41.88), grain yield (3468.59), days to heading (84.52), and minimum total soluble sugars (1.28) were the characteristics of the cluster III. The members of Cluster IV were late maturing (121.93), had longest spike (10.77), highest hectolitre weight (80.07) and grain appearance score (5.92), however, they had minimum number of effective tillers per metre (80.07).Cluster V exhibited longest peduncle length (38.3) and cluster VI revealed smallest (33.22) along with highest crude protein (12.59), total soluble sugars (1.62), and dwarf plant height (90.10), minimum flag leaf length (19.68) and, lowest yield potential (2607.40), biological yield (7950) and hectolitre weight (75.06). Early maturing genotypes (80.50) with high levels of total gluten (48.68) were present in cluster VII.

Table 3: Mean values of different clusters for all the morphological and quality traits in bread wheat under late sown conditions.

Clusters / Traits	DH	PH	NET/m	SL	NS/S	FLL	PL	MSW
I	83.70	94.06	95.77	10.57	17.23	23.32	37.46	3.11
II	81.70	94.26	94.56	9.85	16.63	21.57	35.50	2.74
III	84.52	96.86	97.62	10.69	19.43	25.28	36.98	3.22
IV	84.04	95.52	87.07	10.77	17.56	22.30	34.93	3.03
V	82.74	92.54	96.81	10.53	17.29	23.90	38.30	3.04
VI	83.25	90.10	88.42	10.06	15.92	19.68	33.22	2.57
VII	80.50	92.13	93.67	10.18	16.17	25.28	37.20	3.08

Clusters / Traits	DH	PH	NET/m	SL	NS/S	FLL	PL	MSW
I	83.70	94.06	95.77	10.57	17.23	23.32	37.46	3.11
II	81.70	94.26	94.56	9.85	16.63	21.57	35.50	2.74
III	84.52	96.86	97.62	10.69	19.43	25.28	36.98	3.22
IV	84.04	95.52	87.07	10.77	17.56	22.30	34.93	3.03
V	82.74	92.54	96.81	10.53	17.29	23.90	38.30	3.04
VI	83.25	90.10	88.42	10.06	15.92	19.68	33.22	2.57
VII	80.50	92.13	93.67	10.18	16.17	25.28	37.20	3.08

Clusters / Traits	CP	SV	HW	GAS	WG	DG	TG	TSS
I	11.67	40.58	78.64	5.25	26.42	8.86	35.27	1.36
II	11.37	48.26	78.95	5.50	31.75	10.24	41.99	1.31
III	10.43	41.95	79.84	5.37	30.95	9.03	39.98	1.28
IV	11.75	41.56	80.07	5.92	34.89	11.08	45.97	1.61
V	11.42	45.93	78.99	5.42	37.08	11.54	48.62	1.31
VI	12.59	45.25	75.06	5.35	35.01	10.19	45.20	1.62
VII	10.49	47.00	80.03	5.75	37.44	11.24	48.68	1.51

DH: Days to heading, **PH**: Plant height (cm), **NET/m**: Number of effective tillers per metre, **SL**: Spike length (cm), **NS/S**: Number of spikelets per spike, **FLL**: Flag leaf length (cm), **PL**: Peduncle length (cm), **MSW**: Main spike weight (g), **GW/S**: Grain weight per spike (g), **NG/S**: Number of grains per spike,**NSS**: Number of spikelets per spike,**TGW**: 1000 grain weight (g), **DM**: Days to maturity, **BY/P**: Biological yield per plot (g), **HI**: Harvest Index (%), **CP**: Crude Protein (%), **SV**: Sedimentation Value (ml), **HW**: Hectolitre Weight (Kg/hl), **GAS**: Grain Appearance Score, **WG**: Wet Gluten (%), **DG**: Dry Gluten (%), **TG**: Total Gluten (%), **TSS**: Total Soluble Sugars (%)

Principal component analysis. Principal component analysis highlights the significance of the largest contributor to the total variation at each axis for differentiation. As indicated in Table 4, the first seven of the twenty-three principal components showed eigen values higher than one (significant) and accounted for 70.66% of the total variance. The remaining sixteen PCs (principal components) explained non-significant variation (eigen values <1). The sum of the eigen values is typically equal to the number of variables. As a result, in this analysis, the first factor preserves the data from 5.27 of the original variables. The coefficients illustrating the first five principal components of this data are listed in Table 5. Seven principal components (PC1–PC7), according to the results of the principal component analysis, had corresponding eigen values of 5.27, 3.13, 2.14, 1.60, 1.47, 1.45, and 1.20, respectively. Therefore, these five were given the weightage they ought for the purposes of further discussion. PCA has also been done by numerous researchers *viz.*, Baranwal *et al.* (2013); Fikre *et al.* (2015); Mecha *et al.* (2017) to determine the proportion of variation that can be explained by the different analysed characters.

Table 4: The principal components along with their eigen values, percentage of variance and cumulative percentage of various morphological and quality traits in advance lines of bread wheat under late sown conditions.

Principal Components	Eigen Values	Percentage of Variance	Cumulative percentage of variance
1	**5.27**	22.91	22.91
2	**3.13**	13.62	36.54
3	**2.14**	9.29	45.82
4	**1.60**	6.94	52.76
5	**1.47**	6.40	59.17
6	**1.45**	6.30	65.46
7	**1.20**	5.20	**70.66**
8	0.96	4.17	74.83
9	0.90	3.90	78.73
10	0.77	3.37	82.10
11	0.73	3.19	85.29
12	0.63	2.72	88.01
13	0.57	2.46	90.47
14	0.46	2.02	92.49
15	0.45	1.96	94.45
16	0.32	1.40	95.84
17	0.28	1.20	97.04
18	0.26	1.11	98.15
19	0.19	0.81	98.97
20	0.15	0.66	99.63
21	0.08	0.33	99.95
22	0.01	0.05	100.00
23	0.00	0.00	100.00

According to Chahal and Gosal (2002) clustering is under the ascendancy of traits with higher absolute values near to unity rather than ones with smaller absolute values, within the first principal component. As demonstrated *via* factor loadings in Table 5, the diversification of the genotypes into separate clusters in the current study was caused by the comparatively large contribution of a small number of traits rather than the minor contribution of each character. As a result, the main spike weight, grain weight per spike, number of grains per spike, biological yield per plot, and grain yield per plot were identified as the discriminatory traits that rendered up the first principal component (PC1), which accounted for 22.91% of the variability among genotypes. The second principal component (PC2), which was primarily derived from variations in flag leaf length, crude protein, grain appearance score, wet gluten, dry gluten, and total gluten, also accounted for 13.62% of the overall variability across the evaluated genotypes. Likewise, the major contributing traits for 9.29 % of the total variation in the third principal component were days to maturity, grain appearance score, days to heading, plant height, number of spikelets per spike, and peduncle length. Furthermore, PC4 and PC5 which explained 6.94 % and 6.40 % of the total variations were extracted from the number of effective tillers per metre, total soluble sugars, and hectolitre weight. PC6 and PC7, which account for 6.30 and 5.20% of the variance, respectively, were mostly attained from quality (crude protein and sedimentation value) and morphological traits (spike length, number of spikelets per spike, peduncle length and main spike weight), respectively.

Biplot analysis. The biplot based on PC1 and PC2 depicted in Fig. 3 and 4 elaborated the genetic diversity of advance lines in terms of spatial distance, as also summarised by Baranwal *et al*. (2013); Fikre *et al*. (2015); Mecha *et al*. (2017). The vectors of the traits *viz.* grain yield per plot, biological yield per plot, flag leaf length, grain weight per spike and number of spikelets per spike had longer lengths on both axes indicating a considerable contribution to both components. However, the vectors of total gluten, dry gluten and wet gluten had longer lengths on the y axis depicting their major contribution to PC-2 only. The vectors of traits *viz.*, biological yield per plot, number of effective tillers per metre, flag leaf length and 1000 grain weight made an acute angle with the grain yield vector, indicating their positive correlation, whereas obtuse angles made by the vectors of traits *viz.* crude protein, sedimentation value, dry gluten, total gluten, wet gluten with grain yield vector, indicating their negative correlation.

Table 5: Factor loading of various morphological and quality traits in advance lines of bread wheat under late sown conditions.

Traits	PC-1	PC-2	PC-3	PC-4	PC-5	PC-6	PC-7
DH	0.33	-0.19	**0.46**	-0.09	-0.13	**0.44**	-0.09
PH (cm)	0.16	0.17	**0.47**	0.08	**0.14**	-0.29	0.03
NET/m	0.42	0.13	-0.24	**0.35**	**0.38**	-0.29	-0.08
SL (cm)	0.44	0.06	0.22	0.09	0.07	0.08	**0.57**
NS/S	**0.61**	0.11	**0.45**	0.19	-0.07	0.17	**0.25**
FLL (cm)	**0.77**	**0.33**	-0.25	-0.01	0.04	-0.03	0.12
PL (cm)	0.53	0.09	**-0.40**	**0.28**	-0.05	-0.18	**0.26**
MSW (g)	0.62	0.07	0.17	-0.11	-0.05	-0.01	**0.36**
GW/S (g)	**0.81**	0.08	0.01	-0.20	-0.06	-0.28	-0.03
NG/S	0.55	0.05	-0.13	-0.26	-0.65	-0.03	-0.01
TGW (g)	0.45	-0.04	0.17	-0.08	**0.59**	0.07	-0.23
DM	0.01	-0.27	**0.79**	**0.24**	-0.07	0.01	-0.03
BY/P (g)	**0.76**	0.18	-0.27	-0.11	0.07	**0.27**	-0.12
HI (%)	0.32	-0.07	0.31	**0.63**	-0.01	-0.06	**-0.38**
GY/P (g)	**0.87**	0.11	-0.12	0.18	0.07	0.14	-0.24
CP (%)	**-0.31**	**-0.32**	-0.12	**0.22**	0.05	**0.40**	**0.36**
SV (ml)	-0.27	0.21	0.11	0.14	-0.08	**-0.72**	0.12
HW (kg/hl)	0.32	0.13	0.24	**-0.50**	**-0.18**	-0.10	-0.35
GAS	-0.11	**0.42**	**0.45**	-0.40	**0.10**	-0.20	0.18
WG (%)	-0.28	**0.89**	0.08	0.16	-0.10	0.16	-0.08
DG (%)	-0.28	**0.86**	-0.01	0.13	-0.06	**0.21**	0.01
TG (%)	-0.29	**0.91**	0.06	0.15	-0.09	**0.18**	-0.06
TSS (%)	-0.11	0.24	0.03	-0.42	**0.64**	0.11	0.10

DH: Days to heading, **PH**: Plant height (cm), **NET/m**: Number of effective tillers per metre, **SL**: Spike length (cm), **NS/S**: Number of spikelets per spike, **FLL**: Flag leaf length (cm), **PL**: Peduncle length (cm), **MSW**: Main spike weight (g), **GW/S**: Grain weight per spike (g), **NG/S**: Number of grains per spike, **NSS**: Number of spikelets per spike, **TGW**: 1000 grain weight (g), **DM**: Days to maturity, **BY/P**: Biological yield per plot (g), **HI**: Harvest Index (%), **CP**: Crude Protein (%), **SV**: Sedimentation Value (ml), **HW**: Hectolitre Weight (Kg/hl), **GAS**: Grain Appearance Score, **WG**: Wet Gluten (%), **DG**: Dry Gluten (%), **TG**: Total Gluten (%), **TSS**: Total Soluble Sugars (%)

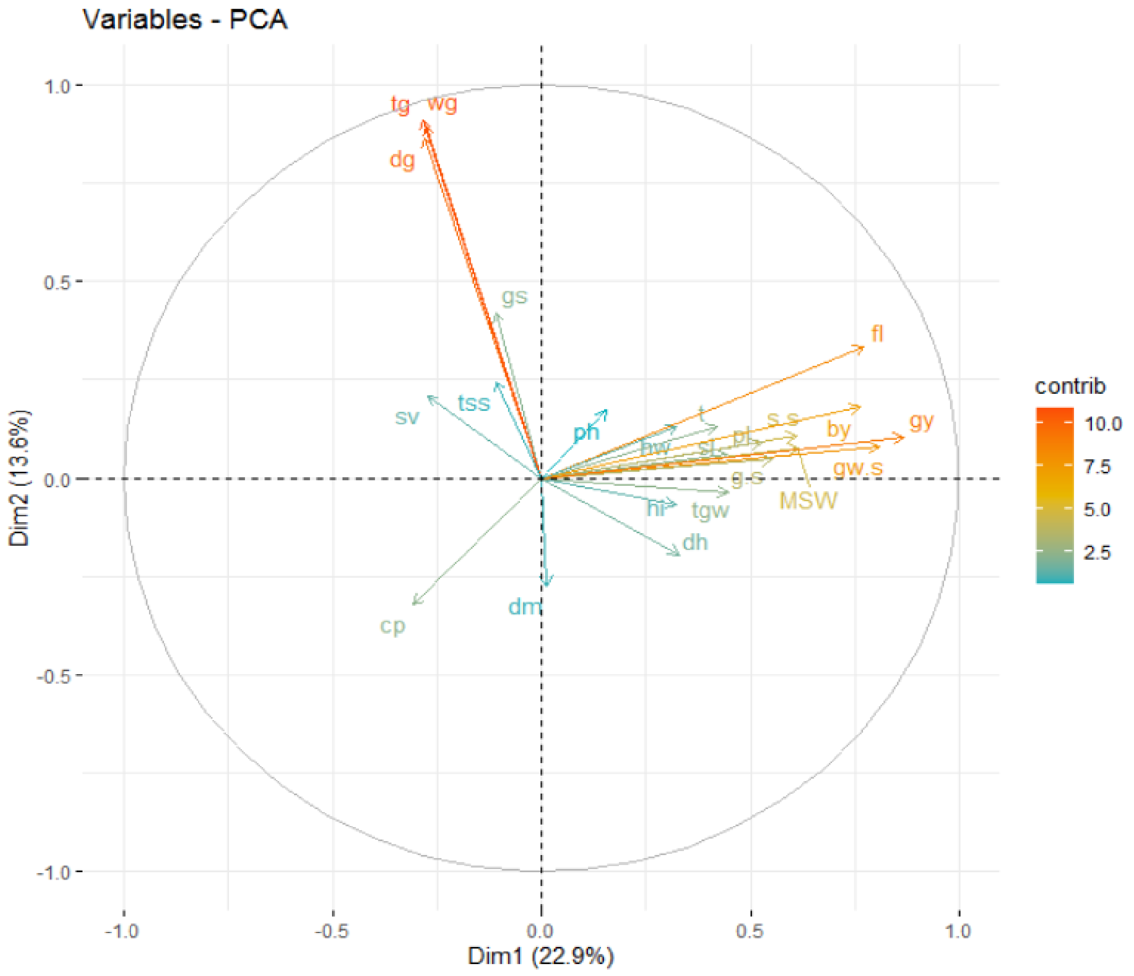

Fig. 3. Biplot of the morphological and quality traits based on PCA analysis under late sown conditions.

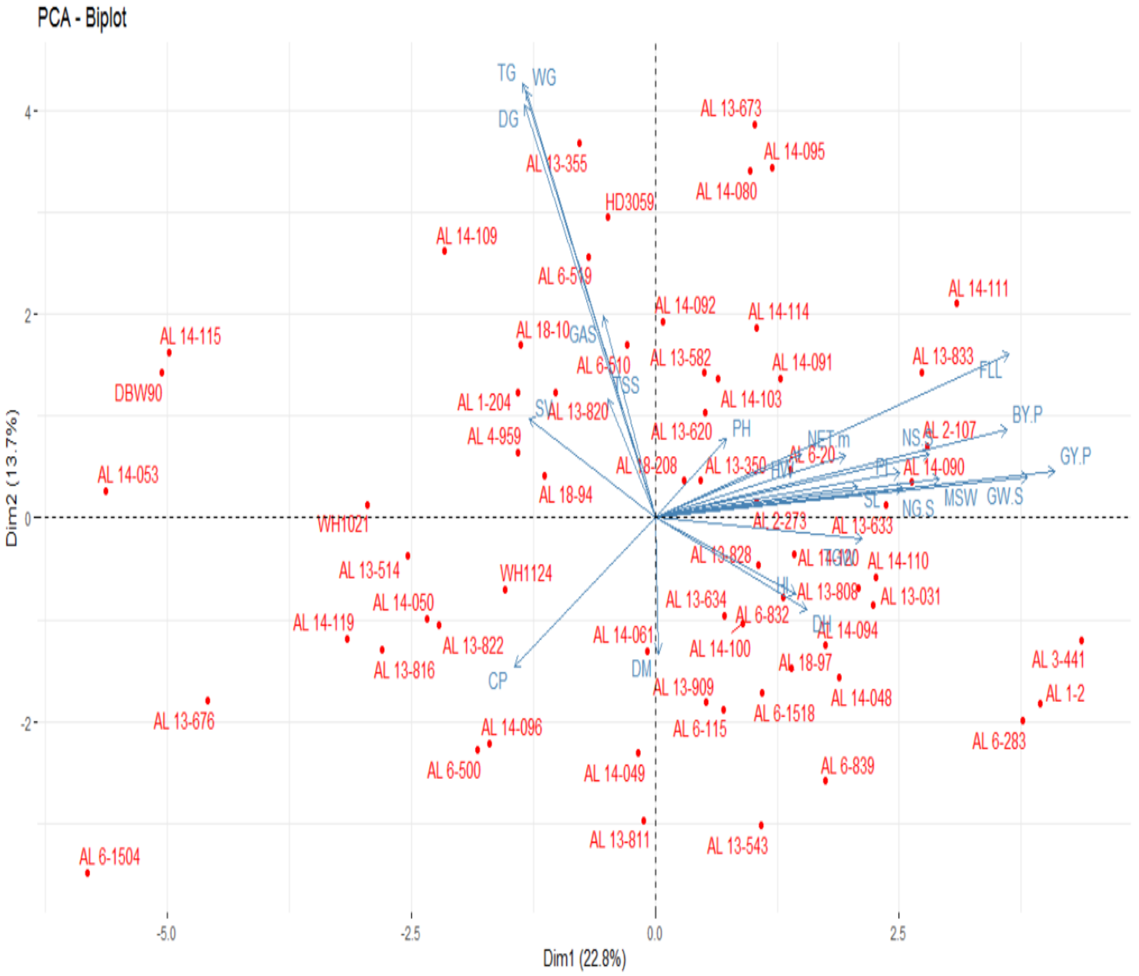

Fig. 4. Biplot of the morphological and quality traits along with advance lines and checks based on PCA analysis under late sown conditions.

CONCLUSIONS

The present study has revealed substantial degrees of variation among the sixty advance lines and four standard checks. The traits *viz.*, biological yield per plot, number of effective tillers per meter, 1000 grain weight, flag leaf length, peduncle length, number of grains per spike, grain weight per spike and hectolitre weight were identified that determine the yield under stress and selection based on which would be efficacious to improve wheat yield. Aforesaid characters have high positive factor loadings and statistically valid differences that contributed more to genetic diversity and were crucial in illustrating the clusters. The grouping of genotypes into seven clusters and reduction of traits to seven major principal components by multivariate approaches would be of practical value to wheat breeders to design experiments for further germplasm collection regarding heat tolerance as well as for hybridization activities to produce heterotic progenies.

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