



## Divergence of bread wheat genotypes (*Triticum aestivum* L.) assessed by Multivariate Biplot analysis based on important markers

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### ABSTRACT

Morphological and molecular markers of fifty seven wheat genotypes had been evaluated for divergence analysis. About 60% to 91% contribution for morphological and 24% to 98% for quality traits had been accounted by genotypes to the total sum of squares. Total of 0.8 to 54 % of genotypic variability and 0.96 to 63 phenotypic variability was recorded for morphological whereas corresponding to quality traits ranged from 0.15 to 25.4 and 0.16 to 25.4 respectively. High heritability and genetic advance as per cent of mean were found in harvest index, biological yield, grain yield/plot, number of grains/ear, wet gluten content, grain hardness, dry gluten content, beta carotene, Mn content. Highly significant positive correlation of grain yield was expressed with ear length, number of grains per ear, number of spikelets per ear, harvest index, biological yield, and number of effective tillers. More over positive and significant correlation of thousand grains weight exhibited with spike length. The first principal component (PC) accounted for 16.4% of the total variation. Major traits for the variations in biplot analysis were wet gluten, dry gluten, Zn content, days to flowering, days to maturity, harvest index, Cu content and protein content. Grain yield, biological yield, plant height, days to flowering, days to maturity, leaf length, number of spikelets per spike, thousand grains weight contributed to second principal component.

**Keywords:** Diversity measures, Biplot analysis, Ward's dissimilarity method, molecular clustering

Wheat, most favored staple food at world level, has been established a cheap source of nutrients and amenable into various liked processed products (Negisho *et al.*, 2021). The prime objective of wheat improvement program has to develop high yielding varieties with desirable quality traits (Lakra *et al.*, 2020). Diversity analysis among the traits and the interrelation among traits recognized as of great relevance in efficient wheat improvement programme (Tilahun *et al.*, 2020). Estimates of variances, coefficients of variation, heritability, genotypic and phenotypic correlations had been provided to account for the proper amount of the variability for the effective selection process (Bartaula *et al.*, 2019; Alemu *et al.*, 2020). Now-a-days, multivariate statistical methods have been recommended over the univariate analytic tools for the analysis of the complex relationship among morphological traits (Ali *et al.*, 2021). Recently hierarchical cluster analysis had been advocated as a simple way to group the genotypes as per similarities among set of traits values (Geleta, 2020).

The estimates based on quantitative, qualitative traits complimented with molecular markers assist for genetic variability among wheat genotypes. Complimentary role of molecular markers to speed up the process of evaluation with less amount of plant material in experiments had been very well appreciated (Devesh *et*

*al.*, 2019). Biotechnological tools based on molecular markers have been relied mostly and played a significant role in recent studies during the period of last 10-12 years (Kandel *et al.*, 2018). Recent studies had observed the augmentation of microsatellite markers for genetic diversity and differentiation of indigenous and exotic introductions (Fu, 2015; Bhandari *et al.*, 2017; Adhikari *et al.*, 2018; Pixley *et al.*, 2018). In this study besides morphological traits, molecular markers were used for diversity analysis among wheat genotypes as per latest analytic tools.

### MATERIALS AND METHODS

Fifty seven wheat genotypes collected from the advanced wheat breeding lines possessing the wide variation for agro-morphological traits had been utilized for the study. The field experiment was conducted at Research Farm, CCS Haryana Agricultural University in Randomized Block Design with three replications during crop season. To harvest the good yield of genotypes the recommended agronomic practices were followed in toto. The genotypes were evaluated for eleven traits namely-days to 50% flowering, days to maturity, plant height (cm), number of effective tillers/m<sup>2</sup>, ear length, number of spikelets/ear, number of grains/ear, grain yield/plot (g), thousand grains weight, biological yield (g), harvest index (%) and twelve quality

parameters *viz.* Fe content (ppm), Zn content (ppm), Cu content (ppm), Mn content (ppm), Beta carotene, protein content (%), dry gluten content (%), hectolitre weight, grain appearance score, grain hardness (kg), sedimentation value (ml), wet gluten content (%). The collected data were subjected to analysis of variance and the mean squares to estimate genotypic and phenotypic variance. Expected genetic advance as part of the mean (GA) for each character at 5% selection intensity ( $K=2.056$ ) was computed. Expected genetic advance as percent of mean (GAM) was calculated to compare the extent of predicted advance of different traits under selection. The establishment of breeding programs and formation of selection indexes had been defined based on heritability of important traits. Estimates of heritability with genetic advance have been appreciated more than heritability alone (Laino *et al.*, 2015). More estimates of genetic advance and heritability suggested the effective conditions of selection (Mengistu *et al.*, 2015). Relationship of yield with contributing components had been explained more appropriately by correlation coefficient analysis.

## RESULTS AND DISCUSSION

### Analysis of variance (ANOVA)

The genotypes diversity for the morphological and quality traits had been depicted by radar charts (Fig. 1). Analysis of variance (ANOVA) observed highly significant differences ( $P<0.01$ ) as presented in Table 1. Tilahun *et al.* (2020) reported the ample scope of selection for further breeding programs. Moreover the percent contribution of genotypes to total sum of squares have been varied from 60% to 91% for morphological characters along with 24% to 98% for quality traits. Genotypic variance ( $\sigma_g^2$ ) and phenotypic variance ( $\sigma_p^2$ ) were estimated for the traits and good amount of variation had been observed for all the characters. High genotypic variability ( $\sigma_g^2$ ) ranged from 0.8 to 54 whereas the phenotypic variability ( $\sigma_p^2$ ) showed the deviation from 0.96 to 63 whereas, for quality traits corresponding variability for quality traits mentioned the values of 0.15 to 25.4 along with 0.16 to 25.4 for  $\sigma_g^2$  and  $\sigma_p^2$  respectively (Table 1). Number of grains, number of effective tillers and plant height expressed more values as compared to ear length, number of spikelets per spike and days to maturity for genotypic variability. The environmental factors had more influence of on these traits as justified by higher values phenotypic variance in comparison to the genotypic variance as mentioned by Tilahun *et al.* (2020) and Alemu *et al.* (2020). More or less similar trends of these traits exhibited for phenotypic variability.

Estimates of phenotypic coefficients of variation (PCV) ranged from 1.1% (days to maturity) to 13.8%

(number of grains/ear) along with 2.85% hectolitre weight to 21.7 for wet gluten content whereas deviation from 1.0% for days to maturity to 12.8% for number of grains per ear seen for genotypic coefficient of variation (Table 1), along with 2.6% of hectolitre weight to 21.7 for wet gluten content. Numerical values more than 15 had been observed for the estimates of PCV and GCV for Cu content, Mn content, dry gluten content, grain hardness and wet gluten content traits. The phenotypic expression of genotypes would be a good indication of the potential of genotypes therefore the selection might be effective for these characters. Other traits *viz* number of effective tillers /m , number of grains/ear, grain yield/plot, biological yield, Zn content, beta carotene and sedimentation value achieved the moderate numerical values (10-15) for PCV and GCV estimates (Table 1). The traits expressed numerical values less than 10 characterized with low phenotypic and genotypic coefficients of variation would be influenced more by the influence of environmental vagaries (Geleta, 2020). The full information about the heritability of the traits accounted by heritability, genetic advance (GA) and genetic advance as per cent of mean. High heritability numerical values more than 60% was seen in plant height, spike length, thousand kernels weight, kernels per spike and in grain yield traits (Lakra *et al.*, 2020). Moderate values of heritability (30-60%) was observed for number of spikelets/ear, Fe content and number of effective tillers/sq. m.

Expected genetic advance was ranged from 2 % for days to maturity to 24.7% for number of grains/ear (Table 1), with 4.9 % of hectolitre weight to 44.7% of wet gluten content indicated that selecting the top 5% of genotypes could result in an advance of 2.3 to 22.5% over the respective population mean. High values of genetic advance are indicative of additive gene action whereas low values were indicative of non-additive gene action (Wouw *et al.*, 2009; Negisho *et al.*, 2021). High heritability and genetic advance as percent of mean were found in harvest index, biological yield, grain yield/plot, number of grains/ear, wet gluten content, grain hardness, dry gluten content, beta carotene, Mn content indicated the heritability was due to additive gene effects and selection may be effective (Tilahun *et al.*, 2020).

### Correlation coefficients

Phenotypic correlation coefficient values among studied traits were presented in Table 2. The highly significant positive phenotypic correlation of grain yield had been observed with ear length, number of grains per ear, number of spikeletes per ear, harvest index, biological yield and number of effective tillers, and negative non-significant correlation was associated with days to flowering, beta carotene, protein, and maturity,

**Table 1: ANOVA, GCV, PCV, heritability on traits of diversity study of wheat**

Traits	Treatments MSS	Significance level	% to total sum of squares	$\sigma^2_g$	$\sigma^2_p$	PCV	GCV	$h^2$	GA	GA as % mean
Days to 50% flowering	19.01	**	85.4	5.90	6.34	2.65	2.56	0.93	4.83	5.08
Days to maturity	6.29	**	82.8	1.90	2.10	1.07	1.02	0.91	2.71	2.00
Plant height (cm)	100.83	**	76.2	30.25	33.61	5.38	5.10	0.90	10.75	9.97
Number of effective tillers/m	461.34	**	70.1	121.28	153.78	11.53	10.24	0.79	20.15	18.73
Ear length	2.87	**	75.2	0.80	0.96	8.01	7.34	0.84	1.69	13.85
Number of spikelets ear <sup>-1</sup>	6.21	**	61.6	1.60	2.07	6.23	5.48	0.77	2.29	9.93
Number of grain ear <sup>-1</sup>	188.24	**	79.1	54.52	62.75	13.80	12.87	0.87	14.18	24.71
Grain yield/plot (gm)	89181.53	**	80.2	26117.88	29727.18	13.44	12.59	0.88	312.05	24.32
Thousand grains weight	18.01	**	71.1	4.88	6.00	6.05	5.45	0.81	4.10	10.12
Biological yield (gm)	373329.62	**	78.3	107488.40	124443.21	11.00	10.23	0.86	627.69	19.58
Harvest index (%)	33.10	**	84.7	10.04	11.03	8.29	7.91	0.91	6.23	15.54
Fe content(ppm)	26.27	**	68.2	6.96	8.76	7.38	6.58	0.79	4.85	12.08
Zn content (ppm)	74.57	**	75.9	21.40	24.86	13.26	12.30	0.86	8.84	23.52
Cu content (ppm)	11.93	**	88.1	3.74	3.98	20.85	20.22	0.94	3.86	40.38
Mn content (ppm)	78.48	**	91.6	24.97	26.16	16.44	16.06	0.95	10.06	32.33
Beta carotene	0.49	**	83.7	0.15	0.16	15.48	14.96	0.93	0.78	29.78
Protein content (%)	3.44	**	84.2	1.06	1.15	8.72	8.39	0.93	2.04	16.63
Dry gluten content (%)	9.45	**	79.2	2.79	3.15	18.17	17.11	0.89	3.24	33.19
Hectolitre weight	15.09	**	24.3	4.25	5.03	2.85	2.62	0.84	3.90	4.96
Grain appearance score	0.54	**	31.9	0.17	0.18	6.98	6.75	0.93	0.81	13.43
Grain hardness (kg)	5.51	**	90.8	1.76	1.84	18.38	18.00	0.96	2.67	36.30
Sedimentation value (ml)	67.87	**	90.6	21.90	22.62	10.71	10.53	0.97	9.49	21.35
Wet gluten content (%)	76.28	**	98.6	25.42	25.43	21.71	21.71	1.00	10.38	44.71

Table 2: Association analysis among traits for wheat genotypes

	Days to maturity	Plant height	No of effective tillers/m	Ear length	No of spikes/ear	No of grain/ear	Grain yield/plot	1000 grain weight	Biological yield	Harvest index	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)	Beta carotene	Protein content	Dry gluten content	Hectolitre wt.	Grain appearance score	Grain hardness	Sedimentation value	Wet gluten content
Days to 50% flowering	0.7532	-0.3476	0.0160	-0.0788	0.0815	-0.0427	-0.1596	-0.1143	-0.2902	0.1266	-0.2073	-0.4749	-0.2265	-0.1334	-0.1041	-0.1860	-0.4361	0.1239	0.1981	0.0328	0.1130	-0.4395
Days to maturity		-0.1727	0.0332	-0.2641	0.0648	-0.0993	-0.2542	-0.1362	-0.0950	-0.2498	-0.2000	-0.1831	-0.1730	0.1302	0.0581	-0.0572	-0.3340	0.1565	0.1538	0.0629	-0.0569	-0.3815
Plant height			0.0453	0.0918	0.1937	0.1276	0.1838	0.1331	0.3816	-0.1959	0.0039	0.4828	0.0807	0.3120	-0.0910	0.2278	-0.0180	0.2199	0.3150	0.1531	-0.0859	-0.0204
No of effective tillers/m				0.1277	-0.0794	-0.0501	0.3038	0.2653	0.2654	0.1127	-0.1428	-0.0410	-0.3347	-0.0598	-0.0847	-0.0108	-0.1912	-0.0743	0.1269	-0.0774	-0.0914	-0.2758
Ear length					0.2658	0.2112	0.3205	0.2917	0.0968	0.3471	-0.2233	0.1002	-0.0402	0.0331	-0.0576	0.2761	0.0376	-0.1425	0.1531	0.1371	0.1332	0.0249
No of spikes/ear						0.3469	0.0686	0.1321	0.2826	0.1047	0.0545	-0.2020	0.1799	0.0745	0.2673	-0.0649	0.1808	0.1289	0.3122	-0.0074	-0.0863	
No of grain/ear							0.3249	0.0741	0.1972	0.2694	-0.0975	-0.1271	-0.0028	0.1566	-0.1270	0.0487	-0.0859	0.2492	0.1250	-0.0014	0.3729	-0.0336
Grain yield/plot								0.3518	0.7544	0.5696	-0.0267	-0.1026	-0.0386	0.0529	-0.2943	-0.0903	-0.0693	-0.0417	0.2404	0.2014	0.1555	-0.1866
1000 grain weight									0.1715	0.3161	-0.1142	0.0039	-0.1167	0.0579	0.0464	-0.0653	-0.1573	0.2592	0.1877	0.1286	0.1091	-0.2044
Biological yield										-0.1076	0.0603	0.1004	0.1997	0.1125	-0.2443	-0.0978	0.0113	-0.0436	0.2530	0.1025	0.1416	-0.1002
Harvest index											-0.1373	0.3331	0.0328	0.2245	-0.1058	0.2939	0.4055	-0.2534	0.1610	0.1291	-0.0855	-0.0531
Fe (ppm)												0.3331	0.1525	-0.0824	0.1197	0.2078	0.0840	0.0417	-0.0933	-0.0179	-0.1871	0.1854
Zn (ppm)													0.0328	0.2519	0.1851	0.4191	0.3652	-0.0284	-0.1496	-0.0425	-0.3077	0.3981
Cu (ppm)														0.2245	-0.2523	0.1184	0.3301	-0.1891	-0.0756	0.1139	0.2120	0.3300
Mn (ppm)															-0.1058	0.2483	0.0165	0.1218	0.1867	0.2825	-0.0489	0.0649
Beta carotene																0.2939	0.4055	-0.2534	0.1610	0.1291	-0.0855	-0.0531
Protein content																	0.4055	-0.0906	0.0607	0.0597	-0.0555	0.4309
Dry gluten content																		-0.2534	-0.0430	-0.0856	0.8858	
Hectolitre wt.																			0.1610	0.1412	0.1032	-0.2511
Grain appearance score																				0.1291	0.2169	-0.1771
Grain hardness																					0.1610	-0.0118
Sedimentation value																						-0.0855
																						-0.0531

**Table 3: Loading of traits as per first two principal components**

Traits	PCA1	PCA2
Days to 50% flowering	0.3239	-0.2608
Days to maturity	0.2161	-0.2532
Plant height	-0.0746	0.3007
Number of effective tillers/m	0.1652	0.1050
Ear length	0.0468	0.2884
Number of spikelets/ear	0.0919	0.2343
Number of grain/ear	0.1219	0.2452
Grain yield/plot	0.1829	0.4180
Thousand grains weight	0.1571	0.2379
Biological yield	0.0513	0.3489
Harvest Index	0.2174	0.1953
Fe (ppm)	-0.1975	0.0112
Zn (ppm)	-0.3294	0.1461
Cu (ppm)	-0.2193	0.0667
Mn (ppm)	-0.0477	0.1847
Beta carotene	-0.1214	-0.1115
Protein content	-0.2436	0.1528
Dry gluten content	-0.3988	0.0787
Hectolitre weight	0.1480	0.0332
Grain appearance score	0.1770	0.1785
Grain hardness	0.0628	0.1732
Sedimentation value	0.1172	0.0938
Wet gluten content	-0.4243	0.0575
% variation	16.38	14.52

hectolitre weight, dry and wet gluten content (Adhikari *et al.*, 2018 ; Mecha *et al.*, 2017. Spike length had positive and significant correlation with thousand grains weight while non-significant correlation values of thousand grains weight exhibited with days to maturity and kernels per spike. Mn exhibited direct correlation with other traits and very small negative values with sedimentation value and beta carotene. Moreover the Cu content expressed positive values of correlation with dry and wet gluten, Mn content whereas negative correlation with sedimentation value (Table 2). Zn content achieved positive correlation with harvest index and plant height only while others were associated in indirect manner. Direct effect was with quality traits protein, dry gluten, Mn and indirect effect was with grain appearance and sedimentation value.

Mostly inverse relationships of Fe content had been seen with morphological traits whereas direct behaviour expressed for Zn content, protein content, Cu content, Beta carotene and negative values for sedimentation value. Harvest index had direct relation with grain yield, thousand grains weight, ear length, number of spikelets, number of grains per ear, Zn content, dry gluten, hectolitre weight and Mn content *etc.* Biological yield expressed direct association with grain yield, plant height, number of effective tillers, grain appearance and inverse with Beta carotene. Thousand grains weight

showed positive correlation with grain yield, ear length, number of effective tillers, harvest index, hectolitre weight and indirect with wet gluten content. Number of grains exhibited positive association with number of spikelets, grain yield, sedimentation value and hectolitre weight.

### Biplot analysis

First two components explained 30.9% of the total phenotypic variation among the 57 genotypes (Table 3). The first principal component (PC) accounted for 16.4% of the total variation. It illustrated the variations in wet gluten, dry gluten, Zn content, days to flowering, days to maturity, harvest index, Cu content, protein content *etc.* Principal component two contributed 14.5% to the total variation. Six variables, including grain yield, biological yield, plant height, days to flowering, maturity length, number of spikelets/spike, number of grains per ear, thousand grains weight were to contribute more to second PC. Out of the 21 traits evaluated, 12 contributed most to the first two principal components (Table 3) and these are considered most desirable to summarize phenotypic variation among the accessions through hierarchical cluster analysis. The biplot analysis is an appropriate method to analyse interaction between genotypes and traits and narrowing down the number of traits to the ones contributing a major portion to the variability as discussed by Devesh *et al.* (2019). The first two components explained 30.9% of the total variation in genotypes (Fig. 2). The high positive correlation has been observed between maturity with flowering, height with Mn content, grain yield with biological yield, length, number of spikelets/spike, thousand grains weight whereas harvest index with sedimentation value, number of tillers, hectolitre weight and grain appearance. Protein had maintained positive with Zn, Cu, dry gluten and wet gluten *etc.* The difference between the biplot origin and genotype position in the biplot is the vector length of the genotype and it is a measure of the distinctiveness of the genotype from other genotypes. In the biplot vectors of traits showing acute angles are positively correlated whereas those showing obtuse or straight angles are negatively correlated and those with right angles have no correlation. The genotypes having long length of the vector have higher or extreme values for one or more characters. Selection among such genotypes may be performed either for further trials or for their use as parents in breeding programs.

### Molecular markers profile for diversity

Wheat entries were also characterized at molecular level to observe the variability in evaluated genotypes. A set of fifty seven genotypes were screened using



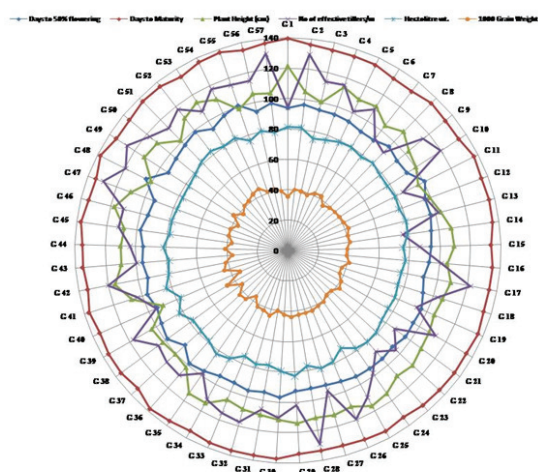


Fig. 1a: Radar chart to show diversity among genotypes for important morphological traits

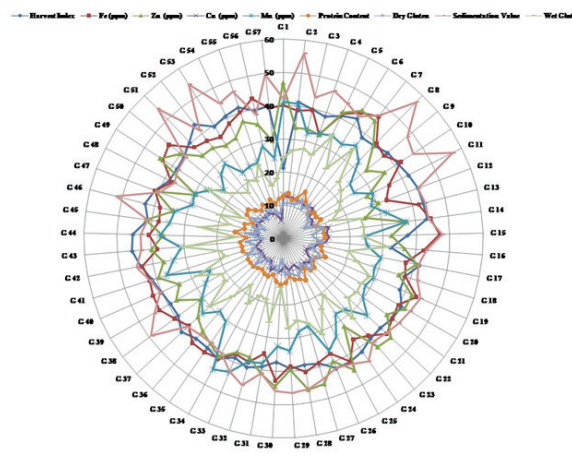


Fig. 1b: Radar chart to depict the diversity among genotypes for important quality traits

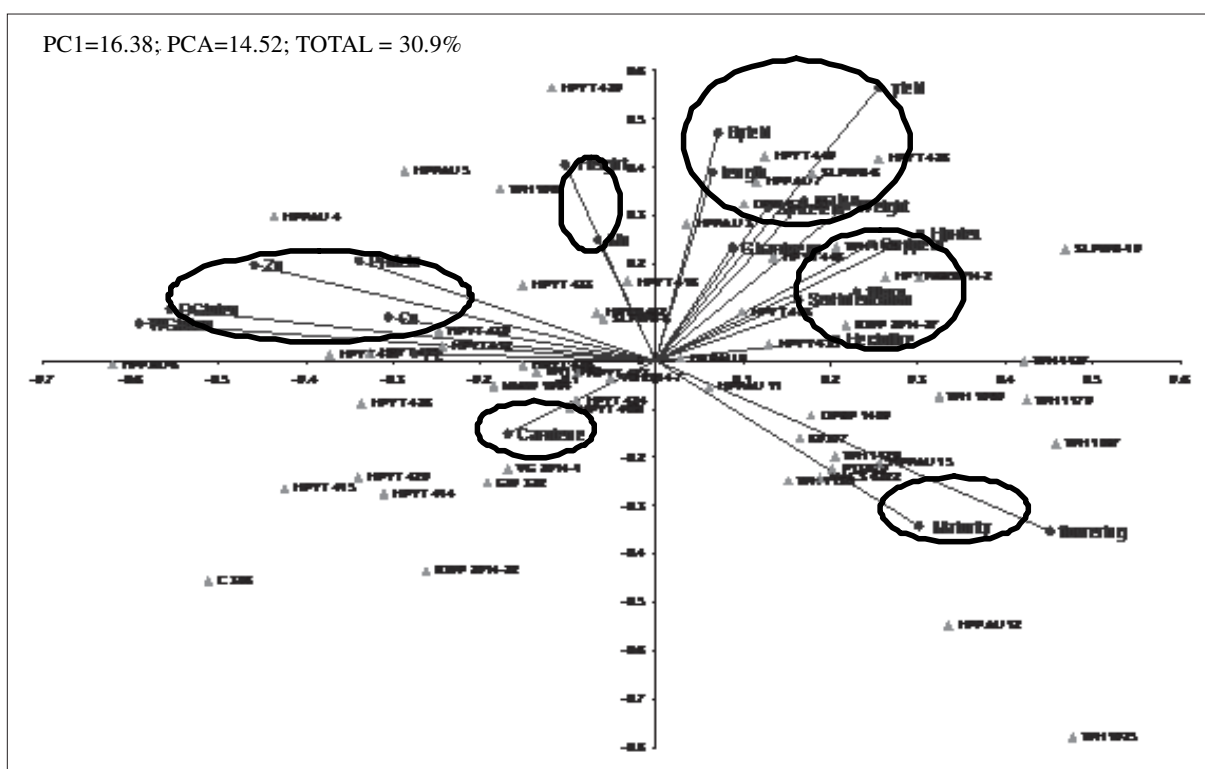


Fig. 2: Biplot analysis of genotypes vis-à-vis traits

specific molecular markers totaled to 46. Allele molecular weight data of amplified profiles were converted to develop binary format (“1” for presence and absence by “0”) for genetic diversity analysis with NTSYS-PC version 2.1. The dissimilarity matrix was input to DARWIN software version 5.0 to construct clustering dendrogram of genotypes to infer relationships.

Two broad groups of genotypes had been depicted in Fig. 3 which further partitioned into five and seven sub groups as classification of genotypes evident from the respective nodes of clusters (Ali *et al.*, 2021).

Sufficient variability existed in the material for most of the traits in the present study. The identification of suitable genotypes for different traits will help in the development of better yielding genotypes in changing

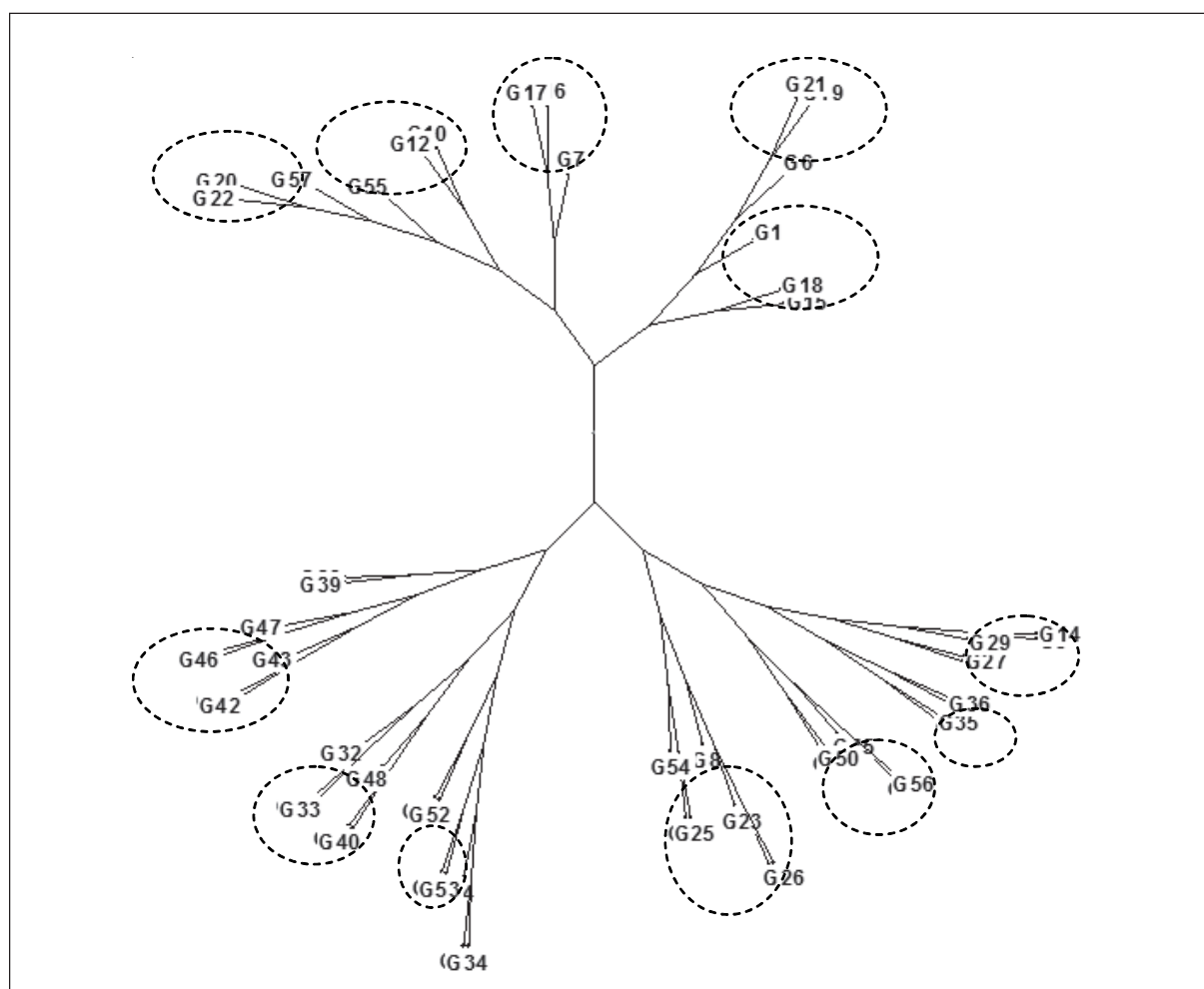


Fig. 3: Diversity of wheat genotypes based on molecular markers by Darwin software

Details of studied wheat genotypes

G 1	G 2	G 3	G 4	G 5	G 6	G 7	G 8	G 9	G 10	G 11	G 12
C 306	DPW 621-50	GW 322	HPPAU 3	HPPAU 4	HPPAU 5	HPPAU 6	HPPAU 7	HPPAU 9	HPPAU 11	HPPAU 12	HPPAU 15
G 13	G 14	G 15	G 16	G 17	G 18	G 19	G 20	G 21	G 22	G 23	G 24
HPYT 403	HPYT 412	HPYT 414	HPYT 415	HPYT 416	HPYT 419	HPYT 420	HPYT 423	HPYT 424	HPYT 425	HPYT 426	HPYT 428
G 25	G 26	G 27	G 28	G 29	G 30	G 31	G 32	G 33	G 34	G 35	G 36
HPYT 429	HPYT 430	HPYT 431	HPYT 432	HPYT 433	HPYT 435	HPYT 436	HPYT 445	HPYT 446	HPYT 449	K0307	MACS 6222
G 37	G 38	G 39	G 40	G 41	G 42	G 43	G 44	G 45	G 46	G 47	G 48
NIAW 1994	P13020	QPBP 1409	RWP 2014-22	RWP 2014-27	SLPWB- 6	SLPWB- 8	SLPWB- 10	VG 2014-1	VG 2014-2	VG 2014-7	WH 1025
G 49	G 50	G 51	G 52	G 53	G 54	G 55	G 56	G 57			
WH 1061	WH 1063	WH 1080	WH 1097	WH 1105	WH 1127	WH 1129	WH 1136	WH 1179			

scenario of biotic and abiotic stress. Multivariate analysis clearly helped in differentiating genotypes into major groups by considering various traits simultaneously especially wet gluten content, grain hardness, dry gluten content, Cu content, Mn content. Identification of contrasting parents based on distance between different clusters of genotypes (G17, G7, G6 to G34, G4, G53) would be used to generate wider variability in the wheat breeding.

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