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Genetic Diversity in Bread Wheat (*Triticum aestivum* L.) Genotypes

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Abstract: Wheat is one most important cereal crops grown in Ethiopia. Yet, keeping in view insufficient information on exotic bread wheat genotypes is limiting the access to useful traits present among the genotypes in the Somali region of Ethiopia. The aim of the study was to assess the extent of genetic diversity among bread wheat genotypes. Twenty six bread wheat (*Triticum aestivum* L.) genotypes obtained from ICARDA-CIMMYT were tested at Gode and Kelafo research sites at three cropping seasons (2009/10, 2010/11 and 2011/12) under irrigation. The experiment was conducted in randomized complete block design with three replications. Ten agronomic traits were included in the study. The mean values, ranges and the coefficient of variation of the 10 characters indicated the existence of sufficient variability among genotypes. Multivariate techniques were used to classify 26 bread wheat genotypes. Principal component analysis showed that the first six principal components explained about 91.87% of the total variation. D^2 analysis showed the 26 bread wheat genotypes grouped into six clusters. This made to become moderate diversity among the genotypes. The crosses between genotypes selected from cluster-III with cluster-VI and cluster V with cluster VI are expected to produce better genetic recombination and segregation in their progenies. Therefore, these bread wheat genotypes need to be crossed and selected to develop high yielding pure line variety.

Key words: Bread wheat, D^2 analysis, principal component, cluster analysis

INTRODUCTION

Wheat is grown on 1.5 million ha with a total production of 3.78 million tons and ranks fourth after Teff (*Eragrostistef*), Maize (*Zea mays*) and Sorghum (*Sorghum bicolor*) both in area and production among cereal crops in Ethiopia (CSA, 2011). In Ethiopia a number of improved bread wheat varieties were released; from 1974 to 1997 thirty varieties and from 1998 seventeen varieties were released and some of them are in production in different agro-ecological zone of the country (Gebre-Mariam, 1991). So far little or no information is generated in genetic diversity of exotic bread wheat genotypes in Somali region of Ethiopia. Therefore, the objective this experiment was to estimate genetic diversity using quantitative traits.

MATERIALS AND METHODS

The experiments were conducted in research sites of Gode (latitude: 5°57".025'N, longitude: 43°33".033'E and altitude: 300 masl) and Kelafo (latitude. 5°35'08.9''N, longitude. 4°11'36.2''E and altitude: 246 m.a.s.l.), located in the Eastern part of Ethiopia during three cropping seasons (2009/10, 2010/11 and 2011/12) using irrigation. The mean annual rainfall of the areas is 300-340 to 150-220 mm. The mean maximum and minimum annual temperature of area is 30-35°C and 22.2-32°C, respectively, (Ayale, 2005). The soil is clay and clay loam in textural type with alkaline PH (Badel, 2012). The experiments were conducted in Randomized Complete Block Design (RCBD) with three replications at two locations, Gode and Kelafo research stations. Plant materials consisted of twenty six bread wheat genotypes from ICARDA-CIMMYT were used in the study (Table 1). Total plot size of 2×3 m consisted of 14 rows per plot and net plot size of 2×2.8 m with 12 harvestable rows were used. Distance of 20 cm and 10 cm were used between rows and plants, respectively. Seed rate of 150 kg ha^{-1} was used and sown by hand drilling at 20 cm row spacing. DAP (18-46-0) was applied basally during planting at the rate of 100 kg ha^{-1} P_2O_5. Total Nitrogen was applied at rate of 100 kg ha^{-1} N as UREA (46.5%) in two splits: first split (2/3) and the second split, (1/3) of the total dose at mid-tillering and flowering stages, respectively (Abdi *et al*., 2009). All experimental plots at both locations were subjected to

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Table 1: Bread wheat genotypes used in the study

Entry	Pedigree	Selection history	Origin
511	PBW343*2/KUKUNA/ /PBW343*2/ KUKUNA	CGSS04Y00099S-099Y-099M-099Y-099M-20WGY-0B	BV2008\C4THEBWYT\12
512	CNDO/R143//ENTE/MEXI_2/3/	CMSS04Y00421S-099Y-099ZTM-099Y-099M-4WGY-0B	BV2008\C4THEBWYT\17
529	WHEAR/SOKOLL	CMSS04Y00201S-099Y-099ZTM-099Y-099M-11WGY-0B	BV2008\C4THEBWYT\52
525	PFAU/SERI.1B//AMAD*2/3/PBW343*2/KUKUNA	CGSS04B00021T-099Y-099ZTM-099Y-099M-22WGY-0B	BV2008\C4THEBWYT\45
522	SW89.5277/BORL95//SKAUZ/3/PRL/*PASTOR/4/	CMSS04M01483S-0TOPY-099ZTM-099Y-099M-1WGY-0B	BV2008\C4THEBWYT\41
519	PRL/2*PASTOR//PBW343*2/KUKUNA	CMSS04Y00086S-0Y-099ZTM-099Y-099M-4WGY-0B	BV2008\C4THEBWYT\29
523	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/PBW343*2/	CGSS04B00018T-099Y-099ZTM-099Y-099M-10WGY-0B	BV2008\C4THEBWYT\43
527	WAXWING*2//PBW343*2/KUKUNA	CGSS04BB00027T-099Y-099ZTM-099Y-099M-3WGY-0B	BV2008\C4THEBWYT\48
530	WHEAR//2*PRL/2*PASTOR	CGSS03B00090T-099Y-099M-099Y-099M-6WGY-0B-1B	BV2008\C4THEBWYT\56
504	SERI/RAYON*2//PFAU/WEAVER	CGSS04Y00001T-099M-099Y-099ZTM-099Y-099M-2WGY-0B	BV2008\C4THEBWYT\1
516	WBLL1*2/KIRITATI	CGSS01B00063T-099Y-099M-099M-099Y-099M-3WGY-0B	BV2008\C4THEBWYT\25
521	KAUZ//ALTAR84/AOS/3/PASTOR/4/ MILAN/CUPE//	CMSS04M01386S-0TOPY-099ZTM-099Y-099M-2WGY-0B	BV2008\C4THEBWYT\39
506	SERI.1B*2/3/KAUZ*2/BOW//KAUZ*2/5/CNO79//	CGSS04Y00058T-099M099Y-099M-099Y-099M-11WGY-0B	BV2008\C4THEBWYT\7
508	WHEAR//INQALAB91*2/TUKRU	CGSS04Y00076S-099Y-099M-099Y-099M-5WGY-B	BV2008\C4THEBWYT\9
505	SAAR/2*WAXWING	CGSS04Y00040T-099M-099Y-099M-099Y-099M-4WGY-0B	BV2008\C4THEBWYT\5
514	KIRITATI//SERI/RAYON	CGSS02Y00152S-099M-099Y-099M-11WGY-0B	BV2008\C4THEBWYT\23
526	PRL/2*PASTOR//PBW343*2/KUKUNA/3/	CGSS04B00025T-099Y-099ZTM-099Y-099M-3WGY-0B	BV2008\C4THEBWYT\46
510	PBW343*2/KUKUNA// PBW343*2/KUKUNA	CGSS04Y00099S-099Y-099M-099Y-099M-18WGY-0B	BV2008\C4THEBWYT\11
528	HUW234+LR34/PRINIA//PBW343*2/KUKUNA/3/	CGSS04B00033T-099Y-099ZTM-099Y-099M-11WGY-0B	BV2008\C4THEBWYT\49
507	PBW343*2/KUKUNA/3/PASTOR//CHIL/PRL/4/	CGSS04Y00060T-099M-099T-099M-099Y-099M-11WGY-0B	BV2008\C4THEBWYT\8
517	WBLL1*2/KIRITATI	CGSS01B00063T-099T-099M-099M-099Y-099M-18WGY-0B	BV2008\C4THEBWYT\26
520	PBW343*2/HUITES/4/YAR/AE. SQUARROSA(783)//	CMSS04M00348S-0Y-099ZTM-099Y-099M-10WGY-0B	BV2008\C4THEBWYT\37
524	PFAU/SERI.1B//AMAD*2/3/ PBW343*2/KUKUNA	CGSS04B00021T-099Y-099ZTM-099Y-099M-15WGY-0B	BV2008\C4THEBWYT\44
518	WBLL1*2/KIRITATI	CGSS01B00063T-099Y-099M-099M-099Y-099M-31WGY-0B	BV2008\C4THEBWYT\27
513	MINO/898.97	CMSS04Y00921S-099Y-099ZTM-099Y-099M-2GWY-0B	BV2008\C4THEBWYT\22
509	PBW343*2/KUKUNA// PBW343*2/KUKUNA	CGSS04Y00099S-099Y-099M-099Y-099M-10WGY-0B	BV2008\C4THEBWYT\10

uniform recommended package of agronomic and plant protection practices to obtain a healthy plants. All samplings were performed from the middle rows of competitive plants. Data on phenological basis was recorded on days to heading, days to maturity and grain filling period. Morphological data on plant height (cm), spike length (cm), number of tillers/plant, number of spikelets/spike, number of grains/spike and 1000-grain weight were recorded on five randomly selected plants in each variety per replication. Grain yield per plot recorded and were converted into quintal/ha.

Principal component analysis was performed by using correlation matrix by employing procedure printcomp corr of SAS version 9.2 (SAS, 2008) in order to examine the relationships among the 10 characters that are correlated among each other's by converting into uncorrelated characters called principal components. Cluster analysis was performed by canonical roots method using procedures of SAS version 9.2 (SAS, 2008) for partitioning a set of objects into groups so that objects within a group are more similar and objects in different groups are more dissimilar (Crossa *et al*.,1995). The genetic distances between clusters were estimated by Mahalanobis's statistics (Mahalanobis, 1936) for the 10 characters and were analyzed using the procedure proc discrim of SAS version 9.2 (SAS, 2008).

D^2 statistics is defined by the following formula:

$$D^2ij = \left(\overline{X}_i - \overline{X}_j\right)^1 COV^{-1}\left(\overline{X}_i - \overline{X}_j\right)$$

where, D^2ij is Total generalized distance between class i and j

$$\left(\overline{X}_i - \overline{X}_j\right)$$

is the difference between the mean vectors of i^{th} and j^{th} and COV^{-1} is the pooled variance-covariance matrix within groups. The significance of D^2ij values for pairs of clusters was tested using the calculated values of chi-square(x^2) at 1% and 5% probability level. The test was done against the tabulated values of x^2 for 'P' degrees of freedom, where P is the number of characters considered (Singh and Chaudhary, 1999).

RESULTS AND DISCUSSION

Variability among bread wheat genotypes: Range, Mean, Standard Error (SE), Coefficient of Variation (CV) and mean square of error of 10 characters of bread wheat genotypes, namely, days to heading, days to maturity and grain filling period, plant height, spike length, number of

Table 2: Range, mean, standard error (SE) with coefficient of variation (CV) for 10 traits across the locations

Source	Minimum	Maximum	Mean±Standard Error	C.V. (%)	Error
Days to heading	32.00	71.00	47.40±7.38	6.42	9.26
Grain filling period	11.00	66.00	29.15±8.35	15.61	20.70
Days to maturity	59.00	105.00	79.17±7.11	4.91	15.13
Plant height	35.00	68.00	53.30±6.30	6.62	12.45
Number of tillers plant^{-1}	1.00	8.00	4.62±1.10	17.35	0.64
Spike length	1.00	13.00	8.15±1.63	12.31	1.01
Number of spikelets spike^{-1}	2.20	17.00	8.92±3.47	14.91	1.77
Number of grains spike^{-1}	131.67	259.00	61.36±69.76	16.64	104.32
1000 grain weight	10.60	54.00	30.05±10.04	10.63	10.20
Grain yield plot^{-1}	12.07	48.21	24.50±6.95	16.29	15.93

Table 3: Eigen values, total variance, percent cumulative variance and eigenvectors for 10 characters studied on 26 bread wheat genotypes

	Eigenvectors					
Characters	Prin 1	Prin 2	Prin 3	Prin 4	Prin 5	Prin 6
DH	-0.3902	0.3908	0.1015	0.4455	0.0696	-0.0447
GFS	0.0995	-0.5240	-0.0340	-0.1298	0.5591	0.0052
MD	-0.3851	0.0045	0.1022	0.4237	0.5692	-0.0469
PHT	0.1071	0.2834	0.4911	-0.4027	0.2846	0.3255
NT	0.4445	0.2317	0.1731	0.3622	-0.0994	0.3132
SL	0.0893	0.2294	-0.5982	0.0336	0.2384	0.6457
NSPK	-0.1427	0.4713	0.1617	-0.4424	0.1972	-0.1479
NSS	0.4563	-0.0826	0.1912	0.1732	0.3389	-0.1056
TGW	-0.1438	-0.3182	0.5337	0.1711	-0.2339	0.4509
YLD	0.4724	0.2365	0.0399	0.2360	0.0488	-0.3722
Eigen value	2.76	2.10	1.45	1.17	1.09	0.62
Total variance explained (%)	27.59	20.95	14.48	11.72	10.95	6.18
Cumulative variance	27.59	48.54	63.02	74.74	85.69	91.87

DH: Days to main heading, GFS: Grain filling period, MD: Days to maturity, PHT: Plant height, NT: No. of tillers plant, SL: Spike length, NSPS: No. of spikelets per spike, NSS: No. of grains per spike, TGW: 1000-grain weight and YLD: Yield per plot, respectively

tillers/plant, number of spikelets/spike, number of grains/spike, 1000-grain weight and grain yield/ha are presented in the Table 2. The mean values and the coefficient of variation of the above characters indicated that existence of sufficient variability among the bread wheat genotypes.

Diversity of bread wheat genotypes

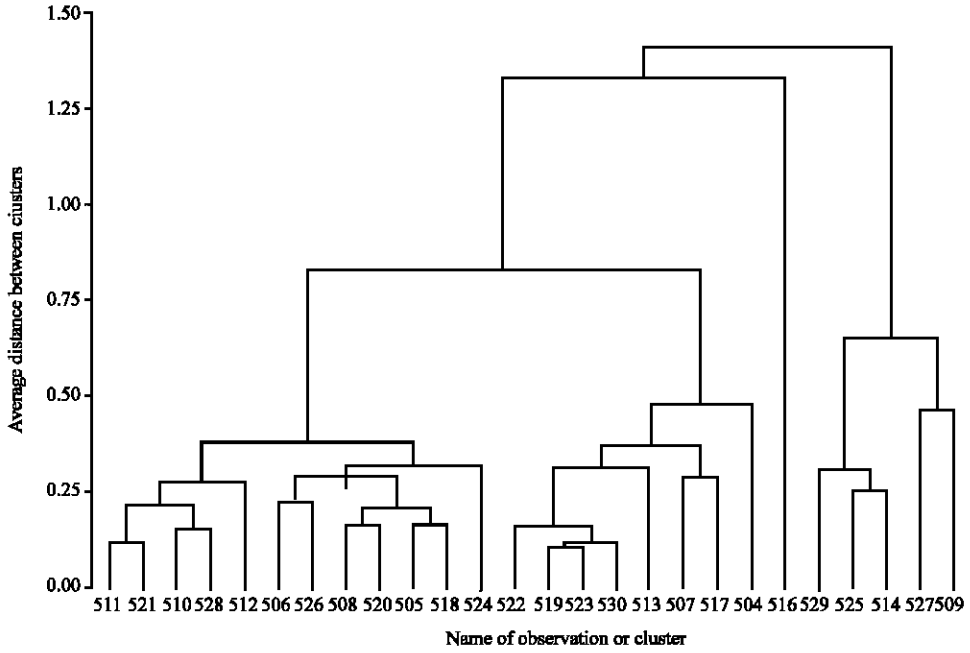
Principal component analysis (PCA): Principal component analysis reflects the importance of the largest contributor to the total variation at each axis for differentiation (Sharma, 1998). In PCA using 26 bread wheat genotypes for 10 traits (Table 3) revealed that six principal components PC1, PC2, PC3, PC4, PC5 and PC6 with eigenvalues 2.76, 2.10, 1.45, 1.17, 1.09 and 0.62, respectively, have accounted for 91.87% of the total variation. The first two principal components PC1 and PC2 with values of 27.59 and 20.95%, respectively, contributed more to the total variation. According to Chahal and Gosal (2002), characters with largest absolute values closer to unity within the first principal component influence the clustering more than those with lower absolute values closer to zero. Therefore, in this study, differentiation of the accessions into different cluster was because of a cumulative effect of a number of characters rather than the contribution of specific few characters (±0.0045-0.6457). Characters having relatively higher value in the first Principal Component (PC1) like grain yield plot^{-1}, number of tillers plant^{-1} and grains spike^{-1} had more contribution to the total variation and they were the ones that most differentiated the clusters. The days to main heading and number of spikelets spike^{-1} in the second Principal Component (PC2); plant height, spike length, 1000 grain weight in the third Principal Component (PC3); days to main heading, days to maturity, plant height, number of spikelets spike^{-1} in the fourth Principal Component (PC4); grain filling period and days to maturity in the fifth Principal Component (PC5); spike length, 1000 grain weight and grain yield in the sixth Principal Component (PC6) were the major contributors to each Principal Components (PC). The present study confirmed that bread wheat genotypes showed wide amount of variations for the character studied and it also suggested that ample opportunities for genetic improvement of bread wheat genotypes through selection directly from bread wheat genotypes and conservation of the germplasm for future utilization. Similar works have done by Maqbool *et al*. (2010) and Sajjad *et al*. (2011) for grouping of germplasm by principal component analysis.

Estimation of squared distance (D^2) and clustering of bread wheat genotypes: The D^2 values based on the pooled mean of bread wheat genotypes resulted in classifying the 26 genotypes in to six distinct clusters (Table 4, 5 and Appendix 1). This indicated that the presence of moderate diversity of genotypes. Distribution of the genotypes showed that 13 bread wheat genotypes in Cluster-I (50%) followed by cluster-II which contained 7 advance lines (26.92%), cluster-III contained 3 genotypes (11.54%), cluster-IV, cluster-V and cluster-VI contained 1 genotypes in each (in each cluster 3.85%), respectively, these cluster had outstanding performance than other genotypes tested in this study. Similar study was done by Noorka and Khaliq (2007) for grouping of 100 wheat genotypes.

Cluster mean analysis: The mean value of the 10 traits in each cluster is presented in Table 5. Cluster I consisted of 13 genotypes having the characteristic of early maturing (78.90), less number of days for grain fill period (29.07), relatively high plant height (53.72) and the highest

Table 4: Grouping of 26 bread wheat lines into different diversity classes

Cluster	Total no. of bread wheat genotypes	Bread wheat advanced line using line entry code	Pedigree	Proportion
Cluster-I	13	511,510,508,505,520,518, 526,528,512,506,524,521,504	PBW343*2/KUKUNA/ /PBW343*2/ KUKUNA, PBW343*2/KUKUNA// PBW343*2/KUKUNA, WHEAR//INQALAB91*2/TUKRU, SAAR/2* WAXWING, PBW343*2/HUITES/4/YAR/AE.SQUARROSA(783)//, WBLL1*2/KIRITATI, RL/2*PASTOR//PBW343 *2/KUKUNA/3/, HUW234+LR34/PRINIA//PBW343*2/KUKUNA/3/, CNDO/R143// ENTE/MEXI_2/3/, SERI.1B*2/3/KAUZ*2/BOW//KAUZ* 2/5/CNO79//, PFAU/SERI.1B//AMAD*2/3/ PBW343* 2/KUKUNA, KAUZ//ALTAR84/AOS/3/PASTOR/4/ MILAN/CUPE//, SERI/RAYON*2//PFAU/WEAVER	50.00
Cluster-II	7	519, 523, 522, 530, 517, 513, 507	PRL/2*PASTOR//PBW343*2/KUKUNA,SERI.1B*2/3/KAUZ* 2/BOW//KAUZ/4/PBW343*2/,SW89.5277/BORL95//SKAUZ/3/PRL/* PASTOR/4/,WHEAR//2*PRL/2*PASTOR, BLL1*2/KIRITATI, MINO/898.97, PBW343*2/KUKUNA/3/PASTOR//CHIL/PRL/4/	26.92
Cluster-III	3	529, 525, 514	WHEAR/SOKOLL, PFAU/SERI.1B//AMAD*2/3/PBW343*2/KUKUNA, KIRITATI//SERI/RAYON	11.54
Cluster-IV	1	527	WAXWING*2//PBW343*2/KUKUNA	3.85
Cluster-V	1	509	PBW343*2/KUKUNA// PBW343*2/KUKUNA	3.85
Cluster-VI	1	516	WBLL1*2/KIRITATI	3.85



Appendix 1: Dendrogram of bread wheat genotypes for 10 characters with average linkage clustering strategy

Table 5: Mean of clusters for 10 characters of bread wheat genotypes

Trait	Cluster-I	Cluster-II	Cluster-III	Cluster-IV	Cluster-V	Cluster-VI
DH	47.07	47.67	44.66	53.83	44.33	54.67
GFS	29.26	29.07	29.92	25.75	35.5	23.17
MD	78.90	79.43	77.17	82.08	82.58	80.58
PHT	53.72	51.99	54.33	54.32	50.78	55.44
NT	4.55	4.38	5.63	5.44	3.59	4.59
SL	8.09	8.17	8.37	8.08	7.91	8.35
NSPK	8.93	8.82	8.91	9.22	8.29	9.82
NSS	187.74	158.48	230.55	207.67	213.33	131.67
TGW	30.77	29.59	28.83	28.89	29.05	29.66
YLD	23.18	22.76	32.30	30.88	22.46	26.15

DH: Days to main heading, GFS: Grain filling period, MD: Days to maturity, PHT: Plant height, NT: No. of tillers plant, SL: Spike length, NSPS: No. of spikelets per spike, NSS: No. of grains per spike, TGW: 1000-grain weight and YLD: Yield per plot, respectively

1000 grain weight (30.77) among all clusters. Cluster II consisted of 7 genotypes. This cluster could be characterized by the lowest number of seeds per spike (158.48) and relatively lower yield per plot (22.76 qt ha^{-1}). Cluster III consisted of 3 genotypes were characterized by the following features: the highest number of tillers $plant^{-1}$ (5.63), spike length (8.37 cm), number seeds $spike^{-1}$ (230.55) and grain yield per plot (32.30 qt ha^{-1}) among all clusters.

Cluster IV, Cluster V and Cluster VI had each one genotypes which was outstanding type by its performance in most of the traits or agronomic characters,

Table 6: Average intra (bold) and inter cluster (off diagonal) D^2 values among six clusters in bread wheat genotypes

Cluster	I	II	III	IV	V	VI
I		35.25**	114.97**	61.00**	78.43**	177.12**
II			258.24**	155.83**	181.25**	70.03**
III				32.65**	96.98**	502.68**
IV					118.13**	319.64**
V						456.27**
VI						

*Significant at $p<0.05$ for $\chi^2 = 18.31$ and ** significant at $p<0.01$ for $\chi^2 = 23.21$, respectively

the cluster-IV characterized by the following essential features, late maturing (82.08), the lower number of grain filling period (25.75), high plant height (54.32 cm), high number of tillers plant^{-1} (5.44), high number spikelets spike^{-1} (9.22), number seeds spike^{-1}(207.67), high grain yield plot^{-1}(30.88 qt ha^{-1}) except cluster III. Cluster V characterized by the lowest days to main heading (44.33), number of tillers plant^{-1} (3.59), grain yield plot^{-1} (22.46 qt ha^{-1}) and the highest days to maturity (82.58) and grain filling period (35.5) among all clusters. Cluster VI characterized by the highest days to main heading (54.67), plant height (55.44 cm), the lowest grain filling period (23.17), the lowest days to main heading (44.33), number of tillers plant^{-1} (3.59), high number spikelets spike^{-1} (9.82), high grain yield plot^{-1} (26.15 qt ha^{-1}) except cluster III and cluster IV.

Estimation of intra and inter cluster square distances (D^2): The average intra and inter cluster D^2 values are presented in Table 6. Maximum average intra cluster D^2 was obtained in cluster VI (D^2 = 456.27) followed by cluster III and cluster V (D^2 = 118.13). The lowest D^2 was recorded in cluster IV (D^2 = 32.65), which shows the presence of less genetic diversity within this cluster. The chi-square test for the six clusters indicated that there was a statistically significant difference in all characters. The highest average inter cluster D^2 was recorded between cluster III and cluster IV (D^2 = 502.68) followed by cluster V and cluster VI (D^2 = 456.27) and cluster IV and cluster VI (D^2 = 319.64) which had shown these clusters were genetically more divergent from each other than any other clusters.

Minimum inter cluster distance was observed between cluster III and cluster IV (D^2 = 32.65) indicating that the bread wheat genotypes in these clusters were not genetically diverse or there was little genetic diversity between these clusters. This signifies that, crossing of genotypes from these two clusters might not give higher hetrotic value in F_1 and narrow range of variability in the segregating F_2 population. Since maximum genetic recombination and variation in the subsequent generation is expected from crosses that involve parents from the clusters characterized by maximum distances, crosses between genotypes selected from cluster III with cluster VI, cluster V with cluster VI and cluster IV with cluster VI are expected to produce relatively better genetic recombination and segregation in their progenies. However, the selection of parents should be also considering the special advantages of each cluster and each genotype within a cluster depending on the specific objective of hybridization program.

CONCLUSION

The progress of crop improvement program depends on the choices of material, the extent of genetic diversity present and the knowledge of quantitative characters with grain yield and among themselves. The combined analyses of variation for two locations were showed significant differences among all the characters 10 studied. The genotypes were grouped into six clusters which make them to be moderately divergent. There was statistical difference between most of the clusters. The maximum inter-cluster distances were observed between cluster-V with VI may exhibit high heterotic values and could give transgressive segregants. On the other hand, the shortest inter-cluster distance was observed between cluster-III with IV, showing less divergence between these clusters. In principal component analysis was showed that six principal components PC1, PC2, PC3, PC4, PC5 and PC6 accounted for 91.87% of the total variation. The first two principal components PC1 and PC2 with values of 27.59 and 20.95%, respectively, contributed more to the total variation indicating hybridization breeding program can be initiated by the selection of genotypes from the PC1 and PC2. Of all characters evaluated, the days to main heading, number of spikelets spike^{-1}, plant height, spike length, days to maturity, grain filling period, 1000 grain weight and grain yield in each principal component contributed more to the total variations. This result also further confirmed the presence of considerable genetic diversity for use in the bread wheat genotypes improvement program. In conclusion, the crosses between genotypes selected from cluster-III with cluster-VI and cluster V with cluster VI are expected to produce better genetic recombination and segregation in their progenies. Therefore, these bread wheat genotypes need to be crossed and selected to develop high yielding pure line variety.

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