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Analysis of Genetic Divergence for Grain Yield and Grain Quality Traits in Bread Wheat (*Triticum aestivum L.)* Genotypes at Axum, Northern Ethiopia

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Abstract

This study was intended to assess genetic diversity for yield and grain quality traits at Axum Agricultural Research Center in 2016. Forty-nine wheat genotypes were tested using 7 x 7 triple- lattice design in which data were collected for 17 agronomic and grain quality traits. The near infrared transmittance (NIT Infratec 1241 Grain analyzer, Sweden) was used to analyze wheat samples for their protein, wet gluten, zeleny sedimentation and starch content on dry weight basis. The data were subjected to analysis of variance and exhibited significant difference among genotypes. New entries viz .ETBW8486, ETBW8480 and ETBW9016 produced grain yield ranged from 4.52 to 5.44 t ha^{-1} and protein content 13.1 to 14.67%, which are higher than the check variety (King bird= 4.38 t ha^{-1} with protein content=11.93%). These genotypes were also early maturing than the check varieties, thus they might serve as good candidates for variety development. Genetic divergence estimated by squared distance (D^2) and clustering conducted using Ward's method grouped the 49 bread wheat genotypes in to five clusters. Cluster mean values and squared distance showed that there is negative association between grain yield and grain quality traits. Hence, for the improvement of grain yield with grain quality, genotypes from cluster V and I might be crossed. PCA analysis also showed that the first five PC's accounted for 80.12% of total variation and suggested these traits could be used as useful sources of genetic variation for future improvement. Generally, the information obtained from this study can be used to plan hybridization among potential genotypes to maximize genetic diversity and expression of heterosis for feature wheat improvement.

Key words: Clustering, ETBW, genetic divergence, grain quality, principal component

Introduction

Wheat (*Triticum aestivumL.)* is considered as a 'King of cereals' because of its largest area coverage, high productivity and the observable position it holds in the international food grain trade (Shashikala, 2006). It is an important staple food of many countries in the world and occupies a unique position as used for the preparation of wide ranges of food stuffs. Wheat is agronomically and nutritionally most important cereal essential for food security, poverty alleviation and improved livelihoods (Sharma *et al*., 2011). It is one of the most important small cereal crops in Ethiopia, which ranks fourth both in area coverage (1,663,845.63 hectares) and in total annual production (4,231,588.716 tons) (CSA, 2015). However, productivity of the crop remains low (2.54 t ha^{-1}) (CSA, 2015) in the country as compared to the world average yield (3.19 t ha^{-1}) (FAO, 2013). The low yield per hectare was attributed to many factors, such as less availability of quality kernel for varieties that are high yielding as well as adapted to wide range of agro-ecologies of the country. Selection of desirable genotypes for hybridization program over the past century has been found as an effective operating implement in developing high yielding crop varieties up on which, the present agriculture can rely. For successful wheat breeding program the presence of genetic diversity is required as a prior condition. Thus, having knowledge about the existing genetic variability is very important for the development of wheat varieties with the desirable traits through crossing (Kahrizi et *al.*, 2010). The D^2 statistic measures the forces of differentiation at intra and inter-cluster levels and determines the relative contribution of each component trait to the total divergence (Sharma, 1996). Those clusters which are separated by the largest genetic distance (D^2) will have maximum divergence; where as individuals in the same group are less divergent (Singh and Chaudary, 1977).

Several genetic diversity studies have been conducted on wheat crop based on quantitative and qualitative traits in order to select genetically distant parents for hybridization (Negash and Grausgruber, 2013; Adhiena, 2015; Dutamo *et al.*, 2015; Shah *et al.*, 2015). Thus, the presence of such kind of variability among the germplasm is crucial to develop desirable recombinants for developing high yielding bread wheat varieties through crossing between superior germplasm. Hence, the present study was conducted with the objectives of identifying genetically divergent bread wheat genotypes with desirable traits for hybridization particularly for yield and grain quality characters (protein, gluten, zeleny sedimentation and starch contents).

Materials and Methods

Testing location

Field experiment was conducted at Axum Agricultural Research Center (AxARC), Northern Ethiopia during 2016. The experimental site is located at latitude of 13^0 15′ 40.2′′ N, and 38^0 34′45.8′′ E longitudes with an altitude of 2148 meter above sea level. The mean minimum- maximum annual rain fall is 500-782.8 mm per annum with minimum and maximum temperatures of 12.6^0C and 25.51^0C, respectively. The soil type of the site is clay type with pH ranged from7.5-8.3.

Planting materials

A total of 49 bread wheat genotypes introduced from ICARDA-CIMMYT through Kulumsa agricultural research center (Table1) were used in the study. For each of the test entries, samples of 500 g were taken from each plot for quality analysis. The near infrared transmittance (NIT Infratec 1241 Grain analyzer, Sweden) was used to analyze wheat samples for their protein, wet gluten, zeleny sedimentation volume and starch content on dry weight basis.

Experimental design and management

The experiment was laid out in 7x7 triple lattice designs. Each genotype was planted in a plot consisting of six rows of 2.5 m long and 1.2 m width, a total of 3 m^2 with spacing of 20 cm between rows. Seed rate of 150 kg/ha was used. Recommended fertilizer rate of 100/100 kg ha^{-1} N/P2O5 in the forms of Urea and DAP (di-ammonium phosphate) was used. All other agronomic practices (land preparation, sowing and weeding) were applied as recommended for wheat production.

Statistical analysis

The mean values of the genotypes from the central rows of each plot were subjected to analysis of variance based on triple lattice design. Squared distances (D^2) for each pair of genotype was measured by generalized mahalanobis D^2 statistics using SAS computer software. Principal component analysis (PCA) was performed using correlation matrix method by employing SPSS 18 computer software in order to evaluate relationships among characters and cluster analysis was performed using SPSS 18 computer software. Squared distances (D^2) for each pair of genotype combination were computed as per the following formula:

$D^2_{ij}= (Xi–Xj)' S^{-1}(Xi –Xj)$ Where, D^2ij = the distance between any two groups i and j;

Xi and Xj = the vector mean of the characters for the i^{th} and j^{th} groups, respectively;

S^{-1}= the inverse of the pooled covariance matrix.

Testing the significance of the squared distance values obtained for a pair of clusters was taken as the calculated value of χ^2 (chi-square) and tested against the tabulated χ^2 values at p-1 degree of freedom at 1% and 5% probability level, where p = number of characters used for clustering the genotypes (Singh and Chaudary, 1985).

Table 1.Genotypes used in the study

Name	Pedigree
ETBW8484	MUTUS//WBLL1*2/BRAMBLING/3/WBLL1*2/BRAMBLING
ETBW8486	SNLG/3/EMB16/CBRD//CBRD/4/KA/NAC//TRCH
ETBW9019	MUTUS//KIRITATI/2*TRCH/3/WHEAR/KRONSTAD F2004
ETBW9026	AGUILAL/FLAG-3
ETBW9027	REYNA-29
ETBW9028	MUTUS//ND643/2*WBLL1
ETBW9029	ND643/2*WBLL1/4/CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92/5/BECARD
ETBW9033	DANPHE #1*2/CHYAK
ETBW9034	MUTUS*2/HARIL #1
ETBW9040	T.DICOCCON CI9309/AE.SQUARROSA (409)// MUTUS/3/2*MUTUS
ETBW9042	HUW234+LR34/PRINIA//PFAU/WEAVER/3/CMH83.30
ETBW8489	VORB/6/CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (208)/5/2*WESTONIA/7/ CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (208)/5/2*WESTONIA
ETBW8492	KRICHAUFF/2*PASTOR//CHONTE
ETBW9015	SUP152//ND643/2*WBLL1/3/ND643/2*WBLL1
ETBW9016	SWSR22T.B./2*BLOUK #1//WBLL1*2/KURUKU
ETBW9017	SWSR22T.B./2*BLOUK #1//WBLL1*2/KURUKU
ETBW9018	SWSR22T.B.//TACUPETO F2001*2/ BRAMBLING/3/2*TACUPETO F2001*2/ BRAMBLING
ETBW9041	T.DICOCCON CI9309/AE.SQUARROSA (409)//MUTUS/3/2*MUTUS
ETBW9051	CROC-1/AE.SQUARROSA (224) //OPATA/3/QAFZAH-21/4/SOMAMA-3
ETBW 8471	WEEBILL-1/BOCRO-3
ETBW 8472	SANOBAR-4
ETBW 8473	SUNCO.6/FRAME//PASTOR/3/PAURAQ
ETBW 8474	1447/PASTOR//KRICHAUFF/3/PAURAQ
ETBW 8475	WORRAKATTA/2*PASTOR//DANPHE #1
ETBW 8476	1447/PASTOR//KRICHAUFF/5/2*SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92
ETBW 8477	C80.1/3*BATAVIA//2*WBLL1/3/EMB16/CBRD//CBRD/4/CHEWINK #1
ETBW 8478	SLVS/3/CROC_1/AE.SQUARROSA(224)//OPATA/5/VEE/LIRA//BOW/3/BCN/4/KAUZ/6/2*KA/NAC//TRCH
ETBW 8479	METSO/ER2000//MUU
ETBW 8480	KA/NAC//TRCH/3/DANPHE #1
ETBW 8481	EMB16/CBRD//CBRD/4/BETTY/3/CHEN/AE.SQ//2*OPATA
ETBW 6861	WAXWING*2/HEILO
ETBW 8506	AGUILAL/FLAG-3
ETBW 8507	DURRA-4
ETBW 7120	QAFZAH-23/SOMAMA-3
ETBW 8508	REYNA-8
ETBW 7213	CHAM-4/SHUHA'S'/6/2*SAKER/5/RBS/ANZA/3/KVZ/HYS//YMH/TOB
ETBW 8509	REYNA-29
ETBW 7038	ATTILA/3*BCN//BAV92/3/TILHI/5/BAV92/3/PRL/SARA//TSI/VEE#5/4/CROC_1/AE.SQUARROSA (224)//2*OPATA
ETBW 8510	HIJLEEJ-1
ETBW 8511	BOW #1/FENGKANG 15/3/HYS//DRC*2/7C
ETBW 7147	CROC-1/AE.SQUARROSA(224)// OPATA/3/QAFZAH-21/4/SOMAMA-3
ETBW 8512	BABAX/LR42//BABAX*2/3/KURUKU/4/KINGBIRD #1
ETBW 7871	PAURAQ/4/PFAU/SERI.1B//AMAD/3/WAXWING
ETBW 8513	MUTUS//WBLL1*2/BRAMBLING/3/WBLL1*2/BRAMBLING
ETBW 6940	UTIQUE 96/FLAG-1
King bird	THELIN # 2/TUKURU
Kakaba (PICAFLOR#1)	Kititati//Seri/Rayon
Shorima (ETBW5483)	UTQE96/3/PYN/BAU//Milan
Ogolcho(ETBW552)	WORRAKATTA/2*PASTOR

Table 2. Mean squares from analysis of variance for the 17 characters of 49 bread wheat genotypes

Traits	Replication (DF= 2)	Genotype (DF= 48)	Error mean square (DF= 78)	CV (%)	Efficiency relative to RCBD
Days to heading	51.76	83.48**	0.98	1.71	102.12
Days to maturity	40.62	169.58**	8.34	2.83	95.82
Grain filling period	56.63	43.42**	5.28	5.22	103.08
Plant height (cm)	60.41	86.58**	15.66	4.92	99.11
Number of effective tillers	0.07	0.21*	0.13	18.75	105.98
Kernels per spike	9.69	82.98**	22.44	10.13	93.74
Spikelets per spike	0.17	3.80**	0.83	5.63	101.69
Spike length (cm)	0.26	0.76**	0.25	5.98	95.55
Biomass yield (t ha^{-1})	20.89	3.71**	1.26	12.00	96.40
Grain yield (t ha^{-1})	5.28	0.97**	0.25	12.53	97.20
Harvest index (%)	33.55	62.61*	35.45	13.91	98.59
Thusand kernel weight (g)	58.07	30.59**	5.67	7.00	103.24
Hectoliter weight (kg hl^{-1})	4.85	10.34**	2.27	1.92	101.70
Grain protein content (%)	5.12	1.70**	0.47	4.95	101.23
Wet gluten content (%)	23.78	14.13**	3.41	5.85	100.03
Zeleny sedimentation volume (%)	78.77	46.49**	11.91	7.15	94.37
Starch content (%)	1.89	2.20**	0.42	1.02	98.50

ns= non-significant,* and ** = significant at 5% and 1% probability level, respectively, DF= degree of freedom.

Mean performance of bread wheat genotypes

Highly significant differences ($P<0.01$) were observed among genotypes for all traits, except for number of effective tillers per plant and harvest index, where genotypes exhibit significant difference ($p<0.05$) for these two traits. Yield and yield components: Grain yield ranged from 2.37 to 5.44 t ha^{-1} with mean value 3.95 t ha^{-1}. The genotypes had above ground biomass yield ranged from 5.5 to 13 t ha^{-1} with a grand mean of 9.29 t ha^{-1}. Spikelets per spike ranged from 12.80 to 18.70 with a mean value of 16.09. The lowest (36.2) and highest (59.5) number of kernels per spike were obtained from ETBW8479 and ETBW8481, respectively while harvest index ranged from 26.35% (Ogolcho) to 51.55% (ETBW8481) with mean value of 42.49%. Moreover, 48.99 % of the tested genotypes had grain yield above the grand mean (3.98 t ha^{-1}). Most of these high yielding genotypes were also early in maturity than the check varieties. This suggested, these genotypes could be considered to in variety development the study area and similar agro ecologies in which terminal moisture stress is a crucial constraint to produce high yield. Iqbal *et al.* (2007), Adhiena (2015) and Tilahun *et al.* (2016) also reported similar results for early genotypes of higher yield potential.

Grain quality traits: Analysis of variance revealed highly significant differences among genotypes for 1000 kernel weight with minimum and maximum mean values of 27.2 and 41.9 g, respectively and a mean of 33.87 g. Grain protein content ranged from 11.93% for the check variety King bird to 15.43% for ETBW8489 with a mean value of 13.79% (Table 3).

Wrigley (1994) categorized wheat flour having higher protein content of >13%, 11-13% as medium and 8-10% with low protein content. Accordingly 81.63% of the genotypes had higher protein content, while 18.37% of the genotypes categorized as having medium protein content. Therefore, these genotypes with high protein content could be used as donor parents for protein content characters in future bread wheat improvement program of Ethiopia. NaiK *et al.* (2015) reported protein content ranged 12.2 to 17.2%, nearly similar with this study. Wet gluten content obtained from the grains of genotypes ranged between 27.23 for Kakaba and 36.47% for ETBW7038 with a mean value of 31.49. The lowest Zeleny sedimentation volume was 34.35% for Kingbird and the highest was 53.13% for ETBW7213. Starch content varied from 65.03% for ETBW8481 to 61.13% for ETBW9042.

Table 3. Mean values of 17 traits of 49 bread wheat genotypes tested at Axum agricultural research center in 2016

Name	DH	DM	GFP	PH	NT	KPS	SKPS	SL	BY	GY	HI	TSW	HLW	GPC	WG	ZSV	SC
ETBW8484	57.33	101.7	44.33	82.40	1.86	39.20	15.5	7.80	9.67	4.16	44.22	33.73	79.33	12.93	28.30	34.95	63.30
ETBW8486	55.33	100.0	44.67	77.30	1.84	47.27	16.0	8.20	11.00	4.56	41.64	35.80	79.27	14.30	31.93	51.93	62.73
ETBW9019	57.33	98.3	41.00	73.77	2.60	48.37	15.2	8.00	8.33	3.84	48.15	35.23	79.13	13.57	30.57	49.33	63.20
ETBW9026	51.67	93.7	42.00	84.53	2.01	43.10	14.9	8.47	9.50	4.49	47.36	38.67	81.60	13.17	29.83	48.87	63.43
ETBW9027	52.67	94.7	42.00	74.93	1.89	41.33	15.3	8.10	10.00	4.35	43.54	37.07	81.40	14.00	32.63	50.63	63.67
ETBW9028	65.67	112.3	46.67	83.37	1.79	57.23	18.5	8.70	9.33	3.71	39.18	29.70	76.27	13.30	30.70	48.80	63.10
ETBW9029	51.67	92.3	40.67	75.40	2.02	48.83	16.5	8.47	9.50	4.24	44.93	36.30	80.67	14.03	31.67	51.50	63.23
ETBW9033	53.00	96.7	43.67	81.67	2.19	54.10	16.9	9.10	11.00	4.09	37.33	38.23	80.10	13.87	31.33	49.60	63.37
ETBW9034	52.33	90.7	38.33	79.53	1.76	49.93	15.6	8.00	8.83	4.15	48.00	34.00	79.30	14.33	31.80	50.63	62.13
ETBW9040	54.33	94.3	40.00	74.17	1.53	51.37	16.6	8.27	8.50	3.26	38.19	29.57	77.93	14.37	30.50	50.60	61.73
ETBW9042	52.67	94.7	42.00	80.37	2.07	50.77	16.3	8.40	9.00	3.68	42.55	33.93	77.83	15.30	33.90	50.03	61.13
ETBW8489	53.67	98.3	44.67	69.47	1.86	36.47	15.0	6.93	5.50	2.59	47.59	35.60	79.20	15.43	33.53	51.33	62.33
ETBW8492	54.67	100.3	46.33	82.87	2.38	47.07	15.4	8.47	9.33	4.19	44.96	39.50	79.70	13.57	30.87	46.60	62.13
ETBW9015	58.33	98.7	40.33	86.53	1.74	51.43	15.8	7.10	10.33	4.46	43.19	30.90	78.20	13.00	27.87	44.40	62.77
ETBW9016	59.33	105.0	45.67	88.13	2.13	50.70	16.7	8.27	13.00	5.44	41.82	33.10	76.90	13.30	30.40	44.37	63.67
ETBW9017	62.33	104.7	42.33	85.10	1.58	43.23	16.1	8.57	9.33	3.64	38.90	30.33	76.17	14.57	33.90	51.70	62.57
ETBW9018	59.67	102.3	42.67	77.43	1.86	51.30	16.6	8.10	8.67	3.64	42.11	30.93	77.20	14.70	31.40	51.07	61.37
ETBW9041	59.33	99.3	40.00	78.03	1.60	54.53	17.7	8.57	8.00	3.82	48.08	29.63	77.13	14.53	31.63	50.50	61.57
ETBW9051	61.67	110.3	48.67	91.83	2.17	51.20	16.8	7.63	10.33	4.36	42.25	31.70	77.03	12.97	31.47	47.45	64.27
ETBW8471	59.33	100.7	41.33	75.63	1.56	49.07	16.7	8.30	8.75	2.43	27.78	28.50	76.50	13.93	30.47	45.30	61.30
ETBW8472	64.33	114.0	49.67	89.63	1.76	41.93	15.5	9.23	9.00	3.46	38.40	31.53	75.30	13.53	30.97	48.87	62.93
ETBW8423	57.00	101.3	44.33	73.43	1.95	45.47	16.5	8.27	9.33	3.85	41.30	30.97	77.40	14.53	33.90	51.93	62.17
ETBW8474	54.00	99.3	45.33	81.80	1.71	42.93	16.4	8.37	9.83	4.21	43.11	37.90	81.10	14.70	33.03	51.43	62.53
ETBW8475	53.67	112.3	58.67	81.53	1.68	49.60	16.5	8.53	11.00	4.64	42.19	33.80	81.30	13.10	31.53	47.30	63.80
ETBW8476	63.00	110.3	47.33	78.27	1.85	42.50	14.7	7.63	10.67	4.42	41.70	33.00	79.60	12.70	29.43	44.57	63.73
ETBW8477	60.67	106.7	46.00	86.07	1.95	50.57	15.9	7.80	10.00	4.43	44.55	33.67	79.33	12.23	27.50	35.90	63.63
ETBW8478	61.67	107.3	45.67	90.43	1.81	50.97	16.4	8.20	9.67	4.04	41.65	35.83	78.27	13.70	30.30	49.43	62.77
ETBW8479	52.00	92.7	40.67	82.07	2.07	36.20	12.8	7.53	8.67	3.75	42.94	37.63	77.87	14.77	35.40	52.40	62.90
ETBW8480	56.67	97.3	40.67	89.43	1.96	41.13	15.7	7.87	11.00	5.37	49.68	36.47	80.13	13.53	28.20	49.97	62.47
ETBW8481	55.67	105.3	49.67	86.37	2.02	59.50	17.6	9.00	8.67	4.47	51.55	41.90	80.80	12.80	30.30	44.50	65.03
ETBW6861	59.33	99.3	40.00	79.60	2.38	52.13	16.6	8.00	9.00	3.84	43.18	32.60	77.90	13.63	29.40	49.87	62.07
ETBW8506	63.00	108.7	45.67	90.93	1.71	45.63	16.6	8.00	10.67	4.12	38.53	32.33	78.37	13.20	31.83	46.83	63.63

ETBW8507	49.33	87.0	37.67	77.97	2.52	36.93	14.0	8.07	9.50	4.21	44.37	35.40	81.00	14.67	34.83	52.03	63.03
ETBW7120	60.67	104.3	43.67	72.90	1.94	46.03	15.5	8.60	8.33	3.12	37.43	28.97	75.90	14.33	33.70	51.00	62.67
ETBW8508	54.00	93.7	39.67	69.03	2.04	37.47	14.1	7.43	7.33	3.54	48.15	33.50	77.73	14.00	31.80	50.27	63.13
ETBW7213	53.00	94.7	41.67	82.33	2.18	43.33	15.2	8.47	10.33	4.38	42.37	40.10	81.50	14.20	33.73	53.13	63.20
ETBW8509	59.33	106.7	47.33	80.83	1.62	46.57	18.6	9.60	7.00	3.28	46.92	34.57	76.60	13.90	32.20	50.27	63.77
ETBW7038	55.00	98.0	43.00	66.87	2.51	39.07	16.0	8.33	8.00	3.23	40.68	35.13	81.27	15.40	36.47	52.47	62.50
ETBW8510	72.67	118.0	44.67	82.07	1.66	51.80	18.7	9.33	10.00	3.83	38.49	36.97	79.40	13.93	34.67	50.37	64.03
ETBW8511	64.67	113.0	51.00	82.20	1.69	53.83	18.7	8.83	9.67	4.51	46.75	29.67	78.37	14.07	33.87	49.47	63.20
ETBW7147	49.00	88.0	39.00	74.43	2.29	49.03	16.0	8.13	7.67	3.70	47.61	37.07	79.83	14.00	31.07	51.03	62.17
ETBW8512	56.33	100.0	43.67	76.73	1.93	48.07	17.1	8.80	9.67	4.24	44.15	32.87	80.67	13.10	28.20	43.63	63.53
ETBW7871	66.33	114.7	48.33	82.40	1.87	44.33	17.7	8.97	9.50	4.31	45.46	27.20	73.95	15.17	36.10	51.20	61.53
ETBW8513	73.33	118.0	44.67	77.63	1.55	43.90	16.3	9.07	9.67	3.79	38.93	34.90	74.77	13.77	32.93	47.87	63.67
ETBW6940	52.67	94.0	41.33	82.20	1.69	46.53	14.8	7.47	10.00	4.52	45.21	37.70	80.70	13.53	32.20	47.13	64.50
Kakaba	57.67	101.3	43.67	81.60	1.62	47.10	17.2	8.67	9.00	3.57	39.52	31.23	78.53	12.60	27.23	43.23	63.33
Shorima	61.00	108.0	47.00	78.13	2.33	42.03	14.8	8.13	8.67	4.38	51.27	36.57	79.60	13.57	31.57	46.60	62.93
Ogolcho	72.67	117.0	44.33	78.80	1.60	40.60	14.9	8.50	9.33	2.37	26.35	32.40	77.60	13.57	32.30	47.90	64.07
king bird	59.33	104.7	45.33	81.53	2.04	49.80	16.6	8.17	10.00	4.38	43.85	32.20	79.23	11.93	27.77	34.35	64.70
Mean	58.17	102.1	44.02	80.40	1.93	46.8	16.15	8.29	9.37	3.98	42.82	34.0	78.7	13.8	31.6	48.3	62.99
CV(%)	1.65	2.79	5.48	4.83	20.1	10.1	5.78	5.86	11.69	12.6	13.90	7.21	2.01	4.99	5.89	6.93	1.03
LSD at 1%	2.13	6.19	4.93	8.49	0.81	10.2	1.95	1.06	2.41	1.07	12.78	5.11	3.23	1.45	3.96	7.40	1.38

Genetic divergence analysis

The 49 bread wheat genotypes exhibited significant differences for 17 characters and lead to further calculation of D^2 (Sharma, 1998). The D^2 values based on the pooled mean of traits of genotypes resulted in grouping of the 49 bread wheat genotypes in to five clusters using Wards clustering method (Table 4, figure 2). Cluster II was the largest cluster consisted of 15 genotypes followed by Cluster I comprised of 13 genotypes. Clusters III and IV comprised each eight genotypes; but cluster V had the lowest number of genotypes that comprises only five genotypes (10.20%). Different authors also reported the presence of diversity among bread wheat genotypes classifying in different number of distinct clusters in such a way that the genotypes within a cluster had smaller D^2 values among themselves than with those belonging to different clusters (Degewione and Alamerew, 2013; Negash and Grausgruber, 2013; Dutamo *et al.*, 2015; Shah *et al.*, 2015)

Cluster mean analysis and inter cluster square distance

The mean values of genotypes were computed in each cluster and registered as mean of the respective cluster and results are presented in Table 5. The cluster mean values revealed considerable differences among the clusters for different characters. The χ^2-test for the five clusters showed that there was highly significant difference among the clusters except between cluster II and III.

Table 4. Clustering pattern of 49 bread wheat genotypes in to five clusters based on D^2 analysis

Cluster	No of genotypes	Percentage	Name of genotypes
I	13	26.53	ETBW8484, ETBW9015, ETBW9016, ETBW9051, ETBW8475, ETBW8476, ETBW8477, ETBW8478, ETBW8481, ETBW8506, ETBW8512, Kakaba, King bird
II	15	30.61	ETBW8486, ETBW9019, ETBW9026, ETBW9027, ETBW9029, ETBW9033, ETBW9034, ETBW8492, ETBW8474, ETBW8480, ETBW6861, ETBW7213, ETBW7147, ETBW6940, Shorima
III	8	16.33	ETBW9028, ETBW8472, ETBW8509, ETBW8510, ETBW8511, ETBW7871, ETBW8513, Ogolcho
IV	8	16.33	ETBW9040, ETBW9042, ETBW9017, ETBW9018, ETBW9041, ETBW8471, ETBW8423, ETBW7120
V	5	10.20	ETBW8489, ETBW8479, ETBW8507, ETBW8508, ETBW7038

Inter cluster distance indicated greater divergence between Cluster IV and Cluster V (D^2 = 123.48) followed by Cluster III and Cluster V (D^2 = 94.78) of which cluster IV had highest mean for yield components, while cluster V bearing genotypes that had early maturing time (92.78 days) and superior for grain quality traits. The results of mean and inter cluster distance analyses suggested that genotypes from these clusters could be used in hybridization program as parental lines since crossing between divergent parents is likely to produce wide variability and transgressive segregations with high heterotic effects. Cluster I contained genotypes which had high mean value for biomass yield (10.09 t ha^{-1}) and grain yield (4.31 t ha^{-1}). Hence, if varieties with simultaneously high grain yield and high grain quality accompanied with early maturity are desired, genotypes from cluster I and V might be crossed. Cluster III, is characterized by late maturing genotypes (113 days). Thus, genotypes from this cluster might not be worthy in the study area since it is characterized by terminal moisture stress. Khodadadi *et al.* (2011), Negash and Grausgruber (2013), Kumar *et al.* (2013), Brijendra *et al.* (2014), Adhiena (2015) and Dutamo *et al.* (2015) have also reported that selection of wheat parents for hybridization should be done from two clusters having wider inter-cluster distances to get maximum variability in segregating generations. The smallest inter-cluster distance was observed between cluster II and III (D2 = 20.99) followed by cluster I and III (D2= 27.71). The genotypes of these clusters are relatively close to each other, in comparison to genotypes grouped in other clusters. According to Rama (1992) crossing of genotypes from those clusters might not give higher heterotic value in F_1 and narrow range of variability in the segregating F_2 population. Such analysis was meant to avoid selection of parents from genetically homogeneous clusters and to maintain a relatively broad genetic base.

Table 5. Mean values of five clusters for 17 characters of 49 bread wheat genotypes

	Cluster				
Characters	I	II	III	IV	V
Days to heading (days)	58.67	53.96	66.48	57.56	52.22
Days to maturity (days)	105.07	95.96	113.37	99.55	92.78
Grain filling period (days)	46.07	42	46.78	41.81	40.56
Plant height (cm)	84.22	79.48	81.62	76.66	72. 05
Number of effective tillers (No)	1.87	2.06	1.68	1.74	2.14
Kernels per spike (No)	48.25	46.14	46.75	48.33	37.06
Spikelets per spike (No)	16.05	15.63	17.09	16.39	14.12
Spike length (cm)	8.05	8.17	8.97	8.35	7.54
Biomass yield (t ha^{-1})	10.09	9.48	8.94	8.66	7.42
Grain yield (t ha^{-1})	4.31	4.25	3.51	3.32	3.32
Harvest index (%)	42.69	44.73	38.54	38.24	44.07
Thousand seed weight (g)	33.37	36.61	31.57	30.15	35.13
Hectoliter weight (kg/hl)	78.89	79.99	76.25	76.88	79.13
Grain protein content (%)	12.82	13.82	13.84	14.47	14.71
Wet gluten content (%)	29.24	31.13	32.72	32.21	33.97
Zeleny sedimentation volume (%)	42.52	49.68	49.18	49.71	51.46
Starch content (%)	63.64	62.86	63.09	61.74	62.7

Table 6. Average inter squared distance (D^2) between clusters based on 17 characters of 49 bread wheat genotypes tested in 2016

Cluster	I	II	III	IV	V
I	-	40.65**	27.71*	31.63*	52.48**
II		-	20.99 ns	69.00**	88.19**
III			-	37.88**	94.78**
IV				-	123.48**
V					-

ns= non-significant, * and **, significant (χ^2= 27.59) and highly significant (χ^2= 33.41) at 5 and 1% probability levels, respectively.

Principal component analysis (pcs)

Ordination analysis (Principal Components Analysis, PCA) was executed using 17 quantitative and qualitative traits to validate the grouping of genotypes observed by the cluster analysis (Table 4). Correlation matrix generated using the genotypic mean values of the 17 traits were used as an input and were subjected to the principal components analysis (PCA). Of the 17 principal components (equal number to the original variables) extracted, the first five PC's with an Eigen value > 1 were considered significant. The first five principal components (PCs) accounted for 80.12% of the total variation, suggesting the included traits were important for the observed variation among the genotypes (Table 7). Ali *et al.* (2015) reported the first two principal components which contributed more to the total variance were plotted to observe the relationships between different clusters on the x and y axises.

PCA and cluster analysis are important methods for grouping of wheat genotypes. Cluster analysis based on PCA is a more precise indicator of differences among wheat genotypes than cluster analysis without PCA (Khodadadi *et al*., 2011).

The first two components scores (PC's) are plotted (Figure 1) to aid visualization of the overall variability in the tested genotypes. Traits such as days to heading (DH), days to maturity (DM), grain filling period (GFP), in the positive direction while grain protein content (GPC) and Zeleny sedimentation volume (ZSV) in the negative direction of the PC 1 had the highest loading (Figure 1, Table 7).

In the second axis (PC 2) traits such as grain yield (GY), thousand kernel weight (TKW), hectoliter weight (HLW) and starch content (SC) had a long vector and associated positively with PC 2. The long vectors indicating, the traits have a large contribution to the total variation (Yan and Kang, 2002). Grain protein content (GPC) had the highest negative loading with PC 2. The PC 1 clearly discriminates genotypes in Cluster I and III from the other, where genotypes grouped in to Cluster I and III were mapped in the positive direction while the rest three clusters (Cluster II, IV and V) were located in the negative direction. As compared to genotypes in cluster II, IV and V, genotypes in Cluster I and III (located in the positive direction of PC 1) had above average values in DH, DM, GFP, PH, and SC (Figure 1, Table 5). The second axis (PC 2) differentiates genotypes in Cluster II and I from cluster III, IV and V. Apparently genotypes in the former two clusters had above average GY and BY, unlike the other variants (Cluster III, IV and V). Nevertheless, genotypes in cluster I and II had lower GPC unlike the other groups (particularly genotypes in cluster IV and V had high GPC) as revealed by the negative sign of GPC in the second axis (PC 2). Similar works have been also reported by Dutamo *et al*. (2015), who found that three principal components (pcs) out of 12 contributed 63.2% of variation. Degewione and Alamerew (2013) studied on 26 genotypes, so that five pcs contributed 91% of the variation. Negash and Grausgruber (2013) studied morphological and quality traits variation in tetraploid and hexaploid wheat accessions from Ethiopia using biplot techniques and the first three PCs accounted for 70.9% of the total variation.

Table 7. Loadings of Principal component analysis for the 17 traits of bread wheat genotype

Traits	PC1	PC2	PC3	PC4	PC5
Days to heading (days)	0.331	-0.235	-0.053	0.223	0.012
Days to maturity (days)	0.381	-0.165	0.068	0.274	-0.074
Grain filling period (days)	0.301	0.008	0.237	0.227	-0.172
Plant height (cm)	0.283	0.179	0.092	0.019	0.385
Number of effective tillers	-0.219	0.203	0.128	0.019	-0.042
Kernels per spike(No)	0.234	0.024	0.179	-0.561	-0.063
Spikelets per spike (No)	0.280	-0.147	0.288	-0.415	-0.158
Spike length (cm)	0.200	-0.175	0.421	-0.062	-0.230
Biomass yield (t ha^{-1})	0.225	0.236	0.115	0.110	0.539
Grain yield (t ha^{-1})	0.098	0.381	0.254	-0.063	0.376
Harvest index (%)	-0.139	0.255	0.219	-0.211	-0.142
Thousand kernel weight (g)	-0.159	0.305	0.312	0.210	-0.217
Hectoliter weight (kg/hl)	-0.167	0.360	0.163	0.055	-0.219
Grain protein content (%)	-0.296	-0.313	0.255	0.007	0.179
Wet gluten content (%)	-0.179	-0.277	0.394	0.309	0.109
Zelenysedimentation (%)	-0.245	-0.241	0.384	0.035	0.198
Starch content (%)	0.205	0.265	0.055	0.357	-0.320
Eigen value	4.70	3.83	1.82	1.55	1.21
Individual percentage	27.65	25.53	10.72	9.11	7.11
Cumulative	27.65	53.18	63.90	73.01	80.12

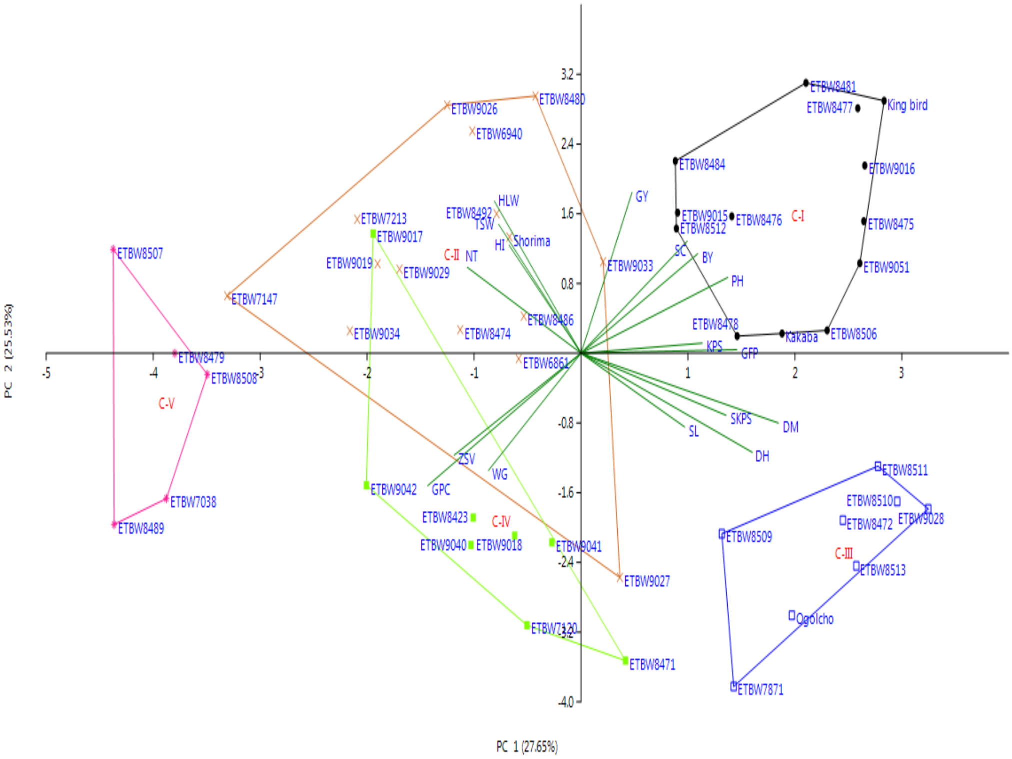

Figure 1. Principal component biplot of 17 quantitative and qualitative characters of 49 bread wheat genotypes

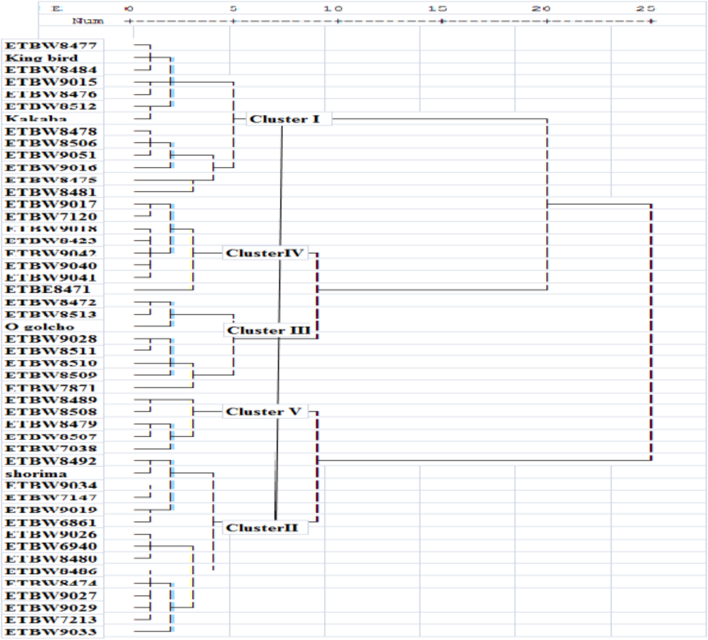

Figure 2. Dendrogram depicting genetic relationships among 49 bread wheat genotypes.

Conclusion and Recommendations

The 49 bread wheat genotypes exhibited significant differences for 17 characters, suggesting the genotypes were phenotypically divergent. Hence, there is good opportunity to bring about improvement through hybridization of relatively distant genotypes in different clusters. The superior genotypes for grain yield and grain quality over the released varieties (Kakaba, Kingbird and Ogolcho) were ETBW9016, ETBW8486 and ETBW8480. These genotypes were also early maturing and hence can be best candidates for release in the study area and similar agro-ecologies. Most pairs of clusters showed highly significant difference, where the highest inter cluster distance was observed between Cluster IV and V (123.48) followed by cluster III and V (94.78) suggesting larger genetic divergence. Hence, crossing of genotypes from these clusters might produce a broad spectrum of segregant in the subsequent generations. PCA analysis showed the first five principal components (PC's) accounted for 80.12% of the total variation, indicating that the investigated traits are useful to consider variation in the bread wheat genotypes. To this end, the presence of such kind of variability among the genotypes is crucial to develop desirable recombinants for developing bread wheat varieties possessing higher grain yield, early maturity and reasonably higher grain quality. Thus, crossing of genotypes with promising quality traits from cluster I with genotypes of higher grain yield in cluster V could be recommended to develop varieties having high grain yield with high quality traits.

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