



Study on Genetic Divergence Using Multivariate Analysis for Bread Wheat (*Triticum aestivum* L.) Genotypes

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Abstract

The research was conducted at KARC in 2023 on 50 bread wheat genotypes to assess traits contributing to phenotypic variation, cluster genotypes into divergent groups, estimate genetic distances between clusters, and evaluate genotypes for yellow and stem rust resistance. Analysis of variance revealed significant genetic variability ($p < 0.01$) for eight out of ten traits, including days to 50% heading, days to 90% maturity, grain yield, and rust disease severity, with plant height and thousand kernel weight also showing significant variation ($p < 0.05$). The genotypes displayed a wide range of values: days to 50% heading varied from 61 to 77 days, with four genotypes heading earlier than the check variety Abay. Thousand kernel weight ranged from 18.0 g to 41.0 g, with 30 genotypes exceeding the check variety. Grain yield also varied significantly, with some genotypes outperforming Abay, making them promising for breeding programs. Cluster analysis categorized the genotypes into five clusters, showing genetic diversity. Significant genetic differences were found between clusters, particularly between Cluster I and Cluster V, suggesting that crossing genotypes from these clusters could yield beneficial recombinants with improved traits. Principal component analysis accounted for 73.45% of the total variation, with key traits like grain yield and thousand kernel weight contributing notably to the observed variation. Disease resistance analysis revealed that 48% of genotypes had high resistance to yellow rust, while 82% showed strong resistance to stem rust. These findings underline the potential for breeding high-yielding and disease-resistant wheat varieties, emphasizing the value of genetic diversity in wheat breeding programs.

Keywords: Bread Wheat, Genetic Divergence, Multivariate Analysis, *Triticum aestivum*.

1. Introduction

Wheat is a globally significant cereal crop, having been one of the first crops domesticated around 10,000 years ago (Feldmann, 2001; Haas et al., 2018). Today, global wheat production primarily relies on two modern species: common or hexaploid bread wheat (*Triticum aestivum* L., $2n=6x=42$, AABBDD) and durum or tetraploid wheat (*T. turgidum* subsp. durum, $2n=4x=28$, AABB) (Feldmann, 2001). Bread wheat (*Triticum aestivum* L.) belongs to the family Gramineae and is a monoecious plant with perfect flowers. It primarily reproduces sexually as an autogamous crop, although limited cross-pollination (approximately 3%) is possible (Mergoum et al., 2009).

In Ethiopia, wheat is one of the most important cereal crops, cultivated across a large portion of the country (Endale and Getaneh, 2015). Wheat production in Ethiopia for the 2021/22 season is projected to reach 5.18 million tons, an increase of 1.6 percent over the estimated production for 2020/21. This growth is attributed to increased government involvement in irrigation, better input supply, and mechanized farming in the lowland and central regions of the country (USDA, 2021). However, wheat production and productivity in Ethiopia remain relatively low compared to global levels. This is primarily due to the fact that wheat is mostly grown by small-scale farmers using rain-fed systems with limited irrigation

(Adugnaw and Dagninet, 2020). Production is further constrained by various infectious diseases, including rust and Septoria leaf blotch, which pose significant challenges to wheat cultivation in Ethiopia (Kasa et al., 2015; Endale and Getaneh, 2015; Tadesse et al., 2017).

Genetic relationships among breeding materials can be identified and classified using multivariate grouping methods, which categorize breeding materials into distinct and variable groups based on genotype performance (Aremu, 2012). Cluster analysis is a widely utilized and highly effective multivariate statistical method for grouping genotypes based on their characteristics. It involves the organization of individuals or items into clusters, where each cluster contains members that are more similar to one another than to members of other clusters. Among the various methods available for cluster analysis, Ward's method is particularly common and effective. Introduced by Ward (1963), this method aims to minimize the total variance within each cluster. Essentially, it seeks to create clusters that are internally homogeneous, meaning the members of a cluster are as similar as possible to each other, while simultaneously ensuring high heterogeneity between clusters, meaning that the clusters are distinct from one another (Karamanos et al., 1998). When plotted geometrically, individuals within a successful cluster analysis will appear closer together, while distinct clusters will be farther apart (Akhilesh and Gulshan, 2005).

A key aspect of cluster analysis is the concept of genetic distance, which refers to the degree of genetic difference between two entities. Genetic distance is often described by variations in alleles, which are different forms of a gene (Nei, 1973). The Mahalanobis generalized distance, introduced by Mahalanobis (1936), is a statistical measure that accounts for multiple variables simultaneously, providing a comprehensive measure of the distance between genotypes. This approach is particularly valuable for identifying genetically divergent genotypes, which can then be grouped based on their agronomic and morphological traits (Morishima and Oka, 1960). Understanding genetic distance is also critical in breeding programs, as it enables the selection of genetically diverse parents. These diverse parents are more likely to produce desirable recombinants, which are offspring that inherit favorable traits from both parents, in subsequent generations (Osiru et al., 2012).

In addition to cluster analysis, Principal Component Analysis (PCA) is another powerful tool used to study genetic diversity. PCA helps to identify the main contributors to variation within a dataset. By reducing the dimensionality of the data, PCA simplifies the complexity while retaining the maximum possible variation. Eigenvalues, which are derived from the PCA process, help determine the number of significant components or factors that should be retained (Jolliffe, 2002). Therefore, this research was conducted to identify traits that contribute significantly to total phenotypic variation, to cluster genotypes into genetically divergent groups, and to estimate the genetic distance between clusters of bread wheat genotypes and evaluate genotypes for yellow and stem rust disease resistance.

2. Materials and Methods

Experimental site and Year

The research was conducted at Kulumsa agricultural research center in 2023 main cropping season. The experimental site is located at 08°01'10"N longitude and 39°09'11"E latitude at an altitude of 2200 meters above sea level. The mean annual rain fall of the site is 820 mm with an average annual temperature of 16.5°C.

Experimental materials and design

The experiment was laid out in an 8 x 5 simple lattice design with two replications. Materials used for study were forty nine (49) bread wheat genotypes introduced from International Maize and Wheat Improvement Center (CIMMYT) and one (1) released variety (Abay) presented in Table 1.

Data collected

Data were collected characters such as days to heading, days to maturity, grain filling period plant height, grain yield, hectoliter weight, thousand kernels weight, agronomic score, yellow rust and stem rust severity and reaction

Disease Scoring

To evaluate these genotypes for yellow and Leaf rust diseases scoring were made for both yellow and stem rust. Host responses to both rusts were recorded based on the modified Cobb scale (Peterson *et al.*, 1948). This scale combines several infection types; resistant (R), moderately resistant (MR), moderately susceptible (MS), moderately Resistant to Moderately Susceptible (MRMS) and susceptible (S). Severity was recorded on 0-100% scale where 0% was considered as immunity while 100% was completely susceptible.

Table1. Materials used in Study

GENOTYPE	PEDIGREE
EBW232340	NINGA #1
EBW232341	BORL14//BECARD/QUAIU #1
EBW232342	MUCUY*2//SUP152/BAJ #1
EBW232571	MUCUY*3//RL6077/AOC-YR
EBW232572	MISR 1//KACHU/KIRITATI
EBW232573	MUTUS*2//TECUE #1*2//KFA/2*KACHU
EBW232574	MUCUY*2/5//FRNCLN/4//WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1
EBW232575	KACHU/DANPHE/3/2*KACHU//KIRITATI/2*TRCH SHA7//PRL/VEE#6/3//FASAN/4//HAAS8446/2*FASAN/5//CBRD/KAUZ/6//MILAN/AMSEL/7//FRET2
EBW232576	*2//KUKUNA/8//KINGBIRD #1/9/2*BORL14
EBW232577	CIRO16*2//KINGBIRD #1//2*KABILU #1
EBW232578	T 2003 (CRE7)//MUNAL*2//WESTONIA/3//NADI#2 BORL14/5//WAXWING*2//KRONSTAD
EBW232579	F2004/3//TRCH/SRTU//KACHU/4//SAUAL/YANAC//SAUAL
EBW232580	KACHU/SAUAL/5//KACHU/3//WHEAR//2*PRL/2*PASTOR/4//BOKOTA
EBW232581	NADI#2//MUCUY
EBW232582	UP2338*2//VIVITSI/3//FRET2//TUKURU//FRET2/4//MISR 1/5//NADI
EBW232583	KAKURU/NADI#2 CROSBILL #1//DANPHE/7//CNDO/R143//ENTE/MEXI_2/3//AEGILOPS SQUARROSA
EBW232584	(TAUS)/4//WEAVER/5/2*KAUZ/6//PRL/2*PASTOR/8//NADI
EBW232585	PAURAQ/KENYA SUNBIRD//PAURAUQUE #1/3//BORL14 CROC_1/AE.SQUARROSA (205)//BORL95/3//PRL/SARA//TSI/VEE#5/4//FRET2/5//CIRO16/6//SWSR22T.B./2*BLOUK
EBW232586	#1//WBLL1*2//KURUKU
EBW232587	SOKOLL/3//PASTOR//HXL7573/2*BAU/4//BECARD/5//BORL14 BECARD*2/5//BAV92//IRENA/KAUZ/3//HUITES/4//DOLL/6//SW2148/2*ROLF07/3//HUW234+LR34
EBW232588	//PRINIA*2//SNLG FRANCOLIN #1/3//PBW343*2//KUKUNA*2//YANAC/4//KINGBIRD #1//INQALAB
EBW232589	91*2//TUKURU/5//CHIPAK ATTLA*2//PBW65/5//CNO79//PF70354//MUS/3//PASTOR/4//BAV92/6//KINGBIRD
EBW232590	#1/7//COPIO/8//BORL14 CROC_1/AE.SQUARROSA (205)//BORL95/3//PRL/SARA//TSI/VEE#5/4//FRET2/6//MTRWA92.161//PRINIA/5//SERI*3//RL6010/4
EBW232591	*YR/3//PASTOR/4//BAV92/7//BORL14
EBW232592	MUCUY*2//AMUR
EBW232593	Pavon 76, 20i ₁ ½i ₂ ½ + 7A.7S-L7//BORL14//KASUKO
EBW232594	BORL14*2/6//COPIO*2/5//UP2338*2//SHAMA/3//MILAN/KAUZ//CHIL/CHUM18/4//UP2338*2//SHA MA
EBW232595	UP2338*2//VIVITSI/3//FRET2//TUKURU//FRET2/4//MISR 1/5//BORL14/6//KASUKO
EBW232596	FRNCLN/3//ND643//2*PRL/2*PASTOR/4//FRANCOLIN #1*2/5//FRNCLN//NIINI #1//FRANCOLIN #1
EBW232597	MUTUS//ND643/2*WBLL1/3//BORL14/5//MUTUS/DANPHE #1/4//C80.1/3*BATAVIA//2*WBLL1/3//C80.1/3*QT4522//2*PASTOR
EBW232598	PASTOR/KAUZ/6//CNDO/R143//ENTE/MEXI_2/3//AEGILOPS SQUARROSA (TAUS)/4//WEAVER/5/2*KAUZ/7/2*PRL/2*PASTOR//PBW343*2//KUKUNA/8/2*BORL14
EBW232599	MUTUS*2//KIRITATI//BORL14/3//MOKUE #1 PASTOR//HXL7573/2*BAU/3//SOKOLL/WBLL1/4//HUW234+LR34//PRINIA//PBW343*2//KUKUN
EBW232600	A/3//ROLF07/5//WHEAR/SOKOLL/6//BORL14/7//KASUKO

EBW232601	KACHU/SAUAL/3/TACUPETO F2001/BRAMBLING//KIRITATI*2/4/FRET2/TUKURU//FRET2/3/MUNAL #1
EBW232602	KACHU/SAUAL/3/TACUPETO F2001/BRAMBLING//KIRITATI*2/4/FRET2/TUKURU//FRET2/3/MUNAL #1
EBW232603	FRANCOLIN #1/3/PBW343*2/KUKUNA*2//YANAC/4/KINGBIRD #1//INQALAB 91*2/TUKURU*2/5/MUCUY
EBW232604	OASIS/5*BORL95/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI/6/SOKOLL//SUNCO/2*PASTOR/7/SOKOLL//SUNCO/2*PASTOR*2/8/CROSBILL #1/DANPHE/7/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*KAUZ/6/PRL/2*PASTOR
EBW232605	SOKOLL/3/PASTOR//HXL7573/2*BAU*2/6/OASIS/5*BORL95/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI*2/7/FRANCOLIN #1/3/PBW343*2/KUKUNA*2//YANAC/4/KINGBIRD #1//INQALAB 91*2/TUKURU
EBW232606	SOKOLL/3/PASTOR//HXL7573/2*BAU*2/6/OASIS/5*BORL95/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI*2/7/BORL14
EBW232607	SITE/MO//PASTOR/3/TILHI/4/WAXWING/KIRITATI/5/KACHU #1/KIRITATI//KACHU*2/6/KACHU/SAUAL*2//COPIO
EBW232608	SITE/MO//PASTOR/3/TILHI/4/WAXWING/KIRITATI/5/KACHU #1/KIRITATI//KACHU*2/6/KACHU/SAUAL*2//COPIO
EBW232609	SWSR22T.B./5/KAUZ//ALTAR 84/AOS/3/KAUZ/4/SW94.15464/6/2*PRL/2*PASTOR/7/BABE/8/VILLA JUAREZ F2009/DANPHE #1
EBW232610	BABE/2*BORL14
EBW232611	YR57#5474-6/3*BORL14
EBW232612	YR57#5474-6/3*BORL14
EBW232613	Pavon 76, 20i _c ½i _c ½ + 1R.1D5+10-2(1D)/BORL14//2*KASUKO
EBW232614	Pavon 76, 20i _c ½i _c ½ + 1R.1D5+10-2(1D)/3*MUCUY
EBW232615	SERI/T.DICOCCON PI94623/AE.SQUARROSA (1027)/3/MUCUY/4/MUTUS*2/HARIL #1/5/MUCUY
EBW232616	IRAGI/3*NADI#2
ETBW9396	ABAY

3. Results and Discussion

3.1 Analysis of Variance (ANOVA)

Analysis of variance (ANOVA) revealed significant genetic variation ($p < 0.01$) among the 50 wheat genotypes for eight of the ten characters studied. These characters included days to 50% heading, days to 90% maturity, grain filling period, grain yield, hectoliter weight, agronomic score, yellow rust severity, and stem rust severity. Plant height and thousand kernel weight also displayed significant variation ($p < 0.05$) among the genotypes. This finding aligns with previous research by Alemu et al. (2017), Wani et al. (2018), Semahegn et al. (2021), and Getachew et al. (2021), who also reported significant genetic variability among wheat genotypes for days to heading, maturity, plant height, grain filling period, thousand kernel weight, and grain yield.

Table 2: Mean squares, coefficient of variation and R- square for 10 traits of 50 bread wheat genotypes evaluated at Kulumsa Agricultural Research center in 2023.

Traits	Replication (DF=1)	Block (Replication) (DF=8)	Genotype MS (DF=49)	Error MS (DF=41)	CV (%)	R ²
DTH	34.81	2.81	23.68**	1.10	1.56	0.96
DTM	92.16	5.87	17.65**	3.85	1.7	0.86
GFP	0.97	13.69	11.66**	3.73	4.10	0.80
PHT	676.00	38.50	50.43*	26.00	6.17	0.76
AgrSc	0.32	0.09	0.67**	0.18	21.00	0.83
TKW	1.96	42.26	38.61*	20.93	14.23	0.73
HLW	7.75	4.63	10.00**	3.27	2.61	0.80

YrS	1332.25	45.85	11.20**	38.08	19.11	0.97
SrS	7.84	8.32	283.55**	3.60	21.43	0.84
GYLD	92659.36	82061.22	249445.71**	37670.70	15.86	0.89

Note. * and ** indicates significant at (5%) and highly significant at (1%) probability levels respectively. DTH: days to heading; DTM: days to Maturity; GFP: grain filling period; PHT: plant height; AgrSc: agronomic score; TKW: thousand kernel weight; HLW; hectoliter weight; YrS: yellow rust severity; SrS: stem rust severity; GYLD; grain yield; MS: mean square; CV: coefficient of variations; DF: degree of freedom and R²: Coefficient of determination

3.2 Mean Performance of studied Bread wheat Genotypes

This section details the variation observed among 50 wheat genotypes for several key traits (Table 3).

Days to 50% heading: This ranged from 61 to 77 days, with an average of 67.21 days. A nearly even distribution was observed, with 29 genotypes (58%) head earlier and 21 genotypes (42%) heading later than the average. Notably, four genotypes (EBW232612, EBW232594, EBW232341, and EBW232581) head significantly earlier than the check variety Abay (EBW9396), which heads at 62.5 days.

Days to 90% Maturity: This ranged from 108 to 121 days, with an average of 114.94 days. Here, a higher proportion (64%) matured later than the average (18 genotypes matured earlier and 32 matured later). Only one genotype (EBW232581) reached maturity earlier (108 days) than the check variety Abay (109.5 days). All others matured later.

Thousand Kernel Weight: This ranged widely, from 18.0 g to 41.0 g, with an average of 32.14 g. Nearly half (48%) of the genotypes had lower than average kernel weight, while the remaining genotypes (52%) had higher values. Notably, 12 genotypes had lower weight than the check variety Abay (31.0 g), while 7 genotypes matched the check. However, a promising aspect is that 30 genotypes exhibited superior thousand kernel weight compared to Abay.

Hectoliter Weight: This ranged from 64.07 to 73.94 hL/kg, with an average of 69.30 hL/kg. Here, 40% of the genotypes had lower than average hectoliter weight, while 60% had higher values. Compared to the check variety Abay (68.79 hL/kg), 18 genotypes had lower hectoliter weight, but a positive sign is that 31 genotypes displayed superior values.

Agronomic Score: This ranged from 1.0 to 3.25, with an average of 2.02. Interestingly, 60% of the genotypes had a lower score (indicating potentially better agronomic performance) than the average, while 40% had a higher score. However, compared to the check variety Abay (2.75), a high proportion (41 genotypes) had a lower score, suggesting potentially better agronomic traits. Only four genotypes had scores equal to the check, and a small number (4%) even surpassed Abay.

Grain Yield: This exhibited the widest range, varying from 504 to 1865 kg/ha, with an average of 1223.8 kg/ha. A slight majority (56%) yielded less than the average, while the remaining genotypes (44%) produced more. Encouragingly, seven genotypes yielded lower than the check variety Abay (850 kg/ha), but all others exhibited superior grain yield compared to Abay.

Generally, the study revealed substantial genetic diversity among the wheat genotypes for all measured traits. There's potential for breeding efforts to target earlier heading combined with faster maturity, improved kernel weight, and even higher grain yield. Many genotypes displayed superior performance compared to the check variety Abay, highlighting the potential for breeding advancements. These findings are consistent with previous research by Gezahegn et al. (2015), Naik et al. (2015), Alemu et al. (2017), and Muhammad et al. (2021), who also reported significant variations in grain yield and related traits among bread wheat genotypes.

Table 3: Minimum and Maximum values with their corresponding genotype, Ranges, means and standard errors of the means for 8 quantitative traits among 50 bread wheat genotypes tested at Agricultural Research center in 2023.

Traits	Minimum		Maximum		Range	Mean	Standard Error
	Values	Genotype	Values	Genotype			
DTH	61.00	EBW232612	77.00	EBW232593	61.00-77.00	67.21	0.83
DTM	108.00	EBW232581	121.00	EBW232593	108.00-121.00	114.94	1.45
GFP	40.50	EBW232609	51.00	EBW232591	40.50-51.00	47.73	1.28

PHT	70.00	EBW232590	92.50	EBW232583	70.00-92.50	82.70	3.74
AgrSc	1.00	EBW232590	3.25	EBW232599	1.00-3.25	2.02	0.32
TKW	18.00	EBW232613	41.00	EBW232599	18.00-41.00	32.14	3.49
HLW	64.07	EBW232606	73.94	EBW232578	64.07-73.94	69.30	1.32
GYLD	504	EBW232590	1865	EBW232342	504.00-1865.00	1223.80	149.86

Note. DTH: days to heading; DTM: days to Maturity; GFP: grain filling period; PHT; plant height; AgrSc: agronomic score; TKW: thousand kernel weight; HLW; hectoliter weight; GYLD; grain yield

3.3 Cluster analysis

Cluster analysis was conducted following the agglomerative hierarchical clustering ward's method, in order to categorize genotypes into different homogeneous groups. Cluster analysis classified the 50 bread wheat genotypes into five distinct clusters (Table 4). This indicated the presence of diversity among the tested bread wheat genotypes. Cluster III was the largest cluster which consisted of 22 genotypes (44% of the studied genotypes) followed by cluster IV and V contained 9 genotypes (18% of the studied genotypes), cluster I consisted of 6 genotypes (12% of studied genotypes) and cluster II had 4 genotypes (8% of the studied genotypes). Meanwhile cluster II had the lowest number of genotypes that comprises 4 genotypes (8% of the studied genotypes).

Similarly clustering of wheat genotypes were studied by different researchers, Zemedu et al., (2019) reported that 64 wheat genotypes grouped into five distinct clusters with cluster-I contain 7 genotypes, cluster-II contain 4 genotypes, cluster-III contain 35 genotypes cluster-IV contain 8 genotypes and cluster-V contain 10 genotypes. Fikre et al., (2015) observed 64 genotypes were grouped into nine distinct clusters, Kumar et al., (2016) also reported the 55 wheat genotypes were grouped into eight clusters and 50 wheat genotypes were grouped into eight clusters (Poudel et al., 2017). 127 wheat genotypes clustered into four distinct groups (Mohi-Ud-Din et al., 2021) and 25 bread wheat genotypes clustered into four diverse groups (Adilova et al., 2020).

Table 4: Distribution of 50 bread wheat genotypes in to five different cluster groups

cluster No	No of genotypes	Percentage (%)	Genotypes
I	6	12	EBW232572, EBW232576, EBW232590, EBW232594, EBW232587, EBW232597
II	4	8	ETBW9396, EBW232588, EBW232606, EBW232616
III	22	44	EBW232341, EBW232340, EBW232573, EBW232585, EBW232579, EBW232582, EBW232581, EBW232577, EBW232593, EBW232591, EBW232596, EBW232604, EBW232602, EBW232600, EBW232601, EBW232603, EBW232598, EBW232607, EBW232610, EBW232608, EBW232611, EBW232615
IV	9	18	EBW232571, EBW232583, EBW232584, EBW232586, EBW232595, EBW232589, EBW232605, EBW232612, EBW232609
V	9	18	EBW232574, EBW232575, EBW232342, EBW232580, EBW232578, EBW232592, EBW232599, EBW232614, EBW232613

3.3.1 Analysis of inter and intra cluster distance

The analysis of intra- and inter-cluster distances revealed significant genetic variation among the wheat genotypes. All inter-cluster distances were found to be highly significant ($p < 0.01$) compared to the chi-square value ($\chi^2 = 23.21$), and these distances were greater than the intra-cluster distances (Table 5). Among the clusters, Cluster I exhibited the smallest intra-cluster distance ($D^2 = 40.32$), followed by Cluster II ($D^2 = 46.28$). The largest intra-cluster distance was observed in Cluster IV ($D^2 = 66.70$), with Cluster III showing the second largest ($D^2 = 60.40$).

Regarding inter-cluster distances, the greatest genetic distance was recorded between Cluster I and Cluster V ($D^2 = 1156.59$), followed by Cluster II and Cluster V ($D^2 = 895.39$). On the other hand, the smallest inter-cluster distance was found between Cluster I and Cluster II ($D^2 = 263.51$), followed closely by Cluster III and Cluster IV ($D^2 = 272.00$).

This analysis underscores the importance of genetic distance in assessing diversity among genotypes. In genetic studies, the distance between clusters represents the degree of variation or similarity between groups of genotypes. Larger inter-cluster distances indicate a greater genetic difference between the clusters, which reflects wider genetic diversity among the genotypes they contain. When clusters exhibit large genetic distances from one another, it suggests that the genotypes in these clusters have accumulated a variety of genetic differences over time. This variation is valuable in plant breeding because it creates opportunities for introducing new traits and increasing genetic variability in offspring. The wider the genetic diversity between genotypes, the greater the potential for producing novel, advantageous combinations of traits when these genotypes are crossed.

Consequently, the largest inter-cluster distances in this analysis point to clusters with highly diverse genotypes. Crosses between genotypes from these distant clusters are more likely to result in superior genetic recombinants, offspring that inherit beneficial traits from both parents, potentially leading to improved yield, disease resistance, or other desirable agronomic traits. This makes these distant clusters ideal candidates for crossing in breeding programs aimed at enhancing genetic gain and achieving higher crop performance.

In contrast, the smallest inter-cluster distances indicate lower genetic diversity between the clusters, with the genotypes being relatively similar. As a result, crossing genotypes from closely related clusters is less likely to produce genetically diverse recombinants and is generally not recommended (Allard, 1960).

Fikre et al., (2015) reported 64 bread wheat genotypes clustered in to nine clusters in which Maximum inter cluster distance was observed between cluster I and IX ($D^2=5112.1$), followed by that between clusters III and IX ($D^2=4694.4$) and VIII and IX ($D^2=3871.9$) which had shown they were genetically more divergent from each other than any other clusters. Crosses between genotypes selected from cluster I with cluster IX, cluster III with cluster IX and cluster VIII with cluster IX are expected to produce relatively better genetic recombination and segregation in their progenies. And 55 wheat genotypes clustered into eight different clusters in which the maximum inter cluster distance was observed between cluster VI and cluster VIII followed by cluster VI and VI which exhibited high degree of genetic diversity. The minimum intra cluster distance was observed within cluster VI and cluster III followed by cluster I and VIII, which exhibited less genetic diversity and thus may be utilized under population improvement of wheat genotypes (Kumar et al., 2016).

Thirty wheat genotypes clustered into seven clusters in which the maximum intra cluster distance observed in cluster III (162.58) followed by cluster II (102.15) and minimum in cluster IV-VII and maximum inter cluster distance found between cluster VI and VII ($D^2= 1627.88$) followed by cluster V and cluster VII ($D^2= 1270.68$) and between cluster IV and VI ($D^2= 943.23$) and significant difference observed between all inter cluster distances (Kumar et al., 2013).

In general, crossing between genotypes from this significant inter clusters distance will be enhancing the yield and chances of getting better recombinants in segregating generations might be utilized in hybridization in wheat breeding program (Fikre et al., 2015, Koshraj et al., 2019, Fouad, 2020, Adilova et al., 2020).

Table :5 Intra (Bold diagonal) cluster distance and Inter (off diagonal) cluster distance of 50 bread wheat genotypes

Cluster	1	2	3	4	5
1	40.32	263.51**	585.83**	857.74**	1156.59**
2		46.28	325.20**	596.37**	895.39**
3			60.41	272.00**	570.83**
4				66.70	299.35**
5					57.43

Note. * and ** indicates significant at (5%) =18.31 and highly significant at (1%) =23.21 probability levels respectively, ns=non-significant

3.3.2. Cluster mean analysis

The mean values of five clusters for 10 different traits revealed considerable differences among the clusters of 50 bread wheat genotypes (table 6). For most traits genotypes grouped in cluster V had the maximum cluster mean values and except for plant height (second maximum for plant height) and genotypes grouped in cluster V were the best resistance for both yellow and stem rust diseases (first best resistance for yellow rust and second best resistance for stem rust), this indicated that genotypes in cluster V were the late maturity genotypes (116.11 days to 90% matured), the highest agronomic score (2.6), thousand kernel weight (34.56 g), hectolitre weight (71.73), the highest grain yield (1738.56 kg/ha). While genotypes in cluster I characterized by minimum cluster mean values for all characters except day to 50% heading and days to 90% maturity, grain filling period, and genotypes grouped in this cluster (cluster I) showed the most susceptible for yellow rust which scored the maximum value of yellow rust severity, but the genotypes were showed the best resistance for stem rust. Genotypes assigned in cluster II had the minimum cluster mean values for grain filling period, the second minimum cluster mean values for plant height, agronomic score, thousand kernel weight, hectolitre weight, grain yield, and the second most susceptible for stem rust.

The cluster mean analysis indicated that genotypes in cluster III had the second maximum value for days to 50% heading, days to 90% maturity, grain filling period and the most susceptible genotypes for yellow rust. Genotypes grouped in cluster IV characterized the tallest in plant height, the second maximum value for agronomic score, thousand kernel weight, hectolitre weight, grain yield the most susceptible genotypes for stem rust.

Fikre et al., (2015) reported 64 bread wheat genotypes clustered in to nine clusters in which Genotypes in cluster IX were characterized by late maturity (125.50 days), highest 1000 kernel weight (34.06g/plot), highest grain yield (5,322.5kg/ha). Cluster I exhibited the lowest values of 1000 kernel weight (22.83g). Cluster V was characterized by the highest values of hectoliter weight (70.41) and harvest index (29.49). Cluster IV was characterized by highest value of days to 50% heading (67.25). Genotypes in cluster VII showed the highest spike length (9.04cm).

Adilova et al., (2020) reported the cluster analysis revealed the classification of 25 accessions into four diverse groups. The maximum average values for grain yield (742.2 kg/ha), biomass (1756.7 kg/ha), and grains per spike (45.3) were higher in cluster III compared to other clusters. Cluster IV exhibited the maximum thousand-kernel weight (46.6)

Zemede et al., (2019) reported that 64 wheat genotypes grouped into five clusters in which the first cluster genotypes had highest in grain yield associated to high number of kernels and long spike. The second cluster genotypes produced relatively low grain yield and biomass yield. Ten genotypes were found in cluster V which had shortest in their height associated with low biomass yield and high harvest index.

Poudel et al., (2017) Studed 50 bread wheat genotypes clustered in to four groups in which cluster I genotypes having highest value of thousand kernel weight, plant height and grain yield, Cluster 2 had the highest value of days to heading and lowest yield in comparison to other clusters. This cluster had shown moderate value of plant height, number of grains per spike and days to maturity. Cluster III showed moderate values for all traits and cluster IV genotypes had the highest number of grains per spike. Ali et al., (2008) The mean number of effective tillers per plant (14.89), number of spikelets per spike (22.20), spike length (11.49), number of grains per spike (73.67) and yield per plant (31.23) were highest in cluster I. The plant height (113.18) and 1000 grain weight (48.55) were maximum in cluster II.

Koshraj et al., (2019) had clustered 30 genotypes into five clusters. Cluster I represented highest, plant height, spike length, peduncle length and yield per spike. Cluster II had highest grain yield and other yield attributing traits. Yadav et al., (2014) clustered 50 wheat genotypes in to eight clusters in which cluster IV (119.75) is characterized by highest mean performance for days to 50 heading (96.50 days), days to maturity (134.50 days), spike length (12.38) and lowest for plant height (81.00) followed by cluster I with cluster mean values highest for plant height (95.90), cluster VIII with highest cluster mean values for thousand kernel weight (56.58 g), while cluster VI had highest cluster mean values for grains per spike (56.70) and remaining cluster VII, cluster II, cluster V and cluster III were characterised average mean values for all traits.

Tabl2 6: the five clusters mean for 10 traits of 50 tested bread wheat genotypes

Class	DTH	DTM	GFP	PHT	AgrSc	TKW	HLW	GYLD	YrS	SrS
I	66.58	114.33	47.75	78.75	1.13	25.17	66.01	583.92	76.67	4.33
II	67.00	113.63	46.63	82.50	1.81	29.25	67.42	843.50	32.50	12.50
III	67.66	115.41	47.75	82.84	1.96	32.96	69.33	1168.55	40.73	8.91
IV	66.06	113.61	47.56	84.44	2.25	33.67	69.84	1439.72	21.78	17.78
V	67.78	116.11	48.33	83.33	2.64	34.56	71.73	1738.56	10.67	4.67

Note. DTH: days to heading; DTM: days to Maturity; GFP: grain filling period; PHT; plant height; AgrSc: agronomic score; TKW: thousand kernel weight; HLW; hectoliter weight; GYLD; grain yield

Principal Component Analysis

The results of principal component analysis for ten traits of 50 bread wheat genotypes are presented in Table 7 and figure1: Scree plot. The first three principal components (PCs) accounted 73.45% of the total observed variation. Among the three principal components the first principal component (PC) contributed for 35.73% of the total variation with the eigenvalue of 3.57. This was due to the positive loading effect of all studied traits except yellow rust and stem rust, mainly grain yield (0.48), agronomic score (0.46), hectolitre weight (0.46), thousand kernel weight (0.41) and yellow rust (-0.38).

The second principal component shared about 21.83% of the total variation with the eigenvalue of 2.18 and the major contributing characters were days to heading (0.64), days to maturity (0.53) and plant height (-0.36). The third principal component had proportion variability of 15.89% from the total variation with the eigenvalue of 1.59. The main loading effect of days to maturity (0.35), grain filling period (0.65), yellow rust (0.43) and stem rust (-0.45).

Wani et al., (2018) studied on twenty-four bread wheat genotypes for principal component analysis the first three principal components based on the Euclidean similarity matrix explained 76.67 percent of the total variation and had eigenvalue greater than one (eigenvalue < 1) which is PC1, PC2 and PC3 had eigenvalue of 12.32, 1.25 and 1.03 respectively. It is revealed that days to heading, biological yield, and grain yield contribute significantly to the quantum of variation as explained by principal component 1 (PCA1) and It was further observed that days to maturity, plant height and thousand grain weight are the main components of the PCA2.

Fikre et al., (2015) reported for six principal components having Eigenvalue greater than one (significant), accounted 77.20% of the total phenotypic variation. Characters having relatively higher value in the principal component (PC1), like grain yield, harvest index, thousand kernel weight and hectoliter weight. Variation in second principal component (PC2) was chiefly due to days to 50% heading, followed by days to maturity, number of productive tillers. Traits such as plant height, biomass yield per plot and spike length were the major contributors of variation in the third principal component (PC3). Variation in the fourth principal component (PC4) was mainly due to number of spikelets per spike, number of productive tillers per plant, days to maturity. Characters having relatively higher value in the principal component PC5 like plant height, biomass yield, number of spikelets per spike and grain yield had more contribution to the total variation.

Kumar et al., (2016) study on principal component analysis the first six principal components showed Eigen values more than one and explained 81.75% variability. The first principal component explained 20.83% of the total variation and the second and third principal components explained 19.428% and 10.39% variation, respectively. And Beheshtizadeh et al., (2013) Had studied on Principal component analysis indicated that only four first components, which account for 76% of the total variance are important. The first principal component, which accounted for about 38% of the variation, was strongly associated with head weight, number of seed per spike, yield per spike. The second principal component, which accounted for about 15% of the total variation, consisted of days to tillering and plant height. The third principal component was positively correlated with thousand kernel weight and number tillers per plant accounted for 12% of the variation. The fourth principal component, accounted for 11% of the variation. In this component, correlation of yield per spike and its components were highly positive.

Table 7: Principal component analysis for ten traits of Three components of 50 bread wheat genotypes evaluated at Kulumsa Agricultural Research Center in 2023

TRAITS	PC1	PC2	PC3
DTH	0.02	0.64	-0.15
DTM	0.08	0.53	0.35
GFP	0.08	-0.25	0.65
PHT	0.18	-0.36	0.08
AgrSc	0.46	0.05	0.02
TKW	0.41	-0.07	0.02
HLW	0.46	-0.02	0.19

GYLD	0.48	-0.01	-0.09
YrS	-0.38	-0.10	0.43
SrS	-0.03	-0.32	-0.45
Eigenvalue	3.57	2.18	1.59
Proportion (%)	35.73	21.83	15.89
Cumulative (%)	35.73	57.56	73.45

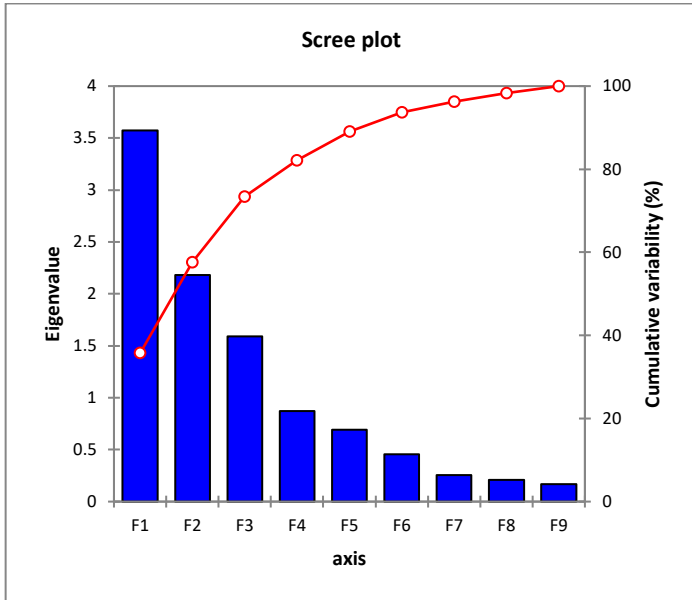


Figure1: Scree plot for 10 traits of 50 bread wheat genotypes

3.5 Diseases

The final rust severity for yellow rust severity showed from immune (0) to 80 %. Based on final rust severity, for yellow rust 24 genotypes (48% of the studied genotypes) showed high adult plant resistance (0-30% of severity), 14 genotypes (28% of studied genotypes) observed as medium resistance (31- 50% of severity) and 12 genotypes (24% of studied genotypes) observed as low adult plant resistance (>51% of yellow rust severity) (table 8). The result line up with Pathan and Park, (2006) who categorized the level of adult plant resistance such as high, medium and low APR based on their severity percentage.

Tested genotypes showed diverse reactions for yellow rust ranging from resistant(R) to susceptible (S) responses. 2 genotypes (4% of the studied genotypes) were observed resistant(R), and 5 genotypes (10% of the studied genotypes) were showed moderately resistant to moderately susceptible (MRMS) reaction type. 13 genotypes (26% of the studied genotypes) were displayed moderately resistance (MR) reaction, 5 genotypes (10 % of the studied genotypes) were showed moderately susceptible (MS) reaction and 25 genotypes (50 % of the studied genotypes) were observed susceptible (S) reactions for yellow rust response (table 8). Similar work also done by different researchers such as Ayele *et al.*, (2021); Bayisa *et al.*,(2023); Shewaye *et al.*,(2021) and Mohammadi *et al.*,(2023), Zewdu and Yimam, (2024), Zewdu *et al.*,(2024) who reported a range of severity and reactions of yellow rust disease observed on Bread wheat genotypes.

The final rust severity for stem rust severity showed from immune (0) to 50 %. Based on final rust severity percentage, for stem rust 41 genotypes (82 % of the studied genotypes) showed high adult plant resistance (0-20 % of severity), 9 genotypes (18 % of studied genotypes) observed as medium adult plant resistance (21-50 % of severity). The result line up with Pathan and Park, (2006) who categorized the level of adult plant resistance such as high, medium and low APR based on their severity percentage.

Tested genotypes showed diverse reactions for stem rust ranging from immune (0) to susceptible (S) responses. 13 genotypes (26 % of the studied genotypes) were observed immune (0), 2 genotypes (4 % of the studied genotypes) were observed moderately resistant (MR), 7 genotypes (14 % of the studied genotypes) were observed moderately resistant to

moderately susceptible (MRMS) reaction type, 17 genotypes (34 % of the studied genotypes) were displayed moderately susceptible (MS) reaction, and 11 genotypes (22 % of the studied genotypes) were showed susceptible (S) reaction for stem rust disease response (table 2). Similar work also done by different researchers such as Shiferaw *et al.*,(2020) and Shewaye *et al.*,(2021), Zewdu and Yimam, (2024) who reported a range of severity and reactions of stem rust disease observed on Bread wheat genotypes.

Table.8: Studied genotypes yellow and stem rust severity and reactions

Entry	Genotype	YrS	YrR	SrS	SrR	Entry	Genotype	YrS	YrR	SrS	SrR
1	ETBW9396	10	MS	15	MS	26	EBW232592	30	MS	5	MS
2	EBW232340	60	S	5	MRMS	27	EBW232593	15	MRMS	0	0
3	EBW232341	70	S	5	MS	28	EBW232594	80	S	5	MRMS
4	EBW232342	1	MR	5	MRMS	29	EBW232595	30	MR	0	0
5	EBW232571	5	MRMS	5	MR	30	EBW232596	10	MRMS	10	MS
6	EBW232572	70	S	5	MS	31	EBW232597	80	S	5	MS
7	EBW232573	10	MR	1	0	32	EBW232598	30	MS	30	S
8	EBW232574	5	MR	10	MS	33	EBW232599	5	MR	1	MRMS
9	EBW232575	5	MR	0	0	34	EBW232600	10	MR	1	MR
10	EBW232576	70	S	10	S	35	EBW232601	50	S	5	MS
11	EBW232577	50	S	20	MS	36	EBW232602	50	S	1	MS
12	EBW232578	5	MR	1	MRMS	37	EBW232603	40	S	5	MS
13	EBW232579	50	S	1	0	38	EBW232604	40	S	0	0
14	EBW232580	30	MRMS	5	MS	39	EBW232605	5	MRMS	30	S
15	EBW232581	50	S	30	S	40	EBW232606	10	MR	5	MS
16	EBW232582	40	MS	30	S	41	EBW232607	60	S	0	0
17	EBW232583	50	S	30	S	42	EBW232608	50	S	5	MRMS
18	EBW232584	30	MS	5	MS	43	EBW232609	5	MR	30	S
19	EBW232585	60	S	1	0	44	EBW232610	50	S	1	0
20	EBW232586	30	MR	0		45	EBW232611	1	MR	30	S
21	EBW232587	80	S	0	0	46	EBW232612	1	MR	50	S
22	EBW232588	50	S	0	0	47	EBW232613	10	R	10	MRMS
23	EBW232589	40	S	10	MS	48	EBW232614	5	R	5	MS
24	EBW232590	80	S	1	0	49	EBW232615	60	S	10	MS
25	EBW232591	40	S	5	S	50	EBW232616	60	S	30	S

Note: MR: Moderately Resistance; MS: Moderately Susceptible; MRMS: moderately Resistant to Moderately Susceptible; S: Susceptible; R: Resistance

4. Conclusion

The analysis of variance (ANOVA) conducted on 50 wheat genotypes revealed significant genetic variability ($p < 0.01$) for eight of the ten studied traits, including days to 50% heading, days to 90% maturity, grain yield, and rust disease severity. Plant height and thousand kernel weight also exhibited significant variation ($p < 0.05$). This indicates the presence of considerable genetic diversity among the wheat genotypes.

The mean performance of the studied wheat genotypes showcased a wide range of values for traits like days to heading, days to maturity, thousand kernel weight, and grain yield. For instance, days to 50% heading ranged from 61 to 77 days, with four genotypes heading significantly earlier than the check variety Abay. Similarly, thousand kernel weight varied from 18.0 g to 41.0 g, with 30 genotypes surpassing the check variety. Grain yield ranged widely, with some genotypes outperforming the check variety Abay, indicating promising candidates for breeding programs.

Cluster analysis grouped the genotypes into five distinct clusters, indicating diversity within the wheat population. Cluster III, with 22 genotypes, was the largest, while Cluster II had the fewest genotypes. The inter- and intra-cluster distance analysis revealed significant genetic differences between clusters, with the greatest genetic distance observed between Cluster I and Cluster V ($D^2 = 1156.59$). Such large distances between clusters suggest that crossing genotypes from these clusters could lead to beneficial recombinants with improved agronomic traits.

Principal component analysis (PCA) explained 73.45% of the total variation among the genotypes, with the first three principal components contributing the most. Traits like grain yield, agronomic score, and thousand kernel weight contributed significantly to the variation observed in PC1. This demonstrates the potential for selecting genotypes with superior performance for these traits.

Finally, disease resistance analysis showed that 48% of the genotypes exhibited high adult plant resistance to yellow rust, and 82% displayed strong resistance to stem rust. This high level of resistance highlights the potential for breeding wheat genotypes that are both high-yielding and disease-resistant. The findings emphasize the importance of genetic diversity in enhancing wheat breeding programs.

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6. Conflict of Interest

The authors have declared that no conflict of interest exists

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