

# **Deciphering Genetic Diversity in Advanced Wheat Lines (*Triticum aestivum* L.) for Yield and Other Yield Contributing Traits across the Locations**

## **Abstract**

This study aimed to explore the genetic diversity among 20 wheat genotypes using principal component analysis (PCA) and cluster analysis, providing a comprehensive understanding of the present genetic variations. In this study, the agro-morphological characteristics of the genotypes were evaluated across two distinct locations using a randomized complete block design (RCBD) with three replications. Three different clusters were formed, with the maximum number of genotypes occurring in the second cluster. A lower p-value indicates that the clusters are statistically significant, suggesting that the observed diversity is not random. High estimates of cophenetic distance (.79) specify a high genetic distance between clusters, indicating that there is diverse genetic material under study. The maximum genetic distance observed was 144.9 between genotypes 5 and 11. These findings suggest that these two genotypes are the most genetically diverse among all the studied genotypes and can be used as parents to develop high genetic variation in the studied traits. The PCA results yielded 18 principal components, with the first seven components accounting for approximately 83% of the total variance, indicating significant genetic diversity. The scree plot affirmed the robustness of the study's results by suggesting that the first eight principal components accounted for a substantial portion of the variance. These genetic distances between genotypes provide valuable insights for crop improvement, aiding in the development of more effective breeding strategies. Therefore, PCA offers valuable insights into the genetic diversity of wheat lines, which could be beneficial for future breeding programs.

**Key words:** Wheat, Genetic diversity, PCA, Cluster and cophenetic distance

## **1. INTRODUCTION**

Wheat (*Triticum aestivum* L.) is a globally significant crop that contributes substantially to food security worldwide, particularly in India. It thrives in warm temperature regions, with temperatures ranging from 21 to 24°C. As the most widely cultivated crop in the world, wheat is cultivated on 220 million hectares annually, yielding a total production of 788.5 million tonnes (FAO, 2023). World wheat production has been lifted by 1.4 million tonnes and now stands at 788.5 million tonnes in 2023

Wheat plants serve as a staple food in many cultures and provide approximately 20% of the total dietary calories and proteins worldwide (Leiva, 2022). Its economic importance is immense, as it serves as

a staple food for millions of people. Genetic diversity is of paramount importance in crop improvement. This approach provides a pool of variation from which plant breeders can select traits that enhance crop yield, disease resistance, and environmental adaptability. However, the use of breeding lines with narrow genetic bases can lead to significant losses (Kumar et al., 2022). Cultivating germplasm with a narrow genetic base carries a risk of genetic vulnerability. Mutations in pest populations or changes in environmental conditions can introduce stresses that the cultivar cannot cope with, potentially leading to severe crop losses. Therefore, maintaining and utilizing a broad genetic base in crop breeding is crucial for sustainable agriculture and food security.

Different algorithmic methods, such as multidimensional scaling, clustering, principal component analysis, and principal coordinate analysis, are currently employed in the assessment of genetic diversity (Rohlf, 1988; Thompson et al., 1998; Melchinger, 1993; Brown-Guedira et al., 2000). Principal component analysis (PCA), a dimensionality reduction technique introduced by Karl Pearson in 1901, identifies the largest variations in the data. Cluster analysis, a method of grouping objects based on similarity, is often used in conjunction with PCA. These techniques play a pivotal role in crop improvement strategies, including in wheat, by determining germplasm variability. These methods assist in identifying lines for desirable traits, segregating progenies with maximum genetic variability for further selection, and introducing desirable genes from diverse germplasms into the available genetic base. These techniques ensure continued improvement in plant selection programs and have been used to study the genetic diversity and relationships of wheat genotypes, which is crucial for planning crosses, assigning lines to specific heterotic groups, and precisely identifying plant varietal protection (Govindaraj et al., 2015; Khodadadi et al., 2011; Szczepanik et al., 2021; Bhandari et al., 2007).

The significance of genetic diversity in wheat is well documented (Gruet et al., 2022; Khan et al., 2015; Yadav et al., 2014). The genetic variability among plants dictates their potential for improving efficiency and consequently, their suitability for breeding programs, which could ultimately result in increased food production (Zedfar and Golabadi, 2013). This diversity paves the way for plant breeders to cultivate improved varieties with desirable traits such as high yield potential, large grains, and resistance to biotic and abiotic stresses (Atsbeha et al., 2023). Therefore, the exploration and utilization of genetic diversity in wheat genetic resources are vital for sustainable production. Genetic diversity is a cornerstone of crop breeding because it augments yield potential by integrating desirable traits from diverse parents (Joshi et al., 2023; Chaudhary et al., 2022; Elahi et al., 2021). Moreover, a diverse genetic base endows crops with enhanced resilience against climatic changes, thereby ensuring sustainable agricultural production amidst environmental uncertainties. This resilience has gained particular importance as climate change poses an increasing threat to agricultural production (Joshi et al., 2023). In this study, we employed cluster analysis and principal component analysis (PCA) to probe the genetic diversity of advanced wheat lines, focusing on their agronomic traits. Our research aimed to identify elite wheat breeding lines, thereby enriching the understanding of wheat genetic diversity and paving the way for future advancements in wheat breeding.

## **EXPERIMENTAL DETAILS**

With a completely randomized block design (RBD), twenty advanced wheat genotypes were assessed with three replications across six field trials during two cropping seasons, Rabi 2020-21 and 2021-22. These trials took place at the N.E. Borlaug Crop Research Centre (NEBCRC), G.B. Pant University of Agriculture and Technology, Pantnagar, District U.S. Nagar, and another location, the Agriculture Research Center Majhera, Nainital, Uttarakhand.

The plants of each genotype were planted in a 4 row plot, each of which was 4 meters long, with rows spaced 20 cm apart. All recommended wheat cultivation practices were followed for a healthy crop. Eight

quantitative traits were observed from five randomly selected plants in each entry. These traits included flag leaf length (FLL), flag leaf width (FLW), exposed peduncle length (EPL), total peduncle length (TPL), spike length (SL), awn length (AL), plant height (PH), and spikelets per spike (SPS). On a plot basis, observations were recorded for germination percentage (GP), seedling vigor (SV), days to anthesis (DA), days to heading (DH), and days to maturity (DM). The total number of productive tillers (TPM) counted from area of one meter in row. 1000-grain weight (TGW) and yield per plot (YPP) were recorded from clean harvest. Brown and yellow rusts were observed as described by Peterson et al. (1948).

Cluster analysis was performed using the unweighted pair group method with arithmetic mean (UPGMA), as proposed by Sokal and Michener (1958), to assemble a phylogenetic tree from a distance matrix. Genetic distances between the genotypes were calculated using the Euclidean method (Gower, 1985).

Principal component analysis (PCA), a statistical tool commonly used in plant breeding to identify trends in multidimensional data (Pearson, 1901 and Hotelling, 1933), was employed. This approach aids in studying the morphological characteristics of germplasm, assessing population differences and breeding potential and reducing data redundancy (Khodadadi et al. 2011; Sewell 2008). PCA and cluster analysis, as well as visualization, were performed using R script (Mojena, 1977; Kassambara, 2020; Le et al. 2008; Husson et al. 2017).

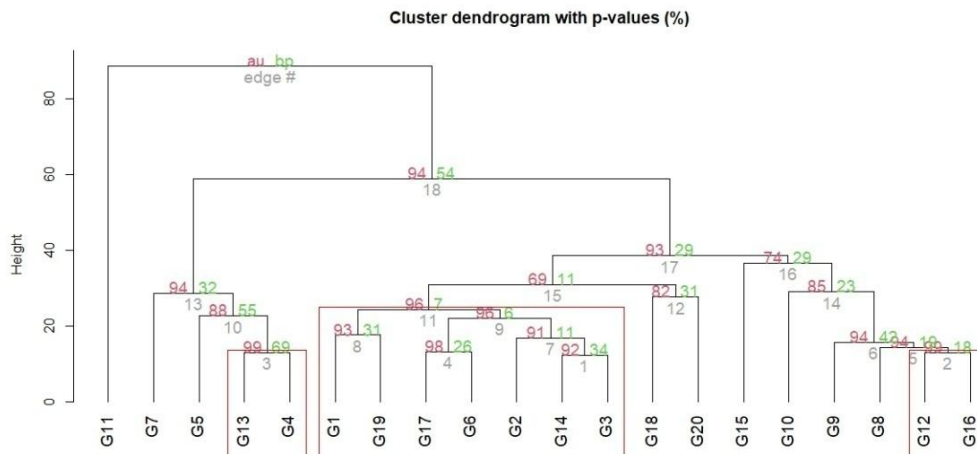
### **3. Results and discussion**

#### **3.1. Cluster analysis**

The results of the cluster dendrogram for genotypes with P values (AU is an approximately unbiased P value computed by multiscale bootstrap resampling, and BP is the bootstrap probability value computed by normal bootstrap resampling) are shown in **Fig.1**. The 20 genotypes were grouped into clusters based on their genetic similarity for the agronomic characteristics under study. The results showed that the whole genotypes were grouped into two groups and that genotype 11 was an outlier. The cophenetic distance was 0.79, which represents the distance between two clusters, indicating that the

studied genotypes are highly diverse in terms of their traits. p values of 7 and 18 for clusters two and three, respectively, inferred that clusters are not formed by chance and that there is genuine diversity in the variables used between them.

The first branch of the dendrogram included genotypes G11, G7, G5, G13 and G4, which formed cluster one with genotypes G13 and G4. These genotypes are closely related, as indicated by the short branch lengths and second branch again grouping into two subgroups, which form two additional clusters. Cluster two contained G1, G19, G17, G6, G2, G14 and G3 and had the highest number of genotypes in this cluster. Genotypes 12 and 16, which form the third cluster indicating 20 advanced genotypes, are genetically diverse. Other genotypes form individual pairs or small clusters, indicating specific close relationships between those genotypes. The results of the dendrogram inferred that there was a great amount of genetic diversity among the 20 genotypes. The three main clusters (cluster one, cluster two and cluster three) represent groups of genotypes that are genetically similar within the group but distinct from each other. This genetic diversity could be leveraged in breeding programs to introduce new traits or improve existing traits. The cluster genotypes, including G13 or G4, can be used as parents to cross genotypes from cluster two (G1, G19, G17, G6, G2, G14 and G3) or from the third cluster (G12 and G16) to maximize genetic diversity and potentially introduce beneficial traits that are present in one cluster but not the other. Alternatively, genotypes within the same cluster could be crossed to reinforce specific traits that are common within that cluster.

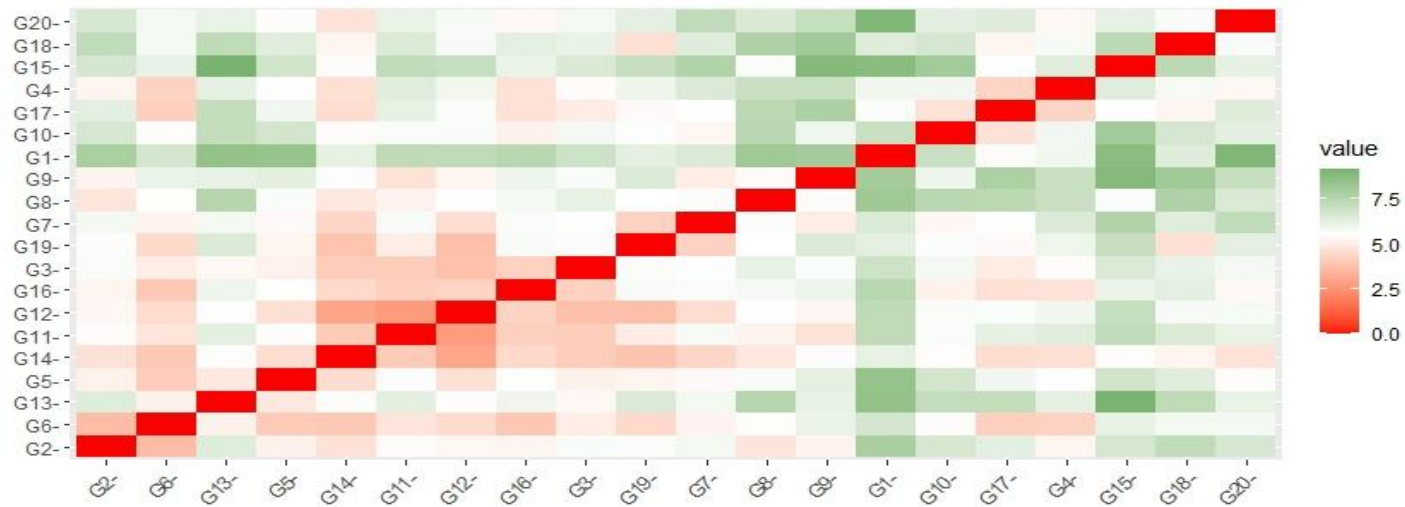


**Fig 1. Hierarchical clustering dendrogram illustrating the genetic diversity among 20 wheat genotypes**

The maximum genetic distance observed was 144.9 between genotypes 5 and 11 (**Table 1**). These findings suggested that these two genotypes are the most genetically diverse among all the studied genotypes and can be used as parents to explain large amounts of genetic variation in the studied traits. On the other hand, the minimum non-zero genetic distance was 12.32 between genotypes 3 and 14, indicating that these two groups are the most genetically similar among all the studied genotypes. The second largest genetic distance was 128.1 for genotype pairs 4 and 11, followed by 113.7, 110.1 and 102.4 for genotype pairs 11&7, 10&5 and 13&10, respectively. A second lowest value of genetic distance was recorded for genotype pairs 13 and 4, followed by 17 and 6.3, 214 and 6, 12 and 18. Similar results were previously reported by Chaudhary et al. (2022), Santosh et al. (2019), Jaiswal et al. (2010). It is

recommended that genotypes that are genetically diverse (i.e., have a large genetic distance) be crossed to maximize genetic diversity in the offspring. Conversely, if the goal is to maintain certain traits, breeders might choose to cross groups that are genetically similar (i.e., have a small genetic distance). By utilizing this information for crop improvement, genetic distance information can provide valuable insights into the genetic diversity and relationships among different genotypes and help to make informed decisions and develop more effective breeding strategies.

Studies conducted previously by Khalid et al. (2022), Braved et al. (2022), Vuset al. (2020) and Shimelis et al. (2018) also implied similar results, suggesting that the variation in genotypes across clusters resulted from the minor impact and cumulative influence of several characteristics and had the potential for hybridization programs to design crosses for the manifestation of heterosis and for improving quality traits. The utilization of cluster analysis in assessing the genetic resources have proven instrumental in identifying valuable starting material for priority breeding areas. The detailed breakdown of clusters in the previous studies highlights the relevance of cluster analysis in delineating distinct groups with specific trait combinations. This knowledge can be applied to wheat breeding, allowing researchers to prioritize and select cultivars with optimal trait combinations for targeted environments.



**Fig 2.** Heatmap representing the genetic distance of 20 advanced lines of wheat

**Table 1.** Genetic distance matrix of 20 wheat genotypes showing genetic diversity for agromorphological characteristics

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	41.3
G1	0.0	26.5	20.1	51.2	71.4	25.8	39.5	34.6	31.1	52.0	85.1	27.9	60.1	22.8	50.0	38.6	32.5	32.8	17.6	41.3
G2		0.0	13.4	32.1	50.5	29.6	24.2	46.2	47.3	70.8	103.3	40.4	41.1	20.4	51.6	50.5	31.3	31.2	32.5	44.5
G3			0.0	36.9	56.3	20.2	26.8	38.3	38.1	60.2	93.8	30.3	46.7	12.3	43.9	40.4	23.0	26.7	23.1	35.9
G4				0.0	24.7	48.8	21.9	70.8	71.3	92.4	128.1	64.4	13.0	38.5	64.0	72.4	42.3	37.7	55.6	52.1
G5					0.0	64.7	34.5	86.8	89.6	110.1	144.9	81.4	20.7	56.1	74.3	88.7	56.1	55.5	73.8	68.2
G6						0.0	34.4	24.5	27.8	46.9	80.6	18.5	58.9	13.4	26.7	25.6	13.2	30.8	16.2	26.4
G7							0.0	55.6	57.3	78.5	113.7	49.7	29.5	24.7	50.0	58.3	27.9	31.3	41.1	43.6
G8								0.0	14.5	31.1	60.1	13.7	81.0	33.4	31.3	15.1	35.7	48.8	21.8	37.9
G9									0.0	25.6	59.1	14.6	81.1	34.9	40.4	18.3	39.3	47.6	19.6	37.2
G10										0.0	39.4	32.9	102.4	56.1	49.6	26.6	55.8	65.3	40.6	49.9
G11											0.0	64.7	138.3	90.7	78.0	57.0	90.2	100.0	74.9	85.2
G12												0.0	74.4	26.7	32.3	13.0	29.3	40.4	13.9	33.2
G13													0.0	48.6	74.3	82.9	52.4	45.9	65.1	61.8
G14														0.0	34.8	36.0	15.0	23.1	18.8	27.2
G15															0.0	30.0	26.3	47.0	36.7	30.9
G16																0.0	34.8	48.1	24.9	35.4

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G17	0.0	28.2	25.6	26.5
G18		0.0	30.4	27.7
G19			0.0	29.6
G20				0.0

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### 3.2 Principal component analysis

Principal component analysis (PCA) revealed that a certain amount of diversity was present in the studied genotypes for the agromorphological traits, resulting in 18 principal components (**Table 2**). The first seven components captured a significant portion of the genetic diversity among the wheat genotypes for the studied traits. These seven components explain approximately 83% of the total variance in the data, suggesting that they account for the majority of the genetic diversity in the wheat genotypes studied. The first principal component explained 20.54% of the total variance, with an eigenvalue of 3.70, while the second principal component accounted for an additional 17.46% of the variance. As a result, the cumulative variance explained 37.99% of the variance by the addition of principal component three, thereby reaching half of the cumulative variance present in the genotypes. Similar results for PCA were also observed by Abdelghany et al. (2023), Adilova et al. (2020), Shivramakrishnan et al. (2016) and Poudel et al. (2017).

The contributions of the various variables to the top five principal components for the variation in the 20 advanced lines of wheat are listed in **Table 3**. In principal component (=Dimension) 1, the spike length had the highest contribution (13.44), while the germination percentage had the lowest (0.14), suggesting that spike length is a significant factor in differentiating the wheat lines along this dimension compared to germination percentage. After spike length, peduncle length (both exposed and total) was the major contributing variable in principal component 1. Days to maturity were the leading contributor, while spikelets per spike were the least contributing factor. Similarly, in PC3, PC4, and PC5 the major contributing variables were brown rust, flag leaf length, and yield per plot respectively, suggesting that these variables are deciding factors for variation among genotypes and that targeting improvements in these characteristics could lead to improvements in the genotypes. The same analysis can be applied to the other dimensions. It is also worth noting that the variables contributing the most to each dimension are different. Earlier studies of principal components in wheat were performed by Shamuyarira et al. (2022); Sharma et al. (2021); Singh et al. (2020); Riaz et al. (2018) and Amin et al. (2014), who concluded that principal component analysis is an excellent tool for determining genetic diversity. This suggests that each dimension captures a different aspect of the variation in the data. Overall, these results provide valuable insights into the genetic diversity of wheat lines, which could be useful for future breeding programs. By understanding which traits contribute most to the variability, breeders can focus on these traits when selecting lines for cross-breeding. Variables such as spike length (0.50), total peduncle length (0.47), and exposed peduncle length (0.41) had high communalities, indicating that these variables were well represented by the extracted factors.

The exploration of Principal Component Analysis (PCA) in wheat research has garnered considerable attention, with several studies delving into its application and implications. Piro et al. (2023), illustrated the potential application of rye chromatin introgression in wheat quality breeding, with the arabinoxylan content of wheat white flour, demonstrating the use of PCA in assessing quality traits in wheat breeding. Kumar et al. (2021), found variability in agro-morphological and physiological traits by PCA, suggesting that identified genotypes can be used for hybridization and improved cultivar development. Saleh et al. (2021) emphasized the use of PCA in assessing genetic variations among wheat genotypes to enhance selection efficiency in breeding programs. Moreover, Ahmad et al. (2017) conducted PCA to examine the suitability of wheat varieties for cookie making quality, demonstrating the use of PCA in correlating physical and rheological parameters of wheat varieties. These studies collectively demonstrate the diverse applications of PCA in wheat research, including assessing wheat variety suitability for specific products, comparing wheat genotypes, and analyzing wheat flour refinement. The use of PCA in these studies highlights its effectiveness in providing valuable insights into the composition, properties, and genetic diversity of wheat, thereby contributing to advancements in wheat breeding and product development.

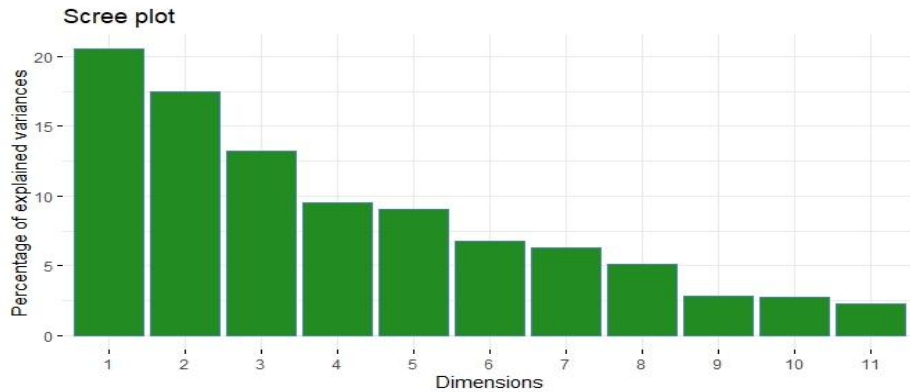
**Table 2.** Eigenvalues, proportions of variance and cumulative proportions of 20 wheat genotypes

<b>Principal Components</b>	<b>Eigen value</b>	<b>Percentage of Variance</b>	<b>Cumulative Percentage of Variance</b>
comp1	3.70	20.54	20.54
comp2	3.14	17.46	37.99
comp3	2.38	13.22	51.22
comp4	1.72	9.56	60.77
comp5	1.63	9.04	69.81
comp6	1.22	6.79	76.60
comp7	1.14	6.32	82.92
comp8	0.92	5.09	88.01
comp9	0.51	2.85	90.86
comp10	0.49	2.73	93.58
comp11	0.41	2.30	95.88
comp12	0.33	1.84	97.72
comp13	0.23	1.28	99.01
comp14	0.11	0.64	99.64
comp15	0.05	0.28	99.92
comp16	0.01	0.07	99.98
comp17	0.00	0.01	100.00
comp18	0.00	0.00	100.00

**Table 3.** Contribution of the top five principal components to the variation in 20 advanced lines of wheat

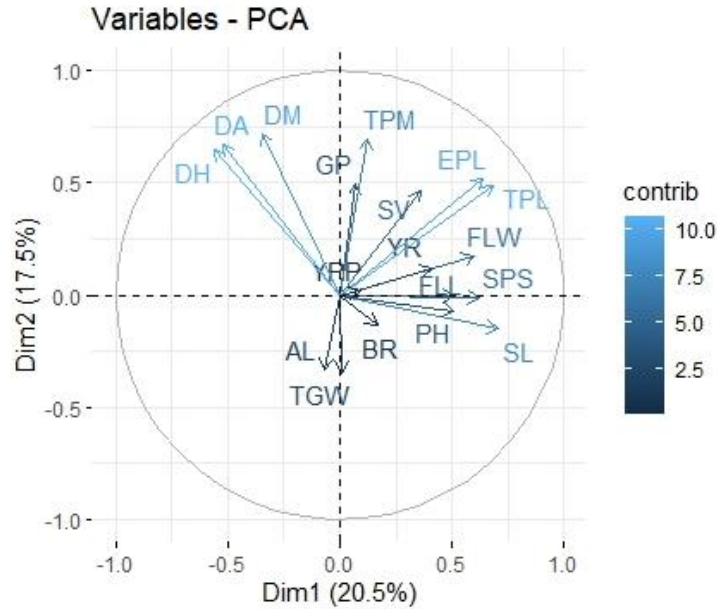
<b>Variable</b>	<b>Dim.1</b>	<b>Dim.2</b>	<b>Dim.3</b>	<b>Dim.4</b>	<b>Dim.5</b>
GP	0.14	7.80	14.19	4.89	0.43
SV	3.56	6.86	8.75	2.08	0.21
DA	7.26	14.18	5.06	0.72	2.80
DH	8.50	13.21	4.07	1.72	2.07
DM	3.21	16.31	1.34	1.28	0.12
FLL	6.99	0.00	1.22	21.71	0.28
FLW	9.63	0.94	2.61	12.29	2.76
EPL	11.08	8.51	0.16	2.84	0.84
TPL	12.60	7.56	3.33	0.80	3.69
SL	13.44	0.74	3.93	8.99	0.00
AL	0.14	3.52	0.35	6.67	5.03
PH	7.03	0.17	3.01	7.83	18.69
SPS	10.44	0.01	13.58	1.79	0.02
TPM	0.43	15.25	3.77	0.14	0.33
TGW	0.00	3.85	0.04	6.50	24.26
YPP	0.22	0.01	7.77	0.65	35.36
YR	4.57	0.48	8.05	18.20	1.88
BR	0.76	0.60	18.77	0.92	1.24

The scree plot (Figure 2) graphically represents the percentage of variance explained by different principal components. The results indicate that the first principal component explains 20% of the variance, while the second principal component accounts for slightly less than 20%. The scree plot suggests that considering the first eight principal components would capture a substantial amount of the variance in the study. These results are consistent with those of Ambati et al. (2020), Mishra et al. (2015), Sarfraz et al. 2021. Therefore, the robustness of the study's results can be attributed to these eight principal components.



**Fig. 3 shows a scree plot diagram built based on eleven principal components.**

Dimension 1 explained 20.5% of the variance in genotypes for traits, while dimension 2 accounted for 17.6% of the variance (Fig 4 & Fig 5). The biplot features multiple vectors, each representing all the characters under study. The direction and length of each vector indicate how each variable contributes to the two principal components. A longer vector indicates a variable that strongly influences the score of the individuals on the corresponding principal component. The results showed that day to anthesis, heading and maturity were positively correlated, and selecting one characteristic directly improved the other characteristics. Similar tiller per meter and germination percentage values are positively associated, suggesting that improving the germination percentage directly benefits the tiller per meter. However, the number of tillers per meter was negatively correlated with awn length and thousand-grain weight. The color scale indicates the contribution of each variable to the principal components, with darker colors representing greater contributions (Fig.4).



**Fig 4. Principal component biplot for variables in 20 advanced genotypes of wheat**

The PCA biplot for individual plots represents an individual, labeled from 1 to 20. The distribution of points across all four quadrants indicates variation among the individuals along these two principal components. The exact position of each point provides information about the individual's scores on the two components. For instance, Genotype 7 was located in the top right quadrant, in which higher values were observed for both dimensions (**Figure 5**). This finding suggested that genotype 7 had high scores for both Dim1 and Dim2. The contributions of genotypes 20, 5, 18 and 4 were negative, and likewise their contributions were lower. Genotypes 1, 2 and 10 positively contributed to dimension 1. This PCA plot can provide valuable insights into the underlying structure of the data, helping to identify patterns and relationships among individuals who might not be apparent from the raw data alone.

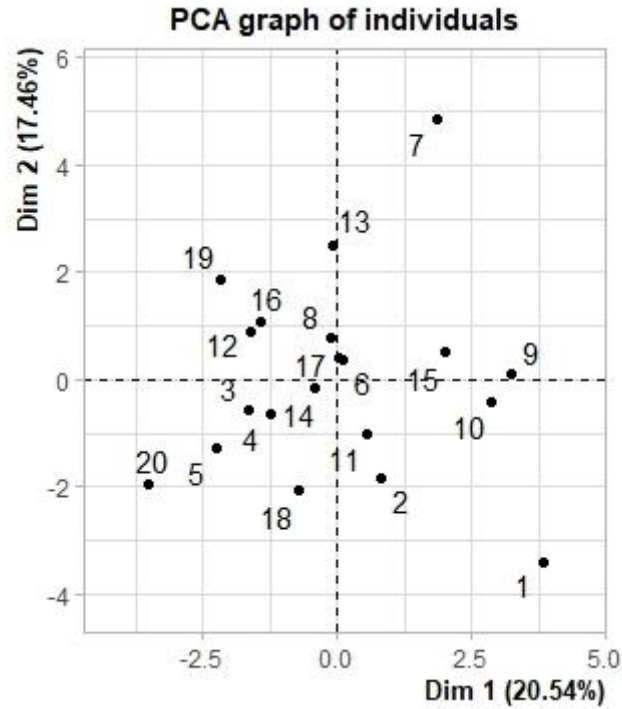


Fig 5. PCA biplot for individuals in 20 advanced genotypes of wheat

### 3.3 Rotated component matrix analysis

The rotated component matrix scores for Principal Component 1 (PC1) across the 20 wheat genotypes revealed a diverse set of characteristics (Fig. 6). The scores ranged from a high of 1.94 (genotype 1) to a low of -1.79 (genotype 20). These scores represent the correlation of each genotype with the first principal component, which is a linear combination of the original variables that captures the maximum variance in the data.

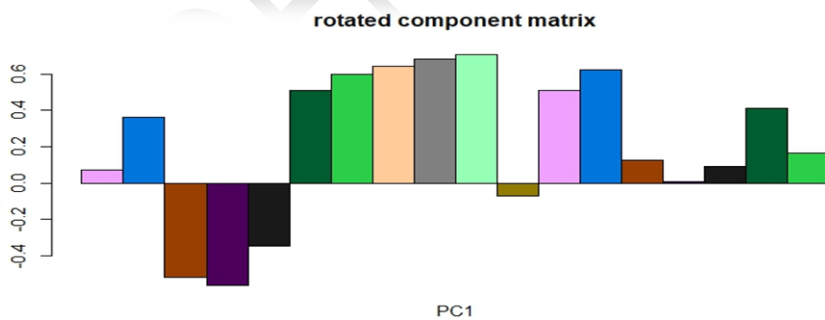


Fig.6. Rotated component metrics (PCs)1 of 20 wheat genotypes.

Positive scores on PC1 (e.g., genotypes 1, 2, 6, 7, 9, 10, 11, and 17) suggested that these genotypes shared certain traits or characteristics. In contrast, genotypes with negative scores on PC1 (e.g., genotypes 3, 4, 5, 12, 16, 18, 19, and 20) indicate different or opposing traits compared to those with positive scores. Similar results were observed by Sheela et al.(2020), Singhet al. (2020),Nachimuthu et al. (2014) and Kumar et al.(2014). These results highlight the genetic diversity among the studied wheat genotypes, which is crucial for crop improvement strategies. The variability in PC1 scores can be used to identify specific traits for further selection and to introgress desirable genes from diverse germplasms into the available genetic base.

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## CONCLUSION

Plant breeding relies on the principle of continuous selection of favorable alleles present in the available germplasm. This selection aims to improve the performance of cultivars or elite lines against major biotic or abiotic stresses in their environment. In this context, the study successfully classified 20 wheat genotypes into three clusters based on their genetic similarity, with a high cophenetic distance of 0.79 and low p-values for the clusters suggesting high diversity for the studied traits. This diversity can be harnessed in breeding programs to introduce new traits or enhance existing traits and can be further elucidated through principal component analysis (PCA). The PCA revealed considerable diversity among the studied wheat genotypes for agro-morphological traits, with the first seven components accounting for approximately 83% of the total variance, indicating significant genetic diversity. The scree plot affirmed the robustness of the study's results by suggesting that the first eight principal components accounted for a substantial portion of the variance. Notably, Genotype 7, located in the top right quadrant, demonstrated high scores on both dimensions, indicating its significant contribution to genetic diversity, while genotypes 20, 5, 18, and 4 contributed less to genetic diversity, with negative values. Genotypes 1, 2, and 10 positively contributed to dimension 1. These genetic distances between genotypes provide valuable insights for crop improvement, aiding in the development of more effective breeding strategies. Therefore, PCA offers valuable insights into the genetic diversity of wheat lines, which could be beneficial for future breeding programs.

## CONSENT (WHERE EVER APPLICABLE)

## ETHICAL APPROVAL (WHERE EVER APPLICABLE)

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