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

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Twenty advanced wheat genotypes were evaluated for yield and yield-contributing traits in six field trials over two cropping seasons (Rabi 2020-21 and 2021-22) using a completely randomized block design (RBD) at two locations: the N.E. Borlaug Crop Research Centre (NEBCRC), G.B.P.U.A&T, Pantnagar, U.S. Nagar district, and the Agriculture Research Center Majhera, Nainital, Uttarakhand. The study aimed to assess genetic diversity among the genotypes via principal component analysis (PCA) and cluster analysis, offering insights into genetic variations. Three 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 diverse genetic material is under study. The maximum genetic distance observed was 144.9 between genotypes G5 and G11. 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.The findings of current study could be exploited in planning and execution of future breeding programme in wheat.

Keywords: Wheat; genetic diversity; PCA; cluster and cophenetic distance; staple food.

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is a globally importantcrop that contribute ssignificantlyto food security worldwide, including India. Wheat is a staple food for millions of people and provide approximately 20% of the total dietary calories and proteins worldwide [1]. It is the most widely cultivated crop in the world, with an area of 220 million hectares and a total production of 788.5 million tones [2]. Global wheat production has increased by 1.4 million tonnes, reaching a total of 788.5 million tonnes in 2023.

It is cultivated in a wide range of environmental conditions, with temperatures ranging from 21 to 24°C. Wheat crop is affected by many abiotic stresses such as terminal heat, drought, salinity, waterlogging, lodging, etc. Therefore, it is essential to identify the genotypes/line for consistent performance under these stresses.Unfortunately, genetic diversity is of paramount importance in any crop improvement program. The presence of genetic diversity provides the opportunity for plant breeders to select promising genotypes with desirable traits that enhance crop yield, disease resistance, and environmental adaptability. However, the use of breeding lines with narrow genetic bases can lead to significant yield losses (Kumar et al., 2022). Occurrence of continuous mutationsin pest populations or uncertainly changes in environmental conditions restrict the real performance of the genotypes and potentially leading to severe crop yield losses. Therefore, maintaining and utilizing a broad genetic base breeding materials in crop improvement 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, [3], Thompson et al., [4] Melchinger, [5] Brown-Guedira et al., [6]. Principal component analysis (PCA), a dimensionality reduction technique introduced by Karl Pearson in [7], 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., [8] Szczepanik et al., [9]. The significance of genetic diversity in wheat is well documented (Gruet et al., [10], Khan et al., [11], Yadav et al., [12]. 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 [13]. 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 [14]. 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., [15] Chaudhary et al., [16], Elahi et al., [17]. 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 [15]. 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.

2. 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. [18].

Cluster analysis was performed using the unweighted pair group method with arithmetic mean (UPGMA), as proposed by Sokal and Michener [19], to assemble a phylogenetic tree from a distance matrix. Genetic distances between the genotypes were calculated using the Euclidean method [20].

Principal component analysis (PCA), a statistical tool commonly used in plant breeding to identify trends in multidimensional data (Pearson, [7] and Hotelling, [21], 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. [22] Sewell [23]. PCA and cluster analysis, as well as visualization, were performed using R script (Mojena, [24], Kassambara, 2016; Le et al. [25], Husson et al [26].

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.

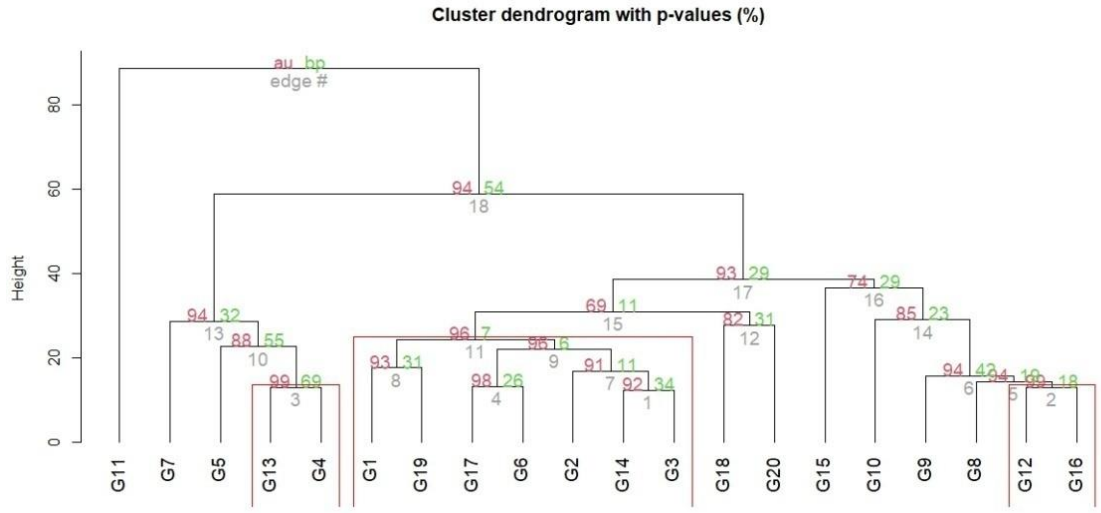

Fig. 1. Hierarchical clustering dendrogram illustrating the genetic diversity among 20 wheat genotypes

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 G13or 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.

The maximum genetic distance observed was 144.9 between G5 and G11 (Table 1;Fig. 2). 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 G3 and G14, 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 pairsG4 and G11, followed by 113.7, 110.1 and 102.4 for genotype pairs G11 & G7, G10 & G5 and G13 & G10, respectively. The second lowest value of genetic distance was 13.0 for genotype pairs G13 and G4, followed by G17 and G6 (13.2), G3 and G2 (13.4), G12 and G18(13.7). The present findings were also supported by several previously reported results (Chaudhary et al [16], Santosh et al. 2019 Jaiswal et al. [27]. Therefore, based on the present findings, the genetically diverse genotypescould be used in the crossing program to maximize genetic diversity in the offspring. Conversely, if the goal is to maintain certain traits, breeders might choose to cross genetically similar groups. The present study helps the breeders to understand the role of genetic diversity, crop improvement, how genetic distance is essential in the selection of genotypes as a parent for further utilization, and also helps to make decisions and develop more effective breeding strategies in the future.

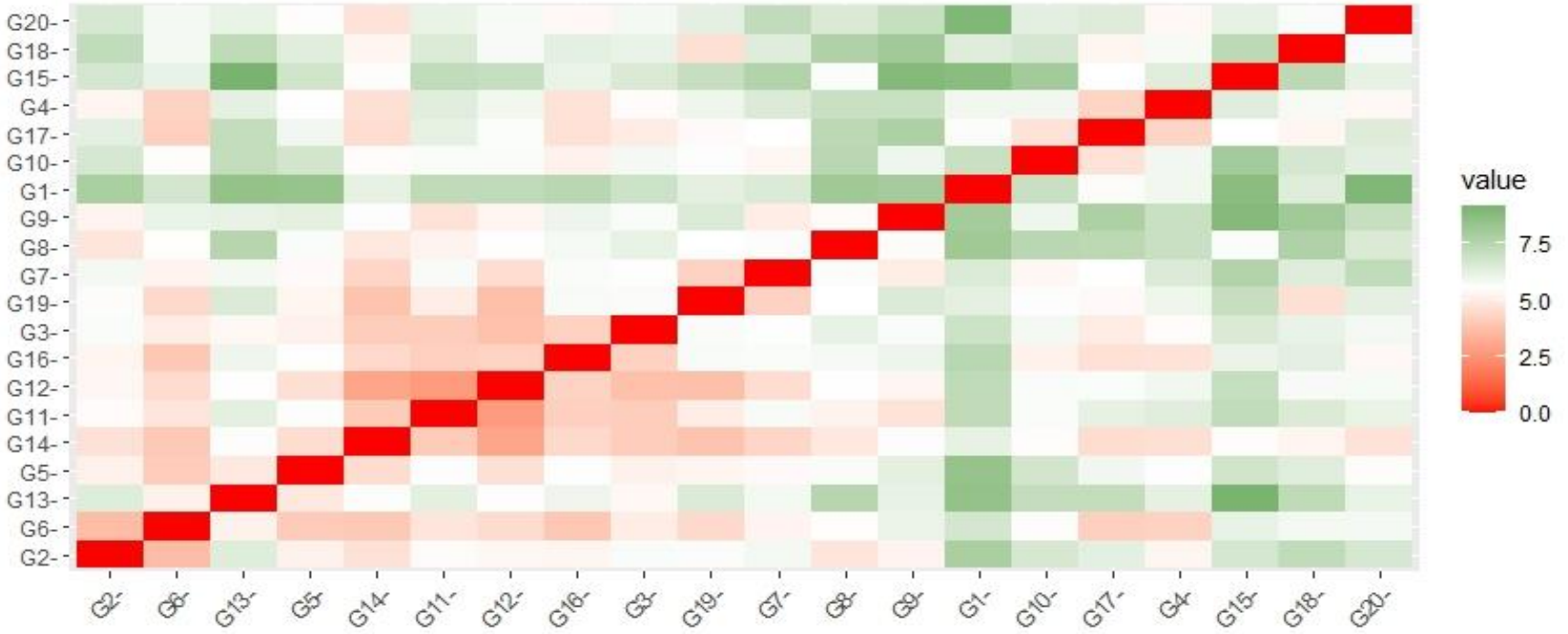

Fig. 2. Heatmap representing the genetic distance of 20 advanced lines of wheat

Table 1. Genetic distance matrix of 20 wheat genotypes showing genetic diversity for yield and yield-contributing traits

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

Studies conducted previously by Khalid et al. [28], Braved et al. (2022), Vus et al. [29], and Shimelis et al. [30] 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 (Very long sentence).The utilization of cluster analysis in assessing the genetic resources has 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.

3.2 Principal Component Analysis

Principal component analysis (PCA) revealed that a certain amount of diversity was present in the studied genotypes for the yield and yield-contributing 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. [31], Adilova et al. [32], Shivramakrishnan et al. [33] 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 themajor 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. [34], Sharma et al. [35], Singh et al [36] and Riaz et al. [37], who concluded that principal component analysis isan 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 werewell 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. [38], 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. [39], found variability in yield contributing traits and physiological traits by PCA, suggesting that identified genotypes can be used for hybridization and improved cultivar development. Saleh et al. [40] emphasized the use of PCA in assessing genetic variations among wheat genotypes to enhance selection efficiency in breeding programs.Moreover, Ahmad et al. [41] 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

Principal Components	Eigen value	Percentage of Variance	Cumulative Percentage of Variance
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

Variable	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5
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 (Fig. 3) 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. [42], Mishra et al. [43], and Sarfraz et al. [44]. Therefore, the robustness of the study's results can be attributed to these eight 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 and 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).

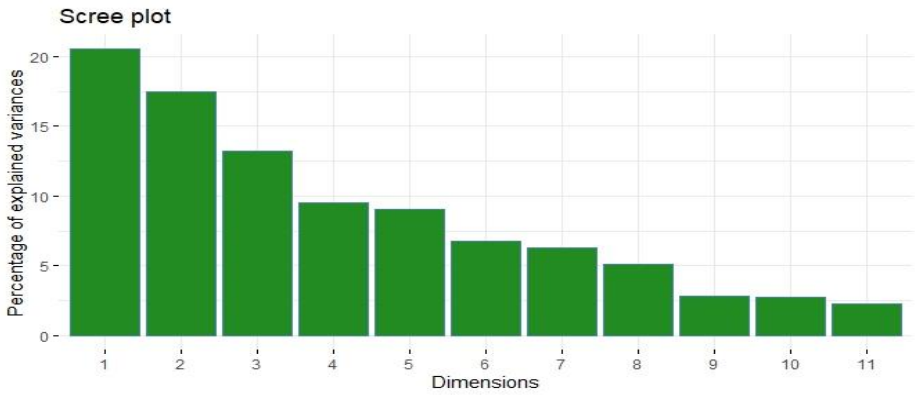

Fig. 3. Shows a scree plot diagram built based on eleven principal components

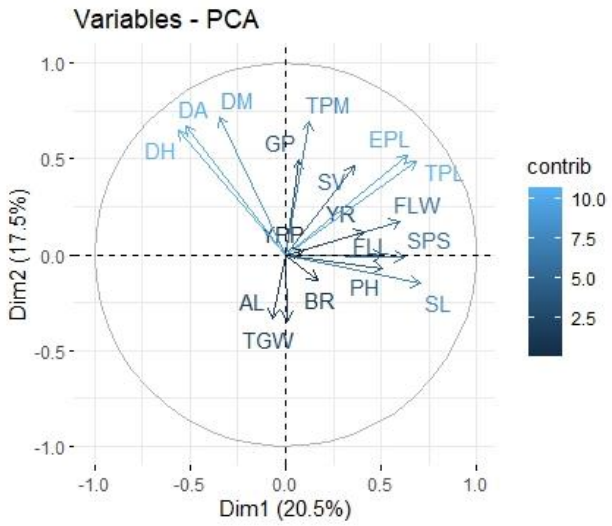

Fig. 4. Principal component biplot for variables in 20 advanced genotypes of wheat

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 (Fig 5). This finding suggested that genotype G7 had high scores for both Dim1 and Dim2. The contributions of genotypes G20, G5, G18, and G4 were negative, and likewise, their contributions were lower. Genotypes G1, G2 and G10 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.

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 (G1) to a low of -1.79 (G20). 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.

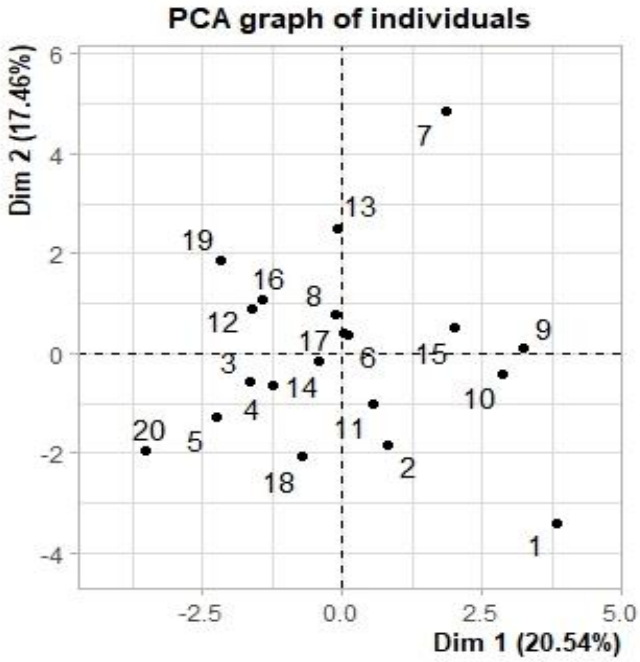

Fig. 5. PCA biplot for individuals in 20 advanced genotypes of wheat

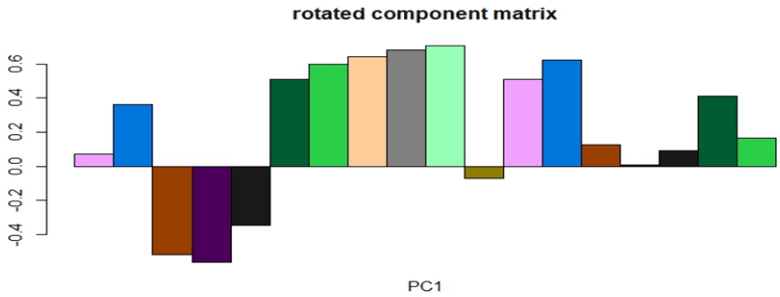

Fig. 6. Rotated component metrics (PCs)1 of 20 wheat genotypes

Positive scores on PC1 (e.g., G1, G2, G6, G7, G9, G10, G11, and G17) suggested that these genotypes shared certain traits or characteristics. In contrast, genotypes with negative scores on PC1 (e.g., G3, G4, G5, G12, G16, G18, G19, and G20) indicate different or opposing traits compared to those with positive scores. Similar results were observed by Sheela et al [45], Singh et al. [46], and Nachimuthu et al. [47]. These results highlight the genetic diversity among the studied wheat genotypes, which is crucial for crop improvement strategies [48-50]. 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 [51-53].

4. CONCLUSION

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 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 yield and yield-contributing 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 G7, located in the top right quadrant, demonstrated high scores on both dimensions, indicating its significant contribution to genetic diversity, while genotypes G20, G5, G18, and G4 contributed less to genetic diversity, with negative values. Genotypes G1, G2, and G10 positively contributed to dimension 1. These genetic distances between genotypes provide valuable insights for crop improvement, aiding in developing more effective breeding strategies. Consequently, PCA yields valuable elucidations regarding the genetic heterogeneity within wheat cultivars, presenting potential benefits for forthcoming breeding initiatives.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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