RESEARCH PAPER

The potential role of quantitative traits for understanding the genetic diversity in Sorghum (*Sorghum bicolor* L.)

Muhammad Ihsan Ullah[1*], Zaib-un-Nisa[1,2], Barkat Ali[1], Guljana Nazir[1], Muhammad Zeeshan Munir[2], Muhammad Amir Siddique[3], Arif Hussain[4], Dilawar Khan[5], Muhammad Atif Muneer[6]

[1]*Sorghum Research Sub-Station, Dera Ghazi Khan, Punjab, Pakistan*

[2]*School of Biological Sciences and Technology, Beijing Forestry University, Beijing 100083, China*

School of Landscape Architecture and Design, Beijing Forestry University, Beijing 100083, China

[4]*Ghazi University, Dera Ghazi Khan, 32200, Pakistan*

[5]*School of Soil and Water Conservation, Beijing Forestry University, Beijing 100083, China*

[6]*School of Grassland Science, Beijing Forestry University, Beijing 100083, China*

Abstract

Sorghum is a popular cereal crop worldwide, and therefore, the understanding and utilization of sorghum accessions play role crucial for improving crop productivity. The in-depth knowledge of genetic variation between the sorghum accessions will allow the plant breeder for precise breeding. Thus, exploration of sorghum crop genetic diversity is inevitable. In this study, we investigated the genetic diversity among the thirty sorghum accessions by using the quantitative morphological traits. The findings were concluded on the basis of ten quantitative traits, among which 07 diverse quantitative traits which maximized the variation, chosen carefully for genetic diversity analysis. We found a significant positive correlation of stalk yield with days to flowering and no. of leaves per plant. Similarly, for grain yield with no. of leaves per plant and panicle width. The PCA revealed that days to flowering, plant height, panicle width, and leaf area contributed maximum towards the divergence. Sorghum accessions were grouped under 4 clusters by using hierarchical analysis. Cluster-2 contained minimum while cluster-4 had maximum number of sorghum accessions. The maximum inter-cluster distance was recorded between cluster-2 and cluster-4. The cluster-4 had the highest mean values for no. of leaves per plant, stalk yield, and days to maturity. Therefore, parent selection must be dependent on best yield related components and wider inter-cluster distance. Therefore, in current study, the quantitative morphological traits showed the broader genetic diversity range among the thirty sorghum accessions, and can be utilized effectively for the improvement of genetic architecture.

* **Corresponding Author:** Muhammad Ihsan Ullah ✉ ihsan7447@gmail.com

Introduction

Sorghum (*Sorghum bicolor* L.) is a C_4 plant with dynamic growth patterns, and higher biomass production is widely grown cereal crop in the world (Hariprasanna and Patil, 2015; Ullah *et al.*, 2016). It is the world's most important cereal crop and ranks at the fifth position after wheat, maize, rice, and barely (Sinha and Kumaravadivel, 2016). It is extensively cultivated as a major food crop in the regions of South Asia and Africa (Bibi *et al.*, 2010). It is commonly grown in tropical and subtropical areas of the world. It can also be cultivated in the changing agro-climatic conditions, e.g., rainfall, temperature, and soil conditions (Reddy *et al.*, 2008). Therefore, besides the importance of sorghum crop for food, forage, and feed purposes, it also supplies the raw material for fiber processing, alcohol, biofuels, starch, , and many other byproducts (Mumtaz *et al.*, 2018; Assar *et al.*, 2020).

In Pakistan, Sorghum is commonly called Jowar, and cultivated for the purpose of fodder and grain. The cultivated area was 274 thousand hectares with annual yield about 161 thousand tons in the year 2015-2016 (Wing, 2016). The shortage of the forage crops in the Pakistan is mostly two times a year, i.e., May- June, and during October-November (Iqbal *et al.*, 2010). Therefore, improvement of sorghum accessions for the quality and yield traits can do a great deal to overcome the scarcity of forage production during the summer season. Sorghum is known as the fast-growing crop that constitutes a considerable proportion of stalk yield as well as grain yield. It possesses carbohydrates about 70%, fat and protein content 0.3%. Therefore, it can be successfully employed in the feeding programs for dairy cattle and poultry (Khan *et al.*, 2007; Ullah *et al.*, 2016). Therefore, it is imperative to explore the genetic diversity in sorghum accessions and should be utilized in the breeding programs.

Genetic diversity is explained as classification or grouping of population or an individual in contrast to the other populations or individuals (Adugna, 2014; Raza *et al.*, 2019). The analysis of genetic diversity is an essential technique for the development of new varieties or cultivars on the basis of genetic distance and similarities. Sorghum was native to the African continent(specifically in the Ethiopia) and then it was introduced globally to the different regions with diverse agro-climatic conditions (Li *et al.*, 2010). Therefore, a wider range of diversity has been reported at both phenotypic and genotypic levels among and within sorghum cultivars (Kong *et al.*, 2000; Hart *et al.*, 2001).

The awareness of a crop's genetic diversity helps the breeder in the breeding programs to choose the suitable parents, and the introgression of genes from distant genetic material produces the superior hybrids having great potential against the biotic and abiotic stresses. By knowing genetic diversity richness in the sorghum crop may allow the improvement of genetic architecture of this crop (Jayaramachandran *et al.*, 2011).

Genetic variability in the sorghum accessions is the gift of nature, and It is caused by spatial separation or genetic cross-compatibility barriers. The morphological characteristics are used as traditional methods in the breeding programs to research genetic variation. In general, the morphological assays need neither the preparatory procedure nor the specialized equipment. These are usually inexpensive and simple to record the data. These quickly identified morphological characteristics are valuable tools for a preliminary assessment, since they provide a practical and straightforward methodology for determining the level of diversity among the cultivars. Over the years, a variety of studies have used the morphological characteristics to predict the genetic variation in the cultivated sorghum (Zongo *et al.*, 1993; Adugna, Rao *et al.*, 1996; Ayana and Bekele, 1998; Dahlberg *et al.*, 2002; Shehzad *et al.*, 2009; Adugna, 2014) .

The most popular method that is used to estimate the associations between the genotypes is the use of morphological traits. The genetic diversity of cultivated varieties/species along with their wild relatives provides a strong base for breeding

improved and new varieties of crops. A better knowledge of genetic variation in sorghum can help us to boost up the crop yield and quality. Therefore, it is of the prime importance to assess the genetic diversity in the available accessions of Sorghum. In this study, we have tried to determine the genetic diversity/variation between the thirty accessions of sorghum crops by analyzing the quantitative traits.

Materials and methods

Plant material

Thirty accessions of sorghum including one check variety were received from Maize and Millet Research Institute (MMRI), Yusafwala, Sahiwal, Pakistan. These accessions were planted at Sorghum Research Sub-Station, D. G. Khan, Pakistan, during Kharif-2018 (Fig. 1).
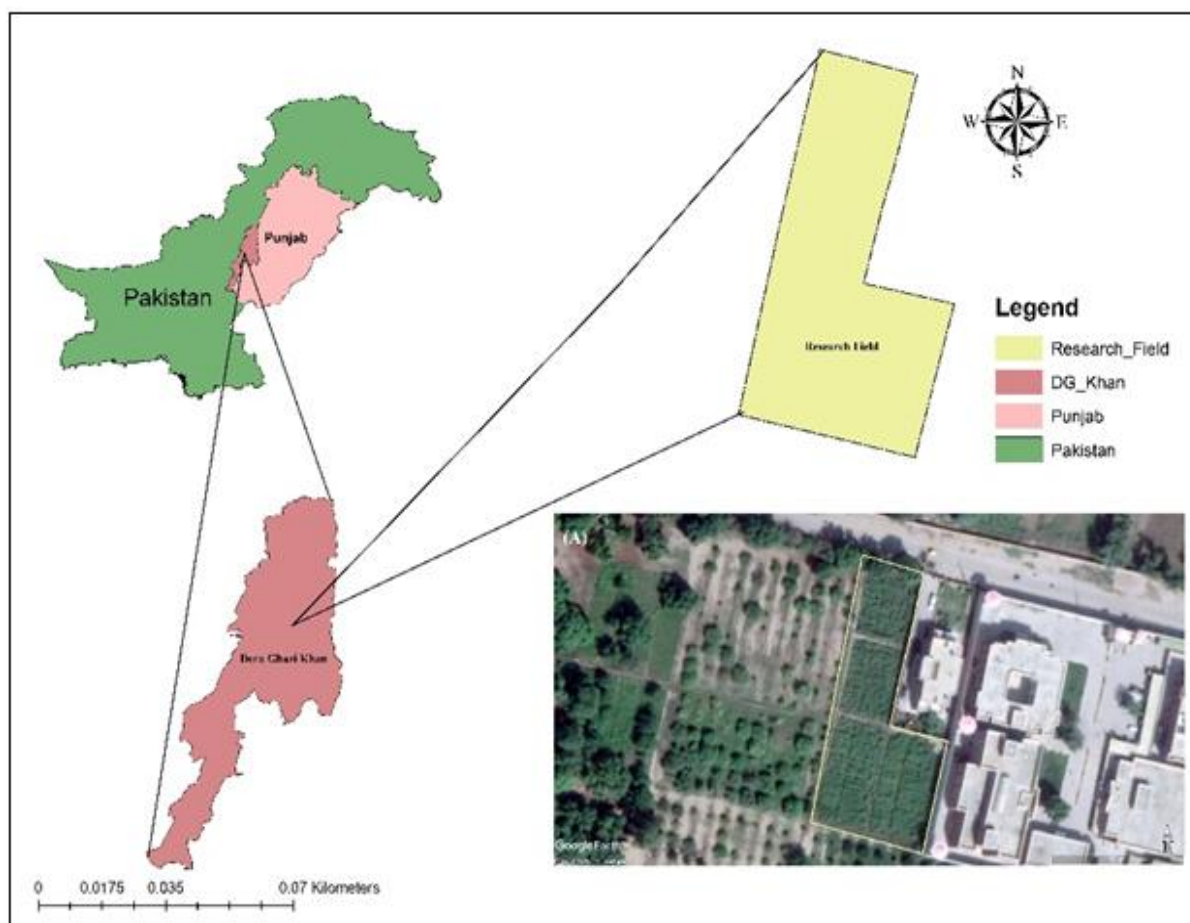

Fig. 1. The geographical location of Sorghum Research-Substation, D.G. Khan. The real image taken by the google earth pro is showing the original site of field trials (A), and sorghum accessions cultivated in this L-shaped field highlighted by yellow color.

These thirty accessions were named as Yusafwala Sorghum Selection (YSS), i.e., YSS-1 (check variety), and YSS-2 to YSS-30 (29 lines) because these were collected form MMRI.

Methodology

The thirty sorghum accessions were raised in a randomized complete block design (RCBD) with three replications during Kharif-2018 at Sorghum Research Sub-Station, Dera Ghazi Khan. The plot size was consisted of two rows of 5m length by the spacing of 75cm × 15cm (row-to-row, and plant-to-plant, respectively), for each accession. The agronomic practices like fertilizer doses, crop protective measures, and irrigation etc., were ensured as and when required during the crop season. Five plants were selected randomly from each replicate, and the observations for quantitative traits were noted at maturity (except for days to flowering). Data were recorded for various quantitative traits like the

number of days to flowering (DFL), plant height (PHT), days taken to maturity (DMA), number of leaves per plant (NPL), Leaf area (LFA), panicle length (PNL), panicle width (PWD), "000" grain weight (TWT), grain yield (GYI), and stalk yield (SYI) tons/ha. For statistical analysis, the mean values were used to determine the genetic diversity of the thirty sorghum accessions.

Statistical analyses

The experiment was performed by following the CRBD (Complete Randomized Block design). The data were analyzed by using the analysis of variance and descriptive statistics with the help of IBM SPSS 25.0 software package, while the person correlation analysis was performed in R programming software.

The factor analysis was conducted to determine what trait contributes to the highest variability.

The PCA analysis was performed to analyze the contribution of each quantitative traits to the overall genetic variation. The hierarchical clustering was conducted on "Euclidean distance matric" using the Ward's linkage method. We performed these analyses by the MINITAB software V.18.

Results and discussion

Variance analysis using a randomized block method revealed a substantial variance for all the traits (results not shown here), showing the presence of a high degree of genetic variability among the 30 accessions.

Table 1. Descriptive statistics of the quantitative traits.

	DFL	DMA	PHT	NPL	LFA	PNL	PWD	TWT	GYI	SYI
Mean	80.08	126.84	243.87	14.44	2.19	25.71	14.33	27.79	515.63	19.63
Standard error	0.49	0.60	5.16	0.16	0.09	0.48	0.19	0.46	10.36	0.73
standard deviation	4.65	5.66	48.98	1.48	0.81	4.52	1.76	4.39	98.28	6.90
Sample variance	21.67	32.02	2399.15	2.20	0.66	20.44	3.11	19.31	9659.18	47.58
Range	20.00	31.00	195.00	6.00	3.06	20.25	7.41	21.00	498.42	27.00

Descriptive statistics

The grain yield provided the highest value of mean (515.63), standard deviation (98.28), standard error (10.36), variance (9659.18), and the range (498.42) (Table 1). The descriptive statistics data of ten quantitative traits confirmed the presence of morphological diversity amongst these thirty

sorghum accessions, providing the potential for crop improvement via selection and hybridization.

The variation coefficients for plant height, and stalk yield seemed to be high, revealing the vulnerability to environmental changes that influence the expression patterns to some extent.

Table 2. Rotated factor loadings of the quantitative traits.

Traits	Factor-1	Factor-2	Factor-3	Factor-4
Days to flowering	0.833	-0.189	-0.172	0.180
No. of leaves per plant	0.812	-0.152	0.232	0.274
Stalk yield	0.762	-0.033	0.048	-0.336
Days to maturity	0.520	0.147	0.230	0.093
Plant height	0.240	0.827	-0.233	0.033
Panicle length	0.204	-0.797	-0.223	0.175
Thousand-seed weight	-0.343	0.499	-0.168	0.218
Panicle width	0.118	0.028	0.922	-0.168
Grain yield	0.147	-0.327	0.688	0.482
Leaf area	0.101	0.031	-0.034	0.915
Total variance	2.468	1.758	1.596	1.407
Variance %	24.681	17.581	15.965	14.067
Cumulative variance %	24.681	42.262	58.226	72.294

Correlation analysis

We found a significant positive correlation for stalk yield (SYI) with days to flowering (DFL) and no. of leaves per plant (NPL) (Fig.2). The grain yield (GYI) was also significantly and positively correlated with NPL, and panicle width (PWD) that could play a significant role in food synthesizing by the plants through early flowering and the process of photosynthesis that have direct effects on stalk yield as well as the grain yield. From these investigations, we concluded that DFL and NPL are associated with SYI, while NPL and PWD are correlated with and GYI. Thus, the selection of these quantitative traits will be of great importance and have a significant impact on stalk yield and grain yield.

Table 3. Principal component analysis showing the contribution of 8 traits among sorghum accessions.

Traits	PC1	PC2	PC3
Days to flowering	0.514	0.131	0.265
No. of leaves per plant	0.526	0.123	0.023
Stalk yield	0.421	0.206	0.433
Days to maturity	0.298	0.253	-0.681
Plant height	-0.065	0.703	-0.055
Panicle length	0.298	-0.540	0.105
Thousand-seed weight	-0.316	0.275	0.513
Eigenvalue	2.593	1.517	1.019
% Variance	37.05	21.67	14.56
% Cumulative variance	37.05	58.72	73.27

Factor analysis

The factor analysis was conducted to lessen the large morphological traits to a lesser but more illustrative set of attributes, and we may interpret which quantitative traits have significant contribution in the maximum variation (Table 2).

The first three factors contributed to72.294% of the total variance that we recorded (Table 2). The factor-1 had the highest factor loadings from days to flowering, no. of leaves/plant, stalk yield, and days to maturity contributed 24.681% of total variance. The factor-2 had the highest loading role from the plant height, the panicle length, and 1000-seed weight attributed 42.262% of total variance. Similarly, factor-3 had the high panicle width and grain yield contribution loadings.

In the loading plot, the distribution of quantitative traits in the first two factors has been shown (Fig. 3). The loading plot made clear explanation of the quantitative traits, i.e., panicle width (PWD), leaf area (LFA), and grain yield (GYI), contributed low variability towards the genetic variation.

Table 4. The inter-cluster distances among the sorghum accessions.

	Cluster-1	Cluster-2	Cluster-3	Cluster-4
Cluster1	0.00			
Cluster2	109.90	0.00		
Cluster3	49.99	61.42	0.00	
Cluster4	44.77	154.24	94.07	0.00

PCA (Principal component analysis)

The principal component analysis (PCA) is interpreted according to the correlations between variables; either it is positive or negative values. Of the ten quantitative traits, a set of seven different quantitative morphological traits was selected, i.e., days to flowering, no. of leaves per plant, stalk yield, days to maturity, plant height, panicle length, and thousand-seed weight used to group the thirty sorghum accessions by PCA analysis. About the contribution made by first three factors was 73.27% of total variance (Table 3).

Table 5. Characteristic means of the four similarity cluster groups of the sorghum accessions.

Traits	Cluster-1	Cluster-2	Cluster-3	Cluster-4
Days to flowering	78.13	78.67	82.11	81.46
No. of leaves per plant	14.54	13.61	13.78	14.92
Stalk yield	18.40	13.94	18.96	23.15
Days to maturity	127.54	122.56	124.67	128.9
Plant height	282.71	173.17	327.11	233.39
Panicle length	23.77	28.89	21.92	26.32
Thousand-seed weight	30.63	28.00	28.56	25.77

The first principal component (PC1) accounting for 37.05% of total variance., and showed maximum contribution of loading factor from the days to flowering, no. of leaves per plant, and stalk yield.

The second principal component (PC2) showed the highest contribution of loading factor from the plant height and panicle length, and made contribution to 21.67% of total variation. The third principal component (PC3) showed the highest of factor loading from days to maturity, thousand seed weight, and contributed to 14.56% of the total variation. Fig. 4 shows the score plot of 30 accessions based on the first two main components. The distributed patterns also showed the presence of a significant amount of variability among the sorghum accessions.
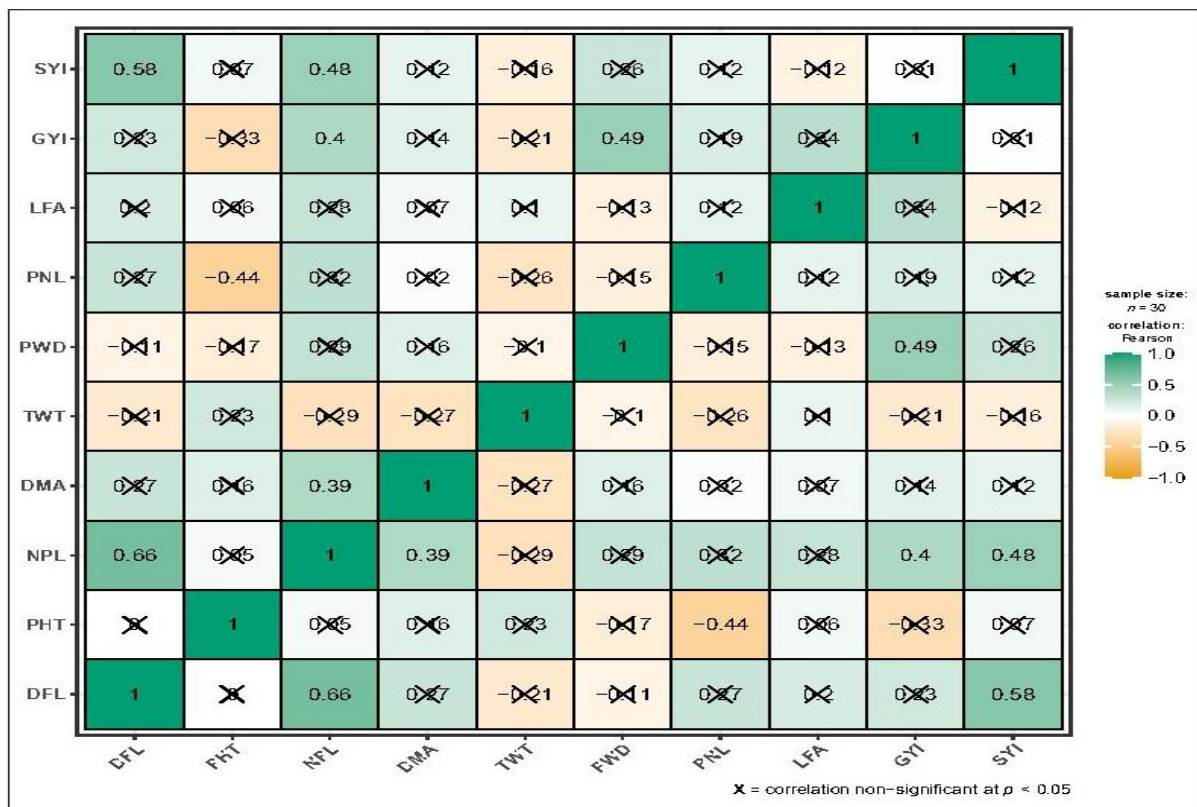

Fig. 2. Pearson correlation coefficients of quantitative traits.

These results indicated that PCA analysis could be instrumental in the evaluation of sorghum accessions for crop production. Makanda *et al.* (2012), Sinha and Kumaravadivel (2016) also found significant differences among the different quantitative morphological traits in the sorghum crop. Shegro *et al.* (2013) found the maximum contribution of the first two principal components in the sorghum

accessions. Our results are in line with previous findings (Sinha and Kumaravadivel, 2016),who also found that the first three PCs contributed the maximum to the genetic diversity among the sorghum accessions. These previous findings also assessed genetic diversity by the contribution of different morphological quantitative traits. Our results are also consistent with the findings of Mumtaz *et al.* (2018), they also investigated the genetic diversity among the sorghum accessions.
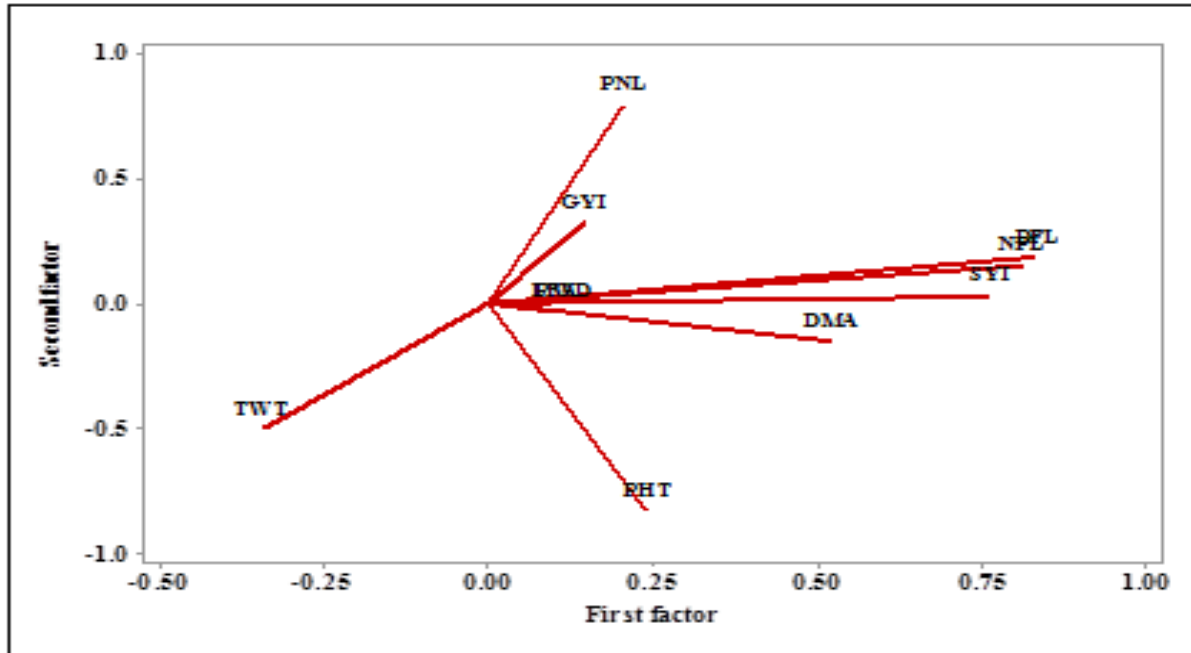

Fig. 3. The loading plot of quantitative traits based on the factor analysis.
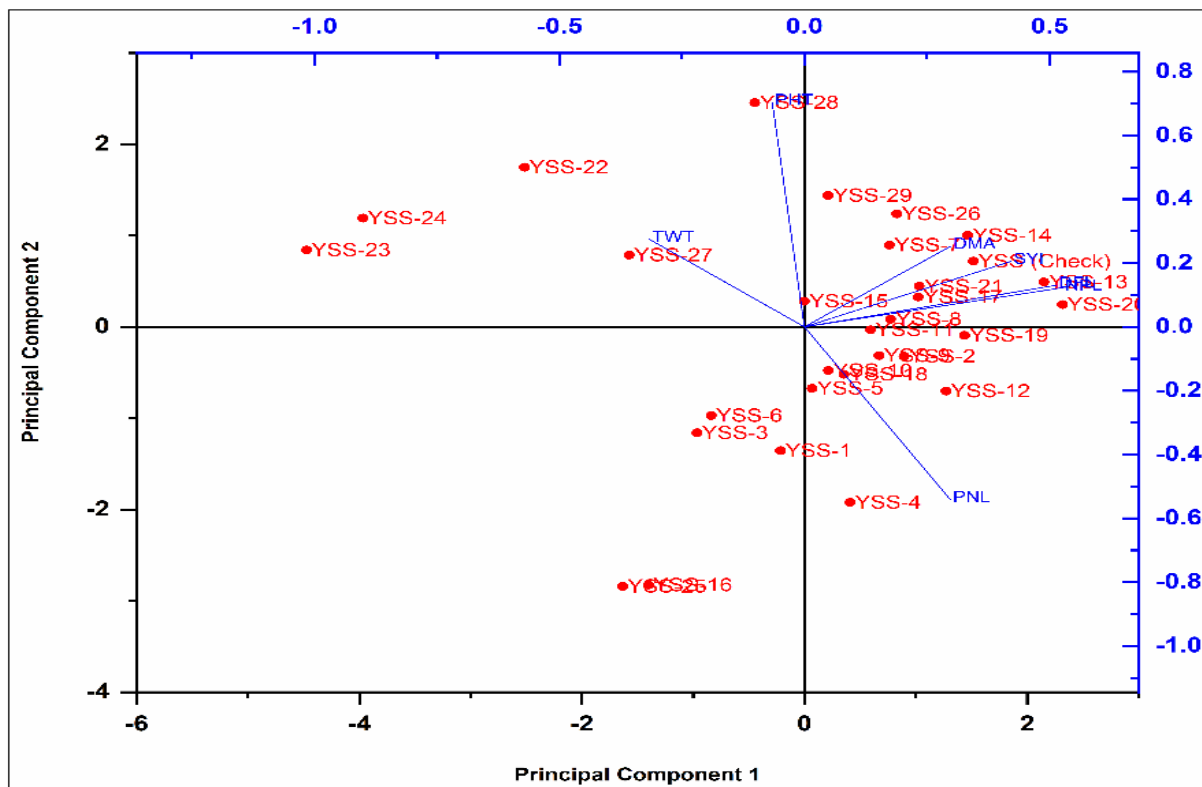

Fig. 4. The distribution of sorghum accessions for the first two principal components based on quantitative traits.

Cluster analysis

The hierarchical clustering study was performed using Ward's linkage method based on the Euclidean distance matrix, and the resulting dendrogram is shown in Figure 5. Thirty sorghum accessions comprised four clusters (Fig.5). The size of clusters varied from three to thirteen of the various groups of clusters. The cluster-1 contained 08 accessions, cluster-2 contained the minimum number of accessions, i.e., 03 accessions, cluster-3 comprised of 06 accessions, while, cluster-4 having the maximum number of accessions about 13. The clustering patterns showed the significant amount of variability in the sorghum accessions.
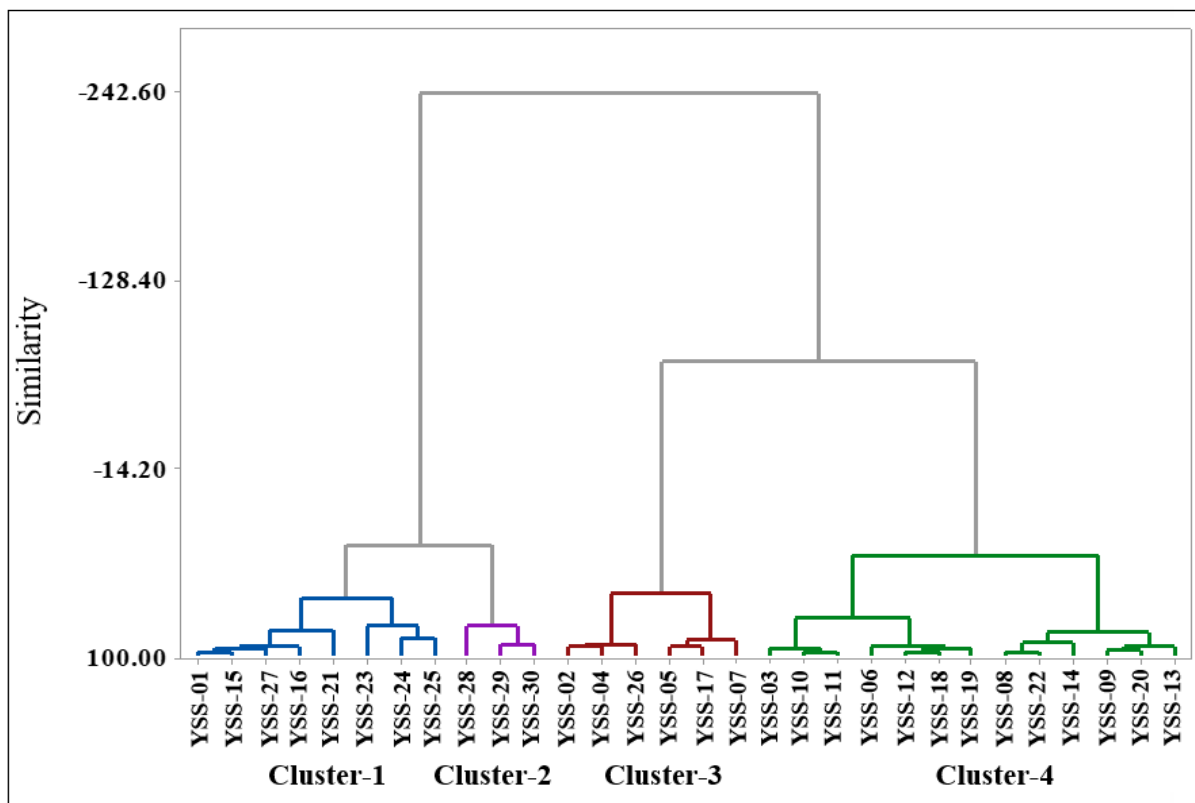

Fig. 5. The dendrogram of sorghum accessions based on seven quantitative traits.

The highest inter-cluster distance was recorded between the cluster-2 and cluster-4 (154.24), it concluded that if the sorghum accessions are selected from these cluster for the hybridization program, may give the broad spectrum of the variability in the segregating generation (Table 4). While, the lowest inter-cluster was noted between the cluster-1 and cluster-4 (44.77). It has been cited in the previous studies that the clusters contributing the maximum divergence to the variability given the greater emphasis for the type of cluster for the purpose of the selection process, and used as parents in the hybridization program (Rohman *et al.*, 2004).

Cluster-1 had the highest mean value for the thousand-seed weight (30.63). Cluster-2 showed the highest mean value for the trait panicle length (28.89). Cluster-3 showed the highest mean values 82.11, and 327.11 for days to flowering, and plant height, respectively. Cluster-4 showed the highest mean values no. of leaves per plant (14.92), stalk yield (23.15), and days to maturity (128.90) (Table 5).

Based on mean values, we found the most crucial cluster is the cluster-4 because of having the highest mean values for no. of leaves per plant, stalk yield, and days to maturity. Thus, the sorghum accessions falling this cluster could be used in the hybridization program as parents. (Shegro *et al.*, 2013) found the five clusters, while (Kisua *et al.*, 2015) observed the three clusters for sorghum accessions on the basis of quantitative traits. Similarly, (Sinha and

Kumaravadivel, 2016) performed the cluster analysis on forty sorghum accession and found the clusters, but in our study, we found the four clusters for thirty accessions based on quantitative characteristics.

Our findings are similar to the results of (Sinha and Kumaravadivel, 2016), because we also performed cluster analysis by using sorghum accessions with their quantitative traits, but there was difference in number of clusters because of difference in number of accessions, and we also found the higher genetic diversity.

Conclusion

These findings suggested that quantitative traits play a crucial role, as well as a useful tool for preliminary assessment of genetic diversity among the various sorghum accessions. The analysis of correlations reveals that the traits, namely, no. of leaves/plant and panicle width, had positive significant correlation with the grain yield. At the same time, stalk yield had also positive significant correlation with no. of leaves/plant, and days to flowering.

The principal component analysis grouped the sorghum accessions into three factors that contributed about 73.27% of the total variance, and the quantitative have great potential in producing the genetic diversity, and selection of sorghum accessions. From cluster analysis, the thirty accessions grouped into four clusters of genetic variability based on selected seven quantitative traits. Thus, the selection of the parents must be on the base of different clusters. Based on the quantitative traits of sorghum, it is concluded that accessions YSS-13 and YSS-20 should be used in future breeding programs for the development of superior verities.

Acknowledgments

Conflict of interest

The authors declare that they have no conflict of interest.

References

Adugna A. 2014. Analysis of in situ diversity and population structure in Ethiopian cultivated Sorghum bicolor (L.) landraces using phenotypic traits and SSR markers. Springer Plus **3(1),** 212.
http://dx.doi.org/10.1186/2193-1801-3-212

Assar AHA, Uptmoor R, Abdelmula AA, Wagner C, Salih M, Ali AM, Ordon F, Friedt W. 2020. Assessment of sorghum genetic resources for genetic diversity and drought tolerance using molecular markers and agro-morphological traits. University of Khartoum Journal of Agricultural Sciences **17(1).**

Ayana A, Bekele E. 1998. Geographical patterns of morphological variation in sorghum (Sorghum bicolor (L.) Moench) germplasm from Ethiopia and Eritrea: qualitative characters. Hereditas **129,** 195-205.
https://doi.org/10.1111/j.1601-5223.1998.t01-1-00195.x

Bibi A, Sadaqat HA, Akram HM, Mohammed MI. 2010. Physiological markers for screening sorghum (Sorghum bicolor) germplasm under water stress condition. International Journal of Agriculture and Biology **12,** 451-455.

Dahlberg J, Zhang X, Hart G, Mullet J. 2002. Comparative assessment of variation among sorghum germplasm accessions using seed morphology and RAPD measurements. Crop Science **42,** 291-296.
https://doi.org/10.2135/cropsci2002.0291

Hariprasanna K, Patil J. 2015. Sorghum: origin, classification, biology and improvement, Sorghum molecular breeding. Springer, p 3-20.
https://doi.org/10.1007/978-81-322-2422-8_1

Hart G, Schertz K, Peng Y, Syed N. 2001.

Genetic mapping of Sorghum bicolor (L.) Moench QTLs that control variation in tillering and other morphological characters. Theoretical and Applied Genetics **103,** 1232-1242.
https://doi.org/10.1007/s001220100582

Iqbal A, Sadia B, Khan A, Awan F, Kainth R, Sadaqat H. 2010. Biodiversity in the sorghum (Sorghum bicolor L. Moench) germplasm of Pakistan. Genetics and Molecular Research **9,** 756-764.
https://doi.org/10.4238/vol9-2gmr741

Jayaramachandran M, Kumaravadivel N, Kandasamy G, Eapen S. 2011. Comparison of genetic variability induced by γ radiation and tissue culture in sorghum. International journal of Bio-resource and Stress Management **2,** 329-333.

Khan A, Nawab K, Khan A, Islam B. 2007. Growth characters and fodder production potential of sorghum varieties under irrigated conditions. Sarhad Journal of Agriculture (Pakistan).

Kisua J, Mwikamba K, Makobe M, Muigai A. 2015. Genetic diversity of sweet and grain sorghum populations using phenotypic markers. International Journal of Biosciences **6,** 34-46.
https://doi.org/10.12692/ijb/6.9.34-46

Kong L, Dong J, Hart G. 2000. Characteristics, linkage-map positions, and allelic differentiation of Sorghum bicolor (L.) Moench DNA simple-sequence repeats (SSRs). Theoretical and Applied Genetics **101,** 438-448.
https://doi.org/10.1007/s001220051501

Li R, Zhang H, Zhou X, Guan Y, Yao F, Song G, Wang J, Zhang C. 2010. Genetic diversity in Chinese sorghum landraces revealed by chloroplast simple sequence repeats. Genetic Resources and Crop Evolution **57,** 1-15.
https://doi.org/10.1007/s10722-009-9446-y

Makanda I, Derera J, Tongoona P, Sibiya J. 2012. Genetic and GGE biplot analyses of sorghum germplasm for stem sugar traits in Southern Africa. African Journal of Agricultural Research **7,** 212-223.
https://doi.org/10.5897/ajar11.989

Mumtaz A, Hussain D, Saeed M, Arshad M. Yousaf MI. 2018. Estimation of genetic diversity in sorghum genotypes of Pakistan. Journal of the National Science Foundation of Sri Lanka **46**.
https://doi.org/10.4038/jnsfsr.v46i3.8479

Rao SA, Rao KP, Mengesha M, Reddy VG. 1996. Morphological diversity in sorghum germplasm from India. Genetic Resources and Crop Evolution **43,** 559-567.
https://doi.org/10.1007/bf00138832

Raza A, Mehmood SS, Ashraf F, Khan RSA. 2019. Genetic diversity analysis of Brassica species using PCR-based SSR markers. Gesunde Pflanzen **71,** 1-7.
https://doi.org/10.1007/s10343-018-0435-y

Reddy BV, Ashok Kumar A, Sanjana Reddy P. 2008. Genetic improvement of sorghum in the semi-arid tropics. Genetic improvement of sorghum in the semi-arid tropics, 105-123.

Rohman M, Hakim M, Sultana N, Kabir M, Hasanuzzan M, Ali M. 2004. Genetic divergence analysis in sorghum (Sorghum bicolor L.). Asian Journal of Plant Sciences **3,** 211-214.

Shegro A, Labuschagne MT, van Biljon A, Shargie NG. 2013. Assessment of genetic diversity in sorghum accessions using amplified fragment length polymorphism (AFLP) analysis. African Journal of Biotechnology **12,** 1178-1188.

Shehzad T, Okuizumi H, Kawase M, Okuno K. 2009. Development of SSR-based sorghum (Sorghum bicolor (L.) Moench) diversity research set of germplasm and its evaluation by morphological traits. Genetic Resources and Crop Evolution **56,** 809-827.
https://doi.org/10.1007/s10722-008-9403-1

Sinha S, Kumaravadivel N. 2016. Understanding genetic diversity of sorghum using quantitative traits. Scientifica 2016.
https://doi.org/10.1155/2016/3075023

Ullah M, Ali B, Muneer M, Munir M, Imran M, Adil S. 2016. Investigation of different Sorghum (Sorghum bicolor L. Moench) hybrids for yield and other associated traits under climatic conditions of Dera Ghazi Khan. Science Letters **4,** 190-192.

Wing EAS. 2016. Finance division. Government of Pakistan,"Pakistan Economic Survey", Varios números.

Zongo JD, Gouyon PH, Sandmeier M. 1993. Genetic variability among sorghum accessions from the Sahelian agroecological region of Burkina Faso. Biodiversity & Conservation **2,** 627-636.
https://doi.org/10.1007/bf00051963