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FIELD-BASED EVALUATION OF SWEET SORGHUM GERMPLASM TO UNVEIL MORPHOLOGICAL DIVERSITY

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Abstract

Sweet sorghum (*Sorghum bicolor*) is an emerging C_4 crop species exploited for its grain, fodder and bio-ethanol purposes. It is recognized as an advanced biofuel feedstock, possessing climate smart attributes like abiotic stress tolerance, low input needs and reduced greenhouse gas emissions. To develop effective breeding program for sweet sorghum in Pakistan, the information on adaptation to local environments, genetic relationships and diversity of sweet sorghum accessions is vital for the identification of better breeding parents. The study used a panel of 200 sweet sorghum accessions obtained from the USDA's worldwide collection for genetic diversity estimation. Eight quantitative traits were evaluated under field conditions for two years. Extensive phenotyping and morphological characterization indicated the presence of significant genetic diversity within the germplasm. High variability was reported in plant height (113.00–321.33 cm), fresh weight (570.67–1058.00 g), dry weight (235.0–450.33 g), and stem thickness (4.00–22.33 mm). A high broad-sense heritability was shown by all the observed quantitative traits. The major 5 principal components having Eigen value (>1) shared 80.09% variability of traits among the genotypes. The correlation analysis showed that plant height was positively correlated with the number of leaves and fresh weight. Days to flowering and days to maturity were also seen to be in strong positive correlation with each other. Cluster analysis classified the genotypes into two classes based on the homology. The explored genetic potential of sweet sorghum collection can be helpful in varietal development and improvement programs. Moreover, this diverse set of sweet sorghums can be evaluated through genome-wide association mapping by using molecular markers for molecular selection of promising genotypes with improved agronomic and quality traits.

Key words: Sweet sorghum, Genetic diversity, Morphological characterization, Quantitative traits.

Introduction

Sorghum is an ancient crop being grown for its grain (grain sorghum), forage (forage sorghum), or sugary sap (sweet sorghum). Several different common names are being used for cultivated sorghum in different regions of the world such as great millet, broomcorn, kaffir corn, durra, milo, jowar or kaoliang. Primarily, sorghum is a self-pollinated summer crop (Djè *et al*., 2004), with a genome size of 740 Mb (Paterson *et al*., 2009). Sorghum is cultivated in a wide range of geographical areas of America, Africa, Asia, and the Oceana. It is the fifth most important cereal in the world after wheat, maize, rice, and barley (Mahmood *et al*., 2008). It is believed that the early domestication of sorghum took place in North-Eastern Africa, whereas, the earliest known record of sorghums was observed from an archeological dig near the Egypt-Sudan border, dated back to 8,000 B.C. Sorghum spread throughout Africa and adapted along the way to a range of environments. The spread and establishment of five different sorghum races can be attributed to the movement of several African tribes. Sorghum, eventually made its way to India, China, and to Australia (McGinnis *et al*., 2020).

Millions of people living in various regions of Asia and Africa use sorghum grain in the form of different food products such as breads, noodles, cakes couscous, beer, and porridge (Mofokeng *et al*., 2019). Nearly all of the sorghum production (97%) in the western hemisphere is for livestock feed and forage, since it is a low cost alternative to maize and requires less water to grow (Motlhaodi *et al*., 2017). Developing countries also use sorghum and its products for cooking, construction materials, leather dyes, paper making, and as fencing material (Wang *et al*., 2018). An extensive root system and the ability to become dormant during water stress make sorghum drought-resistant (Jabereldar *et al*., 2017), typically requiring only one-half to two-thirds the amount of rainfall as compared to maize (Ortiz *et al*., 2017). Cultivated sorghum is physiologically perennial but typically grown as an annual crop (Geleta *et al*., 2006).

Sweet sorghum is an emerging C_4 grass species, primarily grown for syrup or sap. It has sugar-rich stalks due to the presence of high concentration of soluble carbohydrates. Sweet sorghum is a potential multi-purpose crop providing food in the form of grains and fuel in the form of ethanol from stem's juice. Sweet sorghum's juice contains approximately 17-23% fermentable sugars which can directly be fermented into bioethanol (Sawadogo *et al*., 2020). Being one of the most drought resilient agricultural crops, sweet sorghum is considered a viable option against maize and sugarcane as a potential energy and sugar crop (Bojović *et al*., 2019).

Analysis of diversity at genetic level can explore unique and desired characters that can be used in various crop improvement programs at functional level (Mackay & Powell, 2007). Cross hybrids introduction and loss of landraces are the major causes of diversity depletion in cultivated sorghum specifically in Asia, Africa, and Latin America.

Previous decades witnessed a fast transformation from conventional breeding approaches to molecular selection. Molecular markers have been found to be an excellent and preferable choice for modern breeding experiments. Nucleotide mapping offers tremendous opportunity for plant biologists to practice Marker-Assisted Selection (MAS). Genome-Wide Association Studies (GWAS) play a pivotal role in guiding genomic interventions in sweet sorghum by identifying genetic variants, such as SNPs, associated with key traits like sugar content, drought tolerance, and disease resistance. These studies enable the mapping of traits to specific genomic loci and the identification of candidate genes, which serve as targets for interventions like CRISPR/Cas9 gene editing or transgene introduction. GWAS data are also utilized in breeding programs through

Marker-Assisted Selection (MAS) and Genomic Selection (GS) to enhance breeding efficiency. For example, genes involved in sucrose and lignin biosynthesis, identified via GWAS, have been modified to improve sugar content for bioethanol production, while loci linked to stress tolerance and disease resistance are targeted to enhance crop resilience. Despite challenges such as polygenic traits and environmental influences, integrating GWAS with tools like transcriptomics and metabolomics continues to advance sweet sorghum improvement. Sorghum's diploid nature of genetics, flexibility to inbreed, and manageable levels of polymorphisms among the germplasm makes it amenable to modern molecular approaches (Morris *et al*., 2013).

The very first step towards the Marker-assisted selection is field-based evaluation for diversity analysis in any given germplasm. In this context, the study was planned to acquire and maintain USDA's sweet sorghum germplasm at the University of Agriculture Faisalabad (UAF) and to evaluate the obtained germplasm collection (200 accessions) of sweet sorghum germplasm for agro-morphological sucrose-yield traits at phenotypic level. Field trials were conducted for two years (2019-20 and 2020-21) in which extensive phenotyping was done to get an estimate on the adaptation of the germplasm in local conditions and to acquire morphological data on various agronomic and sucrose yield-related traits. Promising sweet sorghum accessions with better local adaptability and high morphological diversity for sucrose yield potential have been identified by studying eight morphological markers. The main objective of the study was to perform phenotypic assessment of the given germplasm for sucrose accumulation potential which would be useful in mapping important genomic regions as well. Our results indicated that a suitable diversity among the studied morphological markers existed which makes the germplasm suited for evaluation at molecular level. The associated morphological markers can be used to identify the genetic loci through genome-wide association mapping which can be exploited in future for molecular marker assisted breeding of sweet sorghum in Pakistan. Overall, the advances in sweet sorghum breeding and genetics will help secure a key role for sweet sorghum in the emerging bio-energy economy.

Material and Methods

Experimental material: Diverse collection of 200 sweet sorghum accessions from 35 countries was acquired from the United States Department of Agriculture (Table 1). All these accessions were grown in the field during 2019-20 and 2020-21 at the Directorate of Farms, University of Agriculture Faisalabad, Pakistan (Latitude 31.44' N, Longitude 73.07' E).

Layout and phenotyping for agronomic and sugar-related traits: Morphological characterization of sweet sorghum collection was carried out for two years (2019-20 and 2020-21). For both trials, sweet sorghum accessions were sown in three replicates following a Randomized Complete Block Design (RCBD). For each accession, 10 plants were planted in a row. Plant to plant distance was maintained 15 cm while the distance between each row was kept 45 cm. Two seeds were planted in each hole to get a reasonable plant stand. For both field trials, three plants from each accession per replication were chosen and tagged. Data from the tagged plants were taken for different agronomic traits such as Plant Height (PH), Number of Leaves (NL), Days to Flowering (DF), Days to Maturity (DM), Fresh Weight (FW), Dry Weight (DW), Brix (BX), and Stem Thickness (ST).

Data analysis: Recorded data were analyzed by studying the descriptive statistics first, where mean, standard error of means, standard deviation (SD), coefficient of variation (CV), minimum, and maximum values for each trait were calculated. Analysis of variance (ANOVA) was carried out using Minitab version 22.1.0. Principle component analysis (PCA), correlation analysis, and cluster analysis were carried out using XLSTAT package in MS-Excel. Broad sense heritability was also estimated for each observed trait (Mohammed *et al*., 2015).

Results and Discussion

Descriptive statistics and broad-sense heritability: The mean values of each trait, standard error of means, standard deviation, coefficient of variation, and lowest/ highest values were computed in basic statistical analysis (Tables 2 and 3).

The description of the basic stats indicated the highest value of PH was observed in the accession 70 (PI 195754), which was 315 cm, while the minimum observed height was 113 cm in the accession 176 (PI 152961) during the year 1. For the second year, the maximum value of PH was 321.33 cm (PI 173121) and the lowest value was 165.67 cm, observed in the accession 176 (PI 152961), showing that the accession 176 had the lowest value of PH in both years. The descriptive statistics for BX showed that the accessions 48 (PI 251672), 74 (PI 181899), 81 (PI 180004), 83 (PI 179504), 93 (PI 173808), 99 (PI 170787), 105 (PI 167352), 170 (PI 152880), and 174 (PI 152923) had the maximum BX (16%) in the year 1. During the second year, the analysis showed that the maximum BX was observed in the accession 170 (PI 152880), which was 16%. While the lowest BX was observed in accession 197 (PI 52606) and 9 (PI 535785) which is 4%, in year 1 and 2, respectively.

For ST, the maximum thickness was observed 21 mm in the accessions 16 (PI 641815), 40 (PI 260210), 66 (PI 197542), 83 (PI 179504), 90 (PI 179749), 109 (PI 157035), and 167 (PI 152828), while the minimum thickness was 4 mm in accessions 12 (PI 533998), and 14 (PI 641904) during the year 1. In the second year, the highest observed diameter was 22.33 mm in the accession 139 (PI 154750), while the minimum value of stem diameter was observed is 6 mm in accessions 11 (PI 535796), 14 (PI 641904), and 119 (PI 155485). According to the descriptive statistics, accession 50 (PI 250898) had maximum NL per plant in the year 1 (2019-20) i.e., 18.67 NL / plant on an average. The minimum NL per plant observed was 7, in the accessions 41 (PI 257599), 66 (PI 197542), 174 (PI 152923), and 196 (PI 88007). During the year 2, the maximum NL was 16.33, observed in the accession 34 (PI 287625), while the minimum leaves were observed in the accession 104 (PI 167047) which was 6.33. For the year 1, largest value of DF was 124 days, observed in 15 accessions whereas the minimum value of DF was 80, observed in 35 accessions. The maximum number of DF for the year 2 were 120 observed in 55 accessions while the minimum number of DF were observed in 34 sweet sorghum accessions.

Table 1. List of sweet sorghum accessions used in the study.

Sr.	Accession	Origin	Sr.	Accession	Origin	Sr.	Accession	Origin
1.	PI 586541	Australia	39.	PI 584989	US	77.	PI 181080	India
2.	PI 583832	US	40.	PI 260210	Guadeloupe	78.	PI 181083	India
3.	PI 610727	China	41.	PI 257599	Ethiopia	79.	PI 180489	India
4.	PI 586443	Hungary	42.	PI 257600	Ethiopia	80.	PI 180348	India
5.	PI 566819	US	43.	PI 257602	Ethiopia	81.	PI 180004	India
6.	PI 562716	US	44.	PI 255239	Mexico	82.	PI 180005	India
7.	PI 563295	US	45.	PI 253986	Syria	83.	PI 179504	Turkey
8.	PI 535783	US	46.	PI 253795	Iraq	84.	PI 177553	Turkey
9.	PI 535785	US	47.	PI 253796	Iraq	85.	PI 177554	Syria
10.	PI 535792	US	48.	PI 251672	Serbia	86.	PI 177156	Turkey
11.	PI 535796	US	49.	PI 250897	Iran	87.	PI 176766	Turkey
12.	PI 533998	US	50.	PI 250898	Iran	88.	PI 175919	Turkey
13.	PI 511355	US	51.	PI 250521	India	89.	PI 179747	India
14.	PI 641904	Donated	52.	PI 250582	Egypt	90.	PI 179749	India
15.	PI 641909	Sudan	53.	PI 250402	Pakistan	91.	PI 174381	Turkey
16.	PI 641815	Donated	54.	PI 250232	Pakistan	92.	PI 173971	India
17.	PI 641817	Donated	55.	PI 250234	Pakistan	93.	PI 173808	Turkey
18.	PI 641821	Donated	56.	PI 248298	India	94.	PI 173112	Turkey
19.	PI 641834	Donated	57.	PI 247744	Congo	95.	PI 173120	Turkey
20.	PI 641835	Donated	58.	PI 247745	Congo	96.	PI 173121	Turkey
21.	PI 641848	Donated	59.	PI 247136	Serbia	97.	PI 173118	Turkey
22.	PI 641862	Donated	60.	PI 221560	Nigeria	98.	PI 170783	Turkey
23.	PI 641893	Donated	61.	PI 218112	Pakistan	99.	PI 170787	Turkey
24.	PI 641806	Donated	62.	PI 217691	Sudan	100.	PI 170788	Turkey
25.	PI 641807	Donated	63.	PI 217770	Sudan	101.	PI 170799	Turkey
26.	PI 302120	Belgium	64.	PI 201723	Nigeria	102.	PI 170802	Turkey
27.	PI 302122	Portugal	65.	PI 198885	Australia	103.	PI 170805	Turkey
28.	PI 302198	Argentina	66.	PI 197542	Algeria	104.	PI 167047	Turkey
29.	PI 302199	Argentina	67.	PI 196592	Taiwan	105.	PI 167352	Turkey
30.	PI 302252	China	68.	PI 196598	Taiwan	106.	PI 157804	Sudan
31.	PI 302264	Tanzania	69.	PI 196049	Ethiopia	107.	PI 157030	Kenya
32.	PI 303658	Sudan	70.	PI 195754	China	108.	PI 157033	Kenya
33.	PI 302131	Portugal	71.	PI 189114	Nigeria	109.	PI 157035	Kenya
34.	PI 287625	Zimbabwe	72.	PI 183149	India	110.	PI 156890	Congo
35.	PI 287627	Zimbabwe	73.	PI 182303	Turkey	111.	PI 156136	Zambia
36.	PI 273955	Ethiopia	74.	PI 181899	Syria	112.	PI 156356	Zambia
37.	PI 273969	Ethiopia	75.	PI 181971	Syria	113.	PI 156393	Tanzania
38.	PI 267476	India	76.	PI 181077	India	114.	PI 156203	Malawi
115.	PI 156217	Malawi	144.	PI 154846	Uganda	173.	PI 152914	US
116.	PI 156252	Malawi	145.	PI 153871	Kenya	174.	PI 152923	Sudan
117.	PI 156352	Zambia	146.	PI 152596	Sudan	175.	PI 152953	Sudan
118.	PI 155805	Malawi	147.	PI 152629	Sudan	176.	PI 152961	Sudan
119.	PI 155485	Zambia	148.	PI 152630	Sudan	177.	PI 152963	Sudan
120.	PI 155516	Zambia	149.	PI 152633	Sudan	178.	PI 152966	Sudan
121.	PI 155543	Zambia	150.	PI 152646	Sudan	179.	PI 152971	Sudan
122.	PI 155556	Zambia	151.	PI 152650	Sudan	180.	PI 152998	Eritrea
123.	PI 155571	Zambia	152.	PI 152651	Sudan	181.	PI 149830	Somalia
124.	PI 155609	Zambia	153.	PI 152671	Sudan	182.	PI 149832	Somalia
125.	PI 155845	Malawi	154.	PI 152675	Sudan	183.	PI 147573	F. Guiana
126.	PI 155902	Malawi	155.	PI 152676	Sudan	184.	PI 147200	India
127.	PI 155721	Malawi	156.	PI 152683	Sudan	185.	PI 147224	India
128.	PI 155760	Malawi	157.	PI 152692	US	186.	PI 147026	Egypt
129.	PI 155924	Zambia	158.	PI 152714	Sudan	187.	PI 146890	Congo
130.	PI 155336	Kenya	159.	PI 152725	Sudan	188.	PI 145619	South Africa
131.	PI 154929	Uganda	160.	PI 152733	Sudan	189.	PI 145622	South Africa
132.	PI 154943	Uganda	161.	PI 152751	Sudan	190.	PI 145632	South Africa
133.	PI 154944	Uganda	162.	PI 152755	Sudan	191.	PI 145633	South Africa
134.	PI 154962	Uganda	163.	PI 152764	Sudan	192.	PI 144331	South Africa
135.	PI 154980	Kenya	164.	PI 152771	Sudan	193.	PI 144134	South Africa
136.	PI 154987	Eswatini	165.	PI 152813	Sudan	194.	PI 92270	China
137.	PI 154988	Eswatini	166.	PI 152816	Sudan	195.	PI 88000	Korea
138.	PI 154990	Eswatini	167.	PI 152828	Congo	196.	PI 88007	Korea
139.	PI 154750	Uganda	168.	PI 152860	Sudan	197.	PI 52606	South Africa
140.	PI 154787	Uganda	169.	PI 152872	Sudan	198.	PI 48191	Australia
141.	PI 154796	Uganda	170.	PI 152880	Sudan	199.	PI 22913	China
142.	PI 154800	Uganda	171.	PI 152898	India	200.	PI 17548	Australia
143	PI 154844	Uganda	172.	PI 152909	Somalia			

The maximum DM were 178 found in 38 accessions while the lowest value was 160, observed in only 2 accessions (94 and 105) for the year 1 (2019-20). During the second year, 42 sweet sorghum accessions had the maximum DM (194 days), while the minimum observed DM were 173, seen in 35 accessions. During the year 1, the maximum value of FW (biomass) was observed in the accession 87 (PI 176766), and the minimum value was 570, observed in accession 5 (PI 566819). The maximum FW was observed in the accession 195 (PI 88000), which was 1,058 g, during the year 2, while the minimum value was 621 g, observed in the accession 28 (PI 302198). During the year 1, the maximum value of DW was 418.67 g, observed in the accession 182 (PI 149832), and the minimum value was 267.33, observed in accession 27 (PI 302122). The maximum DW was observed in the accession 146 (PI 152596), which was 450.33 g, during the year 2, while the minimum value was 235 g, observed in the accession 189 (PI 145622).

Broad-sense heritability was estimated for each trait in both years. The heritability value for PH was 0.98 and 0.99 in year 1 and 2, respectively. This indicated that a highly significant percentage (98% and 99%) of the observed variation was attributed to genetic factors, while the remaining variation was likely due to environmental or other non-genetic factors. In case of BX, the heritability was 0.99 and 0.94 (year 1 and 2, respectively), indicating that the genetic diversity in the second year was mainly due to genetic influence making the germplasm best suited for studying the trait for genome-wide analysis. While calculating the broad-sense heritability for ST, it was observed that the variation was mainly due to genetic factors, in both years, while the contribution of other non-genetic factors was non considerable. For both the years, each descriptive statistical value for each trait and the value of broad-sense heritability are given in tables 2 and 3.

Table 2. Descriptive statistics of observed traits of sweet sorghum germplasm during the 1st year (2019-20).

Traits	Mean	SEM	SD	CV	Minimum	Median	Maximum	Heritability
PH	233.99	2.52	34.09	14.57	113.00	232.33	315.00	0.98
NL	10.818	0.146	1.978	18.29	7.000	10.667	18.667	0.93
DF	102.18	1.23	16.60	16.25	80.00	105.00	124.00	0.99
DM	169.44	0.464	6.28	3.71	160.00	168.00	178.00	0.99
FW	880.53	7.43	100.56	11.42	570.67	904.33	1057.00	0.70
DW	325.11	2.40	32.40	9.97	267.33	319.33	418.67	0.48
BX	10.355	0.216	2.923	28.23	4.000	10.000	16.000	0.99
ST	10.978	0.285	3.851	35.08	4.000	10.000	21.000	0.99

SEM (Standard error of Means), CV (Coefficient of Variation), SD (Standard Deviation), PH (Plant Height), NL (Number of Leaves), DF (Days to Flowering), DM (Days to Maturity), FW (Fresh Weight), DW (Dry Weight), BX (Brix), ST (Stem Thickness)

Table 3. Descriptive statistics of observed traits of sweet sorghum germplasm during the 2nd year (2020-21).

Trait	Mean	SEM	SD	CV	Minimum	Median	Maximum	Heritability
PH	234.99	2.36	31.93	13.59	165.67	231.00	321.33	0.99
NL	10.914	0.139	1.879	17.22	6.333	10.667	16.333	0.85
DF	103.39	1.06	14.40	13.93	86.00	102.67	120.00	0.99
DM	183.50	0.639	8.65	4.71	173.00	184.00	194.00	0.99
FW	905.86	6.20	83.92	9.26	621.00	924.33	1058.00	0.68
DW	333.45	2.59	35.09	10.52	235.00	329.67	450.33	0.41
BX	9.273	0.180	2.437	26.28	4.000	9.333	16.000	0.94
ST	11.690	0.274	3.705	31.69	6.000	10.667	22.333	0.97

SEM (Standard Error of Means), CV (Coefficient of Variation), SD (Standard Deviation), PH (Plant Height), NL (Number of Leaves), DF (Days to Flowering), DM (Days to Maturity), FW (Fresh Weight), DW (Dry Weight), BX (Brix), ST (Stem Thickness)

Table 4. Mean square values of morphological traits observed in ANOVA during the 1st year (2019-20).

Traits	Replication	Genotype	Error
Df	2	182	364
PH	55.48	3486.04**	45.27
NL	0.73	11.73**	0.82
DF	1.04	826.73**	0.30
DM	0.02	118.27**	0.08
FW	262989	30336**	12697
DW	62295	3149	3282
BX	0.63	25.63**	0.19
ST	0.11	44.52**	0.40

Df (Degree of freedom), PH (Plant Height), NL (Number of Leaves), DF (Days to Flowering), DM (Days to Maturity), FW (Fresh Weight), DW (Dry Weight), BX (Brix), ST (Stem Thickness), ** (Highly Significant at 0.05)

Table 5. Mean square values of morphological traits observed in ANOVA during the 2nd year (2020-21).

Traits	Replication	Genotype	Error
Df	2	182	364
PH	34.57	3058.84**	25.07
NL	1.25	10.59**	1.85
DF	1.59	622.25**	0.35
DM	0.41	224.33**	0.20
FW	53238	21045**	9772
DW	25618	3695	4375
BX	31.90	17.81**	0.97
ST	1.72	41.18**	1.03

Df (Degree of freedom), PH (Plant Height), NL (Number of Leaves), DF (Days to Flowering), DM (Days to Maturity), FW (Fresh Weight), DW (Dry Weight), BX (Brix), ST (Stem Thickness), ** (Highly Significant at 0.05)

Analysis of Variance (ANOVA): Statistical analysis of variance was performed on the morphological data collected for all the agronomic and sugar- related traits. Mean square values for both the years are given in Tables 4 and 5, respectively.

In both years, the variation attributed to replications was not statistically significant, as indicated by the *p*-values (0.295 in year 1 and 0.253 in year 2). This suggests that the replication factor does not have a significant effect on plant height in either year.

The variation due to accessions was highly significant for both years, as evidenced by the low *p*-values (0.000 in both years). This indicated that the genetic factors significantly contributed to the observed differences in plant height in both years. Overall, the results showed that the genetic variation has a significant effect on plant height in both years.

The analysis of variance results for number of leaves per pant revealed a highly significant genetic variation among accessions for this trait for both year 1 and 2. Whereas, for the replication factor, the analysis for number of leaves was not significant in either year.

For days to 50% flowering, the results indicated a highly significant genetic variation but moderately significant effect of replication in both the years.

The results showed a significant difference between the accessions (118.27) and the error term (0.08), suggesting that factors other than genetic differences may be primarily responsible for the variation.

The ANOVA results for fresh weight (both years) indicated that a highly significant variation was present among the accessions attributing to both genotypes and replication factors.

The ANOVA results for dry weight data collected in field trails of both the years indicated non-significant variations among sweet sorghum accessions.

As indicated by the analysis of variance for sugar concentration (brix), the highly significant variation attributed to genotype factor but a less significant difference was observed for the replication. While during the second year, both the factors (replication and genotype) were highly significant for the brix, which means an appropriate variation was existed among the germplasm regarding the concentration of sucrose.

While analyzing the data for stem diameter, a highly significant difference was recorded among sorghum accessions in both the years.

Principle component analysis (PCA): Principle Component Analysis of phenotypic traits was carried out to eliminate the redundancy in the data sets. The analysis divided the variance of all traits into 8 principle components (PCs) as shown by the scree plot in the Figures 1 and 2. Five principle components having Eigen value greater than one (>1) are described in Tables 6 and 7 for year 1 and 2, respectively.

For the first year, the cumulative variability of five principle components (PCs) was 80.26%, where the contribution of each component was 25.72%, 15.74%, 14.11%, 13.11%, and 11.56%, respectively. In the first principle component (PC1), traits such as days to flowering and days to maturity contributed more than 30%, while brix contributed the least (0.88%). In PC2, the only factor that contributed more than 30% was the number of leaves (NL) with 37.03% contribution to the genetic diversity, whereas, days to maturity contributed the least (0.51%). Dry weight (DW) contributed 37.36% in PC3 while the zero contribution of number of leaves was observed in this component. For PC4, dry weight and brix contributed significantly (34.11% and 53.61%, respectively), whereas, the contribution of number of leaves was the least. Stem diameter alone contributed more than 30% in the fifth principle component (PC5), while the least contribution was observed for dry weight. Principle component analysis also studied the correlation between each trait and principle component. Plant height was shown to be negatively correlated with PC3 and PC4, while its correlation with other PCs was found to be normal in the first year. Number of leaves was negatively correlated with PCs 1, 4, and 5. Days to flowering and days to maturity were negatively correlated with all the PCs except PC1. A positive correlation of fresh weight and dry weight was recorded with all the PCs, except PC5. The brix value was negatively correlation with PC2 and PC3 while for other PCs, correlation was positive. Likewise, stem diameter was positively correlated with PCs 1, 3, and 5 while a negative correlation was shown for PCs 2 and 4 against the stem diameter in the first year (2019-20).

During the second year, five principle components showed a cumulative variability of 79.92%. The first component (PC1) was being contributed more than 30% by days to flowering and days to maturity which contributed equally i.e., 44.8%, while the number of leaves contributed least in this principle component. The contribution of (FW) was 38.12% in the second PC, which makes it the highly contributing variable while days to flowering (DF) contributed the least (2.08%). The maximum contribution towards PC3 was exhibited by dry weight (DW) and brix (BX) (32.28% and 41.28%, respectively) while the fresh weight had the lowest contributed value (0.35%). For PC4, the only highest contributing factor was the number of leaves (NL) (46.47%) while the least contribution to this PC was attributed by days to flowering (0.36%). Plant height contributed significantly (57.32%) in the fifth principle component while the number of leaves contributed least (0.86%). The correlation of each trait with the constructed principle components showed that the plant height was positively correlated with all the PCs expect for PC4. Number of leaves was negatively correlated with first three PCs while positive for PC4 and PC5. The days to flowering and days to maturity showed positive correlation only towards PC1 while for all other PCs, these traits exhibited negative correlation. Fresh weight was positively correlated with PCs 1, 2, and 4 while negatively correlated with components 3 and 5. In case of dry weight, the only positive correlation was found to be with PC2 while it was negative with other PCs. Brix was shown to be highly positively correlated with all the PCs except for PC5. The correlation of stem diameter was positive with all the PCs, except PC3.

Table 6. Principle Component Analysis (PCA) for morphological traits of sweet sorghum during the 1st year (2019-20).

Variables	PC1	PC2	PC3	PC4	PC5
PH	0.216	0.336	0.153	-0.155	0.757
NL	-0.029	-0.357	-0.343	0.681	0.093
DF	0.670	-0.144	-0.066	-0.060	-0.147
DM	0.670	-0.149	-0.062	-0.080	-0.136
FW	0.126	0.617	-0.059	0.131	-0.182
DW	-0.118	0.324	-0.568	-0.317	-0.376
BX	0.050	0.251	0.646	0.369	-0.412
ST	0.150	0.409	-0.327	0.498	0.183
Eigen value	2.094	1.291	1.087	1.004	0.918
Variability (%)	26.175	16.136	13.593	12.547	11.472
Cumulative %	26.175	42.310	55.903	68.450	79.922

PC (Principle Component), PH (Plant Height), NL (Number of Leaves), DF (Days to Flowering), DM (Days to Maturity), FW (Fresh Weight), DW (Dry Weight), BX (Brix), ST (Stem Thickness)

Table 7. Principle Component Analysis (PCA) for morphological traits of sweet sorghum during the 2nd year (2020-21).

Variables	PC1	PC2	PC3	PC4	PC5
PH	0.145	0.530	-0.437	-0.099	0.423
NL	-0.100	0.609	0.001	-0.021	-0.087
DF	0.663	-0.106	-0.081	-0.098	-0.112
DM	0.670	-0.072	-0.039	-0.046	-0.155
FW	0.188	0.540	0.163	0.104	-0.217
DW	0.112	0.150	0.611	0.584	-0.045
BX	0.094	-0.135	-0.373	0.732	0.404
ST	0.154	-0.021	0.511	-0.300	0.751
Eigen value	2.058	1.259	1.129	1.049	0.925
Variability (%)	25.729	15.740	14.115	13.113	11.563
Cumulative %	25.729	41.469	55.584	68.697	80.260

PC (Principle Component), PH (Plant Height), NL (Number of Leaves), DF (Days to Flowering), DM (Days to Maturity), FW (Fresh Weight), DW (Dry Weight), BX (Brix), ST (Stem Thickness)

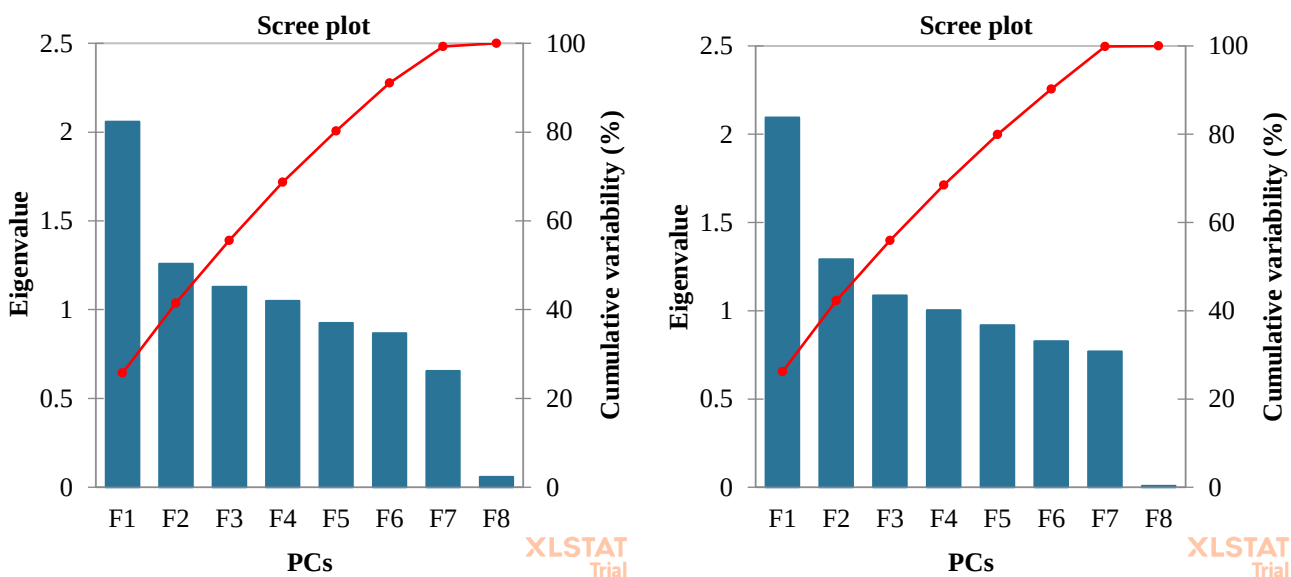

Fig. 1. Scree plot analysis for 8 morphological traits of sweet sorghum during the 2 years (2019-20 and 2020-21)

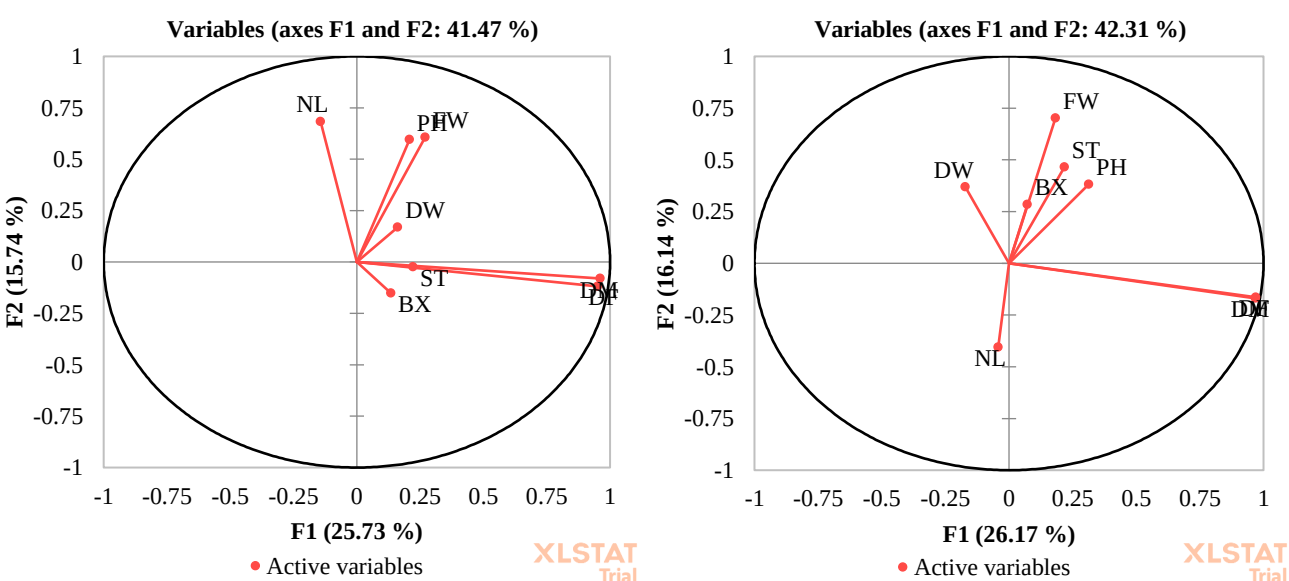

Fig. 2. Biplot analysis related to different morphological traits in sorghum for both years (2019-20 and 2020-21).

Biplot analysis: Biplot analysis represented that variables were superimposed as vector while the relative length of the vector specified as the relative proportion of the variability in each trait. The accessions which were plotted away from origin depicted less similarity as compared to the ones plotted close to the center.

Total variability shown by the first two principal components F1 (25.7%), and F2 (15.7%) for the year 2019-20, was used to establish the biplot which is based on the correlation of variables with principal components. Variables were imposed as vectors whose length revealed the combined variability in both components. Positive and negative factor loading explains the trends of correlation among the factors. Biplot was divided into 4 groups based on loading values. The vectors that were close to the origin such as dry weight, brix, and stem thickness had less variability as compared to other traits, that were located away from the origin (Fig. 2).

Biplot for the second year was constructed by total variability presented by the first two components, F1 (26.1%) and F2 (18.1%), Length of variables revealed the combined variability in both components. Positive and negative factor loadings explained the trends of correlation among the variables. Biplot was divided into four groups based on factor loading values. Vectors that were close to the origin had less variability as compared to the ones away from the origin (Fig. 2).

During the year 1, Plant height (PH) was positively correlated with fresh weight (FW) (0.165). There was a weak positive correlation between PH and number of leaves (NL) (0.147). The correlations between PH and other variables, such as days to flowering (DF), days to maturity (DM), stem diameter (ST), and brix (BX), were relatively weak (ranging from 0.016 to 0.165). The number of leaves (NL) did not show a strong correlation with any of the other variables. There was a weak positive correlation between NL and PH (0.147). NL had weak negative correlations with days to flowering (DF) and days to maturity (DM) (-0.101 and -0.101, respectively).

DF and DM showed a strong positive correlation (0.937). Both DF and DM had weak positive correlations with PH, FW, and ST (ranging from 0.068 to 0.153). There was a weak positive correlation between DF and NL (0.104). The correlation between DF/ DM and other variables, such as brix (BX) and dry weight (DW), was relatively weak.

A weak positive correlation (0.134) was observed between FW (fresh weight) and DW (dry weight) and FW with PH (0.165). The DW had weak positive correlations with DF, DM, and ST (ranging from 0.032 to 0.106). The correlation between FW/ DW and other variables, such as NL and BX, were relatively weak. The BX (brix) and ST (stem thickness) showed a weak negative correlation (-0.073). Both BX and ST had weak positive correlations with DF (0.075 and 0.125, respectively). The correlations between BX/ST and other variables, such as PH and FW, were relatively weak.

Overall, the correlation results for the first year suggested varying degrees of relationships between the variables. Some variables showed weak to moderate positive or negative correlations, while others exhibited weak or negligible correlations.

Correlation analysis: The correlation matrix of year 1 (Table 8) shows the pairwise correlations between different variables. The values in the matrix ranged from -1 to 1, with 1 indicating a perfect positive correlation, while -1 indicated a perfect negative correlation.

For the second year (Table 9), PH had a weak negative correlation with the number of NL (-0.088), while it had weak positive correlations with DF (0.145) and DM (0.155). The PH also had weak positive correlations with FW (0.118) and BX (0.037). The correlation between PH and (ST) was positive but relatively weak (0.088).

Table 8. Correlation matrix between different morphological traits of sweet sorghum during the 1st year (2019-20).

Variables	PH	NL	DF	DM	FW	DW	BX	ST
PH	**1**							
NL	**0.147**	**1**						
DF	0.104	-0.101	**1**					
DM	0.103	-0.101	**0.937**	**1**				
FW	**0.165**	0.079	0.100	**0.153**	**1**			
DW	-0.087	0.055	0.032	0.106	0.134	**1**		
BX	0.070	-0.075	0.075	0.068	-0.045	0.059	**1**	
ST	0.016	-0.044	0.125	0.100	0.017	0.093	-0.073	**1**

PH (Plant Height), NL (Number of Leaves), DF (Days to Flowering), DM (Days to Maturity), FW (Fresh Weight), DW (Dry Weight), BX (Brix), ST (Stem Thickness)

Table 9. Correlation matrix between different morphological traits of sweet sorghum during the 2nd year (2020-21).

Variables	PH	NL	DF	DM	FW	DW	BX	ST
PH	**1**							
NL	-0.088	**1**						
DF	**0.145**	0.001	**1**					
DM	**0.155**	-0.009	**0.991**	**1**				
FW	0.118	-0.092	0.070	0.065	**1**			
DW	-0.025	-0.037	-0.104	-0.106	0.098	**1**		
BX	0.037	-0.058	0.021	0.009	0.101	-0.080	**1**	
ST	0.088	0.020	0.104	0.088	**0.171**	0.045	0.020	**1**

PH (Plant Height), NL (Number of Leaves), DF (Days to Flowering), DM (Days to Maturity), FW (Fresh Weight), DW (Dry Weight), BX (Brix), ST (Stem Thickness)

Table 10. Cluster analysis with variance of two clusters with each principle component for the 1st year (2019-20).

Cluster	F1	F2	F3	F4	F5	Sum of weights	Within-cluster variance
1	-1.015	-0.020	-0.240	0.180	-0.081	97.000	4.896
2	1.144	0.022	0.270	-0.203	0.091	86.000	5.558

Table 11. Cluster analysis with variance of two clusters with each principle component for the 2nd year (2020-21).

Cluster	F1	F2	F3	F4	F5	Sum of weights	Within-cluster variance
1	-0.959	-0.061	0.000	0.072	0.154	110.000	5.222
2	1.444	0.091	-0.001	-0.108	-0.232	73.000	4.701

Number of leaves had a weak negative correlation with PH (-0.088) and a negligible correlation with other variables, as the correlation coefficients were close to zero. The DF and DM showed a strong positive correlation with each other (0.991 for both). Both DF and DM had weak positive correlations with PH (0.145 and 0.155, respectively) and ST (0.104 for both). The correlations between DF/DM and other variables were relatively weak, with coefficients close to zero. The FW had weak positive correlations with PH (0.118), DF (0.070), DM (0.065), and ST (0.171). The correlation between FW and DW was negative and weak. A weak negative correlation of DW was recorded with PH (-0.025) and NL (-0.037), and a weak positive correlation with FW (0.098) and ST (0.045). The correlation between DW and other variables, such as DF, DM, and BX, was relatively weak (Table 9). The BX and ST had weak positive correlations with FW (0.101 and 0.045, respectively). The correlation between BX and PH, as well as ST, was relatively weak (0.037 and 0.020, respectively).

Cluster analysis: For both the years, the agglomerative hierarchical clustering algorithm classified the genotypes into two distinct groups or clusters (C1 and C2) based on their similarities in the measured variables or characteristics. The accessions within each cluster tend to be more similar to one another in terms of their observed traits or attributes as compared to genotypes in the other cluster. The similarity implies that genotypes within a cluster share common features or responses in relation to the traits considered in the analysis (Tables 10-11; Fig. 3).

Discussion

Morphological characterization plays an essential role in exploring and classifying genetic diversity within germplasm, providing a foundational understanding for plant breeding and genetic improvement programs (Boyles *et al*., 2019). In this study, we evaluated eight agronomic traits: plant height (PH), number of leaves (NL), days to flowering (DF), days to maturity (DM), fresh weight (FW), dry weight (DW), Brix (BX), and stem thickness (ST). These traits were systematically analyzed to understand the genetic potential of sweet sorghum accessions obtained from the USDA germplasm collection. The findings revealed that all studied traits were highly significant, underscoring their importance in assessing and improving sweet sorghum's performance. The recorded ranges for plant height, number of leaves, days to flowering, and fresh and dry weights of the accessions were consistent with previous studies (Noor *et al*., 2012; Birhan *et al*., 2022). This consistency suggests that the germplasm under study aligns well with established genetic benchmarks for sweet sorghum. Importantly, broad-sense heritability values for the evaluated traits were above 90% (with the exception of fresh and dry weight), highlighting the robust genetic potential of the germplasm. These findings emphasize the possibility of achieving significant genetic gains through selection, echoing earlier observations reported by Puspitasari *et al*., (2012), Singh *et al*. (2013), El-Abed *et al*., (2021), and Abu-Ellail *et al*., (2023). The application of advanced statistical tools provided further insight into the morphological diversity within the population. These tools revealed cumulative variability of 80.26% for the first five principal components (PCs) during the first year, with individual contributions of 25.72%, 15.74%, 14.11%, 13.11%, and 11.56%, respectively. Similarly, during the second year, the cumulative variability was 79.92%, with the first PC contributing the highest proportion (26.17%), followed by contributions of 16.13%, 13.59%, 12.54%, and 11.47%. These results indicate a high level of variability in the germplasm, which is crucial for identifying and utilizing genetic diversity effectively. Such analyses align with the findings of Morota *et al*., (2022), who emphasized the utility of multivariate approaches in elucidating morphological diversity.

The clustering of germplasm based on principal component analysis (PCA) highlights its potential utility in developing a minicore collection for sweet sorghum in Pakistan. Minicore collections are instrumental in maintaining genetic diversity while reducing the resource demands of evaluating large populations (Wang *et al*., 2018). Furthermore, association mapping of this minicore collection could facilitate the identification of quantitative trait loci (QTLs) linked to desirable traits, enabling the establishment of marker-trait associations. Such efforts would provide a molecular basis for breeding programs aimed at improving sorghum for biofuel production and other agricultural applications. A detailed correlation analysis revealed positive associations between several traits, particularly between plant height, number of leaves, and fresh weight. These interrelationships are critical for the selection of high-performing genotypes with superior biofuel potential. Similar correlation patterns have been reported by Bandara *et al*., (2020) and Sawadogo *et al*., (2023), further validating our observations. The strong positive associations among traits indicate that simultaneous improvement of these characters is feasible, enhancing the efficiency of breeding programs targeting high biomass and sugar yield in sweet sorghum. In summary, the study underscores the genetic richness of the evaluated germplasm and the potential for improving sweet sorghum through targeted breeding. By leveraging statistical tools and understanding trait interrelationships, this research lays the groundwork for developing high-performing genotypes tailored to the specific needs of biofuel production and agricultural sustainability.

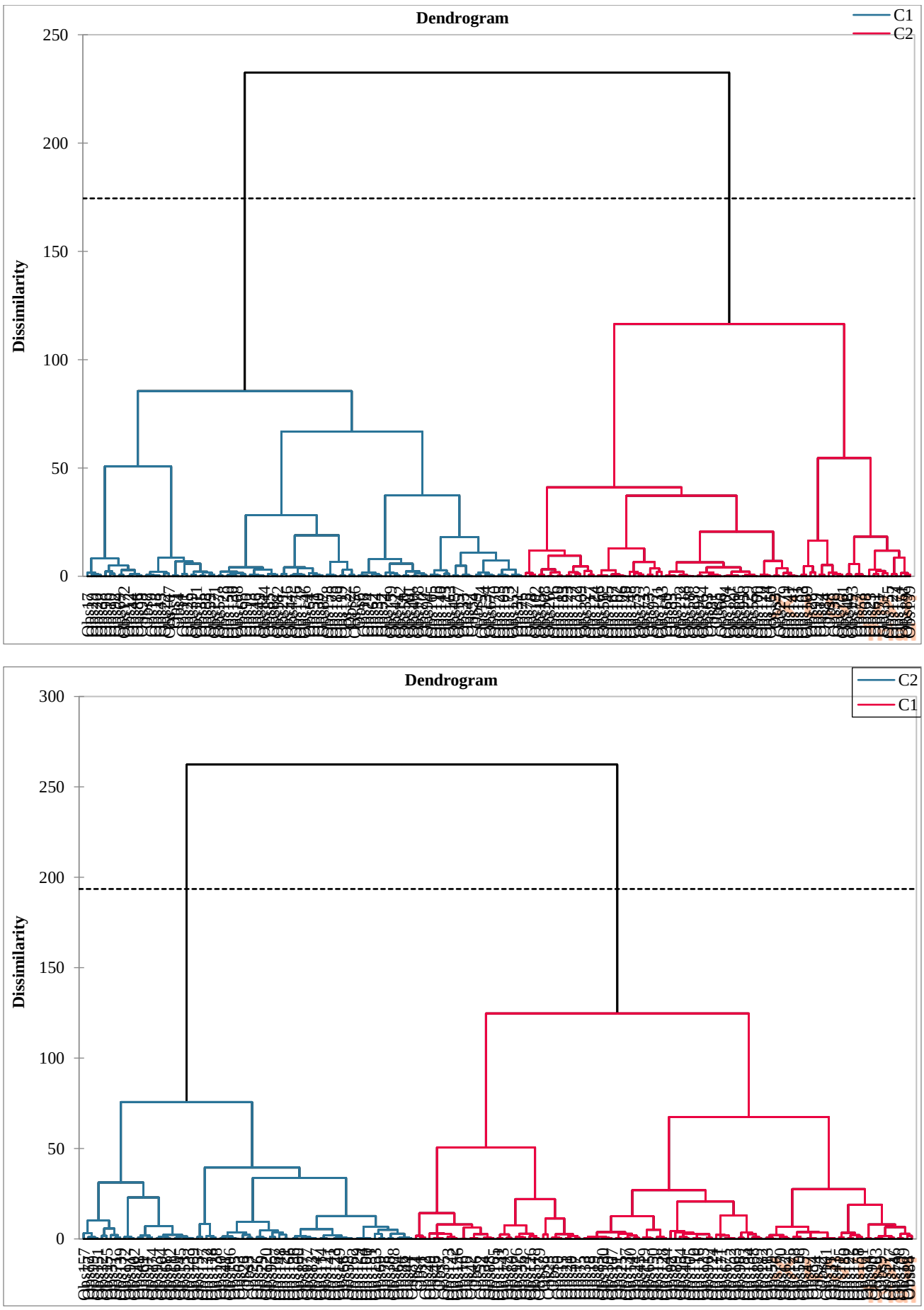

Fig. 3. Classification of sweet sorghum accessions through agglomerative hierarchical clustering (2019-20 and 2020-21.

Conclusion

The findings of our study shed light on the underlying genetic diversity and organization of the population of sweet sorghum for various agronomic traits. The study indicated that considerable variation existed among the accessions, concluding that it can further be exploited in sorghum breeding programs to improve quality traits and yield.

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