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RESEARCH ARTICLE

Estimation of genetic diversity in sorghum genotypes of Pakistan

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Abstract: Livestock and poultry production is increasing steadily in Pakistan but the production of poultry feed, cattle feed and fodder is not increasing at the same rate, and Pakistan is now facing a shortage of quality fodder and poultry feed. Sorghum is a good alternative to meet the growing fodder and poultry feed requirements. The major barrier in enhancing sorghum production is the lack of sufficient information on the genetic diversity of sorghum genotypes in Pakistan. In this study 30 sorghum genotypes from the gene pool maintained by the Maize and Millet Research Institute, Yusafwala, Sahiwal, Pakistan were sown in 2015 in a randomised complete block design. Morphological data such as days to 50 % anthesis, plant height, flag leaf area, brix percentage, panicle length, 1000 grain weight and grain yield were recorded. Data were evaluated for statistical significance using principal component analysis (PCA) and cluster analysis. A highly significant variation was observed among the genotypes. The percentage coefficient of variation in all traits was less than 10 %, which showed that uncontrolled variation was highly unlikely. A close correlation was seen between flag leaf area and grain yield, days to 50 % anthesis and 1000 grain weight, and between panicle length and brix value. Flag leaf area, grain yield and 1000 grain weight indicated the least contribution to the total genetic diversity. The contribution of PC1, PC2 and PC3 was 64.5 % of total variation. According to PCA and cluster analysis genotype YS-9 was important for the character days to 50 % anthesis; genotype YS-22 for grain yield and flag leaf area; genotype YS-27 for plant height; genotype YS-10 and genotype YS-7 for panicle length; and genotype YS-12 for brix value.

Keywords: Biplot, cluster analysis, genetic diversity, PCA, Tukey.

INTRODUCTION

Milk and meat production as well as poultry and egg production can be increased with good quality fodder and feed (FAO, 2017). Livestock and poultry production is increasing steadily in Pakistan: in 2015, livestock production increased by 3.63 % and poultry production by 7.6 %. But the land area used to grow fodder crops has decreased due to the unavailability of quality seeds. As a result, Pakistan is facing a shortage of quality cattle fodder and poultry feed (Govt. of Pakistan, 2016).

The total area used to grow fodder in Pakistan is 2.03 million hectares and the annual production was 4.03 million metric tons in 2015 − 2016. Sorghum [*Sorghum bicolor* (L.) Moench] occupy 0.195 million hectares with a total production of 0.104 million metric tons in 2015 − 2016, accounting for 12.16 % of the total fodder crop production in Pakistan. These figures make sorghum the second largest fodder crop after barseem, which accounts for 43.54 % of the total (Pakistan Bureau of Statistics, 2016). The recommended proportion of sorghum in poultry feed is 15 – 20 % (PARC, 2016). Sorghum is currently considered a good option for meeting the national requirements for high quality cattle and poultry feed in Pakistan.

Further, it is an important coarse grain crop in rain-fed and arid zones. In Pakistan, sorghum is important

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as a grain crop as well as a summer fodder crop (Bibi *et al*., 2010). It is the fifth most important cereal crop worldwide after wheat, rice, maize and barley (Ritter *et al*., 2007; Motlhaodi *et al*., 2014). It is not only a source of fodder for livestock, but its grains are also used in poultry feed as the best substitute for maize. Sorghum is a good source of carbohydrates and protein (Selle, 2011). The crop is thus increasingly valued in view of the expanding poultry industry. It is also gaining importance as a source of starch for textile industries, and is used in gum manufacture (FAO, 2016).

Genetic diversity is a gift of nature for the selection and development of crop varieties. Among the many causes of genetic diversity, the most important are natural mutation, geographical separation and genetic barriers (Zongo *et al*., 1993; Appa *et al*., 1996). Sorghum in Pakistan is not a native crop. It has been introduced from different African countries almost five decades ago and shows a considerable diversity. Although it is widely accepted that the knowledge on sorghum crop diversity is needed for genetic improvement, unfortunately there is little information available in Pakistan. Jain and Patel (2016) performed principal component analysis (PCA) for fodder yield and its components in sorghum accessions in India. It was observed that the first three principal components (PC) scored 70.89 % variation. It was reported that the total variation was mainly due to variation in the fodder yield and their contributing traits i.e. stem girth, dry fodder yield, green fodder yield, days to 50 % anthesis, leaf length, leaf width and number of leaves/plant. The 28 genotypes were grouped into four clusters with the help of cluster analysis on the basis of average linkage, and it was found that the varieties of first and second clusters were superior in terms of green and dry fodder yield, the third cluster for early maturity and the fourth for brix percent. Sinha and Kumaravadivel (2016) estimated the genetic diversity in sorghum genotypes in India. With the help of PCA, it was revealed that the stem girth, leaf breadth and panicle width contributed maximum towards divergence. Fourty accessions were grouped under 6 clusters. Cluster I contained the maximum number of accessions and cluster VI contained the minimum by using hierarchical cluster analysis. The highest mean value was observed for 1000 seed weight and grain yield in cluster III. For yield and yield related components, it was concluded that the selection of parents must be based on the wider inter-cluster distance and superior mean performance. In the present study, the genetic diversity of local sorghum genotypes was determined using morphological traits to provide information for future breeding programmes to develop better varieties and hybrids.

METHODOLOGY

Thirty sorghum accessions, selected from material imported from the International Crop Research Institute for Semi-Arid Tropics (ICRISAT), India were planted at the Maize and Millet Research Institute, Yusafwala, Sahiwal, Pakistan in 2015 in a randomised complete block design with three replicates. The size of each plot was 5 × 3 m. All agronomic practices, i.e., irrigation, fertiliser application, pesticide application, hoeing and thinning, etc. were done at appropriate times. Five plants were selected randomly from each entry for data collection.

Data were collected for the following traits: leaves per plant, days to 50 % anthesis, plant height, flag leaf area, brix percentage, panicle length, 1000 grain weight and grain yield. The statistical significance of differences among accessions was determined according to Steel *et al*. (1998) using Statistix v. 8.1 software. PCA was used to correlate the standardised data of morphological traits recommended by Sneath and Sokal (1973) using XLSTAT software. The determination of genetic similarity was done using hierarchical cluster analysis (Sneath & Sokal, 1973) using SPSS software.

RESULTS AND DISCUSSION

Statistical analysis detected highly significant variations among genotypes. Replication of the comparisons was not effective because of non-significant variations among replication attempts (Table 1). Descriptive statistics of morphological traits of thirty sorghum accessions are summarised in Table 2. The percentage coefficients of variation (CV %) in all traits were less than 10, which indicates that uncontrolled variations were highly unlikely. The largest ranges in values were seen for grain yield (2561.6 − 5190.2 kgha^{-1}) and flag leaf area (135.14 − 315.31 cm). The results of Tukey pairwise comparisons are shown in the Appendix.

Principal component analysis

PCA is interpreted based on the correlations among variables: the further the number from zero in either direction, the greater the positive or negative correlation. Consideration of the magnitude of these numbers is a subjective decision and is needed to determine the level of correlation at which a given value is considered as important (Ringner, 2008). In this analysis three PCs yielded values greater than 1: PC1 26.85 %, PC2 21.03 %, and PC3 16.48 % (Table 3). Together they contributed to

Table 1: Analysis of variance of sorghum accessions for morphological traits

Source	DF	Brix %	D 50 %	FLA	GW	GY	HL	PH
Rep	2	4.01	176.478 ^{NS}	1227.4 ^{NS}	19.3368 ^{NS}	592333 ^{NS}	17.2794 ^{NS}	838.8 ^{NS}
Gen	29	45.40	21.925 **	26213.6 **	88.7054 **	1366555 **	80.90 **	16174.1 **
Error	58	0.0681	3.110	12.8	0.4102	10982	5.7778	9.5
Total	89							

Rep - replication; Gen - genotype; D 50 % - days to 50 % anthesis; FLA - flag leaf area; GW - 1000 grain weight; GY - grain yield; HL - panicle length; PH - plant height; ** - significant; ^{NS} - non-significant

Table 2: Descriptive statistics of sorghum accessions for morphological traits

Variable	Mean	SD	Minimum	Maximum	Range	CV
Brix %	10.61	3.89	3.40	18.12	14.72	2.46
D 50 %	77.60	2.72	72.00	82.00	10.00	2.27
FLA (cm)	263.65	93.48	135.14	450.45	315.31	1.36
GW (g)	26.59	5.44	18.02	39.77	21.75	2.41
GY (kgha^{-1})	4149.20	674.92	2561.60	5190.20	2628.60	2.53
HL (cm)	26.11	5.19	17.02	38.04	21.02	9.21
PH (cm)	239.84	73.43	132.13	380.38	248.25	1.29

D 50 % - days to 50 % anthesis; FLA - flag leaf area; GW - 1000 grain weight; GY - grain yield; HL - panicle length; PH - plant height

Table 3: Principal component analysis of sorghum accessions

	F1	F2	F3	F4	F5	F6	F7
Eigenvalue	1.879	1.472	1.153	0.872	0.627	0.570	0.427
Variability %	26.847	21.029	16.475	12.455	8.953	8.140	6.100
Cumulative %	26.847	47.876	64.352	76.807	85.760	93.900	100.000

Table 4: Squared cosines of the variables for morphological traits

	F1	F2	F3	F4	F5
Brix %	0.021	**0.561**	0.034	0.238	0.001
D 50 %	**0.697**	0.004	0.000	0.007	0.000
FLA (cm)	0.247	0.009	**0.469**	0.010	0.136
GW (g)	**0.574**	0.031	0.004	0.017	0.290
GY (kgha^{-1})	0.114	**0.576**	0.015	0.010	0.135
HL (cm)	0.058	0.271	0.122	**0.473**	0.040
PH (cm)	0.169	0.021	**0.510**	0.116	0.024

D 50 % - days to 50 % anthesis; FLA - flag leaf area; GW - 1000 grain weight; GY - grain yield; HL - panicle length; PH - plant height

The bold values are significant

64.35 % of the total variation, whereas other correlations accounted for the remaining 35.65 %.

The first component correlated strongly with plant height (positive factor loading) and brix value (negative factor loading). The second component showed considerable positive loading for days to 50 % anthesis and panicle length. The third component showed strong correlations with flag leaf area (positive factor loading) and grain yield (negative factor loading) (Tables 4 and 5).

The percentage variations are shown in the scree plot (Figure 1) generated with eigenvalues on the y-axis and PC analysis results on the x-axis. The greatest variation was due to PC1, PC2 and PC3 whereas the other components contributed to much less variation. In addition, PC1 accounted for the largest contribution to total genetic diversity.

Biplot analysis

Biplot analysis aids in selection processes and targeting selection objectives by visualising similarities and correlations between traits. Figure 2 clearly shows close associations between plant height, flag leaf area and grain yield. Flag leaf area and grain yield were strongly correlated (arrows of the same length), as were days to 50 % anthesis and 1000 grain weight, and panicle length and brix value. Flag leaf area and grain yield contributed less to total genetic diversity (very short arrows), as did 1000 grain weight compared to other traits. Biplot analysis was also used by Jain and Patel (2016) to estimate the genetic diversity in sorghum, where a large genetic diversity was observed because the genotypes remained scattered in all four quadrants. The positive values on the vertical axis are linked with plant height, while flag leaf area and grain yield are linked with positive values on both the vertical and horizontal axes. Days to

Table 5: Factor loading of morphological traits

	F1	F2	F3	F4	F5
Brix %	0.144	0.749	- 0.186	- 0.488	0.032
D 50 %	0.835	0.063	- 0.007	- 0.085	- 0.020
FLA (cm)	0.497	0.093	- 0.685	0.100	0.369
GW (g)	- 0.757	0.177	0.061	- 0.132	0.538
GY (kgha^{-1})	- 0.338	0.759	- 0.121	0.099	- 0.367
HL (cm)	0.240	0.521	0.350	0.688	0.200
PH (cm)	0.411	0.143	0.714	- 0.340	0.156

D 50 % - days to 50 % anthesis; FLA - flag leaf area; GW - 1000 grain weight; GY - grain yield; HL - panicle length; PH - plant height

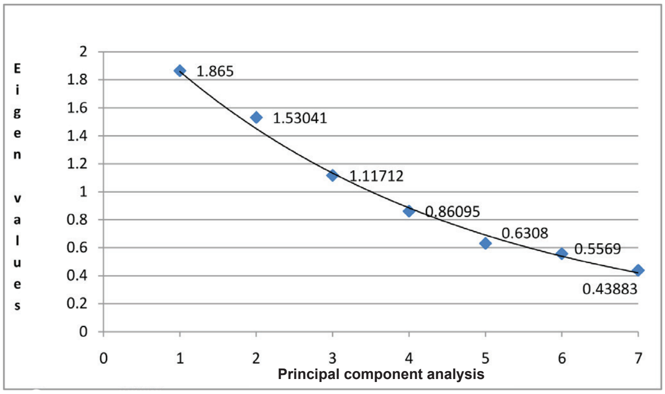

Figure 1: Scree plot resulting from PCA of sorghum genotypes in Pakistan

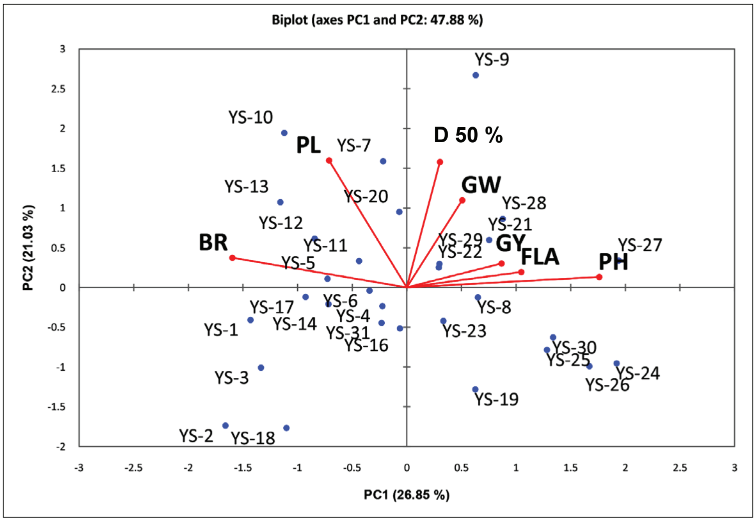

Figure 2: Biplot of sorghum genotypes
BR – brix; PL - panicle length; D 50 % - days to 50 % anthesis; GW - grain weight; GY - grain yield; FLA - flag leaf area; PH - plant height

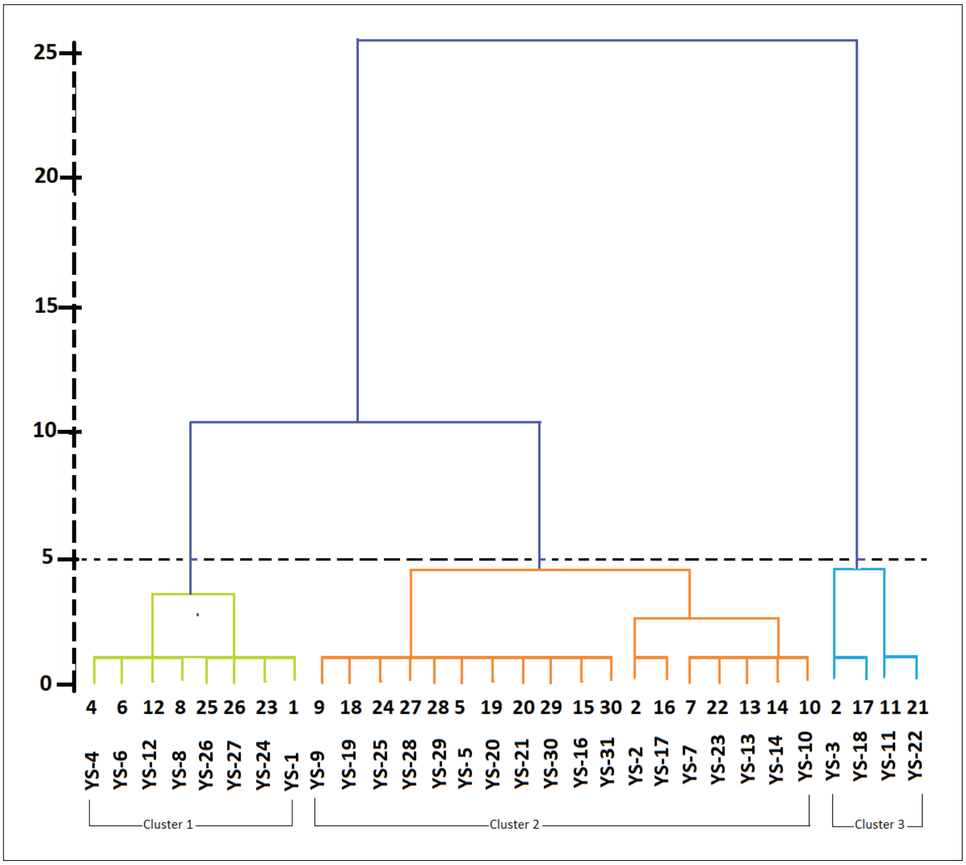

Figure 3: Dendrogram resulting from cluster analysis of sorghum genotypes

Table 6: Contribution of genotypes to factors (%) for variation

	F1	F2	F3	F4	F5
YS-1	6.833	0.549	7.035	1.199	6.983
YS-2	9.188	10.046	0.582	2.794	0.310
YS-3	5.947	3.386	1.779	13.202	0.345
YS-4	0.167	0.180	17.281	3.591	1.170
YS-5	1.760	0.041	0.557	16.526	3.796
YS-6	0.390	0.005	5.662	2.419	6.050
YS-7	0.156	8.427	0.163	12.794	0.122
YS-8	1.409	0.050	3.364	0.150	0.337
YS-9	1.321	23.801	1.025	7.852	2.180
YS-10	4.198	12.606	0.242	0.170	2.890
YS-11	0.638	0.373	3.403	2.173	0.066
YS-12	2.379	1.261	11.797	6.323	8.870
YS-13	4.474	3.848	1.056	4.798	3.790
YS-14	1.715	0.143	0.000	0.037	12.694
YS-16	0.014	0.879	2.255	0.041	1.069
YS-17	2.861	0.047	0.207	4.461	1.824
YS-18	4.052	10.395	3.828	0.233	7.573
YS-19	1.309	5.477	5.550	0.414	5.636
YS-20	0.016	3.018	0.806	0.959	3.551
YS-21	1.887	1.192	0.225	1.388	3.474
YS-22	0.286	0.213	5.880	0.127	1.723
YS-23	0.370	0.589	4.552	0.195	1.551
YS-24	12.285	3.014	1.781	1.122	0.375
YS-25	5.485	2.042	5.794	1.746	13.445
YS-26	9.297	3.268	6.264	0.248	4.914
YS-27	12.542	0.383	2.019	0.374	0.730
YS-28	2.569	2.498	1.274	1.232	0.150
YS-29	0.294	0.296	0.011	12.919	1.599
YS-30	5.977	1.310	2.873	0.157	0.553
YS-31	0.179	0.660	2.735	0.356	2.230

50 % anthesis, 1000 grain weight and panicle length are linked with positive values on the horizontal axis, whereas brix value shows negative vertical axis values. This information is helpful in clarifying trait correlations among genotypes. Performance of genotypes for any trait could be determined with the arrow direction and position of the genotype (Aslam *et al*., 2014). The genotypes which showed better performance for different traits are; genotype YS-9 for days to 50 % anthesis, genotype YS-28 for 1000 grain weight, and genotype YS-21 and YS-29 for grain yield. Genotype YS-22 showed better performance for grain yield and flag leaf area. For plant height, genotypes YS-27 and YS-8 showed better performance. For panicle length, better performance was observed for genotypes YS-10, YS-7 and YS-20. For brix value, the best performing were genotypes YS- 5, YS-6, YS-1, YS-4, YS-11, YS-12, YS-14 and YS-17. Genotype YS-13 showed better performance with both panicle length and brix value (location midway between the two arrows). The genotypes YS-2, YS-3, YS-16, YS-18, YS-19, YS-23, YS-24, YS-25, YS-26 and YS-30 showed no significant improvement for any trait (location at the axes with no trait arrows) (Figure 3). The largest contributions to genetic diversity were from the genotypes YS-27, YS-24 and YS-26 in PC1, YS-9, YS-10 and YS-18 in PC2, and YS-4, YS-12 and YS-1 in PC3 (Table 6).

The results show that PC analysis can be helpful in evaluating crop production data. Makanda *et al*. (2011) and Sinha and Kumaravadivel (2016) also found significant differences among different morphological traits in sorghum.

According to PC analysis, selective breeding would be favoured with YS-9 for days to 50 % anthesis, YS-28 for 1000 grain weight, YS-29 for grain yield, YS-22 for grain yield and flag leaf area, YS-27 and YS-8 for plant height, YS-10 and YS-7 for panicle length, and YS-1, YS-4, YS-5, YS-6 and YS-12 for brix value. Although genotypes YS-1, YS-4 and YS-12 yielded moderate correlations, they made the largest contributions to total genetic diversity in PC3. Similarly, large contributions to the total genetic diversity makes YS-9 the best genotype for days to 50 % anthesis, whereas YS-27 and YS-8 are optimum choices to select for plant height, and YS-10 and YS-7 for panicle length.

Shergo *et al*. (2013) found maximum contribution of the first two PCs in sorghum genotypes. Kisua *et al*. (2014) and Sinha and Kumaravadivel (2016) observed maximum contribution of the first four PCs in sorghum genotypes, and Sinha and Kumaravadivel (2016) observed maximum contribution of the first three PCs to total genetic diversity in sorghum genotypes. The present study is similar to data from Sinha and Kumaravadivel (2016) in contribution of PCs to total genetic diversity because they found maximum contribution of stem girth and leaf breadth towards divergence. The previous study also evaluated the contribution of different morphological traits to total genetic diversity similar to the present study. The results of this study also agree with Felix *et al*. (2015), where widespread varieties were observed in the biplot which represent the large diversity in sorghum germplasm.

Cluster analysis

Cluster analysis is a data exploration (mining) tool that can divide a multivariate dataset into natural clusters (groups). This approach grouped the 30 sorghum genotypes into three clusters of 8, 18 and 4 genotypes (Figure 3, Table 8). The genotypes in the first cluster had high mean values for grain weight and grain yield, while those in the second cluster had high mean values for days to 50 % anthesis, plant height, flag leaf area and panicle length. Members of the third cluster had high brix percentage values (Table 7).

These results are depicted in the dendrogram, which shows two main groups, the first of which is further divided into two subgroups. According to these groupings, selection of genotypes from cluster 2 should be selected to favour days to 50 % anthesis, plant height, flag leaf area and panicle length. The brix value contributes for cluster 3 while grain weight and grain yield contribute for cluster 1.

Cluster 2 contained the largest number of genotypes, followed by cluster 1 and cluster 3, which contained fewer genotypes. Shergo *et al.* (2013) observed five clusters in their study. Kisua *et al*. (2014) observed three clusters and Sinha and Kumaravadivel (2016) observed four clusters in sorghum. The results of this study agree with Kisua *et al*. (2014), as they observed that the accessions which plot in different parts of the biplot would be the most informative for distinguishing accessions with narrow and wide diversity. Also, the selection of traits was similar to those in this study. The results to this study were somewhat different with respect to Shergo *et al*. (2013), as they observed comparatively more genetic diversity. The main reason for this is that they performed cluster analysis on morphological as well as quality traits. The genetic diversity observed by Sinha and Kumaravadivel (2016) was also comparatively higher because they evaluated some complicated traits like leaf length, leaf breadth and number of leaves.

The two statistical methods predicted somewhat different results for 1000 grain weight. According to PCA YS-18 is important for 1000 grain weight, whereas according to cluster analysis YS-4, YS-6, YS-12, YS-8, YS-26, YS-27, YS-24 and YS-1 are important for the same trait.

For most traits there is consensus based on PCA and cluster analysis. For efficient breeding, variety and hybrid development, preference should be given to: genotype YS-9 for short duration plants, genotype Y-22 for high grain yield, genotype YS-27 for medium height plants, genotypes YS-19 and YS-7 for long panicle, and for sweeter stalks preference should be given to genotype YS-12. Days to 50 % anthesis, 1000 grain weight and panicle length are positively correlated highlighting the fact that care is needed for joint selection of these traits. If selection is done considering these genotypes and traits in combination, it will surely lead to a high yielding, medium height and high sweet stalk variety.

Table 7: Cluster analysis of sorghum accessions for morphological traits

Variable	Cluster 1	Cluster 2	Cluster 3
Brix %	9.61	10.33	10.61
D 50 %	77	78.36	77
FLA	201.98	323.67	247.25
GW	29.01	25.16	25.70
GY	4929.80	4269.08	2903.40
HL	22.96	26.57	24.52
PH	252.88	274.82	206.46

D = days to 50 % anthesis; PH = plant height; FLA = flag leaf area; HL = panicle length; GW = grain weight; GY = grain yield

Table 8: Summarisation of sorghum genotypes for cluster analysis

Cluster	No. of genotypes	Name of genotypes
1	8	YS-1, YS-4, YS-6, YS-8, YS-12, YS-24, YS-26, YS-27
2	18	YS-2, YS-5, YS-7, YS-9, YS-10, YS-13, YS-14, YS-16, YS-17, YS-19, YS-20, YS-21, YS-23, YS-25, YS-28, YS-29, YS-30, YS-31
3	4	YS-3, YS-11, YS-18, YS-22

CONCLUSION

Significant variations were found among genotypes, and both PCA and cluster analysis can be helpful in identifying genotypes which merit further evaluation. Flag leaf area correlated closely with grain yield, days to 50 % anthesis and 1000 grain weight, and panicle length correlated closely with brix value. Flag leaf area, grain yield and 1000 grain weight contributed least to total genetic diversity. The results of the two statistical analyses for 1000 grain weight were inconclusive: PCA suggested that YS-18 is important whereas cluster analysis identified YS-4, YS-6, YS-12, YS-8, YS-26, YS-27, YS-24 and YS-1 as important for 1000 grain weight. But for most traits, there is consensus between PCA and cluster analysis; selective breeding will be favoured with genotype YS-9 for short duration plants, genotype YS-22 for high grain yield, YS-27 for medium height plants, genotype YS-10 and YS-7 for long panicles, and genotype YS-12 for sweeter stalks for best variety and hybrid development.

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Appendix

Tukey mean comparisons

	Brix (%)	D 50 %	FLA (cm^2)	GW (g)	GY(g)	HL (cm)	PH
1	17.417 ^{B}	77.000 ^{DEF}	145.15 ^{R}	26.205 ^{IJ}	4670.7 ^{C}	22.023 ^{H}	132.13 ^{Q}
2	14.914 ^{CD}	72.000 ^{H}	158.56 ^{Q}	24.027 ^{K}	3502.5 ^{L}	22.023 ^{H}	140.14 ^{P}
3	13.616 ^{E}	74.000 ^{GH}	224.22 ^{L}	29.560 ^{FG}	2561.6 ^{N}	23.023 ^{FGH}	165.16 ^{NO}
4	10.911 ^{JK}	75.000 ^{FG}	448.45 ^{A}	34.633 ^{C}	5138.1 ^{AB}	23.023 ^{FGH}	167.17 ^{N}
5	15.215 ^{C}	82.000 ^{A}	160.16 ^{Q}	19.358 ^{OP}	4203.2 ^{EFG}	22.523 ^{GH}	190.19 ^{M}
6	8.106 ^{O}	77.000 ^{DEF}	252.25 ^{K}	23.023 ^{KLM}	5190.2 ^{A}	29.530 ^{CD}	165.16 ^{NO}
7	11.113 ^{HIJ}	78.000 ^{CDE}	171.17 ^{P}	37.408 ^{B}	3891.9 ^{IJ}	34.037 ^{B}	198.20 ^{L}
8	9.510 ^{N}	77.000 ^{DEF}	328.33 ^{F}	28.086 ^{H}	5004.0 ^{B}	24.027 ^{EFGH}	270.27 ^{E}
9	11.513 ^{FGH}	81.000 ^{AB}	254.25 ^{K}	39.768 ^{A}	4163.2 ^{FG}	36.037 ^{AB}	280.28 ^{D}
10	14.517 ^{D}	81.000 ^{AB}	405.41 ^{B}	27.907 ^{H}	3736.7 ^{JK}	38.037 ^{A}	225.23 ^{HI}
11	11.914 ^{F}	78.000 ^{CDE}	396.40 ^{C}	28.649 ^{GH}	3269.3 ^{M}	28.027 ^{CD}	220.22 ^{I}
12	18.116 ^{A}	80.000 ^{ABC}	196.20 ^{M}	27.917 ^{H}	5190.2 ^{A}	24.027 ^{EFGH}	207.21 ^{JK}
13	13.715 ^{E}	80.000 ^{ABC}	304.30 ^{G}	20.460 ^{N}	3970.0 ^{HI}	36.037 ^{AB}	202.20 ^{KL}
14	7.705 ^{O}	77.000 ^{DEF}	135.13 ^{S}	22.672 ^{M}	3891.9 ^{IJ}	30.030 ^{C}	167.17 ^{N}
15	7.907 ^{O}	75.333 ^{EFG}	224.22 ^{L}	20.321 ^{NO}	4437.4 ^{D}	30.030 ^{C}	170.17 ^{N}
16	13.616 ^{E}	76.000 ^{EFG}	179.38 ^{O}	29.931 ^{EF}	3589.6 ^{KL}	26.027 ^{DEFG}	160.16 ^{O}
17	10.209 ^{LM}	74.000 ^{GH}	384.38 ^{D}	18.017 ^{Q}	2668.7 ^{N}	24.027 ^{EFGH}	210.21 ^{J}
18	11.811 ^{FG}	74.000 ^{GH}	273.27 ^{J}	19.080 ^{P}	4203.2 ^{EFG}	23.023 ^{FGH}	300.30 ^{C}
19	13.815 ^{E}	80.000 ^{ABC}	180.18 ^{O}	26.835 ^{I}	4337.3 ^{DE}	29.030 ^{CD}	240.24 ^{G}
20	5.603 ^{Q}	81.000 ^{AB}	450.45 ^{A}	26.196 ^{IJ}	4323.3 ^{DEF}	27.027 ^{CDE}	250.25 ^{F}
21	6.705 ^{P}	82.000 ^{A}	344.34 ^{E}	26.556 ^{I}	3114.1 ^{M}	23.023 ^{FGH}	230.23 ^{H}
22	9.808 ^{MN}	78.000 ^{CDE}	280.28 ^{I}	23.593 ^{KLM}	3882.9 ^{IJ}	23.023 ^{FGH}	230.23 ^{H}
23	4.904 ^{R}	76.000 ^{EFG}	280.28 ^{I}	30.931 ^{E}	4670.7 ^{C}	17.017 ^{J}	350.35 ^{B}
24	11.411 ^{GHI}	78.000 ^{CDE}	384.38 ^{D}	22.845 ^{LM}	4087.1 ^{GH}	18.017 ^{IJ}	345.34 ^{B}
25	4.007 ^{S}	75.333 ^{EFG}	294.29 ^{H}	28.229 ^{H}	4770.8 ^{C}	21.020 ^{HI}	380.38 ^{A}
26	3.904 ^{S}	79.000 ^{BCD}	180.18 ^{O}	33.033 ^{D}	4803.8 ^{C}	23.023 ^{FGH}	350.35 ^{B}
27	10.510 ^{KL}	78.000 ^{CDE}	275.48 ^{IJ}	32.934 ^{D}	4283.3 ^{DEF}	29.027 ^{CD}	380.38 ^{A}
28	11.411 ^{GHI}	81.000 ^{AB}	228.03 ^{L}	20.020 ^{NOP}	4203.2 ^{EFG}	26.527 ^{CDEF}	345.34 ^{B}
29	3.404 ^{T}	78.000 ^{CDE}	190.19 ^{N}	23.864 ^{KL}	4281.3 ^{DEF}	22.023 H	250.25 ^{F}
30	11.010 ^{IJ}	74.000 ^{GH}	180.18 ^{O}	25.507 ^{J}	4437.4 ^{D}	29.027 ^{CD}	270.27 ^{E}