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Full Length Article

Estimation of Genetic Divergence among Sorghum Germplasm of Pakistan through Multivariate Tools

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Abstract

Sorghum is an important fodder crop with high biomass production potential all around the world including Pakistan. Genetic divergence was estimated among 208 sorghum genotypes of Pakistan by evaluating fourteen different quantitative traits for one year. High variability was reported in fresh biomass (35.60–629.12 g), dry biomass (23.41–367.72 g), flag leaf area index (37.61–407.39 cm^2), leaf area index (94.71–1061.74 cm^2) and plant height (106.14–298.27 cm). All the quantitative traits showed high broad sense heritability. The first three principal components (PCs) with Eigen value>1 shared 75.39% variability of traits among sorghum genotypes. Positive correlation was observed between plant height and days to maturity, whereas fresh and dry biomass had significant positive correlation with leaf area index, number of leaves per plant, flag leaf area index, days to maturity and 50% days to flowering. Un-weighted Pair-Group Method of Analysis (UPGMA) revealed 141 morphotypes. The germplasm was grouped in to seven classes based on homology. The genotype P-13-2013 gave the highest values for number of leaves/plant, stem thickness, leaf length, fresh biomass, dry biomass and flag leaf area index. The explored genetic potential of sorghum germplasm of Pakistan can be helpful for varietal improvement program. Moreover, the diverse set of genotypes can be screened through principal component analysis for structure and association mapping by using molecular markers.

Keywords: Sorghum; Diversity analysis; Multivariate analysis; Principal components analysis; UPGMA clusters analysis

Introduction

Sorghum belongs to family Poaceae and grouped into five taxonomic subgenera: *Chaetosorghum, Spitosorghum, Hetrosorghum, Parasorghum* and *Eusorghum* (Garber, 1950). It is a multipurpose crop providing food of subsistence, fodder and biofuel (Xin *et al*., 2017). The crop possesses excellent drought and heat tolerance and hence adapts well-to marginal lands (Kausar *et al.,* 2014). With the small genome of 730 Mb, sorghum has emerged as a model crop among tropical grasses due to its C4 photosynthetic pathway (Paterson *et al.,* 2009).

Genetic variation provides the base line for all plant improvement programs (Mohammed *et al.,* 2014). Diversity in crop germplasm enables the breeders to develop varieties specific to both farmer and consumer's preferences. Genetic divergence analysis gives descriptive information of the traits and helps in understanding the similarities and differences among genotypes (Nawaz *et al.,* 2004). The variations in crop germplasm are characterized using different molecular, morphological and biochemical marker systems. Morphological characterization is the simplest and economical approach for exploring these differences (Rakshit *et al.,* 2012).

Various methods of multivariate analyses are being exploited to find the pattern of genetic divergence among crop genotypes such as principal component analysis (PCA), principal coordinate analysis (PCoA), cluster analysis and multidimensional scaling (MDS) (Brown-Guedira *et al.,* 2000). PCA is a statistical data reduction tool which explains the total variance of related characters and their splitting in the form of few uncorrelated new variables known as principal components (PCs) (Wiley, 1981). Estimation of Eigen value is the first step in PCA which describes the total dissimilarity exhibited on PC axis. Maximum variability exists in the first PC. While the second PC covers most of the variations which remained unchecked and uncorrelated with the first PC (Meulman, 1992). Biplot analysis assists to screen genotypes based on performance under different environments and score plots allocate the genotypes in the coordinate system. In cluster analysis, the resultant dendrogram shows high heterogeneity between clusters and high homogeneity within cluster (Hair Jr *et al.,* 1995). Generally, two types of clustering methods

are used (1) Model-based and (2) Distance-based (Johnson and Wichern, 1992). Distance-based clustering methods are further divided into two groups: (1) hierarchical clustering methods in which the most similar individuals are grouped and pooling of these groups is done based on their relatedness. UPGMA is the most common and efficient among different hierarchical methods followed by Ward's minimum variance method (Ward Jr., 1963): (2) Non-hierarchical methods are based on similar threshold or chronological threshold for pooling individuals to certain cluster (Everitt and Dunn, 1992).

Sorghum occupies the prominent place among summer fodders in Pakistan owing to its hardy nature. However, there is an urgent need to develop high yielding and stable sorghum varieties for quality fodder and grain purposes. A steady supply of diverse germplasm in the form of landraces, introductions, weedy and wild relatives is mandatory to achieve this goal. Though several reports are available on morphological diversity assessment of sorghum (Noor *et al*., 2012; Dossou-Aminon *et al.,* 2015); this area is still unexplored. In this context, present study is an attempt to dissect the phenotypic diversity of the largest collection (208) of Pakistani sorghum germplasm so far, with the objective to identify promising high biomass genotypes.

Materials and Methods

Plant Material and Field Layout

The experimental material comprised of 208 sorghum genotypes (Table 1) collected from Fodder Research Substation, Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan and National Agricultural Research Centre (NARC), Islamabad, Pakistan. The experiment was conducted at Postgraduate Agriculture Research Station (PARS), University of Agriculture, Faisalabad (31° - 26' N, 73° - 06' E, 184.4 m) during 2015-2016. The trials were planted in randomized complete block design with three replications in the normal growing season i.e., 2015–16. Each row was 3 m long and inter row and inter plant distances were 75 and 25 cm, respectively. Seeds were sown by dibbler method with two seeds per hole for good plant stand. Thinning was done to keep one plant/hole after germination with fourteen plants per row of each genotype.

Morphological Characterization

At the time of 50% flowering, data were recorded for all parameters except dry biomass and days to maturity from the tagged plants per genotype per replication. Plant height was recorded from ground level to the last node of the plant in cm. Stem thickness was measured with the help of Vernier Caliper and days to 50% flowering was recorded as number of days from the sowing date to the stage when 50% plants flowered (Dossou-Aminon *et al.,* 2015). Flag leaf length was measured from the point of origin to the tip of flag leaf and flag leaf width was calculated at three points i.e., near the base, near the tip and middle point of flag leaf blade. Leaf length was measured from base to tip of each leaf in cm and leaf width was recorded on three points i.e., near the base, mid and near the tip of leaf blade in cm. Leaf area and flag leaf area indices were calculated as product of leaf length and leaf width. Fresh biomass was weighed in grams with the help of weighing balance and brix value was recorded with hand refractometer. The data for traits like dry biomass (sundried single plant samples were weighed in grams with weighing balance) and days to maturity (recorded as number of days from the sowing date to the stage when 100% plants get matured) were calculated at final maturation stage.

Statistical Analysis

The quantitative data were first subjected to descriptive statistics (mean, standard deviation, coefficient of variation) and analysis of variance (ANOVA) using SAS ver. 9.1 (Institute, 2011). To identify the pattern of morphological variation, PCA was conducted on correlation matrix using Minitab 14 statistical software and the significant loading factors (explaining ≥30% variation) were noted (Maji and Shaibu, 2012). Simple Pearson moment coefficients of correlation were computed between pairs of quantitative morphological traits. Broad sense heritability was estimated as a ratio of genetic variance to phenotypic variance (Ijaz and Samiullah, 2013) and Cluster analysis performed using UPGMA (Swofford and Olson, 1990).

Results

Analysis of Variance and Descriptive Statistics of Quantitative Traits

The analysis of variance (ANOVA) showed highly significant differences among sorghum germplasm for all quantitative traits viz., plant height, number of leaves/plant, stem thickness, brix value, days to 50% flowering, leaf length, leaf width, leaf area index, fresh biomass, days to maturity, flag leaf length, flag leaf width and flag leaf area index. The mean square values, heritability estimates and descriptive statistics of quantitative traits are presented in Table 2. All the quantitative traits had heritability greater than 80% which indicates higher contribution of genotypes than environment. While plant height (106.14–298.27 cm), fresh biomass (35.60–627.12 g) and leaf length (33.92–94.49 cm) were more variable than the rest of the traits. Among all quantitative traits, days to 50% flowering depicted the lowest variability (4.36% CV). The mean values of plant height, fresh biomass, dry biomass, leaf area index, flag leaf area index, stem thickness, number of leaves, brix value, 50% days to flowering and days to maturity were 189.43 cm, 199.3 g,

Table 1: List of sorghum genotypes used in multivariate analysis

Sr. No.	Genoptypes	Source	Sr. No.	Genoptypes	Source	Sr. No.	Genoptypes	Source	Sr. No.	Genoptypes	Source
1	9984	NARC	53	10016	NARC	105	9987	NARC	157	JS-26-2	AARI
2	10035	NARC	54	5017	AARI	106	10026	NARC	158	J-100	AARI
3	9967	NARC	55	1563	AARI	107	S-9902	AARI	159	SS-9803	AARI
4	9961	NARC	56	s-2016	AARI	108	FRI-02	AARI	160	NO.4158	AARI
5	10483	NARC	57	9977	NARC	109	Indian-6	AARI	161	9985	NARC
6	9920	NARC	58	9944	NARC	110	Yss-15	AARI	162	Sugroile	AARI
7	9914	NARC	59	9940	NARC	111	Yss-1	AARI	163	SS-98-12	AARI
8	9952	NARC	60	9999	NARC	112	S-167	AARI	164	10028	NARC
9	9949	NARC	61	10444	NARC	113	PARC-SS-1	NARC	165	10031	NARC
10	9925	NARC	62	10021	NARC	114	Indian-1	AARI	166	Indian-13	AARI
11	9978	NARC	63	10449	NARC	115	PARC-SV-1	NARC	167	Sgd-011-2	AARI
12	10027	NARC	64	10453	NARC	116	S-9901	AARI	168	11-011-1	AARI
13	10474	NARC	65	9953	NARC	117	Yss-4	AARI	169	PARC-SV-2	NARC
14	9919	NARC	66	9996	NARC	118	Indian-3	AARI	170	9995	NARC
15	9939	NARC	67	10422	NARC	119	K-94	AARI	171	Ballo	AARI
16	950	NARC	68	10459	NARC	120	80022	AARI	172	Indian-7	AARI
17	9918	NARC	69	9994	NARC	121	F-904	AARI	173	S-145	AARI
18	951	NARC	70	10480	NARC	122	10467	AARI	174	B-203	AARI
19	9922	NARC	71	9983	NARC	123	JS-1	AARI	175	Bm-726	AARI
20	9964	NARC	72	10040	NARC	124	AK-113	AARI	176	Yss-89	AARI
21	9955	NARC	73	10476	NARC	125	559803	AARI	177	PAK-65130	AARI
22	10022	NARC	74	9962	NARC	126	NO.337	AARI	178	1572	AARI
23	10029	NARC	75	9945	NARC	127	SUDAN GRASS	AARI	179	Sgd-2011	AARI
24	10025	NARC	76	10030	NARC	128	A-4009	AARI	180	PARC-SS-2	NARC
25	9958	NARC	77	10430	NARC	129	F-2007	AARI	181	P17-2003	NARC
26	10020	NARC	78	10479	NARC	130	SL-18	AARI	182	p-2-S5-2013	NARC
27	9989	NARC	79	10044	NARC	131	SMALL PAK	AARI	183	p-16-2013	NARC
28	9916	NARC	80	10412	NARC	132	SMALL CHINA	AARI	184	p-11-2013	NARC
29	9913	NARC	81	10446	NARC	133	HOK	AARI	185	p-8-2013	NARC
30	9921	NARC	82	10461	NARC	134	AK-114	AARI	186	p-14-2013	NARC
31	9948	NARC	83	10458	NARC	135	SS-97-12	AARI	187	p-5-2013	NARC
32	9927	NARC	84	10411	NARC	136	NOOR JAWAR	AARI	188	p-15-2013	NARC
33	9941	NARC	85	10460	NARC	137	JS-3	AARI	189	p-7-2013	NARC
34	9946	NARC	86	101448	NARC	138	JS-6	AARI	190	p-4-2013	NARC
35	9942	NARC	87	9934	NARC	139	m-sm-04	AARI	191	p-12-2013	NARC
36	9943	NARC	88	10468	NARC	140	JS-5	AARI	192	p-13-2013	NARC
37	9993	NARC	89	10037	NARC	141	1749	AARI	193	Johar2013	AARI
38	9963	NARC	90	9908	NARC	142	F-6503	AARI	194	p-3-2013	NARC
39	9912	NARC	91	10435	NARC	143	D-169	AARI	195	Sp-1484-1	NARC
40	9947	NARC	92	10432	NARC	144	No.1518	AARI	196	P-10-2013	NARC
41	10033	NARC	93	10024	NARC	145	JS-4	AARI	197	p-19-2013	NARC
42	10039	NARC	94	100453	NARC	146	JS-1914	AARI	198	p-18-2013	NARC
43	9923	NARC	95	100482	NARC	147	SL-146	AARI	199	p-20-2013	NARC
44	10052	NARC	96	9969	NARC	148	NO.293	AARI	200	V-100-t-1-2013	NARC
45	10041	NARC	97	9986	NARC	149	PAK SORGHUM	AARI	201	p-6-2013	NARC
46	10023	NARC	98	9973	NARC	150	NO.162	AARI	202	AK-115	AARI
47	10042	NARC	99	Hegari	AARI	151	Laylpur-44	AARI	203	p-8-R1 2013	NARC
48	9975	NARC	100	Sorghum2011	AARI	152	Marker	AARI	204	p-9-2013	NARC
49	9979	NARC	101	Js-2002	AARI	153	M-sm-03	AARI	205	p-1-2013	NARC
50	9915	NARC	102	Js-263	AARI	154	22-Oct	AARI	206	R-93046-p-2013	NARC
51	997	NARC	103	9991	NARC	155	No.86	AARI	207	N0.87	AARI
52	10036	NARC	104	957	NARC	156	Jandi	AARI	208	R-160-p-9-2013	NARC

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102.60 g, 398.71 cm^2, 148.56, 4.53 cm, 11.45, 8.32 %, 69.66 days and 117.89 days, respectively. The minimum and maximum ranges for different traits are also presented in Table 2, including plant height (106.14–298.27cm), days to 50% flowering (46.33–93.22 days), days to maturity (94–140.89 days), leaf area index (94.71-1061.74 cm^2) fresh biomass (35.60–629.12 g) and dry biomass (23.41–367.72 g).

Principal Component Analysis (PCA)

PCA analysis of various traits was used to eliminate the redundancy in the data set. Three principal components (PC1, PC2 and PC3) having Eigen value >113 were recorded (Table 3). The cumulative variability of three PCs was 75.39%. The PC_1 shared 50.47% of the total variability followed by PC_2 (16.97%) and PC_3 (7.93%). Different quantitative traits contributed more than 30% to the variation factor in PC_1 such as: leaf area index (34.9%), flag leaf area index (32.9%), fresh leaf weight (32.8% of variation factor), leaf length (32.2%), leaf width (31.4%) and stem thickness (30.5%). PC_1 showed weak and positive correlation with the brix value (0.25%), days to 50% flowering (0.82%) and plant height (0.30%).

Table 2: Mean square values, heritability and descriptive statistics for 14 quantitative traits of 208 sorghum genotypes

Traits	Replication	Genotype	Error	Heritability	Minimum	Maximum	Mean	SD	CV (%)
DF	2	207**	414						
NL	11.91	16.05**	0.66	0.83	6.11	16.66	11.45±0.47	2.31	7.13
DTF	80.46	297.03**	6.37	0.86	46.00	93.44	69.62±1.42	9.95	4.36
BV	9.04	6.79**	0.37	0.87	4.84	16.89	8.25±0.35	1.50	7.32
ST	1.71	7.17**	0.09	0.91	1.06	8.63	4.37±0.17	1.54	6.96
LL	855.99	527.42**	0.32	0.90	32.60	90.74	62.85±0.33	13.25	11.54
LW	1.94	6.22**	0.13	0.90	2.49	10.26	5.66±0.21	1.44	6.58
LAI	6923.67	69735.62**	543.83	0.91	97.47	930.56	368.45±13.46	152.46	6.30
FB	542.83	39043.78**	29.86	0.91	32.55	611.80	193.453.15	114.08	15.43
DB	144.08	12391.38**	37.30	0.89	20.40	357.35	99.59±3.52	64.26	6.13
FLL	2.21	166.22**	0.21	0.85	21.23	56.22	34.91±2.68	7.44	11.43
FLW	0.29	4.52**	0.05	0.91	1.83	7.56	3.87±0.12	1.22	5.79
FLAI	474.09	14198.09**	57.37	0.91	44.51	364.26	141.41±4.37	68.79	5.35
PH	9233.19	3981.44**	28.72	0.87	102.40	291.76	185.12±3.09	36.43	18.56
DTM	131.48	270.15**	26.76	0.83	92.55	141.22	115.95±2.98	9.49	4.46

Standard deviation (SD), Coefficient of variation (CV), Degree of freedom (DF) Number of leaves per plant (NL), Days to 50% flowering (DTF), Brix value (BV), Stem thickness (ST), Leaf length (LL), Leaf width (LW), Leaf area index (LAI), Fresh biomass (FB), Dry biomass (DB), Flag leaf length (FLL), Flag leaf width (FLW), Flag leaf area index (FLAI), Days to maturity (DTM), Plant height (PH) and Highly significant (**)

PC_1 represented positive and strong factor with fresh biomass (29.9%), number of leaves/plant (29.8%), dry biomass (29.4%) and flag leaf length (27%). The PC_2 contributed for 16.87% of the total trait variation. While it exhibited strong and positive correlation with the traits such as: plant height (61.7%), days to 50% flowering (61.3%) and days to maturity (37.8%). Negative correlation was observed with the stem thickness (18.3%) and leaf width (11.6%) in PC_2 (Table 3). Brix value represented 85.9% of the factor variation in PC_3. While, strong and negative correlation was observed with days to maturity (48.9% of variation factor) in PC_3. The remaining variables had weak or no discriminatory power. Thus, the most important descriptors were those associated with PC_1, PC_2 and PC_3 (Table 3).

Table 3: Principle component analysis (PCA) of sorghum traits

Variables	PC1	PC2	PC3
NL	0.298	-0.030	0.051
DTF	0.082	0.613	0.018
BV	0.025	0.173	0.859
ST	0.305	-0.183	0.013
LL	0.322	0.070	0.011
LW	0.314	-0.116	-0.003
LAI	0.349	-0.054	-0.017
FB	0.299	0.025	0.092
DB	0.294	0.036	0.088
FLL	0.270	0.016	-0.042
FLW	0.328	-0.098	-0.001
FLAI	0.329	-0.061	-0.034
PH	0.030	0.617	0.032
DTM	0.145	0.378	-0.489
Eigen value	7.067	3.377	1.112
% of total variance	50.479	16.977	7.939
Cumulative variability (%)	50.497	67.456	75.396

Number of leaves per plant (NL), Days to 50% flowering (DTF), Brix value (BV), Stem thickness (ST), Leaf length (LL), Leaf width (LW), Leaf area index (LAI), Fresh biomass (FB), Dry biomass (DB), Flag leaf length (FLL), Flag leaf width (FLW), Flag leaf area index (FLAI), Days to maturity (DTM), Plant height (PH) and Principal Component (PC)

Score Plot Analysis

A scatter plot reflected that close genotypes were perceived as being similar when judged on 14 variables. PCA assembled the accessions into three groups (G1, G2 and G3) based on descriptors. The score plot analysis assembled 56, 49, 48 and 55 sorghum genotypes in G1, G2, G3 and negative coordinate of score plot, respectively. In G2, genotypes were grouped based on plant height, days to 50% flowering, days to maturity, brix value, flag leaf length, fresh biomass, dry biomass and leaf length. Whereas, phenotypic traits like number of leaves, leaf width, flag leaf width, stem thickness and flag leaf area index were accounted for grouping the genotypes in G3 (Fig. 1). The genotypes which were allocated away from the central point to the coordinate system, proved more variable as compared to the ones plotted near the central point. The promising genotypes grouped in G1 included 10023, 9923, 9922 and 10030. The genotypes K-94, PARC-SV-1, P-11-2013, F-6503-PARC-SS-2, Noor jawar, S-167 and D-169 showed good performance regarding biomass related traits in G2. While in G3, P-13-2013, Johar 2013, P-7-2013, P-4-2013 and P-5-2013 were better performing than the rest in relation to biomass traits. These genotypes could be recommended for efficient response under biotic/ abiotic stresses. The more variable genotypes in G0 were 10453, 9940, 9999 and 10037.

Biplot Analysis

This analysis depicted that variables were super imposed as vector; relative length of the vector was characterized as the relative proportion of the variability in each variable. The genotypes which were plotted away from origin represented less similarity and more variation in comparison with the ones plotted close to the central point.

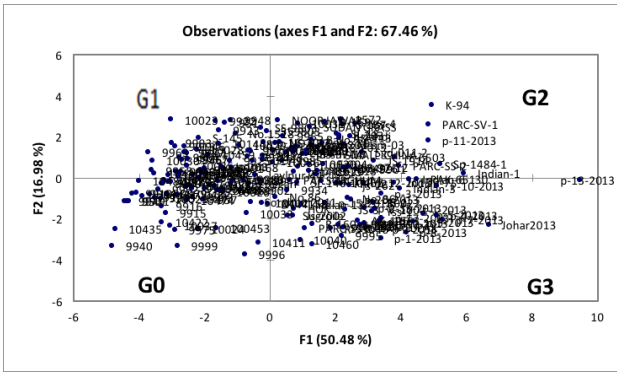

Fig. 1: Score plot grouping of 208 sorghum genotypes

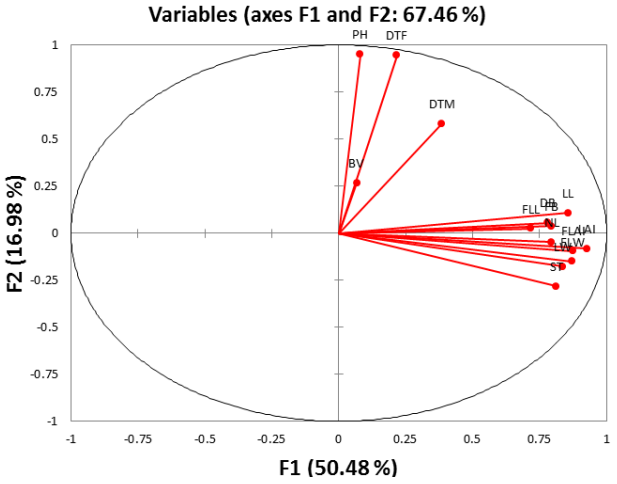

Fig. 2: Biplot analysis of sorghum genotypes for 14 quantitative traits

All the traits were well represented and exhibited high variability (Fig. 2). Quantitative characters including plant height, days to 50% flowering, leaf length, fresh biomass, dry biomass, flag leaf length, number of leaves and brix value were allocated at positive and positive coordinate in biplot analysis. While, flag leaf area index, leaf area index, flag leaf width, leaf width and stem thickness were represented at second positive and negative coordinate. The high variability in the traits representing the divergence among genotypes could be used in sorghum breeding program effectively.

Correlation Analysis

Significant association between quantitative traits was derived from the Pearson correlation analysis. The trait 50% days to flowering exhibited highly significant and positive correlation with leaf length, fresh biomass, dry biomass, plant height and days to maturity. The phenotypic correlation of brix value was highly significant and positively correlated with plant height only. Stem girth revealed positive phenotypic correlation with majority of the traits except plant height (negatively correlated). Whereas, days to 50% flowering showed non significant negative correlation pattern with stem thickness. Leaf area index was positively correlated with number of leaves, leaf length, leaf width, flag leaf length, flag leaf width, fresh biomass, dry biomass, flag leaf area index, stem thickness and days to maturity. Flag leaf area index showed positive correlation pattern with leaf area index, fresh biomass, stem thickness, number of leaves, leaf length, leaf width, flag leaf width and dry biomass. Plant height showed highly significant and positive correlation with brix value and days to 50% flowering. Fresh and dry biomass were positively correlated with all traits except brix value and plant height. Days to maturity showed highly significant and positive correlation with all quantitative traits except brix value (Table 4).

Cluster Analysis

UPGMA analysis generated 141 morphotypes of 208 sorghum genotypes (Fig. 3). Here, main cluster was divided into seven sub clusters. Cluster C1 comprised of 96 genotypes and 67 morphotypes. The only genotype P-13-2013 present in cluster C7 was placed in the separate class. The C3 accommodated 27 genotypes and 18 morphotypes. Cluster C2 was made up of 58 genotypes and 37 morphotypes and four genotypes belonged to C4. There were 45 genotypes and 3 morphotypes in C5. The C6 contained 18 genotypes in which 12 were morphotypes. Based on similarity, seven classes were formed (Fig. 4). The class centroids and their characterization is given in Table. 5. The genotypes 10444, 11-011-1, 1749, S-167, K-94, PAK-65130, P-13-2013 were screened as central centroids of class I, II, III, IV, V,VI, and VII, respectively. The VII class nominator (P-13-2013) showed better genetic variability or genetic potential for different morphological characters including stem thickness, number of leaves/plant, leaf area index, fresh biomass, dry biomass and flag leaf area. Variance decomposition indicated 26.29% and 73.71% variance within and between classes, respectively.

Identification of Promising Genotypes for Sorghum Improvement Program

Morphological characterization led to the selection of twenty genotypes for sorghum improvement program, considering the economic preference traits (fresh biomass and days to flowering) of Pakistani farmers. The screened genotypes were also identified in positive coordinates of principal component analysis. Their fresh biomass ranged from 363.6 to 611.8 g; the highest value (611.8 g) was recorded in SP-1484-1. The genotypes BM-726 and Johar-2013 showed intermediate range for both characters i.e., fresh biomass (470.05 and 440.3 g) and days to 50% flowering (67 and 58), respectively.

Table 4: Correlation matrix for 14 quantitative traits of sorghum

Variable	NL	DTF	BV	ST	LL	LW	LAI	FB	DB	FLL	FLW	FLAI	PH
DTF	0.126												
BV	0.079	0.252											
ST	0.676**	-0.065	0.012										
LL	0.665**	0.263**	0.096	0.682**									
LW	0.642**	0.029	0.045	0.782**	0.654**								
LAI	0.715**	0.12	0.042	0.814**	0.871**	0.928**							
FB	0.605**	0.185**	0.061	0.561**	0.611**	0.538**	0.635**						
DB	0.586**	0.196**	0.057	0.548**	0.599**	0.529**	0.626**	0.986**					
FLL	0.442**	0.175*	0.028	0.473**	0.634**	0.449**	0.572**	0.453**	0.429**				
FLW	0.651**	0.079	0.032	0.670**	0.623**	0.749**	0.762**	0.627**	0.607**	0.661**			
FLAI	0.602**	0.118	0.013	0.634**	0.665**	0.672**	0.739**	0.607**	0.584**	0.873**	0.929**		
PH	0.019	0.929**	0.233**	-0.181**	0.166*	-0.07	0.017	0.069	0.088	0.087	-0.065	-0.009	
DTM	0.275**	0.559**	-0.143	0.181**	0.364**	0.261**	0.328**	0.26**	0.266**	0.225**	0.232**	0.244**	0.46**

Number of leaves per plant (NL), Days to 50% flowering, Brix value, Stem thickness, Leaf length, Leaf width, Days to maturity (DTM), Plant height (PH). Highly significant (**) and Significant (*)

Table 5: Characterization of 7 class centroids in sorghum genotypes

Variable	1 (10444)	2 (11-011-1)	3 (1749)	4 (S-167)	5 (K-94)	6 (PAK-65130)	7 (p-13-2013)
NL	9.00	15.45	13.44	10.66	14.55	16.11	16.45
DTF	64.78	73.78	72.33	90.22	93.22	75.78	75.44
BV	7.67	8.11	7.89	6.22	7.56	8.67	6.56
ST	2.87	3.90	5.10	5.63	6.63	7.13	8.33
LL	44.95	71.70	73.51	78.97	76.73	82.75	90.75
LW	5.23	5.86	5.99	7.26	6.36	7.57	10.26
LAI	235.15	420.28	440.56	573.17	488.09	626.20	930.56
FB	101.50	167.30	320.95	94.15	515.20	314.30	581.35
DB	46.55	80.50	193.55	47.25	271.60	164.85	357.35
FLL	28.11	37.64	31.50	41.63	46.73	40.59	48.14
FLW	3.56	3.79	5.57	4.97	4.73	5.76	7.57
FLAI	99.95	142.79	175.36	206.69	221.04	233.84	364.26
PH	178.60	193.61	201.10	259.72	271.26	199.58	197.75
DTM	112.78	120.66	122.78	134.89	141.22	114.78	133.66

Number of leaves per plant (NL), Days to 50% flowering (DTF), Brix % value (BV), Stem thickness (ST), Leaf length (LL), Leaf width (LW), Leaf area index (LAI), Fresh biomass (FB), Dry biomass (DB), Flag leaf length (FLL), Flag leaf width (FLW), Flag leaf area index (FLAI), Days to maturity (DTM) and Plant height (PH)

Days to 50% flowering ranged from 58–93 days. The genotype Johar-2013 proved early flowering (58 days) while K-94 took the longest to flower (93 days) (Fig. 5). The selected genotypes from the available sorghum germplasm can be added in national breeding program.

Discussion

Morphological characterization is the vital step to explore and classify the genetic diversity of available germplasm (Rakshit *et al.,* 2012). Present study provides the comprehensive information on exploiting multivariate analysis tools to evaluate sorghum germplasm of Pakistan. As reported earlier (Jadhav *et al.,* 2011) our results indicated that all 14 quantitative traits were highly significant. The range of values recorded for days to flowering, plant height, number of leaves, leaf area index, fresh biomass and dry biomass of sorghum germplasm are also in accordance with the earlier reports (Jain *et al.,* 2011; Noor *et al.,* 2012). The broad sense heritability value of all the traits had greater than 90% which indicated the higher genetic potential of sorghum germplasm. Similar observations have also been made in previous experiments (Jain *et al.,* 2010; Puspitasari *et al.,* 2012; Singh *et al.,* 2013). Majority of Pakistan sorghum genotypes possessed higher biomass character with intermediate height, leaf area index and number of leaves as observed in Ethiopian sorghum land races (Adugna, 2014). We suggest that highly diverse sorghum germplasm of Pakistan may share the common ancestor as of Ethiopian sorghum. Such a diverse germplasm might be a good candidate for varietal development (Jain *et al.,* 2010).

Multivariate statistical tools offer valuable information to find morphological diversity within and between given germplasm (Shrestha, 2013). Present study reported that three PCs contributed 75.39% of overall diversity among the genotypes. PC_1 being the highest contributor having 50.47% share in the total variability. Among 208 sorghum genotypes, 122 showed maximum contribution in the variability of different characters such as number of leaves, stem thickness, leaf length, leaf width, leaf area index, fresh biomass, dry biomass, flag leaf width and flag leaf area index in first axis, 46 genotypes contributed more expression in characters like days to

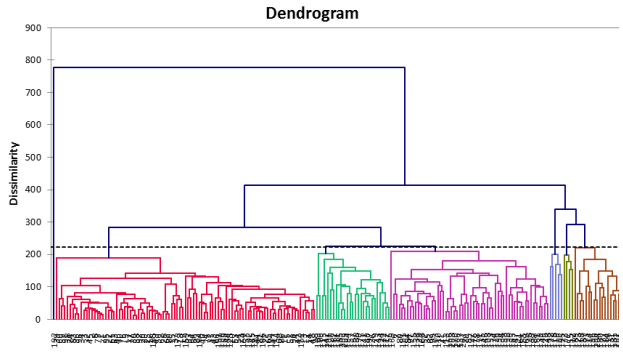

Fig. 3: Classification of 208 sorghum genotypes into 141 morphotypes using UPGMA cluster analysis

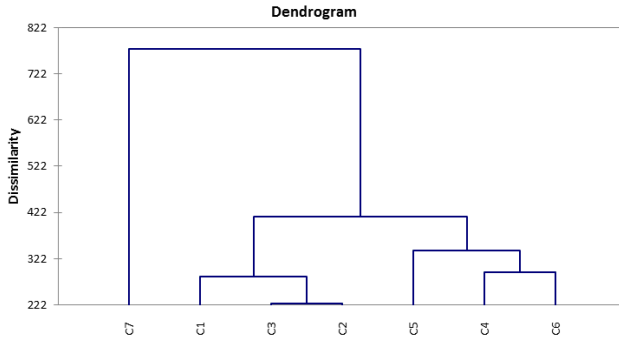

Fig. 4: Cladogenesis of 208 sorghum genotypes of Pakistan

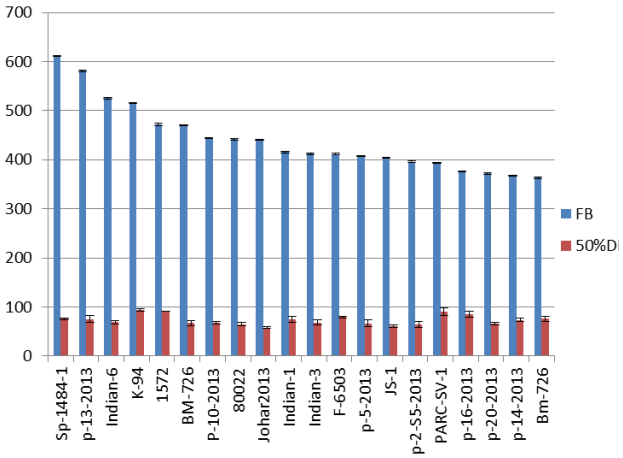

Fig. 5: Comparison of 20 selected sorghum genotypes for fresh biomass and 50% days to flowering

50% flowering, plant height and days to maturity in PC_2. Sixteen genotypes in PC3 exhibited maximum variation in brix value. The genotypes with maximum variability of traits in first axis can be used for high biomass production. While the genotypes in PC_2 can aid in the development of early maturing varieties. The 3rd axis has potential for the biofuel breeding program. Similar results were observed in Ethiopian sorghum land races (Maji and Shaibu, 2012; Adugna, 2014). The genotypes away from the origin (K-94, PARC-SV-1, P-11-2013, SP-1484-1 and Indian-1) in G2 can be used in heterosis breeding program due to their efficient discriminatory power related to examined parameters and maximum diversity among them. Generally, for the varietal development, structure analysis and association studies, genotypes are screened from the first two PCs. Grouping of the germplasm in the coordinates can be useful in determining minicore collection (Rakshit and Patil, 2013) for sorghum in Pakistan. Association mapping can be done on the germplasm of minicore collection to identify the quantitative trait loci (QTLs) and thereby to find out the marker trait associations.

We report that different traits associated with each other in a positive manner e g., plant height was positively correlated with brix value and days to 50% flowering, such correlated characters are vital for the selection of high biofuel genotypes. Similar correlation pattern was observed in the previous studies (Abubakar and Bubuche, 2013; Jain and Patel, 2013). UPGMA analysis yielded 141 morphotypes of 208 sorghum genotypes under study. The genotype P-13-2013 exhibited maximum variability for all the traits and hence, indicated the presence of synonymies within germplasm as earlier reported (Dossou-Aminon *et al.*, 2014).

The twenty elite genotypes that belonged to different groups or morphotypes can be crossed in diallel mating fashion to get more variability in breeding program (Jain and Patel, 2014). These screened genotypes can be subjected to mass selection for three generations to enhance homozygosity of the useful quantitative characters (Rakshit and Patil, 2013). Moreover, these can directly be grown to increase sorghum production due to their better adaptability to changing climate, fresh biomass and earliness. In addition, crossing experiments can be designed involving these early maturing genotypes (Johar-2013, JS-1, P-2-S5-2013 and P-5-2013) and the ones with high fresh biomass (SP-1484-1, P-13-2013, Indian-6 and K-94) to get high biomass and early maturing varieties, as earlier suggested by Dossou-Aminon *et al*. (2015).

Conclusion

Present study explored the largest collection of Pakistan sorghum genotypes so far, for phenotypic diversity assessment. It will enrich the existing sorghum breeding program with the best performing genotypes identified in the present research. The genotypes revealed high variability with the pronounced broad sense heritability. Principal component analysis can lead to diverse set of genotypes for the association mapping. The cluster analysis extracted class nominators from the seven classes, which can provide the novel material with natural resistance against abiotic and biotic stress. Two sorghum genotypes P-10-2013 and P-13-2013 2013 can be used for direct cultivation in multi-location trials.

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