Research Article

Multivariate Analysis in Determining Morphologically Diverse Sugarcane Genotypes (*Saccharum officinarum* L.) and their Flowering Response at Arja, Azad Kashmir

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Abstract | Sugarcane is the most important sugar crop in Pakistan. However, its improvement through hybridization is limited because of its flowering and viable fuzz production. New avenues suitable for flowering were explored using 20 genotypes for initiation of breeding program in the country. A site near Bagh district (Arja) Azad Kashmir was found suitable and used for morphological evaluation, phenotypic diversity analysis and their flowering behavior sugarcane under local conditions. Two year data revealed that genotype HSF-242 showed maximum mean values for, internodes length, brix percentage and reducing sugar while S-08-FSD-19 showed maximum values for number of tillers and stem girth, whereas S-05-FSD-307 revealed maximum values for plant height and number of nodes. Analysis of variance revealed highly significant differences for plant height, number of tillers, leaf area, brix percentage, reducing and non-reducing sugar. A total of 79.75% cumulative variance was estimated in genotypes with PCA analysis at eigenvalue >1 and grouped 20 genotypes into five clusters at Euclidean distance (ED) 7. Cluster analysis revealed that the members of clusters I, cluster III and Cluster V diverse genotypes that should be utilised to initiate breeding program. The genotype HSF-242 was the outlier at ED 8. Out of twenty genotypes tested only five (S-03-US-694, S-05-FSD-307, S-05-FSD-317, S-08-FSD-23 and S-08-FSD-19) are responded flowering and viable fuzz production at new site. More germplasm need to be evaluated for selection of suitable parents that can be used for future breeding program.

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Introduction

Sugarcane (*Saccharum officinarum* L.) is the most important sugar and cash crop not only in Pakistan but also in various parts of the world (Deho et al., 2002). It is cultivated primarily for its sucrose in the internodes of the stem and raw material for industrial products such as alcohol and ethanol as a biofuel (Martin, 1982). Sugarcane is grown predominately in tropics and sub-tropics between 30° N and 35° S (Nazir, 1999) and accounts for approximately 75 percent of the total world sugar production (Henry and Kole, 2010). Sugarcane production in Pakistan for the year 2014-15 was 62.7 million tons against 67.5 million tons during 2013-14 showing a decrease of 7.1 percent (MNFS and R, 2015). These yield changes may be due to poor yielding varieties frequently adopted for cultivation coupled with adverse climatic factors that hinders cane yield.

Without adaptation of promising sugarcane varieties production cannot be enhanced. Current situations warrant the evaluation and assessment of genetic diversity in local and exotic sugarcane germplasm that

can withstand biotic and abiotic stresses (Khalid et al., 2014). Plant genetic resources provide raw material for development of new varieties that can cope with pests and climatic influences (Sajjad and Khan, 2009).

Genetic diversity assessment among cultivars is a robust tool for initiation of plant breeding program. Genetic diversity provides sugarcane breeders a means to identify more diverse germplasm to introduce within their breeding programs (Aitken and McNeil, 2010). To facilitate the appropriate analysis of genetic diversity in sugarcane several methods have been utilized that include: morphological data (Brown et al., 2002), pedigree data (Lima et al., 2002) and data of agronomic attributes (Skinner et al., 1987). For genetic diversity analysis various statistical approaches are used depending on data set used. Multivariate data analysis techniques are widely used in the sugarcane genetic diversity analysis by using morphological and molecular data (Mohammadi and Prasanna, 2003). Cluster analysis using hierarchical method (Sneath and Sokal, 1973) and Principal Component Analysis (PCA) are frequently used approaches for sugarcane diversity assessment (Aitken et al., 2006). Hierarchical clustering methods have been mostly used coupled with Ward's method (Milligan, 1980)

Sugarcane is a short day plant and flower at some locations in the world like Coimbatore (India), Barbados (West Indies), Canal point, Florida and Louisiana (USA), Taiwan, Natal (South Africa) Java (Indonesia), Brisbane (Australia), (Moore and Nuss, 1987) and to some extent at Thatta and Murree (Pakistan). Fuzz, the sexual seed is fibre like structure produced in sugarcane at arrow (spike) is a dilemma in the country because of unavailability of suitable geo-climatic conditions. Development of new cane varieties in country is totally dependent on import of sugarcane fuzz (true seed) from USA, Australia, Brazil, Barbados, South Africa, Mauritius and Sri Lanka (Nadeem et al., 2011) which is not only cost effective but the breeding material raised from exotic fuzz affected by number of factors like G x E interaction, stability in the performance, lower yield, biotic and abiotic stresses. Flowering with less viable fuzz necessitates for searching more suitable sites for sugarcane breeding and improvement in Pakistan. For this purpose, various places at Azad Kashmir were visited and their climatic conditions were assessed and available climatic data was reviewed and finally Arja Azad Kashmir (District Bagh-150 Km from Islamabad) was taken into consideration. Sugarcane is not a commercial crop grown at anywhere in Azad Kashmir and this would be the introduction. Climate of Arja, Bagh Azad Kashmir is mild, generally warm and temperate with significant rainfall throughout the year. The average annual rainfall is 1051 mm and average annual temperature is 20.2 °C and altitude is 797m while latitude North 33.97°- 21 minutes and longitude East 73.97°-42 minutes (http://climate.org/). The soil of Arja, Azad Kashmir is loam with medium to low fertility (Zafar et al., 2013). High levels of nitrogen in soil during flowering initiation phase may reduce or delay flowering (Nuss and Berding, 1999). Flowering in sugarcane is affected by many factors like temperature, photoperiod, humidity, altitude and latitude. Sugarcane is a short day plant (Burr, 1957) ideal latitude for flowering ranged from 11°N to 29°S (Moore and Nuss, 1987). Self-pollination does occur in sugarcane (McIntyre and Jackson, 2001) and seed set mostly with cross-pollination. Optimum photoperiod for flower induction is 12 hours and 35 min and flowering decline in any decrease in day length by ±5 min (Coleman, 1959). At night a period of 11 hours 32 min is very conducive for flowering (Clements and Awarda, 1964). At 0° equators 11:50-12:00 hours nycte period of 49 days is required for profuse flowering but at local conditions a nycte period of 24 days is available, however some variables are negatively influencing the flowering under this situation. Flowering is abandon where night temperature drops below 18°C (Coleman, 1963). Ten continuous nights with temperature below 18°C prevent flowering induction (Coleman, 1968).

Aim of this research work included morphological evaluation, assessment of genetic variability among the adopted sugarcane genotypes and their flowering response with viable fuzz production to identify best parents for future hybridization program at Arja, Bagh Azad Kashmir.

Materials and Methods

Sugarcane germplasm containing twenty adopted varieties/genotypes (Table 1) were collected from Sugarcane Research Institute and Sub-Station Murree, Ayyub Agricultural Research Institute Faisalabad, Pakistan and were sown in March 2013 at Arja (District Bagh Azad Kashmir) in three replications. Germplasm contained commercial varieties, local adopted varieties and exotic genotypes.

Table 1: *Sugarcane genotypes, nativity and their mean performance of morpho-physological attributes from pooled data obtained during the years 2013-14*

S. NO	Variety/ Genotype	Nativity	Plant height	Tillers/ Plant	Stem Girth (cm)	No. of nodes	Inter-nodes length (cm)	No. of leaves	Leaf area (cm^2)	Brix %age	Reducing sugar (mg/ml)	Non-reducing sugar (mg/ml)
1	HSF-242	Sindh, Pakistan	180abcde	6.3 abc	2.58abcd	12.3abc	15.6a	17.7ab	560bcde	19.9a	10a	8.0b
2	SPF-213	Sao Paulo, Brazil	195 ab	5.7abcd	2.43abcd	9.3cd	14.8ab	17.3ab	642abc	12.3k	7.0e	9.1a
3	CPF-237	Canal Point, America	190abcd	6.7ab	2.22cd	12.7ab	14.8ab	18.7ab	450ef	19.1b	5.0i	3.7j
4	BF-162	Barbados, West Indies	180bcde	3.7f	2.54abcd	11.3abcd	14.7ab	19.0ab	410f	15.7i	5.3h	2.3l
5	S-03-US-694	Canal Point, America	192abc	4.3def	2.47abcd	12.0abcd	14.6ab	20.3a	556bcde	19.2b	5.0i	4.7g
6	S-06-SP-321	Sao Paulo, Brazil	178abcde	5.3bcde	2.68abc	10.7abcd	14.3ab	18.7ab	672ab	18.2de	6.0g	6.1e
7	S-05-FSD-307	Murree, Pakistan	202a	6.0abc	2.67abcd	13.0a	14.3ab	19.0ab	508def	14.9j	4.9i	4.9g
8	S-05-US-54	Canal Point, America	164bcdef	6.0abc	2.43abcd	10.7abcd	14.1ab	18.3ab	468def	17.9ef	3.3k	7.0d
9	S-06-US-300	Canal Point, America	172abcde	6.7ab	2.26bcd	11.7abcd	14.0ab	16.0b	481def	17.9ef	3.9j	4.1h
10	S-03-SP-93	Sao Paulo, Brazil	160cdef	5.7abcd	2.18d	9.7bcd	14.0ab	18.0ab	568abcde	16.1h	6.0g	6.3e
11	S-06-US-272	Canal Point, America	175abcde	4.0ef	2.83a	11.0abcd	13.4ab	17.3ab	479def	16.0h	2.3l	7.3c
12	S-03-US-127	Canal Point, America	153ef	3.7f	2.46abcd	11.0abcd	13.3ab	17.3ab	502def	19.7a	2.3l	6.3e
13	S-06-US-658	Canal Point, America	158def	5.7abcd	2.43abcd	9.0d	13.2ab	15.7b	540cde	18.7c	9.7b	9.0a
14	S-03-US-778	Canal Point, America	153ef	6.7ab	2.30bcd	11.3abcd	13.2ab	18.3ab	688a	19.8a	6.3f	3.9ij
15	S-05-FSD-317	Murree, Pakistan	175abcde	5.0cdef	2.43abcd	11.7abcd	13.1ab	18.7ab	466def	18.3d	4.0j	2.3l
16	SPF-232	Sao Paulo, Brazil	182abcde	4.0ef	2.54abcd	11.0abcd	12.9ab	18.0ab	658abc	12.1k	7.7d	5.3f
17	LHO-83153	Not known	179abcde	5.7abcd	2.57abcd	11.0abcd	12.7ab	15.7b	577abcd	17.1g	5.0i	4.0hi
18	S-08-FSD-23	Murree, Pakistan	139f	5.0cdef	2.75ab	11.0bcd	12.5ab	18.0ab	509def	19.3b	10a	2.9k
19	S-08-FSD-19	Murree, Pakistan	176abcde	7.0a	2.33bcd	11.3bcd	11.9b	19.7ab	481def	19.3b	9.0c	2.1l
20	HSF-240	Sindh, Pakistan	175abcde	6.3abc	2.47abcd	10.7abcd	11.9b	17.3ab	493def	17.7f	4.8i	2.2l
		Grand mean	173.95	5.46	2.48	11.12	13.66	11.54	535.55	17.45	5.87	5.07
		S.E	11.41	1.86	0.25	1.51	1.55	1.69	93.84	0.15	0.12	0.12

LSD: *α 0.05*

Field experiment

The experiment was conducted for two years under irrigated conditions. The field was ploughed two times and then beds were prepared. The experiment was conducted in three replications with Randomized Complete Block Design (RCBD). Borders of the experimental field was covered with non-experimental line. Three meter rows of each entry were sown. Stem setts of almost 1.5 ft from each sugarcane genotype containing 2 to 3 nodes were placed in furrows prepared in beds 2 ft apart. DAP at the rate of 100 kg per hectare spread with broad cast method, later setts were covered with soil and light irrigation was applied. After almost 25 days of germination hoeing of plant were carried out followed by irrigation. Frequent weeding and hoeing carried out throughout crop season. During second season ratoon crop was raised from crop grown in the previous season by earthen up the stubbles. Frequent irrigations applied and all agronomic practices were carried out with the plants raised to maturity. At maturity crop was harvested and only mother shoots were left for flowering and frequent irrigations were applied with no fertilizer application until flowering to curtail the vegetative growth phase. After DAP at the rate of 100 kg per hectare were spread by broad cast method.

Data collection

Data were recorded from 10 guarded randomly selected plants at maturity level for two cropping seasons during years 2013 and 2014. Parameters recorded at maturity included; plant height (cm), number of tillers per plant, stem girth (cm), number of nodes, inter-nodes length (cm), numbers of leaves, leaf area (cm^2), brix percentage, reducing sugar (mg/ml) and non-reducing sugar (mg/ml). For plant height, internodal length and leaf area estimation centimetres scaled steel measuring tape was used. Leaf area was recorded in centimetre from three places for width (cm) and average was then multiplied with length and then with factor 0.72 according to Sinclair et al., (2004).

$$Leaf\ Area = (length \times width\ from\ three\ places) \times 0.72$$

Brix percentage was estimated from the juice extracted for each genotype at maturity by using the digital refractometer by putting a 2 to 3 drops of juice on the lens of refractometer and readings were recorded. Reducing sugar was estimated by using Benedict's method (AOAC, 1990). 2 gram of anhydrous sodium carbonate was added to 5 ml of Benedict solution in 250 ml flask. Mixture was shake well and gently warmed at 100°C and finally titrated against the sugarcane juice drop by drop through burette until colour was changed to bricks read. Volume of sample solution was recorded in duplicate. Final calculations were based as follows:

1 ml of juice used in titration = 2 mg of reducing sugar

Non-reducing sugar was determined by using Benedict's method (AOAC, 1990). In this method sugarcane juice sample of 20 ml was taken in a beaker and 5 ml of 2% HCl was added and boiled for 30 minutes in a water bath. It was cooled down and its pH was adjusted to 7.0 with NaOH (0.1N). Then it was titrated against the 5 ml boiled Benedict's reagent containing 2 gm anhydrous sodium carbonate drop by drop through burette and continue shake until the colour was changed to brick red. Volume of juice used in titration was recorded and finally calculations were recorded as follows:

1 ml of juice used in titration = 2 mg of non-reducing sugar

Statistical analysis

Data collected from above mentioned parameters was subjected to some basic statistics *i.e.* mean, least significant difference (LSD), standard error (SE), coefficient of variation (CV) (Table 1) and analysis of variance (ANOVA) by using software STATISTIX 8.1 (Table 2). Analysis of variance was performed according to the Steel and Torrie (1980). Principal Component Analysis was performed by using PAST Statistical software, version 2.17c (Hammer et al., 2001). As measuring units of various parameters were not same means the data were standardized according to the Hair et al. (2006). Cluster analysis based on Ward's method using Euclidean distance (Kumar et al., 2009) was performed by using the statistical software STATISTICA version 5.0. Euclidean distance, identifies parameters that are close to each other when PCA is performed using Euclidean distance (Elmore and Richman, 2001).

Results and Discussion

Mean performance and analysis of variance (ANOVA)

Twenty sugarcane genotypes were used to quantify the genetic divergence by using various quantitative

Table 2: *Analysis of variance (mean squares) for different morpho-physiological traits in 20 sugarcane genotypes*

Source	d.f	Plant height	Tillers/ Plant	Stem Girth (cm)	No. of nodes	Inter-nodes length (cm)	No. of leaves	Leaf area (cm^2)	Brix %age	Reducing sugar (mg/ml)	Non-reducing sugar (mg/ml)
Reps	2	2001.5	4.933	0.403	8.316	3.486	4.200	3266	2.97	0.01	0.054
Genotypes	19	742.7*	64.27**	0.09NS	3.06 NS	3.08 NS	4.50 NS	18712**	16.01**	17.18**	15.15**
Error	38	390.6	37.733	0.090	3.404	3.609	4.287	5399	0.039	0.02	0.024
Total	59										
CV		11.36	18.23	12.16	16.60	13.90	17.95	13.72	1.06	2.44	3.09

*: *Significant at p 0.05;* **: *Significant at p 0.01;* **NS:** *Non-significant*

traits. Basic statistics for various morphological traits are presented in Table 1. Maximum mean values for plant height (202 cm) showed by genotype S-03-US-127 and minimum showed by the genotype S-08-FSD-23 (139 cm), Number of tillers recorded in the range from 3.7 to 7 cm. maximum tillers recorded from genotype S-08-FSD-19. Average 2.5 cm stem girth was recorded in all the genotypes ranged from 2 to 2.8 cm. Average 11 internodes per plant were recorded followed by 13.6 cm average inter-nodal length. Average 11 leaves per plant with leaf area 535 cm^2 were recorded per plant. The genotype HSF-242 showed maximum value (19.9) for brix percentage and reducing sugar contents while genotype SPF-213 showed maximum values for maximum value for non-reducing sugar but minimum value for brix percentage. HSF-242 showed maximum value for reducing sugar contents while minimum value for number of tillers, internode length and number of leaves, S-03-US-778 showed maximum leaf area but minimum plant height. The genotypes S-03-US-127 and S-06-US-321 were found batter on the bases of mean performance for most of the important agronomic characters.

Singh et al. (2008), Junejo et al. (2010), Dalvi et al. (2012), Islam and Begum (2012), Sobhakumari (2012), Seema et al. (2014); Khan et al. (2015), Beghum et al. (2015) and Gaddkh et al. (2015) reported plant height in sugarcane ranged from 58 cm to 286 cm, number of tillers in ranged from 2 to 8, stem diameter ranged from 1.4 cm to 2.8 cm, brix percentage ranged from 15% to 21%. Sood et al. (2006), Dalvi et al. (2012), Khan et al. (2015) and Gaddkh et al. (2015) reported number of internodes ranged from 11 to 24, Sood et al. (2006) reported internodes length in the range from 9cm to 12.5 cm. These finding are very much in accordance with our findings however some differences may be due to differences in plant material used or environment.

Results for analysis of variance (ANOVA) presented in Table 2 revealed significant results for plant height while highly significant differences for number tillers per plant, leaf area, brix percentage reducing and non-reducing sugar. The results indicated that these parameters have a pivotal role in the variability among genotypes. Non-significant results were recorded in stem girth number of nodes, and intermodal length. Bakshi and Hemaprabha (2005), Cardozo et al. (2014) and Khan et al. (2015) also reported significant differences for those traits.

Principal component analysis

Principal component analysis was performed to assess the variability among 20 sugarcane genotypes, using quantitative traits to reveal the outlier genotypes. The primary purpose of PCA was to define the underlying structure in a data. As a data reduction or exploratory methods, these procedures were used to reduce the number of variables and to detect structural relationship between these variables. PCA is a technique for finding putative variables which gives interpretation for as much of the variables in a multivariate data as possible. PCA is a unique mathematical solution; it performs simple reduction of the data set to a few components, for plotting and clustering purposes, and can be used to assume that the most essential components have association with some other underlying variables (Acquaah, 2012). A data matrix was constructed using the determined quantitative traits as columns and the sugarcane genotypes as rows. Principal components analysis was performed on auto-scaled data. Significance of Principal Components (PCs) were determined with Jolliffe cut off value >0.7. The Jolliffe cut-off value for eigenvalues, used in the PAST software, is a method to determine how many principal components (PCs) should be considered significant (Jolliffe, 1986). The first four principal components were chosen for modelling the

Table 3: *Principal Components of quantitative traits in 20 Sugarcane Genotypes their eigenvalues and variance generated by each component. Factor loadings of PC1 and PC2 for parameters studied*

Principal Components	Eigenvalue	Percentage Variance	Cumulative variance %age	Parameters	Factor loading PC1	Factor loading PC2
PC1	3.26*	32.67	32.6	1	0.414	-0.330
PC 2	2.19*	21.95	54.63	2	1.359	0.430
PC 3	1.29*	12.94	67.57	3	-0.322	-0.300
PC 4	1.21*	12.17	79.75	4	0.461	-0.188
PC 5	0.61 NS	6.14	85.89	5	0.242	0.199
PC 6	0.56 NS	5.68	91.58	6	0.422	-0.095
PC 7	0.31 NS	3.18	94.76	7	-0.122	0.300
PC 8	0.29 NS	2.97	97.74	8	0.284	0.454
PC 9	0.12 NS	1.26	99.00	9	-0.380	0.201
PC 10	0.10 NS	1.00	100.00	10	-0.175	-0.356

*:*Significant at Jolliffe cut off value = >0.7;* **:*Highly significant;* *:*Significant;* **NS :** *Non-Significant*
Parameters: 1. Plant height, 2. No. of tillers, 3. Stem girth, 4. No. of nodes, 5. Internodes length, 6. No. of leaves, 7. Leaf area, 8. Brix %age, 9. Reducing Sugar, 10. Non-reducing Sugar

data, which communally accounted for 79.75% of the variation in the traits (Table 3). The remaining variance of other principal components did not have significant eigenvalues. First four principal components (PCs) have significant eigenvalues for all 10 quantitative traits compared, hence they all included in the model. PC1 contributed maximum variance (32.6%) in the data set followed by the PC2 (21.9%) while the PC3 has generated variance of 12.9% followed by the PC4 that produced 12.1% variance in the data set.

Geminet al. (2006) obtained three Principal Components with 82 percent cumulative variance in *S. Spontaneum* L. while studying 7 quantitative traits. Al-Sayed et al. (2012) computed 85 percent variance by Factor analysis on morphological traits of sugarcane with maximum variability generated by Factor I was 34 percent. Ajirlou et al. (2013) Factor analysis in sorghum genotypes and elucidated 86% total variability with first main Factor contained 33 percent total variability. Tahir et al. (2013) obtained two Principal components with cumulative variability of 88 percent. James et al. (2014) found 97 percent total variance in sugarcane germplasm evaluated by doing PCA analysis. Our results were nearly similar to the findings of previous reports except Tahir et al. (2013) and James et al. (2014) whom reports were more included to first few components.

Factor loadings of PCs for quantitative traits Loadings of PC1: Factor loading is defined as the correlation coefficients between the PC scores and the variables (Yamamoto et al., 2014). Loading of first principal component (PC1) presented in the (Table 2), which depicted that number of nodes per plant showed maximum positive loadings (0.461) followed by the plant height (0.414) and number of leaves per plant (0.422). Reducing sugar showed minimum loadings (-0.38) followed by stem girth (-0.322). From the results it can be inferred that plant height, number of nodes per plant and number of leaves per plant have positive correlation among themselves while these parameters have negative correlation with stem girth and reducing sugar.

Loading of PC2: Loadings of the PC2 presented in the Table 3. Brix percentage has maximum loading (0.45) in this PC which means that its contribution in the generation of variance is more in this PC followed by the number of tillers per plant and leaf area with the loadings 0.43 and 0.3 respectively. Non-reducing sugar showed minimum loadings (-0.3567) followed by the plant height (-0.33) and stem girth (-0.30). Plant height, leaf area and brix percentage have negative correlation with number of tillers per plant, stem girth and non-reducing sugar. Sanjay and Devendra (2014) found that yield was significantly correlated with number of tillers, stem diameter, plant height, number of inter nodes, intermodal length and number of leaves.

PC1 versus PC2 biplot for 10 quantitative traits of 20 sugarcane genotypes: The first two PCs; *i.e.* PC1 and PC2 genetared 54.63 percent of the total variance (Table 3) among the 20 genotypes for the 10 quantita-

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tive traits under study and is resperented in the Figure 1. Plant height, number of nodes per plant and number of leaves falls on opposite axis with respect to leaf area and reducing sugar in the biplit diagram which means that these parameters have negative correlation. Brix percentage, inter-nodes length number of tillers per plant have negative correlation with stem girth and non-reducing sugar while plant height has negative correlation with leaf area. These results are very much in accordance with the loadings of the PC2 (Table 3). Biplot diagram of 20 genotypes for 10 quantitative traits has that five genotypes; *i.e.* S-08-FSD-19, S-03-US-778, HSF-242, S-06-272 and S-03-US-127 fall outside the range of the center of origen of axis as compare to the rest of other genotypes hence, these genotypes are considered to be outliers, which means that these genotypes are more morphologically divergent as compare to other genotypes under study. Biplot diagram detpected that S-03-US-778 has maximum leaf area, S-08-FSD-19 has maximum value

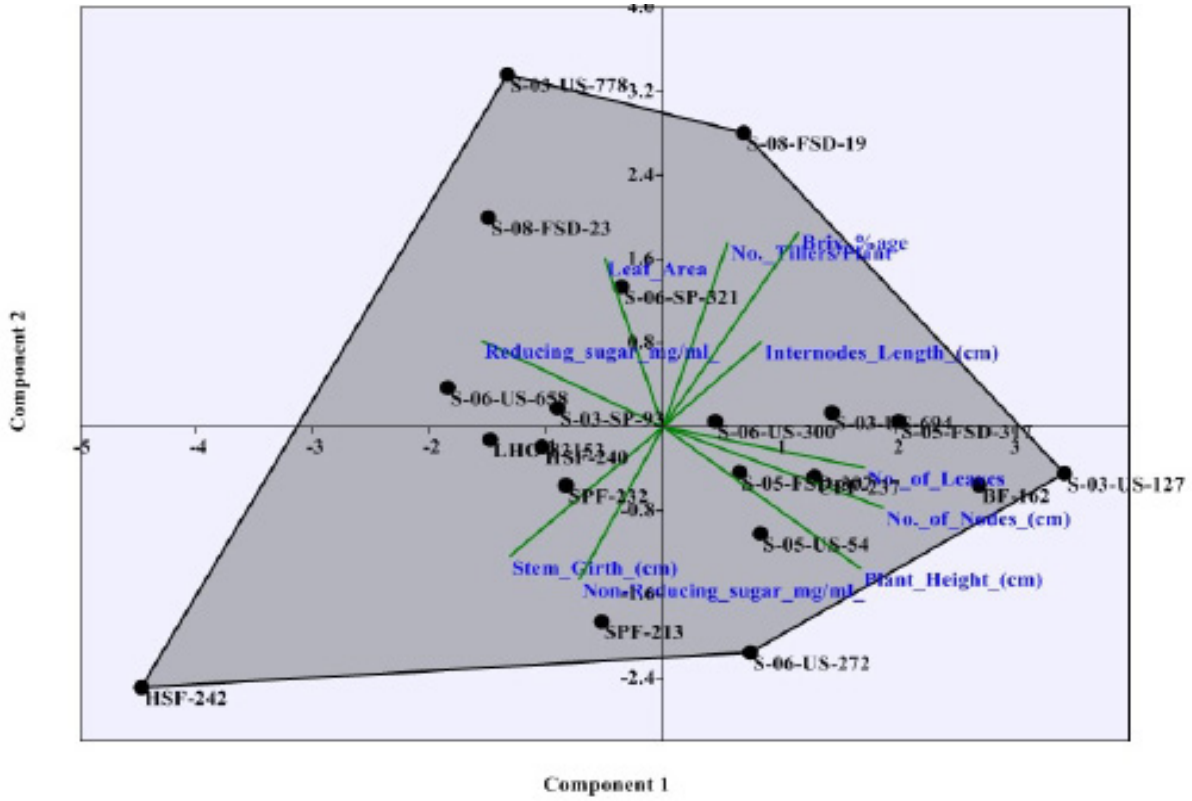

Figure 1: *Plot of (PC1) versus (PC2) for 10 Quantitative traits and 20 Sugarcane genotypes*

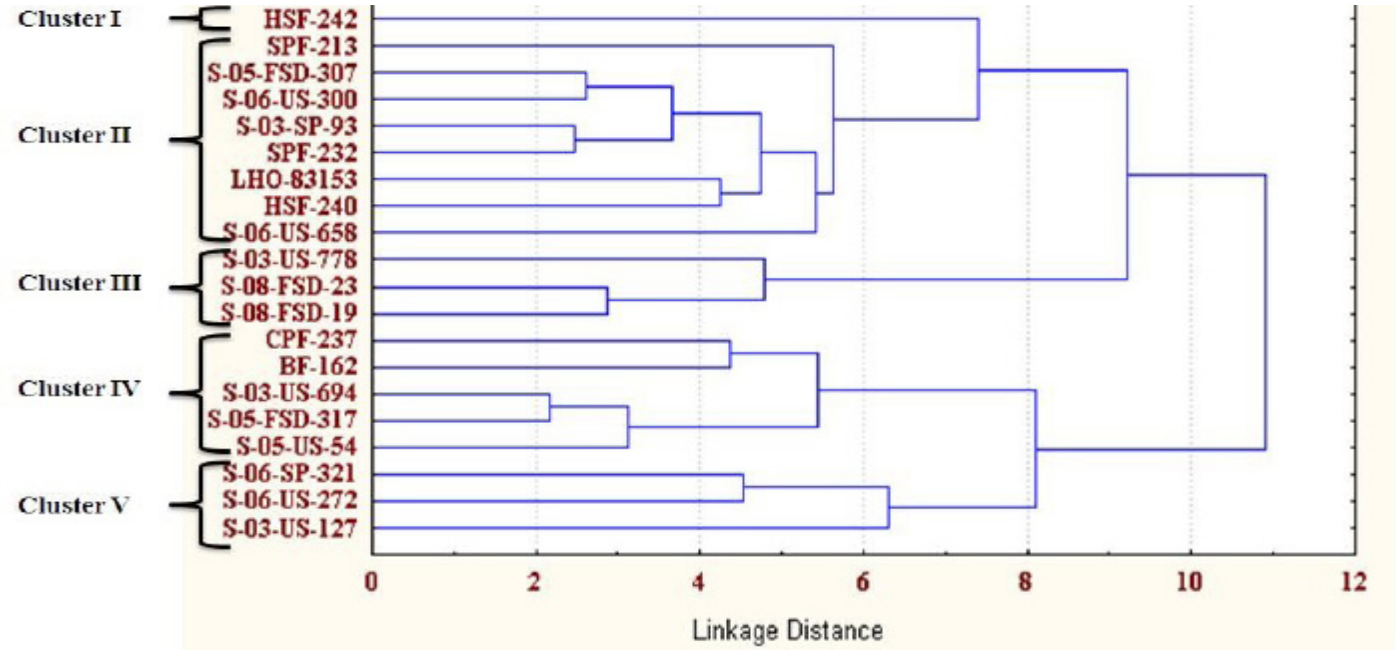

Figure 2: *Cluster Diagram of 20 Sugarcane Genotypes on the bases of 10 morpho physiological traits*

for brix percentage, number of tillers per plant and inter-nodes length, S-03-US-127 and S-06-US- 272 have maximum plant height, number of nodes and leaf area while HSF-242 has maximum stem girth and non-reducing sugar. Contribution of these parameter in the generation of variance was high, therefore during selection these parameter must be given due consideration. It can further be inferred for the biplot diagram that the genotypes used in this study make three groups on the bases of variability present in the traits. First group comprised of genotypes; S-06-US-658, S-03-SP-93, LHO-83153, HSF-240, SPF-232 and SPF-213. Second groups consisted of genotypes; S-06-US-300, S-03-US-694, S-05-FSD-317, S-05-FSD-307, CPF-237 and BF-162.

Cluster analysis

For cluster analysis hierarchal clustering with Ward's method was used. Data was subjected to the cluster analysis that generated five clusters at Euclidean distance (ED) of 7 by following Ward's method. Euclidean distance is the distance present in the genotypes on the bases of similarity present among the genotypes this is also called linkage distance. Range of linkage distance or ED were between 0-12 (Figure 2). Cluster I contained only one genotype; HSF-242 which is an outlier in the cluster diagram. This genotype is also an outlier in a biplot diagram (Figure 2) of PC1 and PC2. Cluster II comprised of eight genotypes; SPF-213, S-05-FSD-307, S-06-US-300, S-03-SP-93, SPF-232, LHO-83153, HSF-240 and S-06-US-658. SPF-213 and S-06-US-658 are the outliers in this cluster. Cluster III composed of three genotypes; S-03-US-778, S-08-FSD-23 and S-08-FSD-19. Two of these genotypes S-03-US-778 and S-08-FSD-23 are the outliers in the biplot diagram and also form a separate group along with S-08-FSD-19. Genotypes; CPF-237, BF-162, S-03-US-694, S-05-FSD-317 and S-05-US-54 are included in cluster number four. These genotypes have close association with the genotypes included in the cluster V and cluster III. Biplot of PC1 and PC2 also showed same genotypes in the second group. These genotypes have association with genotypes of cluster V but much divergent as compare to the genotypes of cluster I, cluster II and cluster III. Only three genotypes (S-06-SP-321, S-06-US-272 and S-03-US-127) included in Cluster V. S-03-US-127 is an outlier in this group which can be confirmed from the biplot diagram of PC1 versus PC2 where S-06-US-272 and S-03-US-127 are the outliers. It clearly depicts that these genotypes are more divergent in the overall genotypes compared and can be used for future crop improvement program.

To compare our results, findings of some previous researchers was reviewed. Bakshi and Hemaprabha (2005) done cluster analysis on sugarcane genotypes containing 13 traits and grouped genotypes into 9 clusters. Gemin et al. (2006) obtained 4 clusters on the bases of sugar contents by doing cluster analysis. Kashif and Khan (2007) determined genetic diversity in fourteen sugarcane genotypes on the bases of 12 quantitative characters and obtained 4 clusters while Ahmed and Obeid (2010) found genotypes clustered into six groups with higher genetic distance between two clusters was 83 percent. By using Ward's method Tahir et al. (2013) distinguish sugarcane genotypes and revealed 3 clusters with linkage distance of 4.5 while Kang et al. (2013) partitioned sugarcane genotypes into eight clusters. Sanghera et al. (2015) assessed genetic diversity by using cluster analysis in sugarcane based on eighteen quantitative traits and found genotypes grouped into five clusters with maximum genetic distance between two clusters as much as 89. Above studies supports the authentication of our findings. Similar findings were reported by Gemin et al. (2006), Kashif and Khan (2007), Ahmed and Obeid (2010), Tahir et al. (2013) and Sanghera et al. (2015).

Flowering response of varieties

At Arja Bagh Azad Kashmir we have conducted experiment containing twenty exotic and local sugarcane genotypes (Table 1) for two consecutive years during 2013-14 and 2014-15 to observe their flowering behaviour. Sugarcane flower at few more locations in Pakistan including Charra Pani (Murree) and Thatta (Sind) but the viable fuzz (seed) production is very low. Annual temperature ranges and cane flowering times at Arja (Azad Kashmir), are presented in Table 4. According to climatic data of Arja available on net (http://climate.org) during November rainfall is lowest with average 23 mm but during July rainfall is generally high with an average almost 202 mm. July is the hottest month of the year with average temperature 30°C while in January average temperature is as low as 9°C. Between the driest and wettest months the difference in precipitation is 179 mm. During the month of May cone formation (inflorescence packed in top leaves not yet emerge) and the panicle inflorescence in sugarcane called arrow (Govindaraj and Sreenivasa, 2014) start emergence and anthers dehiscence. At Arja, Bagh Azad

Table 4: *Average annual temperatures, rainfall ranges and sugarcane flowering time at Arja (Azad Kashmir)*

Months	Av. Max (°C)	Av. Min (°C)	Average sunshine hrs	Rainfall (mm)	Flowering time/conditions
September	25.8	19.6	11	84	Growth phase of cane plant
October	21.2	13.6	11	38	Maturity of cane plant
November	15.3	7.6	09	22	
December	10.9	4.0	08	43	Cold air wave, Cold and rain, growth and development activity became slow
January	9.2	3.3	08	65	
February	11.5	5.4	08	85	
March	16.1	9.8	10	110	Period of flowering induction
April	21.1	14.5	11	85	Flower differentiation
May	25.8	18.8	13	59	Cone formation, arrow emergence, anther dehiscence
June	30.0	23.1	13	62	Possible seed setting, problem of synchronization
July	28.6	23.3	12	210	Growth phase of cane plant
August	27.1	22.2	11	184	

Sources: www.Climate-data.org; www.holiday-weather.com

Table 5: *Flowering response of sugarcane genotypes during 2014 and 2015 at agro-climatic conditions of Arja, Bagh Azad Kashmir*

S. No	Genotypes/varieties	Flowering response	Arrow emergence during 2014	Arrow emergence during 2015
1	S-03-US-694	Yes	15, May	11, May
2	S-05-FSD-307	Yes	05, May	05, May
3	S-05-FSD-317	Yes	20, May	17, May
4	S-08-FSD-23	Yes	10, May	15, May
5	S-08-FSD-19	Yes	08, May	10, May

Kashmir flowering time was observed during May under this study. Average night temperature at Arja during flowering time was recorded 18.8° C respectively (Table 4). Panicle development and male fertility takes place at temperature 21°C (Berding, 1987) decline in fertile pollen production is reported by Heinz and Tew (1987) where night temperatures remain between 14.5 and 16 °C for a period of 5-10 days. Average sunshine during flowering period was 12.5 to 13 hours. Optimum photoperiod for flower induction is 12 hours and 35 min (Coleman, 1959) and our experiment site meet this requirement. A period of 11 hours 32 min at night is very conducive for flowering (Clements and Awarda, 1964).

Among the twenty exotic and local sugarcane genotypes only five genotypes (S-03-US-694, S-05-FSD-307, S-05-FSD-317, S-08-FSD-23 and S-08-FSD-19) flowered for two years testing (Table 5). Arrows, the sugarcane inflorescence containing anthers and stigmas are presented in Figure 3. A little bit synchronization differences in genotypes were recorded. Five days difference in cone emergence was observed among genotypes S-03-US-694 and S-05-FSD-307, S-05-FSD-307 and S-08-FSD-19, S-08-FSD-23 and S-08-FSD-19. Maximum ten to twelve days difference were recorded in synchronization in genotypes S-05-FSD-307, S-08-FSD-23 and S-05-FSD-317 respectively. Synchronization problems can be overcome by sowing date and fertile seeds can be obtained at Arja Bagh. More genotypes/cultivars are needed to be included in future experiments to find out wider germplasm responsive to flowering and viable fuzz production. The fuzz collected from the flowers at Arja was tried under nutrient medium for germination and about 50% viability was recorded (data not presented) in the laboratory.

Conclusion

The sugarcane is an important cash crop of Pakistan, however, its conventional hybridization and improvement for yield and percentage sugar accumulation is limited due to its particular behaviour for photoperiod, altitude and longitude. The varieties/ genotypes of sugarcane compared indicated ample variability, which could be utilized for crop improvement once

Figure 3: *Flowering response of sugarcane genotypes at Arja, Bagh AJK. Where (a) represents flowering arrow of variety S-03-US-694, (b), S-08-FSD-23 (c) anthers and stigmas on arrow of S-08-F*

particular niche is identified for flowering and viable fuzz production. Out of twenty genotypes/varieties only five (S-03-US-694, S-05-FSD-307, S-05-FSD-317, S-08-FSD-23 and S-08-FSD-19) responded to flowering and fuzz production at Arja Bagh Azad Kashmir, Pakistan, which is encouraging. It can be concluded that this location is ideal for flowering and large amount of germplasm must be put under evaluation on national level for selection of suitable parents that can be synchronised timely for future hybridization and improvement program.

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Author's Contribution

Muhammad Shahzad Ahmed contributed for site selection, germplasm collection, experiment conduction, data collection and manuscript write-up. Dilnawaz Ahmed Gardezi generated idea of this research, arranged financial support for experiment and help in writing.

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