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Genetic Diversity among Sugarcane Cultivars in Pakistan

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Abstract: The present research was carried out in the experimental area of the Department of Plant Breeding and genetics, University of Agriculture, Faisalabad, to group forty cultivars into different clusters using Mahalanobis D^2 statistic technique. The Randomized Complete Block Design (RCBD) was used with three repeats and block size of 10m x 5m. The characters measured were number of tillers, cane length (cm), cane weight (g), cane diameter (cm), leaf area (cm^2), juice content (%), brix value (%), pol value (%), purity %, fibre % and commercial cane sugar (CCS) (commercial cane sugar). The data was subjected to Mahalanobis D^2 analysis that provided information regarding genetic divergence among forty sugarcane accession. value was significant, therefore D^2 analysis was proceeded to calculate D^2 values. The contribution of each character in genetic diversity was also calculated. The most contributing character was fibre% fallowed by cane weight, pol% and brix value, CCS and juice contents. The cane length contributed zero. According to Tocher' method the forty genotypes were grouped into sixteen cluster. Cluster XII was the smallest one consisting of only one genotype. The largest cluster was VI consisting of four genotypes. Geographical origin did not influenced grouping. Genotypes from different provinces fell in the same cluster.

Key words: Genetic diversity · Mahalanobis D^2 · *Saccharum officinarum* L.

INTRODUCTION

Modern sugarcane varieties that are cultivated for sugar production are complex interspecific hybrids (*Saccharum* spp.) that have arisen through intensive selective breeding of species within the *Saccharum* genus primarily involving crosses between the species *Saccharum officinarum* L. and *S. spontaneum* L. [1].

In Pakistan sugarcane crop serves as a major raw material for production of white sugar and gur. Their share in value added of agriculture and GDP are 3.4 percent and 0.7 percent, respectively. For 2005-06, the area under sugarcane crop was targeted at 955 thousand hectares as against 966 thousand of last year. However, sugarcane has been sown in the area of 907 thousand hectares that is 5 percent below the target and 6.1 percent less than previous year. Sugarcane production for the year 2005-06 is estimated at 44.3 million tons against the 47.2 million tons last year. Thus sugarcane production is estimated to be lower by 6.2 percent over the last year. Factor responsible for decline in sugarcane production include water shortage , farmer's shifting to other competing crops and frost affecting the crop in Punjab and NWFP. [2].

Genetic diversity gives species the ability to adapt to changing environments, including new pests and diseases and new climatic conditions. Plant genetic resources-that component of genetic diversity of actual or potential use to humanity-provide the raw material for breeding new varieties of crops. These, in turn, provide a basis for more productive and resilient production systems that are better able to cope with biotic and abiotic stresses

Knowledge about genetic diversity is a prerequisite of any breeding programme. Inclusion of diverse parents in hybridization programmes serves the purpose of combining desirable genes in new recombinations. There are several methods of studying genetic diversity. These include morphometric, biochemical and molecular methods. For this study, only the morphometric method was used.

Mahalanobis D^2 statistic is a powerful tool in quantifying the degree of divergence at genotypic level. Several workers have used this method to quantify the degree of divergence based on phenotypic observations in different crops. These studies have shown that accessions from the same geographical region may differ genetically as well as phenotypic ally and also

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in adaptability. In the present study, Mahalanobis D^2 statistic was used to determine the genetic diversity among 40 sugarcane clones existing in Pakistan.

MATERIALS AND METHODS

The present study was conducted in the experimental area of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, during the year 2006-2007.

Forty cultivars (Table 1) were planted in the research field on February 26, 2006 and harvested on March 3, 2007. A Randomized Complete Block Design was employed with row to row distance of 1m, set to set distance 4 inches and a block area of 10m x 5m and with three replications. Sets with two buds each was sown, practicing all agronomic and plant protection recommendations. From each experimental unit ten guarded stools were selected randomly for phenotyping eleven traits. Number of millable canes was counted separately. Cane length was measured from bottom to the tip of cane after removing top of cane, by using of meter rod in centimeter. Cane diameter was measured in centimeter at three different places by using of vernier caliper viz. top, middle and base. Leaf area was measured in "m^2" by using of "meter rod". Leaf width was measured at three different points viz. near the tip, middle and base of the leaf. The value of leaf length was multiplied with mean width of leaf and then multiplied with correction factor "0.75". Each of ten selected plants from each experimental unit was crushed in an electric powered cane crusher for juice extraction. Juice in % was calculated as; Juice%= Juice wt. x 100/ cane wt. From extracted juice, brix reading (%) was recorded by "hand refrectometer" standardized at 20 C. Brix value in cane was calculated using following formula; Brix in cane = brix in 1 juice x (100 (% fibre + 3))/100. Sucrose content is often referred to as per cent pol, with pol being derived from the name of the machine that measures the sucrose content, a polarimeter. A polarimeter works by sending polarised light through a known quantity of sugar juice (for example, a 100 mm or 200 mm column filled with juice) and measuring the rotation of the light after passing through the liquid. Standards have been developed by mixing known quantities of sucrose with water and measuring the light rotation. The resulting rotation figure can then be converted to a figure that estimates sucrose (or pol) per cent in juice.The fallowing formula was used to obtain the sucrose per cent in juice.

Table 1 : Cultivars names

S. No	Genotype/Population
1	HSF-240
2	CPF-237
3	HSF-242
4	CPF-243
5	SPF-245
6	SPF-213
7	CP-85-1491
8	S-2000-US-400
9	S-2002-US-104
10	S-2003-US-624
11	S-98-SP-108
12	SPF-234
13	S-96-SP-302
14	CP-81-1254
15	S-96-SP-646
16	CP-HS-35
17	CP-72-2086
18	CP-77-400
19	COJ-84
20	CP-43-33
21	BL-4
22	BF-162
23	ABT super
24	SPSG-26
25	T-10
26	NSG-311
27	NSG-555
28	S-95-HS-185
29	HS-12
30	Malakand-16
31	CoL-1148
32	CoL-54
33	CoL-29
34	Trition
35	BF-129
36	BL-19
37	Mardan-92
38	Bannu-1
39	JN-88-1
40	IM-61

Per cent pol (sucrose) in juice = {-6.517 + (25.3 x PR) 0.0118 x (PR x PR) + (2.937 x brix) 0.207 x (brix x brix)} / 100

Where, PR = Pol reading

Per cent pol (sucrose) in cane = pol in 1 juice x (100 (% fibre + 5)/100

Cane purity is a measure of the level of sucrose present in cane relative to the total level of soluble solids. Purity is generally expressed in percentage terms.

Purity of cane = (pol in cane/brix in cane) x 100

Over a period of 24 hours samples were collected from a clone immediately after the cane had passed through the shredder. These samples were combined and a ½ kg sub sample was taken.

- Sub sample was put through a cutter grinder.
- The ground sample was then placed into a fibre machine where it is washed to remove brix (soluble solids) and fine dirt.
- The sample was then dried using hot air and weighed on an electric balance.

The final weight divided by the initial weight provides a fibre percentage.

Fibre percentage = (final weight / original weight) x 100

CCS provides an estimate of the percentage of recoverable sucrose from cane.

Commercial cane sugar (CCS) = Pol in Cane - 0.5x impurities in cane

Impurities in cane= Brix in cane- Pol in cane

Statistical Analysis: A measure of group distance based on the multiple characters was given by Mahalanobis [3, 4]. Mahalanobis D^2 statistic is defined as: ${}_pD^2 = b_1d_1 + b_2d_2 +----+ b_pd_p$

Here, the bi values are to be estimated such that the ratio of variance between the populations to the variance within the population is maximized. In terms of variances and covariance, the D^2 value is obtained as fallows:

$$ {}_pD^2 = W_{ij} (X^1{}_i - X^2{}_i) (X^1{}_j - X^2{}_j) $$

Where, W_{ij} is the inverse of estimated variances covariance matrix. After calculating the D^2 values test of significance was performed. The D^2 value obtained from pair of population was taken as calculated value of chi-square and was tested against tabulated value for all characters at 5% and 1% level of significance. Contribution of each individual character towards divergence was calculated by ranking each character on the basis of $d_i = Y^j{}_i - Y^k{}_i$ values. Rank one was given to highest mean difference and rank P was given to lowest mean difference. These genotypes were grouped into various clusters. Average intra-distance was calculated as:

$$ \text{Average intra-cluster } D^2 = D^2{}_i/n $$

Where, $\Sigma\ D^2{}_i$ is the sum of distances between all possible combination (n) of the population included in the cluster. The number of all possible combinations was calculated by n(n-1)/2. The average inter-cluster distances were calculated as:

$$ \text{Average inter-cluster distance } D^2 = \sum D^2{}_{ij} /ni.nj $$

Where, $\sum\ D^2{}_{ij}$ is the sum of distance between all possible combinations ($n_i\ n_j$) between the population.

The formation of clusters, to group genetically similar genotypes in the same cluster, was done by Tocher's method [5].

RESULTS AND DISCUSSION

As calculated χ^2 value (23246.52) is greater than tabulated value (176.14) as calculated by $V_{(stat)}$ using Taples 12 and 13, therefore, further D^2 analysis can be performed. ANOVA Table 13 also depicts that variety variance is significant and D^2 analysis can be proceeded. Tables 2-12 show that clones are significantly different among themselves for all the characters, therefore, they all be included for D^2 analysis.

Punia *et al*. [6] found that tillers/clump and internodes/can contributed most towards the high genetic divergence observed, followed by cane weight, cane yield/clump, brix, sucrose content and percentage purity of commercial cane. Table 3 shows that number of tillers significantly different among the populations hence it have a share in genetic divergence among the clones used in this study. It can also be concluded that this character should be used in divergence analysis. Table 1 also reveals insignificance in case of replication, this means that soil conditions and other agronomic practices are enough uniform to have ignorable variation among blocks. Table 5 depicts that contribution of this character is 0.77% and it appears six times in first ranking. It seems according to Punia *et al*. [6] findings as it contributes a share in genetic divergence.

Table 2: ANOVA for variety and error variance and covariance

S.OV	d.f	S.S	M.S	F.
Cultivars	39	12.79098	0.327974	5.63E-12**
Error	741	4.31E+13	5.82E+10	
Total	780	4.31E+13		

Table 3: Mean squares of 11 trairs of bread wheat (*Triticum aestivum* L.)

SoSoV	df	No. of tillers	Cane length	Cane diameter	Leaf area	Cane weight	Juice%	Brix%	Pol%	Purity%	Fiber%	CCS
Genotypes	39	0.89**	702.34**	6.64**	1.40**	99.93**	133.6**	3.54**	0.49**	64.9**	97.7**	0.98**
ReRep.	2	0.017**	27.6^{NS}	0.07^{NS}	0.19**	7.4^{NS}	2.89**	0.01^{NS}	0.01^{NS}	0.68^{NS}	0.23^{NS}	0.06^{NS}
EEError	76	0.18	94.72	2.18	0.25	20.27	1.66	0.12	0.05	2.94	8.17	0.09

** indicates highly significance at p<0.01

^{NS} indicates non significant

Table 4: Clusters including genotypes.

Clusters	Populations
I.	12, 21
II.	14, 27, 38
III.	2, 26
IV.	32, 30
V.	16, 28, 25
VI.	40, 3, 10, 23
VII.	13, 8, 20
VIII.	29, 35
IX.	33, 22, 17
X.	5, 9
XI.	37, 6, 11
XII.	4
XIII.	18, 34, 39
XIV.	24, 1, 36
XV.	7, 31
XVI.	15, 19

Nair *et al*. [7] reported that cane height contributes the maximum towards divergence. This can also be inferred from this significant value that it is useful to include this character in divergence analysis. Table 3 also reveals insignificance in case of replication, this means that soil conditions and other agronomic practices are enough uniform to have ignorable variation among blocks. But results are opposite to Nair *et al*. [7] as shown in Table 5. It shows that there is no contribution of cane length to divergence. This may be because the genotypes used in this study are identical in loci controlling cane length character.

Singh *et al*. [8] assessed that cane girth has the maximum contribution in genetic dissimilarity among then used clones. The result shown in Table 5 supports Singh *et al*. [8] assessment. Cane diameter is the highest contributor to genetic divergence after fibre%. It contributes 23.08% and appears 180 times in first ranking. It is clear from Table 3 that leaf F value is significant both at 5% and 1% uncertainty level, therefore it too have a role in genetic diversity among the clones of sugarcane. So, it is right to use it for divergence study. Table 3 also reveals insignificance in case of replication, this means that soil conditions and other agronomic practices are enough uniform to have ignorable variation among blocks. Table 5 shows that contribution of this character to divergence is 0.38% and it appear three times in first ranking.

Rao *et al* [9] and Nair *et al*. [7] narrated that clump weight significantly adds to genetic diversity among sugarcane clones. This came true in the present research as is clear from Table 5. As it contributes 0.38 to divergence and appears three times in first ranking.

Silva *et al*. [10] pointed that juice and brix contents contributes the most in genetic variation among 129 sugarcane clones. Here Table 3 is in agreement with them. Therefore it is quite fruitful to include this variable while studying genetic diversity. Table 5 reveals that though it is not the highest contributor yet it contributes a major share that is 7.82 to genetic diversity among 40 sugarcane clones used in this research. It appears 61 times in first ranking.

Silva *et al*. [10] reported brix % as one of the variables that contributed the most in genetic divergence among Quban sugarcane clones. Table 5 indicates that using this character for genetic diversity study is favourable. As it is clear from the table that it is third most contributor after fibre and cane diameter. It contributes 13.21% to genetic diversity and appears 103 times in first ranking.

Rao *et al* [9] concluded that pol value was the second most contributor in genetic diversity among 51 sugarcane genotypes. Hence it is useful to this variable for genetic divergence analysis. The results shown in Table 5 are in agreement with their findings but here it is the third highest contributor rather than second one. It contributes equal to Brix value. It contributes 13.21% and appears 103 times in first ranking.

Purity% contributes zero in genetic divergence this may be because genetic similarity among the varieties regarding this character. Punia *et al*. [6] reported that among twelve characters purity percentage was the least contributor in genetic diversity. Here, the result is somewhat close to that as purity percentage does not contribute in genetic divergence.

Table 5: Contribution of each character to divergence

Character	No. of Tillers	Cane length	Cane diameter	Leaf area	Cane weight	Juice%	Brix%	Pol%	Purity%	Fiber%	Commercial cane sugar	Total
Number of timesappearing first in ranking	6	0	180	3	3	61	103	103	0	236	85	780
Percent contribution	0.77	0.00	23.08	0.38	0.38	7.82	13.21	13.21	0.00	30.26	10.90	1000

Table 6: Number of genotypes in clusters and average intra and inter-cluster distances.

		Cluster															
Cluster	No. genotypes	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
I	2	*533*	3724	4387	2176	4302	6034	5136	2538	5139	5182	4415	13553	2709	2964	6754	4435
II	3		*769*	4560	3433	5156	4762	6938	2647	4688	5408	5352	2377	4236	3421	4106	4991
III	2			*580*	2576	3988	4433	5824	2885	6581	4805	12222	4406	4118	12910	4203	3181
IV	2				*610*	3039	6675	8947	1854	6695	2897	4688	2100	6741	1945	4770	6666
V	3					*1624*	4807	8678	3949	3925	5588	2920	6791	4040	4972	3844	3235
VI	4						*1540*	4986	28409	8048	5380	4624	7597	5386	4466	2344	5687
VII	3							*1687*	24667	5427	6913	5711	3744	6457	4900	5997	6140
VIII	2								*629*	5792	2897	3068	12056	7166	4740	2149	6768
IX	3									*1091*	6425	4974	8308	3893	2254	6554	1754
X	2										*707*	3335	2509	4287	7218	4608	7775
XI	3											*904*	5592	4074	3444	4278	3665
XII	1												*0*	7524	4543	5960	1609
XIII	3													*1549*	6139	5738	4233
XIV	3														*919*	4897	5027
XV	2															*1256*	5469
XVI	2																*2087*

Note: figures in italics denote intra-cluster distance

As it is shown in Table 5 that fibre % is the highest contributor to genetic diversity. It contributes 30.26% and it appears 236 times in first ranking. As Table 5 shows that commercial cane sugar adds 10.9% to the genetic divergence. It is clear that it is the third largest sharer of divergence. It means that the loci controlling this character are different in all forty genotypes used. It appears 85times in first ranking. According to Tocher' method [5] the forty genotypes were grouped into sixteen clusters that are discussed as fallows.

Cluster I includes SPF-234 and BL-4. Intra cluster distance is 533; therefore, it is not suggested to cross these cultivars with each other for hybrid sugarcane production. The cluster has maximum distance (13553) with cluster XII that includes only one variety CPF-234. It can be concluded that a potential hybrid may be developed by crossing SPF-234 with CPF-234 and or BL-4 with CPF-234. The closest cluster is cluster IV (2176) having Malakand-16 and CoL-54. It is not wise to cross these two clusters because the varieties in these clusters are not enough genetically diverse to produce a good hybrid. It can also be seen from the cluster that the varieties in cluster I belong to different breeding stations. So, it can be hypothesized that these varieties would have been developed from parents that are not too much divergent to cast that diversity to these varieties.

Cluster II consists of CP-81-1254, NSG-555 and S-95-HS-185. The intra cluster distance is 769 that reveals that these cultivars are uniform enough not to be used as parents to produce a valuable hybrid having desirable results for the characters studied in this research. The most divergent cluster is VII that includes S-96-SP-302, S-2000-US-400 and CP-43-33. It is suggested that one parent may selected from each of these two clusters to produce a hybrid having desirable results for the eleven characters used in this study. The closest cluster is XII (2377). Therefore, the cultivars in these two clusters may not be good hybrid producing parents. Cluster III consists of two populations viz. CPF-237 and NSG-311. Intra-cluster distance is 850 indicating the similarity between these two varieties. The most divergent cluster is XIV (12910) fallowed by cluster no XI (12222). The crosses of cultivars in cluster III with cultivars in clusters XIV and XI may result into a promising hybrid.

The nearest cluster is IV (2576) that denotes the similarity among the cultivars in these two clusters. Cultivars in these clusters may not be good parents to develop a potential hybrid.

Cultivars Malakand-16 and CoL-54 fall in cluster IV. Intra-cluster distance 610 indicates the genetic similarity between these two varieties. The most dissimilar cluster

is VII as inter-cluster distance between IV and VII, 8947, is highest compared to other cluster. The second most divergent cluster is IX having inter-cluster distance 6695. The crosses of cultivars in cluster IV with cultivars in VII may produce valuable results. The most genetically identical cluster is XIV having inter-cluster with cluster IV, 1945.

Cluster V consists of CP-HS-35, S-95-HS-185 and T-10. The intra cluster distance is 1624 that reveals that these cultivars are uniform enough not to be used as parents to produce a valuable hybrid having desirable results for the characters studied in this research. The most divergent cluster is VII that includes S-96-SP-302, S-2000-US-400 and CP-43-33. It is suggested that one parent may selected from each of these two clusters to produce a hybrid having desirable results for the eleven characters used in this study. The closest cluster is XI (2925). Therefore, the cultivars in these two clusters may not be good hybrid producing parents.

Cluster VI consists of four populations viz. IM-61, HSF-242, S-2003-US-624 and ABT super. Intra-cluster distance is 1540 indicating the similarity between these two varieties. The most divergent cluster is VIII (28409) fallowed by cluster no IX (8048). The crosses of cultivars in cluster VI with cultivars in clusters VIII and IX may result into a promising hybrid. The nearest cluster is XV (2344) that denotes the similarity among the cultivars in these two clusters. Cultivars in these clusters may not be good parents to develop a potential hybrid.

Genotypes S-96-SP-302, S-2000-US-400 and CP-43-33 fall in cluster VII. Intra-cluster distance 1687 indicates the genetic similarity between these two varieties. The most dissimilar cluster is VIII as inter-cluster distance between VII and VIII, 24667, is highest compared to other cluster. The second most divergent cluster is X having inter-cluster distance 6913. The crosses of genotypes in cluster VII with genotypes in clusters VIII and X may produce valuable results. The most genetically identical cluster is XII having inter-cluster with cluster VII, 3744.

Cluster VIII includes BF-129 and H-12. Intra cluster distance is 629; therefore, it is not suggested to cross these genotypes with each other for hybrid sugarcane production. The cluster has maximum distance (12056) with cluster XII that includes only one variety CPF-234. It can be concluded that a potential hybrid may be developed by crossing BF-129 and H-12 with CPF-234. The closest cluster is cluster XV (2149). It is not wise to cross these two clusters because the varieties in these clusters are not enough genetically diverse to produce a good hybrid. It can also be seen from the cluster that the varieties in cluster I belong to different breeding stations. So, it can be hypothesized that these varieties would have been developed from parents that are not too much divergent to cast that diversity to these varieties.

Three genotypes CoL-29, BF-162 and CP-72-2086 come under this cluster. The genetic diversity among these three genotypes is 1091 that is not sufficient to cross them. The most distant cluster is XVI with inter-cluster distance 17548 fallowed by XII having inter-cluster distance with cluster IX, 8308. A promising hybrid can be produced by crossing genotypes in cluster IX with genotypes in clusters XVI and XII. The most similar cluster is XIV having inter-cluster distance with cluster IX, 2254. AS the genotypes in these two clusters are genetically more similar, therefore, they are not suggested to be crossed to breed a sugarcane hybrid having desirable characters used in this study.

Cluster X consists of SPF-245 and S-2002-US-104. The genetic similarity regarding number of tillers, cane length (cm), cane weight (g), cane diameter (cm), leaf area (cm^2), juice content (%), brix value (%), pol value (%), purity %, fibre % and CCS % (commercial cane sugar) between these two genotypes is 707. Genetically the most distant cluster is XVI having inter-cluster distance with cluster X, 7775. The genotypes in these clusters may produce a good hybrid when crossed. The closest cluster is XII that has inter-cluster distance with cluster, 2509.

Three genotypes Mardan-92, S-98-SP-108 and SPF-213 fall in this cluster. The genetic diversity among these three genotypes is 904 that is not sufficient to cross them. The most distant cluster is XII with inter-cluster distance 5592.A promising hybrid can be produced by crossing genotypes in cluster XI with genotypes in clusters XII. The most similar cluster is XIV having inter-cluster distance with cluster IX, 3344. AS the genotypes in these two clusters are genetically more similar, therefore, they are not suggested to be crossed to breed a sugarcane hybrid having desirable characters used in this study.

Cluster XII is the smallest cluster consisting of only one genotype. The only genotype in this cluster is CPF-243. As only one genotype is present in this cluster, therefore, the intra-cluster distance is zero. The most divergent cluster is XVI with inter-cluster distance, 16309 and the most similar cluster is XIV with inter- cluster distance, 4543.

Genotypes CP-77-400, Triton and JN-88-1 fall in cluster XIII. Intra-cluster distance 1549 indicates the genetic similarity between these two varieties. The most dissimilar cluster is XIV as inter-cluster distance between XIII and XIV, 6139, is highest compared to other cluster. The crosses of genotypes in cluster XIII with genotypes in XIV may produce valuable results. The most genetically identical cluster is XVI having inter-cluster with cluster XIII, 4233.

Cluster XIV includes HSF-240, SPSG-26 and BL-19. The intra-cluster distance is 919. The most divergent cluster is XVI having inter-cluster distance with cluster XIV, 5027. The crosses between genotypes in cluster XIV with genotypes in cluster XVI may produce satisfactory results.

Cluster XIV includes CoL-1148 and CP-85-1441. The intra-cluster distance is 1256. The most divergent cluster is IX having inter-cluster distance with cluster XV, 6554. The crosses between genotypes in cluster XV with genotypes in cluster IX may produce satisfactory results.

Cluster XVI consists of S-96-SP-646 and COT-84. The genetic similarity regarding number of tillers, cane length (cm), cane weight (g), cane diameter (cm), leaf area (cm^2), juice content (%), brix value (%), pol value (%), purity %, fibre % and CCS % (commercial cane sugar) between these two genotypes is 2087. Genetically the most distant cluster is IX having inter-cluster distance with cluster XVI, 17548. The genotypes in these clusters may produce a good hybrid when crossed. The closest cluster is III that has inter-cluster distance with cluster, 3181.

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