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# Genetic diversity of *Saccharum officinarum* accessions in Pakistan as revealed by random amplified polymorphic DNA

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**ABSTRACT.** Genetic diversity of 20 sugarcane accessions in Pakistan was studied using 21 random amplified polymorphic DNA markers. The mean genetic distance between the cultivars was 39.03%, demonstrating that a large part of the genome is similar among the accessions. This probably arises from a lack of parental diversity, with few clones, which are themselves related, contributing to the parentage of these varieties. Among the varieties, none was found to be totally distinct and divergent from the others. We conclude that the current Pakistan commercial varieties have a limited genetic base and that there is a need to diversify commercial sugarcane lines in Pakistan by introducing new germplasm sources.

**Key words:** Sugarcane; Random amplified polymorphic DNA; Genetic diversity; Polymerase chain reaction

 

## INTRODUCTION

Commercial sugarcane varieties are interspecific hybrids and are derived mostly from crosses involving *Saccharum officinarum* L. (the "noble cane") and *S. spontaneum*. The number of parental clones involved in these crosses is limited (Price, 1965; Arceneaux, 1967). Consequently, the genetic base of the modern varieties appears to be very narrow, and this is reflected in the slow progress in sugarcane breeding at present. In this context, information on the phylogeny and genetic diversity of available germplasm is essential for the identification of potential germplasm groups and for optimizing hybridization and selection procedures.

Genetic diversity had been studied in many crops using molecular markers such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), and microsatellites (Wang et al., 1992; Stiles et al., 1993; Orozco-Castillo et al., 1994; Hokanson et al., 1998; Nakajima et al., 1998; Aggarwal et al., 1999; Angiolillo et al., 1999; Barker et al., 1999). Polymerase chain reaction (PCR)-based methods such as RAPD are increasingly being used in the analysis of genetic diversity in crop plants, because of the relative ease with which PCR assays can be carried out compared to RFLPs. Besides, prior knowledge about the genome is also not a prerequisite, which makes RAPD the method of choice for such studies. These studies have been useful in resolving the phylogenetic relationships in the *Saccharum* complex and in assessing the genetic diversity among *Saccharum* species and hybrids. This paper reports the results of a study on the genetic diversity among 20 currently cultivated sugarcane varieties as revealed by RAPD.

## MATERIAL AND METHODS

### Plant material

This genetic diversity study involved 20 sugarcane genotypes (Table 1) that were collected from the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, and Agricultural Biotechnology Research Institute, AARI, Faisalabad. RAPD analysis was performed at the Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad.

**Table 1.** Different sugarcane accessions used for the study of genetic diversity.

| SR. # | Sugarcane genotype | SR. # | Sugarcane genotype |
|---|---|---|---|
| 1 | SPF-213 | 11 | COJ-64 |
| 2 | CPF-237 | 12 | HSF-242 |
| 3 | CPF-243 | 13 | HSF-240 |
| 4 | SPF-245 | 14 | SPF-234 |
| 5 | S-97-US-297 | 15 | CP-77-400 |
| 6 | CP-72-2086 | 16 | BF-162 |
| 7 | SPSG-26 | 17 | BF-129 |
| 8 | LCP-81-10 | 18 | COJ-54 |
| 9 | SPF-232 | 19 | CPF-235 |
| 10 | Triton | 20 | COJ-84 |

SR. # = serial number.

## Morphological studies

Genotypes were also planted in rows to study different morphological traits, namely plant height, number of tillers/plant, number of leaves, cane diameter, leaf area, internodal distance, cane weight, dry matter content, juice content, sucrose content, Brix value, and pol value. The mean values of all the traits were regressed against mean genetic distance shown by genotypes. The traits showing a significant relationship with genetic distance are shown in Figure 2 (see Results).

## DNA extraction

DNA from leaf cylinders of these accessions was extracted by the modified CTAB method (Doyle and Doyle, 1990). DNA concentration was determined spectrophotometrically, and the quality of DNA was determined by 0.8% agarose gel electrophoresis. Some samples were brown colored, apparently due to phenolic compounds, which inhibit the RAPD reaction, and thus, these were discarded.

## RAPD amplification and agarose gel electrophoresis

The amplification conditions were as described by Williams et al. (1990). Amplifications were carried out in a 25-µL reaction mixture containing 12 ng template DNA using 1 U Taq DNA polymerase. Twenty-one random primers obtained from Gene Link (USA) were used for amplification. PCR consisted of 40 cycles of 94°C for 1 min, 36°C for 1 min, and 72°C for 2 min, with a final extension step of 72°C for 10 min.

Amplification products were mixed with 3 µL 6X tracking dye and spun briefly in a centrifuge before loading. PCR product, 10 µL, was loaded in each lane of a 1.2% agarose gel made with 0.5X TBE buffer containing 0.5 µg/mL ethidium bromide. Samples were electrophoresed at 80 V for 2 h, and gels were observed under a UV transilluminator and photographed. Clearly resolved bands were scored as present (1) or absent (0).

In order to access the overall distribution of genetic diversity, genetic distance was calculated by the POP gene software, version 1.31, for RAPD datasets (Nei, 1978). In addition, population relationships were inferred using the unweighted pair group with arithmetic means (UPGMA) clustering method based on Nei's (1978) unbiased genetic distance with POP gene, version 1.31. Relationships between accessions were displayed graphically in the form of a dendrogram.

## RESULTS

Approximately 86.8% polymorphisms were shown by 188 of 210 polymorphic fragments resolved by the use of 21 primers among the 20 sugarcane accessions. The rest of the 22 bands were monomorphic in all 20 accessions. Each primer, thus, produced on average 10 bands ranging from 5 to 24, although none of the primers individually was so informative as to differentiate all the accessions. Therefore, it may be concluded from the present results that RAPD markers can be used for identification of sugarcane accessions.

The genetic distance of RAPD data for the 20 sugarcane accessions was constructed

according to Nei (1978), and relationships between accessions were displayed graphically in the form of a dendrogram in Figure 1. The pairwise genetic distances between the varieties ranged from 4.88 to 16.53%. The lowest genetic distance of 4.88% was seen in genotypes SPF-213 and COJ-64. The genotypes CPF-237 with HSF-242 and CP-72-2086 with BF-162 shared the highest value of genetic distance, i.e., 16.53%. The second highest value of genetic distance, 14.315%, was also shared by four genotypes, SPSG-26 with BF-129 and Triton with COJ-84. The rest of the genotypes showed a common genetic distance of 11.6%.

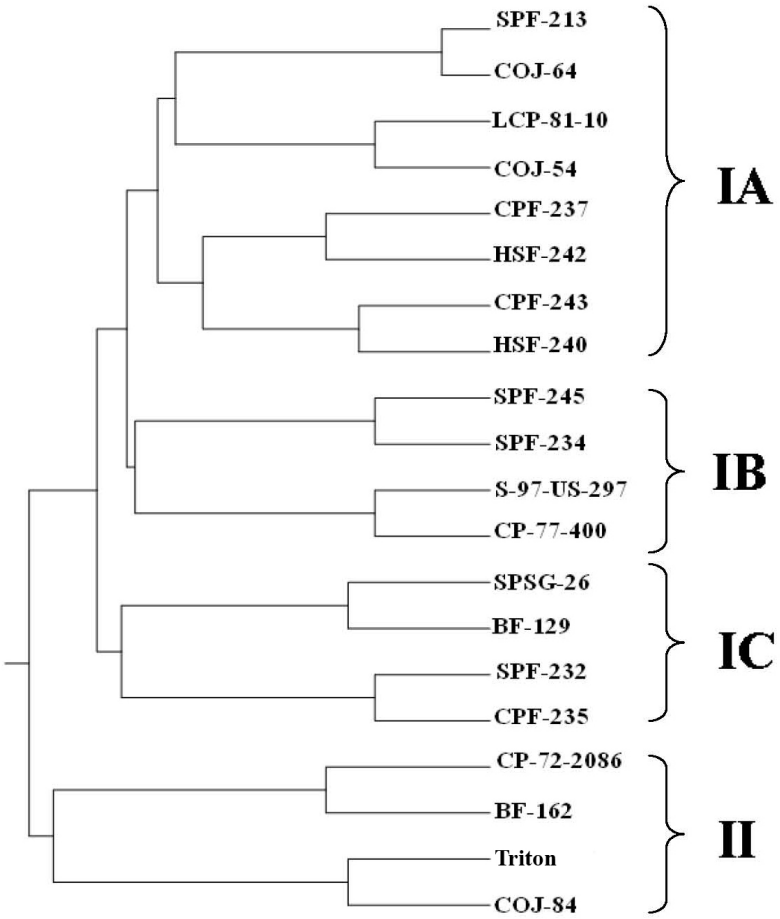


**Figure 1.** Dendrogram of 20 sugarcane accessions constructed from random amplified polymorphic DNA (RAPD) data using unweighted pair group method with arithmetic means (UPGMA) based on Nei's (1978) genetic distance.

The UPGMA clustering method based on Nei's (1978) unbiased genetic distance with POP gene, version 1.31, classified all the sugarcane accessions into two major groups (I and II) comprising four clusters (Figure 1). The first major group consisted of 16 geno-

types of the 20 studied, and was further divided into three subgroups, IA, IB, and IC. Group IA comprised 6 genotypes, namely SPF-213, COJ-64, LCP-81-10, COJ-54, CPF-237, HSF-242, CPF-243, and HSF-240.

Group IB included the genotypes SPF-245, SPF-234, S-97-US-297, and CP-77-400, while the genotypes SPSG-26, BF-129, SPF-232, and CPF-235 constituted the third group IC. The major group II was the most distinct one as only 4 of the 20 genotypes studied are included in it.

Traits showing significant relationship with genetic distance are shown in Figure 2. Mean genetic distance showed highest relationship with number of tillers/plant. Similarly, this relationship was also found significant with the traits sucrose content and cane diameter. However, other morphological traits did not show any relationship with mean genetic distance.

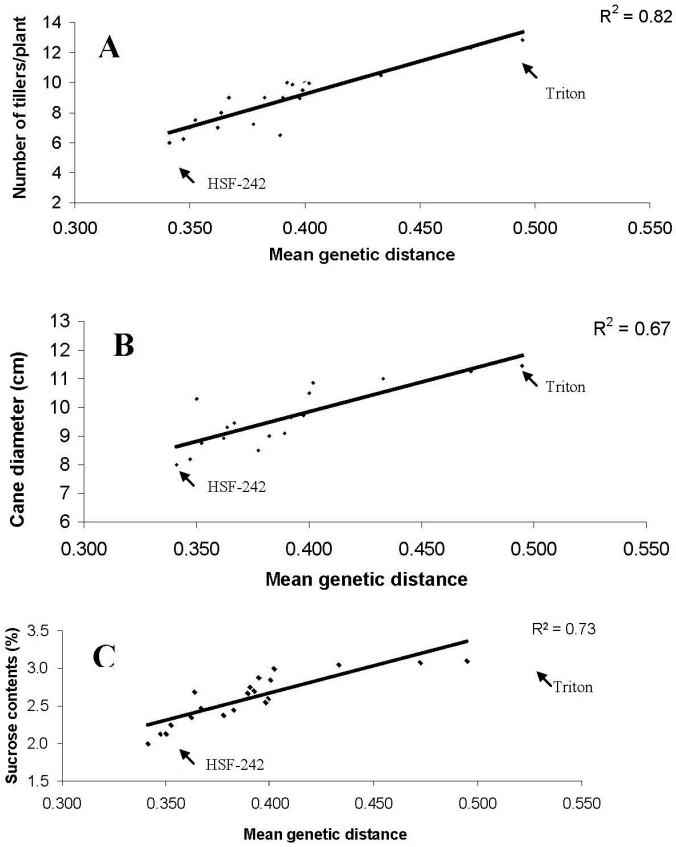


**Figure 2.** Relationship of genetic distance with different morphological traits. **A.** Number of tillers/plant. **B.** Cane diameter. **C.** Sucrose content.

Genotype Triton showed the highest mean genetic distance, tillers/plant, cane diameter, and sucrose content, whereas the genotype HSF-242 was the opposite.

## DISCUSSION

Using RAPD as genetic markers, 87.59% polymorphic bands were detected in 20 accessions of sugarcane. Nair et al. (2002) investigated genetic diversity in prominent Indian sugarcane varieties with 63.74% polymorphism. Burner et al. (1997) compared the genetic diversity of North American and Old World members of *Saccharum* with 31.44% polymorphism across the taxa. Polymorphism revealed by RAPD could be a result of nucleotide changes at the primer annealing site, or due to addition or deletion between two priming sites, which results in different lengths of the amplification products (Williams et al., 1990).

Genetic diversity is commonly measured by genetic distance or genetic similarity, both of which imply that there are either differences or similarities at the genetic level (Weir, 1990). The genetic distance of 20 accessions ranging from 0.048 to 0.59 with an average of 0.39 suggested that the level of genetic diversity among the sugarcane accessions is low. In several other studies, elite sugarcane (*Saccharum* hybrids) germplasm showed genetic diversity as well (Arceneaux, 1967; Harvey et al., 1994; Harvey and Botha, 1996). Harvey and Botha (1996) reported similarities as high as 77-95% among 20 elite varieties. This low value of genetic distance is an indication that a large part of the genome of the accessions under study is identical. This is primarily due to the lack of parental diversity, because all the accessions may share somewhat similar parents in the pedigree. Furthermore, a closer view of Figure 1 revealed that 16 of 20 accessions under examination were grouped together in the major group I comprising three clusters. However, 4 genotypes of group II were the most distinguishable ones, as this group had the highest genetic distance.

Thus, mean genetic distance among the 20 accessions in this study was 39.03%, implying that genetic diversity among the genotypes is limited. From the above mentioned results one can easily foresee that genotypes that have high values of genetic distance and fall into different clusters can be crossed to obtain maximum variability of good combinations of characters. Similarly, a specific character that is desirable or otherwise weak in a genotype can be improved by selection in segregating generation resulting from these crosses.

Mean genetic distance showed significant relationship with traits such as tillers/plant and sucrose content. The cultivars at extreme ends in all figures may be utilized in cross-combinations to produce a highly diverse $F_2$ generation. Future studies may be carried out with these segregation generations to find molecular markers for these traits.

Thus, conscious efforts need to be made to diversify the parental genetic base to ensure high genetic variability among the cultivated varieties. New sources from the interspecific/intergeneric hybrid gene pool need to be used along with proven parents to generate the variability that will be both commercially viable and genetically diverse.

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