Characterization of twenty-six genotypes of sugarcane using SSR markers

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ABSTRACT

Twenty-six genotypes of sugarcane, including elite lines, commercial cultivars of *Saccharum officinarum* L. and clones of *S. barberi* fingerprinted with 53 SSR markers using a PCR-based marker assay. Nei's genetic distances for SSR, data were determined and relationships between accessions portrayed graphically in the form of a dendrogram. Genetic distance values ranging from 0.69 to 0.98 observed among the 26 sugarcane accessions. The shortest genetic distance of 0.69 seen between genotypes NSG-555 and CSSG-668, these two genotypes was very close with each other. The most dissimilar of the accessions were HSF-240 and S-2002-US-133, with a genetic distance of 0.98. SSR fingerprints can help sugarcane breeders to clarify the genetic pedigree of commercial sugarcane varieties and evaluate the efficiency of breeding methods.

Key words: SSR marker; Genetic distance; Fingerprinting; Saccharum officinarum L.

1 INTRODUCTION

Saccharum is a complex genus consist of at least six distinct species, *S. oficinarum*, S. *barberi*, *S. sinensi*, *S. spontaneous*, *S. robustum*, and *S. ed*ule (Daniels and Roach, 1987; Naidu and Sreenivasan, 1987; Grain, 2004) and categorized by high ploidy levels. Being an allopolyploid, modern cultivated sugarcane has approximately 80-140 chromosomes with 8-18 copies of a basic set (i.e., X = 8 or x = 10 haploid chromosome number) (D'Hont *et al.,* 1995; Ha *et al.,* 1999; Ming *et al.,* 2001). *S. oficinarum* consults the genes for high sucrose content, low fiber, thick stalks, sparse pubescence, rare flowering, and limited tillering (Ming *et al.,* 2001) and thought to include a large part of the cultivated sugarcane genome. The wild relative, S. spontaneum, credited with informing the needed pest and disease resistance and abiotic stress tolerance due to its wide eco-geographical adaptive circulation (Sreenivasan *et al.,* 1987) and comprises about 10% of the cultivated sugarcane as showed from in situ hybridization (D'Hont *et al.,*1996).

Plant breeders always saw characterization of germplasm genetic diversity as the most accurate tool in improving the genetic make-up of a cultivated crop species. The first wave of molecular marker applications in plant development saw big work concentrated on dissecting and characterizing genetic diversity using random molecular markers. Among the range of DNA-based molecular marker techniques, a promising polymerase chain reaction (PCR) -based technique used lengthily for genetic mapping (McIntyre *et al.,* 2001) as well as fingerprinting of sugarcane clones (Piperidis *et al.,* 2000; Pan *et al.,* 2002). Microsatellites have developed the

marker system of choice due to their high reproducibility, abundant in the genome, hyper variability, and co-dominance. Due to their hyper variability and efficiency in polymorphism detection, SSR markers have become ideal for genetic map construction (Devey *et al.,* 1996; Paglia *et al.,* 1998), identification of clones (Dayanandan *et al.,* 1998), identification of species and hybrids, determination of paternity (van de Ven and McNicol, 1996), and marker-assisted selection (Weising *et al.,* 1997).

The present research reports the results of a study on the genetic diversity among 26 genotypes including currently cultivated sugar cane (*S. oficinarum*) varieties. The objective of the present study was to regulate whether polymorphism is satisfactory or not to distinguish sugarcane genotypes and to assess the patterns of genetic diversity among a selected group of *Saccharum oficinarum* L. In order to provide more information to assist breeding programs and to surpass the output levels presented today.

2 MATERIAL AND METHODS

2.1 PLANT GENOTYPES AND DNA EXTRACTION

For genetic diversity studies 26 sugarcane genotypes including elite lines, commercial cultivars were selected from sugarcane growing centers of Pakistan. Parameters like yield potential, maturity trend, ratoonability, drought tolerance, salt tolerance and disease resistance were used as selection criteria for the above-mentioned genotypes. DNA was extracted from shoot apical meristems. Genomic DNA extracted by the ABBAS DNA extraction method (Abbas *et al.,* 2012).

2.2 PCR AMPLIFICATION

PCR conditions optimized in a Gene Amp 2700 thermal cycler (Applied Biosystems, Foster City, CA, USA). PCR for Simple Sequence Repeat (SSR) analysis performed in a 20-µL reaction volume containing 5.8 µl d3H2O, 2.0 µl 10X PCR buffer, 3.0 µl MgCl2, 3.0µL of each dNTP (Fermentas Inc., Hanover, MD, USA), 3 µl of both forward and reverse primers, 0.2 µl Taq DNA polymerase (Fermentas) and 3.0 µl template DNA in each reaction mixture. The reactions subjected to the following profile. Initial denaturation at 94°C for 5 min, followed by 35 cycles, each of which consisted of 45 sec denaturation at 94°C, 45 sec annealing at 55°C and 1 min extension at 72°C, with a final extension at 72°C for 7 mins. Primers that did not show any amplification at 55°C submitted to annealing temperatures from 48-65°C. Amplification products were mixed with 3µl 6X loading dye (0.25% bromophenol blue, 0.25% xylene cyanole, and 40% sucrose). PCR products (7 µl) were loaded in each well of 2.0% agarose gel made with 1.0X TBE buffer and 0.5 µg/ml ethidiumbromide, and electrophoresed at 90 W until bands fully opened. The gels photographed under UV in gel documentation system.

All segregating bands that were well resolved and unambiguous scored for the presence (1) or absence (0) in the 26 genotypes.

2.3 DATA ANALYSIS

The data on bands generated by 70 primers selected for analysis of genetic diversity. From these 70 primers, 53 showed results and 17 primers did not show any result. The bands counted by starting from the top and ending with the bottom of the lanes. All segregating bands that were well resolved and unambiguous scored for the presence (1) or absence (0) in the 26 genotypes. The data of the primers were used to estimate the dissimilarity based on number of unshared amplified products and a dissimilarity matrix was generated using Nei's similarity indices (Nei, 1972). In addition, population relationships inferred using the un weighted pair group of arithmetic means (UPGMA) clustering method using the NTsys software.

3 RESULTS

3.1 SSR POLYMORPHISM

The polymorphism rate estimated to be 88.70%.110 out of 124 loci were polymorphic with 53 primers used among the 26 sugarcane accessions. The remaining 14 bands from the 26 accessions were monomorphic. In the present study, the 26 sugarcane accessions appeared to show difference/variability with the 53 primers used. Some SSR primers showed polymorphism on silver nitrate staining shown in (Fig-1, 2) and monomorphic results on agrose gel in (Fig-3). Therefore, it concluded from the present results that SSRs could be use for identification of genetic diversity and the relationship between the members of the complex genome of sugar cane.

3.2 GENETIC DISTANCE BETWEEN THE ACCESSIONS

The genetic distance from SSR data using 26 sugarcane accessions constructed based on Nei (1972), and relationships between accessions portrayed graphically in the form of a dendrogram in Figure 4. Genetic distances ranging from 0.69 to 0.98 observed among the 26 sugarcane accessions. The lowest genetic distance of 0.69 has seen between genotypes NSG-555 and CSSG-668. These two genotypes showed high similarities and the most dissimilar of all the accessions were HSF-240 and S-2002-US-133 with a genetic distance of 0.98.

3.3 CLUSTERING PATTERNS

The cluster analysis, based on dissimilarity values, classified all the sugarcane accessions into five major clusters (Figure-5). Cluster I and cluster II was not further sub grouped. Cluster I contains three genotypes HSF-240, S-2003-US778 and CPHS-35 while cluster II contains CPF-247, S-2002-US-160 and SPF-234. Cluster III further sub grouped into sub clusters III-A and III-B. III-A contains CPF-198, Lho 83-153 and III-B consists of S-2003-US-114, SPF-238, CO-1148, Rb-72 and S-2003-US-694. The major cluster was cluster IV and further divided into three sub clusters. IV-A contains only one genotype, which has CPF-213 and IV-B, consists of CP-77-400, CP-43-33, CPF-246, and IV-C contains HSF-242, NSG-60and NSG-45. Cluster V has two sub clusters, which were V-A containing three genotypes, namely CPF-237, NSG-555, CSSG-668 and V-B consist of three genotypes, namely S-2003-US-633, S-2003-US-718 and S-2002-US-133.

	Clusters	Sub clusters		
Cluster I	HSF-240, S-2003-US778, CPHS-35	I A	3	HSF-240, S-2003-US778, CPHS-35
Cluster II	CPF-247, S-2002-US-160, SPF-234	II A	3	CPF-247, S-2002-US-160, SPF-234
Cluster III	CPF-198, Lho 83-153, S-2003-US-114,	III A	2	CPF-198, Lho 83-153
	SPF-238, CO-1148, Rb-72, S-2003-US-	III B	5	S-2003-US-114, SPF-238, CO-1148,
Cluster IV	SPF-213, CP-77-400, CP-43-33, CPF-	IV A	1	SPF-213
	246, HSF-242, NSG-60, NSG-45	IV B	3	CP-77-400, CP-43-33, CPF-246
		IV C	3	HSF-242, NSG-60, NSG-45
Cluster V	CPF-237, NSG-555, CSSG-668, S-2003-	V A	3	CPF-237, NSG-555, CSSG-668
	US-633, S-2003-US-718, S-2002-US-133	V B	3	S-2003-US-633, S-2003-US-718, S-

4 DISCUSSION

As genetic markers, SSRs are usually measured co-dominant markers although several issues regarding their use have documented, Including the tendency for Taq polymerase to add an adenosine nucleotide to the 3′ end of products. The incapability of the marker to distinguish between homology of fragments that run at the same band size, and mutations in the binding region of microsatellite primers ensuing in the loss of the PCR product (null-alleles) (Hu, 1993; Callen et al., 1993). These matters are compounded in the highly polyploid sugarcane genome, predominantly where the difficulty in distinguishing alleles from homologous chromosomes makes it difficult to determine heterozygosity or homozygosity at any particular locus. Hence, for the determinations of this study, SSRs have been deliberated dominant markers. It is however, the goal of this discussion to investigate the utility of SSRs for the identification of genetic diversity and the interactions between members of the complex. Hence, an attitude that looks at genetic distances between individuals, rather than any attempt at elucidating the evolutionary history, was accepted.

The genetic distance of 26 accessions ranging from 0.69 to 0.98 with an average of 0.84 proposed that, the level of genetic diversity among the sugarcane genotypes is high. In several other studies, elite sugar cane (Saccharum hybrids) germplasm showed genetic diversity as well (Selvi *et al.,* 2003; Cordeiro *et al.,* 2003). Selvi *et al.,* (2003) exposed a broad range (0.324- 0.8335) of pair wise similarity values when tested on 30 or 40 commercial sugarcane cultivars.

The complex banding patterns met in sugarcane is due to its high level of polyploidy and heterozygosity as compared to other genera. Reports from the International Sugar Cane Microsatellite Consortium demonstrate the amplification of several fragments per clone with a theoretical maximum of 12 fragments.

Furthermore, isozyme analysis has exposed complex banding patterns in relation to their high ploidy level (Glaszmann et al., 1989). In spite of the high polyploidy and heterozygosity of the Saccharum genome, few primers amplified a single separate band across the members of the Saccharum complex, suggesting that these allelic regions or primer binding sites are highly conserved and no SSR expansion or reduction has taken place during the evolution of Saccharum oficinarum and Saccharum barberi. Another reason for fewer bands having shaped is that the primers range in size from 300-420 bp.

A high degree of similarity between *S. oficinarum* and *S. barberi* has exposed in the present study has been recognized by another marker system (Glaszmann *et al.,* 1989; Nair *et al.,* 1999). The proximity between the two species is predictable, since *S. barberi* is considered to be the progenitor species of *S. oficinarum.*

The observation made in this study has maintained by the results of RFLP and RAPD profiles where S. barberi and *S. sinense* portion the nuclear DNA pattern of *S. oficinarum* and *S. spontaneum* (Lu *et al.,* 1994; Nair *et al.,* 1999). *S. barberi* and *S. sinense* thought to be of secondary origin resulting through hybridization between *S. oficinarum* and *S. spontaneum* (Daniels and Roach, 1987).

In the present study, two genotypes showed very close similarities; it means these have the same ancestors. These were NSG-555 and CSSG-668. Cluster I and Cluster V having a long genetic distance with each other. Thus, it concluded that, estimations of genetic diversity based on molecular markers might be deliver information that is more accurate to plant breeders than the pedigree method. It could assist breeders in making reliable crossings on a short-term basis or strategically plan as compared to long-term breeding programme. Genetic analysis has delayed in sugarcane due to lack of satisfactorily informative markers. Less information exists about the genetic diversity within and among *Saccharum* cultivars, which have based mainly on the morphological characteristics. Thus, it concluded that, estimation of genetic similarity based on molecular markers might be delivering, more accurate information for plant breeder.

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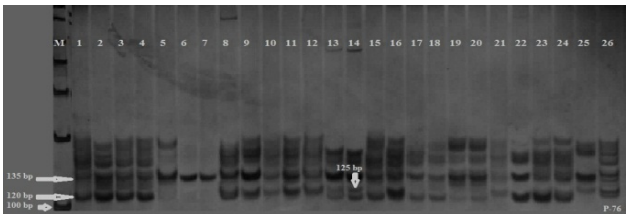

Fig.1. Silver nitrate staining polymorphism (P-76)

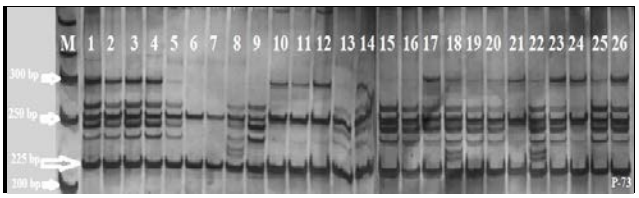

Fig. 2. Silver nitrate staining polymorphism (P-73)

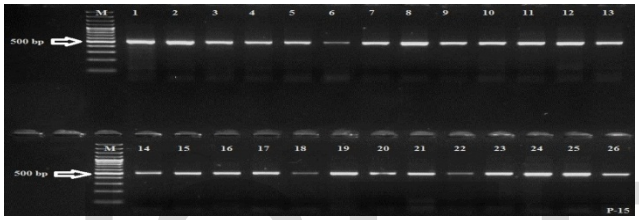

Fig. 3. Agrose gel pic (P-15)

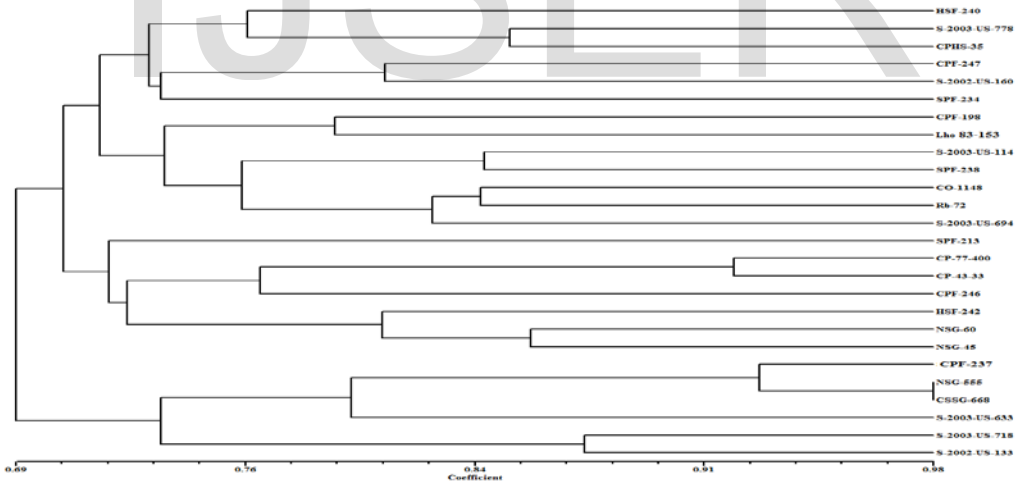

Fig. 4. Dendrogram of 26 sugarcane accessions developed from simple sequence repeat data using the un weighted pair group of arithmetic means (UPGMA) based on Nei's (1972) genetic distance.