Assessment of Genetic Diversity Among *Saccharum Spontaneum* Genotypes using SSR Markers

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Abstract

Sugarcane is a genetically-complex crop of major economic importance in Sri Lanka. It is mainly used for sugar production. Considering the current needs of cane sugar industry it is imperative to breed high sugar producing varieties with other desirable agronomic traits. Genome of modern sugarcane varieties is a complex that derived from introgression of genomes of several *Saccharum* species particularly *Saccharum officiarum* and *Saccharum spontaneum* through nobilization (inter-specific or inter-generic hybridization). The involvement of *Saccharum spontaneum* in sugarcane crop improvement turned out almost all modern commercial sugarcane varieties with high sugar and resistance to most of biotic and abiotic stresses. *Saccharum spontaneum* is the most variable and the diverse among *Saccharum* species with varying somatic chromosome number from 2n = 40 to 128. It is characterized by low sugar content, high biomass production, thin stalks, high fiber, high ratooning ability and high resistance to biotic and abiotic stresses. Characterization of sugarcane germplasm provides essential information in genetic diversity which are vital in crop improvement.

The objective of this study was to identify genetically-diverse *Saccharum spontaneum* accessions to expand the breeding population of sugarcane through characterization of *Saccharum spontaneum* accessions using molecular markers.

Twenty-four *Saccharum spontaneum* clones used in this study included16 imported and 8 locally-collected accessions. Young leaf samples were crushed with liquid nitrogen and DNA was extracted using modified CTAB method. The quality of extracted DNA was assessed on 1% agarose gel. SSR analysis was carried out in a total of 15µl volume containing 2µl template DNA, 1.5µl of each forward and reverse primer, 0.5µl of 10mM dNTPs, 0.1µl of taq DNA polymerase, 3µl of 5 x buffers, 1.5µl of 25mM $MgCl_2$ and 4.9µl distilled water. A total of 25 SSR primers were used to determine the diversity among the genotypes. Amplified products were separated in 6%

polyacrylamide gel. Visualization of bands was done after silver staining. Bands were scored for the presence (1) or absence (0) in all 24 genotypes and the distance matrix was used to construct phylogenetic tree by using Un-weighted Pair-Group Arithmetic Mean (UPGMA) using the software NTSYS pc 2.2.

Out of 25 SSR primers, 9 primers produced significant output from which a total of 53 loci were generated for 24 genotypes. The dendrogram constructed for the accessions using these 9 SSR primers is shown in Figure 1.

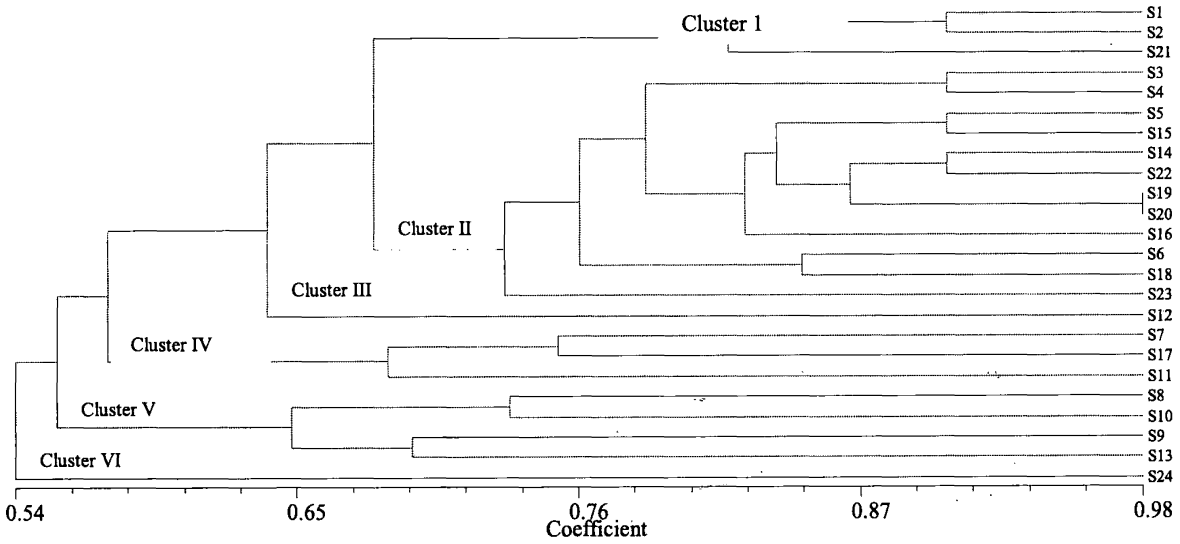

Figure 1: The dendrogram constructed for 24 *Saccharum spontaneum* genotypes using 9 SSR primers

The dendrogram shows the existence of six major divergent groups differentiated in between 55.5% - 98% similarity levels. Analysis of SSR data using similarity coefficient measures genetic identity and genetic distance between accessions. In this study, genetic similarity values are in the range of 0.39 to 0.98. The maximum genetic similarity 98% falls between the accessions S20 (NG 77 159) and S19 (SES 07). The minimum genetic similarity (39.6%) is observed between the accessions S14 (IS 76 215) and S9 (ISD 20) that are in clusters 2 and 5, respectively.

The results revealed that there are six divergent groups among 24 *Saccharum spontaneum* accessions tested and therefore some selected accessions from each group could be used for inter-specific crossing programs expecting more variability in the resulting progenies.

Key words: Genetic diversity; *Saccharum spontaneum*; Similarity coefficient; SSR primers