ASSESSMENT OF GENETIC DIVERSITY IN THE SACCHARUM GERMPLASM OF SRI LANKA

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ABSTRACT

Two hundred accessions of Saccharum germplasm repository of Sri Lanka were assessed for genetic diversity using 23 SSR primers. The accessions comprised typical Saccharum officinarum, Sacchurum spp. hybrids, Saccharum spontaneum and Erianthus arundinaceous. Similarity matrix and dendrogram were constructed using the NTSYS pc 2.2. The values of polymorphic information content (PIC) were calculated for each primer to evaluate their efficiency. Based on genetic distances, analysis of molecular variance (AMOVA), principal coordinate analysis, Nei's gene diversity, Shannon's information index, number of polymorphic loci and their percentages for different types of accessions were performed to assess genetic relationship using GenAlEx-6, -6.5 and Popgene-1.31. A total of 439 SSR alleles were scored for tested primers. At the genetic similarity coefficient of 79%, the accessions were classified into four major clusters. The genetic similarity among accessions ranged from 0.957 to 0.665. The average PIC value of primers was 0.926. AMOVA confirmed that the variation of the accessions within population was higher (84%) than between populations (16%). Based on the results of principal coordinate analysis, the accessions clustered into 3 major groups: i). hybrids imported from Pakistan, ii). Erianthus arundinaceous with all other hybrids and iii). Saccharum officinarum with Saccharum spontaneum. Almost similar clustering pattern of accessions was obtained from principal coordinate analysis and dendrogram based on similarity indices. The highest Nei's gene diversity and Shannon's information index values confirmed that high genetic diversity observed within imported sugarcane hybrids. The information derived from this study is important in devising mating systems for maximizing efficiency of sugarcane breeding in Sri Lanka.

Keywords: Analysis of molecular variance, Genetic diversity, Simple sequence repeats, Sugarcane

INTRODUCTION

Sugarcane is native to South-East Asia and cultivated in tropical and sub-tropical regions in the world (Santos et al., 2012). It is cultivated in more than 90 countries around the world including Sri Lanka.

Modern sugarcane cultivars have complex polyploidy genomes resulted and carried from introgression through the crosses among several species of the genus, Saccharum (Besse et al., 1998), process during nobilization. Cross breeding is the most effective way for the development of new sugarcane varieties (Jackson, 2005). However, the narrow genetic base of modern sugarcane cultivars restricts the improvement in cane yield and sugar content (Deng et al., 2006; Dal-Bianco et al., 2012; Wu et al., 2019). Therefore, better understanding of genetic diversity of the accessions in the Saccharum germplasm is vital in sugarcane crop improvement. In this scenario, characterization of germplasm and conducting studies on the assessment of genetic diversity in the germplasm are imperative in efficient utilization of Saccharum germplasm. Morphological characterization is the important first step towards the assessment of sugarcane

diversity (Prakash and He, 1996). However, morphological markers are influenced by the environment and, are not abundant. PCR-based DNA markers are the cheapest and the most efficient method to reveal the genetic diversity and phylogenetic relationship at the molecular level (Ramu et al., 2013). Simple Sequence Repeat (SSR) markers provide a better technology for genotyping in sugarcane (Chandra et al., 2014) as they are abundant, co-dominant, reliable and multi-allelic.

Imported and locally-collected 1,660 sugarcane and its related accessions belong to Saccharum officinarum, Saccharum spp. hybrids, Saccharum spontaneum, Erianthus arundinaceous and Miscanthus spp., are preserved and maintained in the Saccharum germplasm repository in Sri Lanka. Previous studies conducted by Pakistan scientist on diversity of locally-bred sugarcane varieties have shown that genetic diversity of locally-bred sugarcane varieties is narrow and all the accessions are grouped in one cluster (unpublished data). The broad genetic variation existing in a germplasm collection is a resource for sugarcane breeders as it provides more opportunities in developing genetically diverse new varieties. Hence, studying the genetic diversity of the sugarcane accessions in the germplasm has become a prime requirement and therefore, this study was planned and carried out to investigate the genetic diversity of accessions in the Sri Lanka Saccharum germplasm collection using SSR markers.

Material and Methodology

Accessions used in the genetic diversity analysis

Two-hundred out of 1,660 accessions from the Saccharum germplasm collection in Sri Lanka, which can be used as parental clones in sugarcane crop improvement were selected for genetic diversity analysis. These accessions were selected representing five different populations viz, 1) S. officinarum (32), 2) S. spontaneum (10), 3) E. arundinaceous of local and exotic origins (10), 4) Imported Saccharum spp. hybrids (98) and 5) Locally-bred Saccharum spp. hybrids (50). Samples were selected randomly to represent all the sub populations. Accessions selected for this study are depicted in Table 1.

Table-1 Type and name of accessions and the places of introduction to Sri Lanka or origin of the accessions used in the assessment of genetic diversity

Type of accessions	Accession name	Place of introduction /origin
(1) *Saccharum* spp. hybrid – Imported	Ajawa, LF 5104, LF 51753, LF 6376, LF 7126, LF 765285	Fiji
	Akoki 22, BF 166	Unknown
	Co 1001, Co 1148, Co 245, Co 321, Co 527, Co 540, Co 622, Co 658, Co 740, Co 775, Co 789, Co 853, Co 896, Co 975, Co 977	Coimbatore, India
	COJ 84	Coimbatore-Jullundur, India
	CP 691052, CP 701548, CP 70360, CP 70414, CP 77400	Canal point, USA
	CPSG 1607, CPSG 2402, CPSG 2423, CPSG 25, CPSG 26, CPSG 2713, CPSG 2875, CPSG 3453, CPSG 3481, CPSG 437	Canal point-Shakaganj, Pakistan
	F 146, F 167, F 7213, ROC 09	Taiwan
	H 442772, H 593775, H 700144	Hawaii
	HoSG 1257, HoSG 315, HoSG 529, HoSG 795	USA-Shakaganj, Pakistan

	Accession name	Place of introduction /origin
	HSF 240, HSF 242, SPF 213, SPF 234, SPF 237	Pakistan
	M 115666, M 118686, M 12459, M 1358, M 139486, M 155770, M 33758	Mauritius
	N 11	South Africa
	NCO 310, NCO 339	Natal-Coimbatore
	Ni 09	Japan
	NSG 555, NSG 59	Natal-Shakaganj, Pakistan
	PH 5333, PH 831528	Philippine
	POJ 64, PS 36	Indonesia
	Q 101, Q 63, Q 68, Q 70, Q 73, Q 83, Ragnar	Australia
	QSG 1741	Queensland-Shakaganj, Pakistan
	RB 70141, RB 70151, RB 70194, RB 705051, SP 853877	Brazil
	S-2002-US312, S-2002-US628, S-2003-US247, S-2003-US618, S-2003-US623, S-2003-US633, S-2003-US694, S-2003-US718, S-2003-US778, S-2005-US54	United States – SRI- Pakistan
	UCW 5463	Cuba
(2) *Saccharum* spp. hybrid – Locally-bred	SL 020061, SL 6301, SLC 0901, SL 7103, SL 7130, SL 7177, SL 7771, SL 8109, SL 8301, SL 8306, SL 8506, SL 8518, SL 8613, SL 8615, SL 8619, SL 87219, SL 8735, SL 87360, SL 8756, SL 88116, SL 89111, SL 891675, SL 892227, SL 905599, SL 905695, SL 906237, SL 924918, SL 924997, SL 925588, SL 93305, SL 933370, SL 943325, SL 943360, SL 954033, SL 954430, SL 954443, SL 96061, SL 96128, SL 96328, SL 96771, SL 971239, SL 971442, SL 982118, SL 982524, SL 982528, SLT 8415, SLT 88238, SLI 121, SLT 4920, SLT 4921, SL 00529, Hellamulla	Sri Lanka
(3) *Erianthus arundinaceous*	MOL 4503, Mindanaoo, NG 7794, IK 76218	Fiji
	SLC 9277, SLC 9137, SLC 9280	Sri Lanka
	SES 218, SES 03, SES 356	Indonesia
(4) *Saccharum spontaneum*	NG 77162, IK 7792, IK 7688	Fiji
	SLC 9295, SLC 8951, SLC 9297	Sri Lanka
	IND 81164, IS 76220, IS 7673	Indonesia
	ISD 20	Bangladesh
(5) *Saccharum officinarum*	SLC 1201, SLC 1202, SLC 1203, SLC 1204, SLC 1210, SLC 1216, SLC 1218, SLC 1219, SLC 1220, SLC 1236, SLC 1268, SLC 1271, SLC 1273, SLC 1276, SLC 1291, SLC 1292, SLC 1294, SLC 1004, SLC 1008, SLC 1022, SLC 1301, SLC 1302, SLC 1303, SLC1 310, SLC 1320, SLC 1325, SLC 1327, SLC 1368, SLC 9272	Sri Lanka
	Badila, Korpi, Pindar	Australia

Table-2 The nucleotide sequences of the forward and reverse primers used and their annealing temperatures

Primer	Nucleotide sequence 5'- 3' Forward	5'- 3' Reverse	Anneal temp.°C
P5	GAGGACGAGGACGAGAAG	GAGAGAGTCAGAGAGAGCGA	56
P7	AGGCAGGTCGGGATGGAG	AGCACGCACACACGAGCA	60
P13	CGGCTTCTTGAGTTTGAC	CGTTCTTGCTTGCTTGAC	57
P14	CTCTCTGTCCTCCGCCTC	GAAACCCATCCTTCTCCTC	58
P16	ACAAGAGGAGGTTCAGGG	AGCAGAGACACACGCACA	58
P17	GACGAAACCCTATCCTCC	ATGTCGCTCTCCACACTC	56
P19	CTTCCACAACCAGAGCAG	GGAGACAGAGGCGAACAG	55
P38	GCAAATCCTCCTCTGACC	CTCCCAAGTCCCAACAAC	59
NKS 15	AACCCATTGACCAGATCCAG	TAGTGGCCCTAGGCGTAAAA	59
mSSCIRI[a]	CTTGTGGATTGGATTGGAT	AGGAAATGGATTGCTCAGG	54
mSSCIR10[a]	ACACCACTCACATCCACTTG	TGATACACCATTGTTGATGC	56
mSSCIR53[a]	TGGTCTACTGAAGTTCGTG	TGCTTCTAAGTCAACCAAA	52
mSSCIR58[a]	CTCACTCAGGCACAAGAAT	TGGGGTCTAACAATCAACT	54
mSSCIR74[a]	GCGCAAGCCACACTGAGA	ACGCAACGCAAAACAACG	54
SMC1420FL[a]	GAGCCAAA ATCTCTTTTGAAG	CCTATCAAACCATAACCAGACAG	62
SMC1604SA[a]	AGGGAAAAGGTAGCCTTGG	TTCCAACAGACTTGGGTGG	58
SMC18SA[a]	ATTCGGCTCGACCTCGGGAT	AGTCGAAAGGTAGCGTGGTGTTAC	62
SMC1751CL[a]	GCCATGCCCATGCTAAAGAT	ACGTTGGTCCCGGAACCG	60
SMC1814LA[a]	GGTTGACGATGAGAAGGACGTG	CACCCACATAGTGCCCAACG	64
SMC39BUQ[a]	CGTCTGGCGGATGAAATTGAG	CCTATCGGCATCAAATGGTCG	64
SMC 486CG[a]	GAAATTGCCTCCCAGGATTA	CCAACTTGAGAATTGAGATTCG	58
SMC 569CS[a]	GCGATGGTTCCTATGCAACTT	TTCGTGGCTGAGATTCACACTA	62
SMC 640CS[a]	TTAAGAGACCCGCCTTTGGAA	TGCCAGAAGTGGTTGTGCTCA	62

DNA extraction and SSR analysis

Total DNA was extracted from 3 to 4-month old accession leaf tissues using modified Cetyltrimethylammonium bromide (CTAB) method (Rogers and Bendich, 1989). Quality and quantity of DNA were measured by 1% agarose gel electrophoresis and nano-drop spectrophotometry, respectively.

PCR amplification was carried out using 23 highly polymorphic SSR primers selected from 50 primers initially tested on genomic DNA of 20 randomly selected Saccharum spp. and E. arundinaceous genotypes designed by the International Sugarcane Microsatellites Consortium (ISMC) and STMS markers for sugarcane designed by National Research Center on Plant Biotechnology of Indian Agricultural Research Institute (Table 2).

PCR reactions were carried out with 3 µl of 5× PCR buffer, 1.5 µl of 25 mM MgCl2, 0.5 µl of 10 mM dNTP's, 1.5 µl of 10 mM forward and reverse primers, 0.1 µl (5u/µl) Taq polymerase and 4.9 µl of de-ionized water. Amplifications were performed for 35 cycles as initial denaturation at 94 °C for 5 minutes, one cycle and denaturation at 94 °C for 45 seconds, annealing at specific temperature for appropriate primer for 45 seconds, extension at 72 °C for 1 minute and final extension at 72 °C for 7 minute (one cycle), (Applied Bio-systems 2720 Thermal Cycler).

PCR product of each primer was confirmed by running on a 1.5% agarose gel. Then, the products were separated in 6% polyacrylamide gels (PAGE) and Silver staining protocol was adopted to the gels to visualize the bands. The amplified products in PAGE were shown in Figure 2.

Data recording and statistical analysis

Bands of PAGE were scored as presence (1) and absence of bands (0). Similarity matrix was developed using the NTSYS pc 2.2 and the Un-weighted pair group method with arithmetic mean (UPGMA) based phylogenetic tree was constructed using

the binary data generated. Polymorphic information content (PIC) values for markers were calculated by using following formula (Cordeiro et al., 2003).

$$PIC = 1 - \sum_{j=1}^{N} p_{ij}^2$$

Where Pijis the frequency of the jth allele for marker i and submission extends over n alleles. Analysis of molecular variance (AMOVA) was carried out to find out the genetic differentiation within and among populations by GenAlEx 6, 6.5 (Peakall and Smouse, 2006; 2012). The popgene 1.31 (Yeh et al., 1999) was used to calculate observed number of alleles (na), effective number of alleles (ne), Nei's gene diversity (h), Shannon's Information index (I), number of polymorphic loci (NPI), percentage of polymorphic loci (PPI). Principal Coordinate Analysis (PCoA) was performed based on the genetic distance of SSR banding patterns of accessions by GenAlEx 6, 6.5.

RESULTS AND DISCUSSION

The accessions collected/ imported from twenty-one countries were subjected to this study and the geographical distribution of the accessions is shown in Figure 1. The dendrogram developed using the genetic similarity matrix generated from 439 amplified fragments produced by 23 SSR primers is illustrated in Figure 3. The number of alleles per primer ranged from 11 (for SMC1751CL andSMC39BUQ) to 27 (for mSSCIR10) with an average of 19 alleles per primer. SSR markers used, size range of the fragments and PIC value of relevant primers are given in Table 3. Fragment size ranged from 75 bp (for P5 and P7) to 500 bp (for P5, P7, P14, P19 and mSSCIR58) in length. The Polymorphic Information Content (PIC) value indicates the effectiveness of a marker varied from 0.85 (for SMC39BUQ) to 0.98 (for SMC640CS) with an average of 0.926 for all the markers (Table 3).

Table-3 Name of the primers, size range of bands and polymorphic information content (PIC) values produced by primers

Primer	Size range of bands (bp)	PIC value
P5	75-500	0.939
P7	100-500	0.970
P13	75-400	0.928
P14	175-500	0.966
P16	110-350	0.925
P17	180-310	0.881
P19	120-500	0.921
P38	100-400	0.921
NKS15	140-300	0.872
SMC640CS	175-425	0.976
mSSCIR10	115-400	0.959
SMC1420FL	120-275	0.890
SMC1814LA	110-300	0.924
-*mSSCIR1	110-300	0.977
mSSCIR53	150-425	0.958
mSSCIR58	80-500	0.944
SMC18SA	130-280	0.917
mSSCIR74	200-400	0.920
SMC1604SA	90-300	0.938
SMC486CG	75-450	0.953
SMC1751CL	125-220	0.860
SMC569CS	165-400	0.914
SMC39BUQ	140-450	0.846

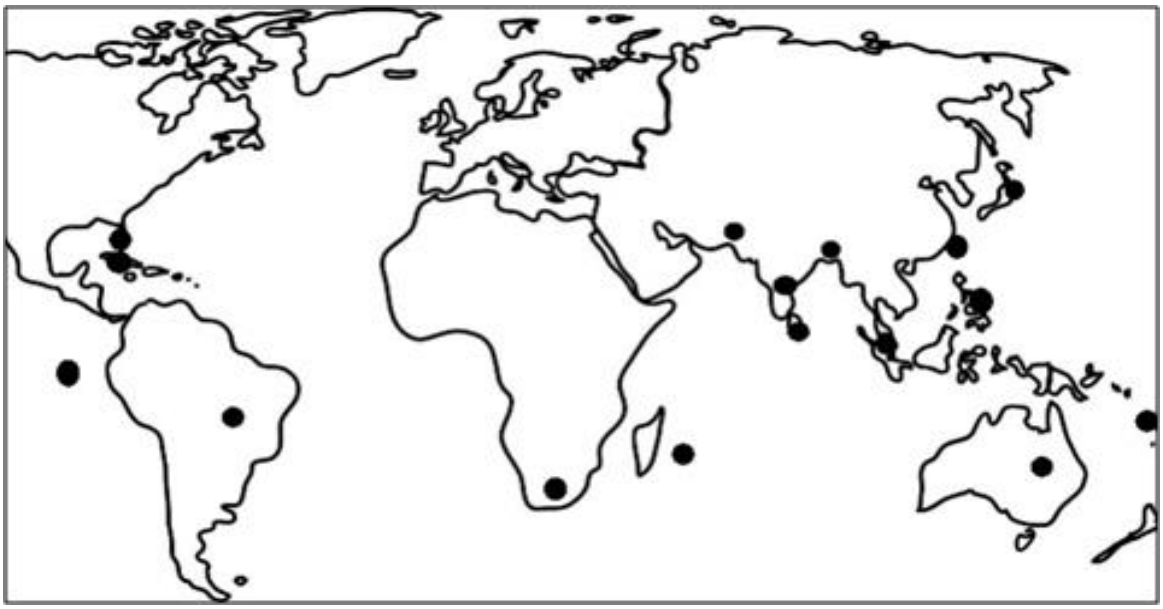

Figure 1.Places of introduction or origin of the accessions used in the study

Therefore, 23 markers used in this study have been proven to be polymorphic and as effective markers for diversity studies. The primers which did not show amplification and produced only monomorphic bands were excluded from the study. The percentage of polymorphic loci was high in imported *Saccharum* spp. hybrids (82.46%) followed by locally-bred *Saccharum* spp. hybrids (65.83%) and *S. officinarum* (48.97%), (Table 4). Among the populations, the number of alleles was in the range of 1.82 in imported *Saccharum* spp. hybrids to 1.36 in *E. arundinaceous* and with a mean of 1.544.

The number of effective alleles between the populations ranged from 1.17 in *E. arundinaceous* to 1.22 in imported *Saccharum* spp. hybrids and with an average of 1.20. The highest Nei's gene diversity index (0.14) was shown by *Saccharum* spp. hybrids of both imported and locally-bred origins. The highest Shannon's Information index value (0.24) was observed in imported *Saccharum* spp. hybrids followed by 0.22 in locally-bred *Saccharum* spp. hybrids, with an overall mean of 0.2. This study confirmed a considerable SSR polymorphism between the populations, with a mean of 54.35% polymorphic loci (Table 4). In general, higher percentage of polymorphic loci, higher number of alleles, higher number of effective alleles, high level of Nei's gene diversity and highest Shannon's information index values were observed between the accessions in the population of imported *Saccharum* spp. hybrids, indicating the presence of the highest genetic diversity within this population. The highest genetic variation among the varieties imported from different countries could be attributed to the geographical variation. The lowest values for all of these parameters observed in the *E. arundinaceous* population demonstrate that the accessions of *E. arundinaceous* are genetically not more diverse (Table 4).

At the genetic similarity coefficient of 79%, the accessions were classified into four major clusters (Figure 3). Cluster 1 comprised varieties imported from India and Brazil. All the accessions of “Co-series”, 20 in number, originated from Coimbatore, India were fallen into Cluster 1 with accessions imported from Brazil. This cluster was subdivided further into two sub clusters; 1a and 1b. The sub cluster 1a comprised 11 accessions originated from Coimbatore while the rest of the accessions of “Co-series” (9) and all the RB accessions imported from Brazil are clustered in sub-cluster 1b. Sugarcane varieties developed in similar climatic conditions tends to cluster in same group (Singh *et al.,* 2017). The Brazilian varieties grouping with the varieties developed in India revealed that the varieties developed in similar climatic conditions tend to cluster in one group.

Table 4: Observed number of alleles, Effective number of alleles, Nei's gene diversity, Shannon's Information index, number of polymorphic loci and percentage of polymorphic loci (PPl) of different types of cane

Type of cane	Sample size	Naa	neb	Hc	Id	NPle	PPlf (%)
Imported hybrids	98	1.82	1.22	0.14	0.24	362	82.46
SL hybrids	50	1.66	1.21	0.14	0.22	289	65.83
*Erianthus*spp.	10	1.36	1.17	0.10	0.16	160	36.45
S. spontaneum	10	1.38	1.21	0.12	0.18	167	38.04
S. officinarum	32	1.50	1.21	0.13	0.20	215	48.97
Mean		1.54	1.20	0.13	0.20	238.6	54.35

aObserved number of alleles, b Effective number of alleles, cNei's gene diversity, d Shannon's Information index, e number of polymorphic loci

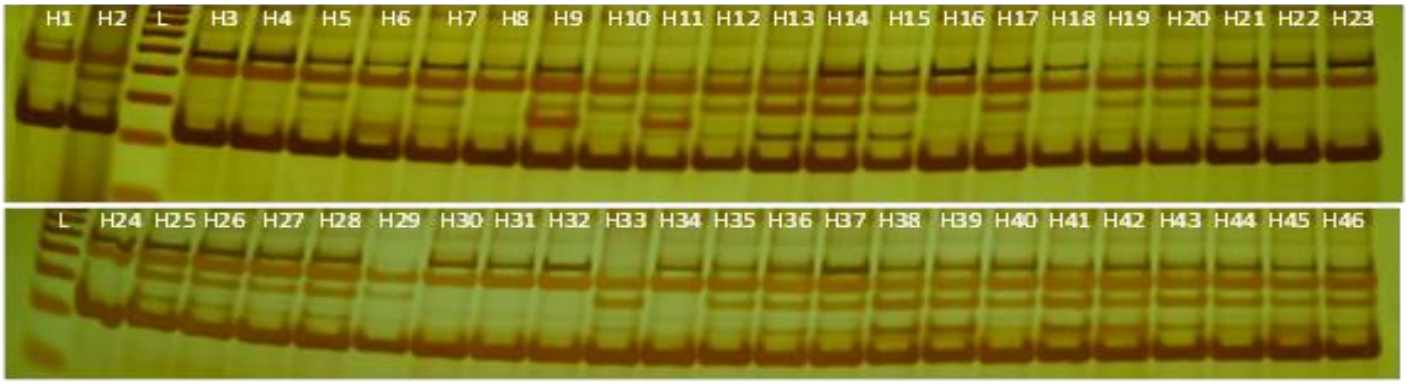

Figure 2.Amplified products for *Saccharum* spp. hybrids using P16 SSR primer in PAGE (L – 50 bp ladder; H1-H46 *Saccharum* spp. hybrids)

The clustering of Indian hybrid varieties in one cluster confirms the high genetic similarity among the varieties developed in India. Hemaprabha *et al.* (2006) states that, high genetic similarity observed among the sugarcane hybrid verities grown in India. Cluster 2 comprised the accessions of *E. arundinaceous* together with all *Saccharum* spp. hybrids with a few *Saccharum* spp. hybrids imported from Pakistan. The cluster 2 divided into 4 sub-clusters 2a, 2b, 2c and 2d. Out of the 148 *Saccharum* spp. hybrids, 102 accessions were classified into sub clusters 2a, 2b and 2d. All the accessions of *E. arundinaceous* except MOL 4503 were grouped into sub cluster 2c. MOL 4503 has been classified in sub cluster 2a with *Saccharum* spp. hybrids. According to the Daniels *et al.* (1975) origin of *Erianthus* accession is genetically closer to *Saccharum* spp. hybrids. The *Erianthus* and *Saccharum* belongs to different genera but both are included in family Poaceae. Therefore, it shares a common evolutionary ancestry within the grass family. Hence, resulting similarities in their DNA sequences in molecular level. Cluster 3 comprised most of the accessions imported from Pakistan and locally-collected *S. officinarum* accessions including SLC 92 72, Badila, Pindar and Korpi which were imported from Australia. Cluster 3 is further divided into two sub-clusters, 3a and 3b. Sub cluster 3a comprised the accessions imported from Pakistan. All locally-collected *S. officinarum* accessions were grouped in sub cluster 3b together with the olden-day commercially cultivated sugarcane varieties of *S. officinarum*; Badila, Pindar and Korpi of which Badila and Korpi have been confirmed as typical *S. officinarum* with the somatic chromosome number 2n = 80 (Cai *et al.,* 2005). Hence, all 29 accessions in

sub-cluster 3b can be considered as related accessions with the typical *S. officinarum* and they express some genetic relatedness with the *Saccharum* spp. hybrids imported from Pakistan (Bakker, 1999). The cluster 4 is a separate cluster identified in 0.76 similarity coefficient level. This cluster consisted of all *S. spontaneum* accessions and they are morphologically different form all other accessions used in this study. The genetic similarity values among tested accessions ranged from 0.665 to 0.957 indicating that there was a moderate to low variation among accessions collected from separate geographical locations across the world. The highest genetic similarity of 0.957 was observed in cluster 4, between SLC 89 51 and SLC 92 97 which were locally collected *S. spontaneum* accessions. The lowest genetic similarity of 0.665 was observed between accessions IS 76 220 (*S. spontaneum*) and S-2003 US 247 (*Saccharum* spp. hybrids) which was imported from Pakistan and they are located in the sub clusters 4 and 3a, respectively. These two genotypes differed from each other and were easily distinguishable based on the morphology. The three species *E. arundinaceous, S. officinarum and S. spontaneum* accessions were separately grouped into the sub-clusters 2c, 3b and cluster 4, respectively. Therefore, as per the UPGMA clusters, four distinguish populations are identified as; 1) *Saccharum* spp. hybrids (Coimbatore and Brazil origin), 2) *Saccharum* spp. hybrids (locally-bred and imported) and *E. arundinaceous*, 3) *Saccharum* spp. hybrids (introduced from Pakistan) with *S. officinarum* (locally-collected and imported) and 4) *S. spontaneum*.

The accessions of the five populations used in this study were subjected to analysis of molecular variance (AMOVA). It indicated that proportion of variation attributable to within population difference is high (84%) whereas only 16% occurred between populations (Table 5).

Table 5: Molecular variance between populations and within populations

Source of variation	Degree of freedom (df)	Sum of squares (SS)	Mean square (MS)	F - value	Prob>F	% of variation
Between populations	4	1162.195	290.549	7.12	<0.0001	16
Within populations	195	7956.835	40.804			84
Total	**199**	**9119.030**				

The genetic differences between populations were significant (p<0.001).

These results suggested that narrow range of genetic diversity existed between populations while existence of considerably high genetic variation within the populations could be due to the diverse origin of the individuals within populations. Principal coordinate analysis (PCoA) was conducted to estimate genetic diversity existing in the accessions based on covariance matrix developed with standardized data using GenAlEx 6, 6.5. All the accessions were clustered into three major groups including most of the *Saccharum* spp. hybrids imported from Pakistan in cluster 1, *E. arundinaceous* and other *Saccharum* spp. hybrids in cluster 2 and *S. officinarum* and *S. spontaneum* accessions in cluster 3 (Figure 4). A clustering pattern of *E. arundinaceous* with *Saccharum* spp. hybrids in cluster 2 confirmed that origin of *Erianthus* accession is genetically closer to *Saccharum* spp. hybrids (Daniels *et al.,* 1975) and common evolutionary ancestry within the grass family. As per the clustering patterns obtained from the PCoA (Figure 4) and the UPGMA-based phylogenetic tree (Figure 3), most of the accessions clustered similarly except few.

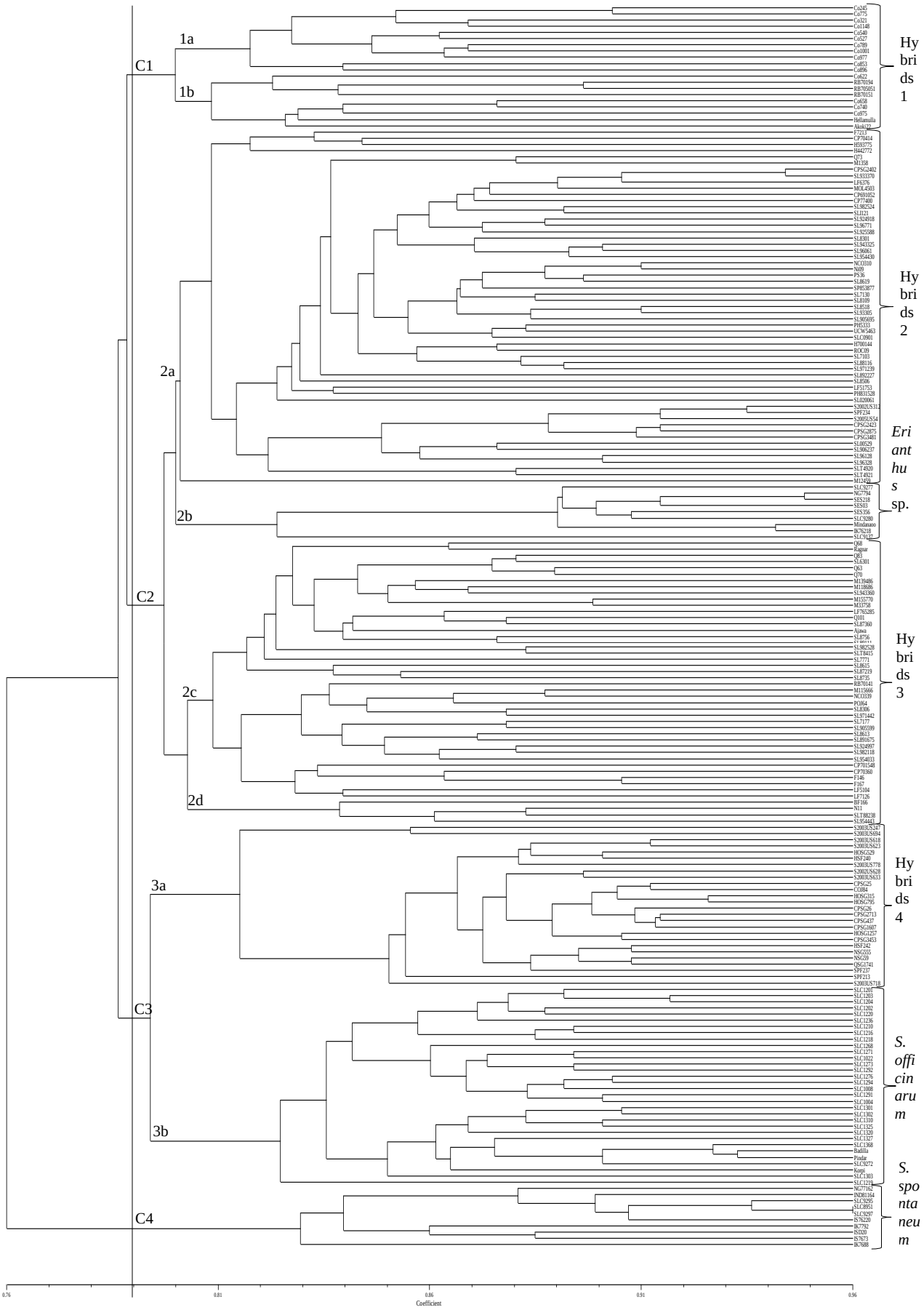

Figure-3:Dendrogram developed from the similarity matrix using group average method for 200 accessions

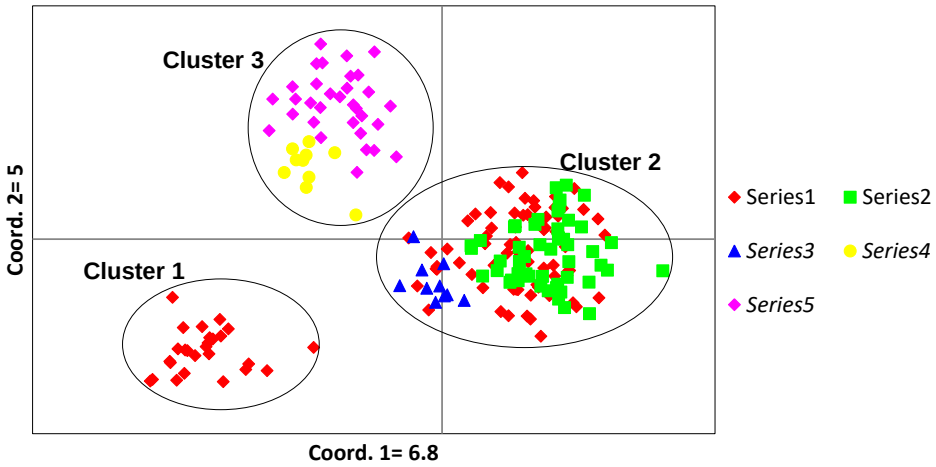

Figure 4.The scatter plot developed through principal coordinate analysis (PCoA) of the accessions based on similarity matrix using GenAlEx 6, 6.5.

In clustering pattern of PCoA, *Saccharum* spp. hybrids imported from Pakistan were not clustered with *S. officinarum* accessions as it was in the phylogenetic tree in Figure 3. The *S. spontaneum* accessions are cluster together with typical *S. officinarum* accessions. The clustering of *S. spontaneum* and typical *S. officinarum* together revels a genetic relationship between genus *Saccharum*. Grouping of parental accessions in germplasm is important in sugarcane hybridization and it is course to broaden the genetic base of the cultivated varieties (Selvi *et al.,* 2005). Therefore, identification of genetic variability of germplasm accessions using PCR-based DNA markers is highly informative in sugarcane crop improvement program of Sri Lanka.

CONCLUSION

Evaluation of genetic diversity among 200 accessions using 23 highly polymorphic SSR markers provided genetic information to be utilized in sugarcane breeding in Sri Lanka. The SSR markers used in this study showed a high PIC values and they characterized the *Saccharum* germplasm effectively. The values for percentage of polymorphic loci, Nei's gene diversity and Shannon's Information index indicated the presence of a higher variation within imported *Saccharum* spp. hybrids, and lower genetic variation within *E. arundinaceous* accessions. At the genetic similarity coefficient of 79%, the accessions were classified into four major clusters and in principle coordinated analysis, accession were divided into 3 major groups. The clustering pattern obtained from phylogenetic tree and PCoA was similar for *E. arundinaceous* and all other *Saccharum* spp. hybrids. The genetic variation within populations, is higher than the between populations indicating the opportunity for exploiting of such variation for further genetic improvement of sugarcane. The genetic information gathered and clustering patterns of tested accessions disclosed by this study can effectively be used in devising crossing systems in sugarcane breeding in Sri Lanka to meet the demand of commercial sugarcane cultivation and industry needs.

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