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Analysis of genetic diversity and population structure among exotic sugarcane (*Saccharum* spp.) cultivars in Ethiopia using simple sequence repeats (SSR) molecular markers

Esayas Tena[1], Firew Mekbib[2*] and Amsalu Ayana[3]

[1]Sugar Corporation of Ethiopia, Research and Training Ethiopia.
[2]Haramaya University, College of Agriculture and Environmental Sciences, School of Plant Sciences, Plant Breeding and Genetics Program, Ethiopia.
[3]Integrated Seed Sector Development Ethiopia Program Addis Ababa, Ethiopia.

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The present study was performed to study genetic relationships and population differentiation of 90 introduced sugarcane accessions in Ethiopia by means of 22 SSR molecular markers. The 22 SSR markers amplified a total of 260 alleles, of which 230 were polymorphic with a mean of 10.45 alleles per SSR locus. The range in allele number was 4–22. A high level of polymorphism with a mean of 60.51% polymorphic loci within the genotypes was detected. The polymorphic information content (PIC) ranged from 0.231 to 0.375 with an average of 0.303. Measures of effective number of alleles and genetic diversity on average were 1.55 and 0.317, respectively, across all the 22 markers evaluated. The SSR genetic profiles obtained using the 22 markers enabled complete discrimination among all the 90 introduced sugarcane cultivars. The neighbor- joining unweighted pair group method with arithmetic mean (UPGMA) cluster analysis based on the simple matching dissimilarity indices unambiguously distinguished all sugarcane genotypes with three major clusters and 11 groups. The same clustering pattern was also found in the PCoA analysis. In all the geographical populations, genotypes from the same country were often in different clusters and likewise accessions from different countries often clustered together indicating the possibility of exchange of materials between countries. Population genetic differentiation showed F_{st} values among pairs of populations ranging from 0.0024 to 0.5134 with an overall average of 0.0590. The average gene flow (Nm) among populations was 1.7213. Nei's unbiased genetic distance ranged from 0.018 to 0.279 with an overall average of 0.053. Genetic identity values were in the range of 0.756 to 0.992 with overall average of 0.950. The genetic relationship information of the cultivars will help sugarcane breeders to select the appropriate parents in their breeding programs to maximize yield as well as to maintain genetic diversity.

Key words: Sugarcane, Ethiopia, simple sequence repeats (SSR), genetic diversity, population genetic differentiation.

INTRODUCTION

Sugarcane is an important food crop of the tropics and sub tropics that is cultivated in about 74 countries between 40°N and 32° 5´S (Anonymous, 1998). A wide variety of sweet cane types existed from which were

selected thick barrelled, high sucrose soft sticks and this crude method of selection eventually produced better cane types. Sugarcane produces numerous valuable by-products like alcohol used by pharmaceutical industry, ethanol used as a fuel, bagasse used for manufacturing paper and chipboard and press mud used as a rich source of organic matter and nutrients for crop production (Muhammad and Farooq, 2007). The genome of modern sugarcane cultivars is a complex blend of aneuploidy and polyploidy derived from the interspecific hybridization involving different *Saccharum* species particularly, *S. officinarum* and *S. spontaneum* (Swarup et al., 2009). Use of an efficient molecular marker system is essential for sugarcane genome for understanding the genetic and taxonomic complexity, and broadening the genetic base of sugarcane cultivars, thereby improving sugar yield and its stabilization against abiotic and biotic stresses.

Since the start of the sugar industry, Ethiopia has been relying on importation of sugarcane varieties from many source countries to satisfy the varietal requirements of the sugarcane plantations. So far more than 300 sugarcane varieties have been imported and preserved in germplasm conservation garden located at Wonji. Importing variety *per se* is not an easy task, besides this all introduced varieties might not become successful commercial cultivars. For instance, among these introduced varieties, currently only 6-7 varieties are grown widely and commercially for sugar production in the three old sugar estates namely Wonji, Metehara and Fincha and the recent ones like Tendaho, Kesem, Beles, Kuraz and Wolkayit. These varieties are of very old generation and are contracted with many problems and not satisfactorily adapted to the different sugarcane growing areas of the country. This could be because of the reason that the varieties were released to suit the growing conditions of the country of source. At present, in Ethiopia sugarcane plantations are increasing at large and the demand for new superior varieties for each sugarcane growing region is expected to rise than ever. Accordingly, preparation to launch sugarcane breeding program in the country is underway to generate high yielding varieties that can adapt to different agro ecologies of sugarcane plantations and withstand different biotic and abiotic stresses. As the first step in this endeavour to broaden the genetic base of germplasm, exploration and collection of local sugarcane germplasm growing in the country in the back yards of small holder farmers since ancient times has been conducted and more than 200 clones were collected and preserved (Esayas et al., 2012).

There is lack of information on the imported varieties vis a vis pedigree, identity of the varieties etc., which is very difficult to trace as many of the clones are of old generation and significant number are of unknown sources. In spite of a long history of introduction, no systematic effort has been made to understand the genetic relationship of these cultivars. For better use of these materials in the breeding program and for broadening the genetic base characterization of these germplasm, efficient molecular marker is a must. The modern genetic breeding requires crosses between productive and genetically divergent parents, in order to have better heterotic effect and variability in the segregant generations (Cruz, 2001).

Genomic microsatellite markers are capable of revealing high degree of polymorphism. Sugarcane (*Saccharum* spp.), having a complex polyploid genome requires such informative markers for various applications in genetics and breeding. Microsatellite markers have gained considerable importance in plant genetics and breeding owing to their many desirable genetic attributes including hyper variability, wide genomic distribution, co-dominant inheritance, reproducibility, multi-allelic nature, and chromosome specific location (Singh et al., 2010). These markers are amenable to high throughput genotyping and are thus suitable for paternity determination, construction of high density genome maps, mapping of useful genes, marker-assisted selection, and for establishing genetic and evolutionary relationships (Swarup et al., 2009). SSRs are an ideal means for the identification of the genetic constitution of modern sugarcane cultivars of inter-specific origins (Giovanni et al., 2003).

Hence, this study was conducted to quantify genetic variability/diversity among introduced sugarcane germplasm collections using SSR markers and to assess genetic associations within and among populations of clones from different source countries.

MATERIALS AND METHODS

Plant materials

A total of 90 accessions of sugarcane introduced from a range of sugarcane producing countries around the world were used in this study. These materials were selected amongst more than 300 introduced sugarcane varieties found at the field conservation garden of Sugar Corporation of Ethiopia, Research and Training located at Wonji (Table 1). Selection was made taking into consideration the variation in place of origin that is source countries and different periods of introductions to the country. For genomic DNA isolation, young immature leaves of sugarcane genotypes was taken and stored in zip lock plastic bags containing Grade 12, blue silica gel. The silica was added in the ratio 1:5 which was sufficient enough to dry the leaves completely. This way, the leaf samples were transported to Sugarcane Breeding Institute, Coimbatore, India and total DNA isolation was conducted using the method described by Walbot (1988) in the laboratory of department of biotechnology between November 2012 and February 2013.

*Corresponding author. E-mail: firew.mekbib@gmail.com.

Table 1. Introduced sugarcane varieties in Ethiopia used for SSR diversity analysis.

Number	Cultivar	Country of origin	Year of introduction	Number	Cultivar	Country of origin	Year of introduction	Number	Cultivar	Country of origin	Year of introduction
1	B3172	Barbados	1983	17	CB 41-76	Campos (Brazil)	1970	33	CP 36/111	Do	1970
2	B37172	Do	1956	18	C 105-73	Do	1974	34	CP44/101	Do	1957
3	B 4098	Do	1960	19	CB 56-20	Do	1983	35	CP 48/103	Do	1960
4	B41211	Do	1970	20	CO 245	Coimbatore(India)	1970	36	CP 52/68	Do	1974
5	B 4425	Do	1974	21	CO 331	Do	1954	37	H48/4605	Hawaii	1965
6	B 45154	Do	1957	22	CO 434	Do	1970	38	H49/5	Do	1965
7	B 49119	Do	1962	23	CO 440	Do	1963	39	H49/3533	Do	1974
8	B 5364	Do	1965	24	C120-78	Do	1970	40	CP 65/357	Canal point	1983
9	B 62347	Do	1974	25	CO 475	Do	1956	41	CP 8/1026	Do	1984
10	BO 10	Bihar-Orissa (India)	1960	26	CO 513	Do	1960	42	CP 1/441	Do	1983
11	BO 14	Do	1974	27	CO 740	Do	1962	43	CP 71/443	Do	1984
12	BO 29	Do	1974	28	CO 957	Do	1965	44	COS 109	Unknown	1965
13	BO 3	Do	1970	29	CO 1208	Do	1987	45	COS 443	Do	1965
14	CB 36-14	Campos,(brazil)	1974	30	CP 29/116	Canal point	1953	46	COS 510	Do	1962
15	CB 38-22	Do	1959	31	CP 29/291	Do	1954	47	COK 30	Do	1970
16	CB 40-35	Do	1983	32	CP 36/105	Do	1959	48	D 42/58	Demerara, (Guyana)	1974
49	D 141/46	Demerara, (Guyana)	1974	63	H 39/3633	Hawaii	1960	77	N 14	Natal (South Africa)	1980
50	D 188/56	Do	1974	64	H 44/3098	Do	1960	78	N 50/93	Do	1965
51	DB 95/57	(Demerara) X Barbados	1974	65	L 60-14	Louisiana, USA	1974	79	WD II	Do	1953
52	DB 377/60	Do	1974	66	L 60-25	Do	1974	80	C86-12	Cuba	2003
53	DB 386/60	Do	1974	67	L 60-35	Do	Unknown	81	C132-81	Do	2003
54	DB 414/60	Do	1974	68	L 60-40	Do	1974	82	NCD 310	Natal	1953
55	DB 414/66	Do	1983	69	M 31/45	Mauritius	1957	83	NCD 349	Do	1970
56	Ebene 1/37	Unknown	1957	70	M 53/263	Do	Unknown	84	PR 905	Puerto Rico	1959
57	E 88/56	Do	1974	71	M 112/34	Do	1960	85	PR 980	Do	1965
58	E 188/53	Do	1974	72	M 147/44	Do	1957	86	PR 1000	Do	1960
59	E 188/56	Do	1974	73	Mex 52/29	Mexico	1970	87	PR 1007	Do	1970
60	F 134	Formosa, (Taiwan)	1970	74	Mex 59/1828	Do	1983	88	PR 1059	Do	1974
61	F 141	Do	1970	75	N 6	Natal (South Africa)	1983	89	PPQK 1604	Do	1958
62	H 38/4443	Hawaii	1960	76	N 11	Do	1987	90	Pindar	Unknown	1957

DNA isolation

Approximately 500 mg leaves of each genotype were separately ground to fine powder in liquid nitrogen using pre-cooled sterilized mortar and pestle. Fine powder was transferred into sterilized centrifuge tube containing 10-15 ml of grinding buffer and centrifuged at 6500 rpm for 6 min at 4°C. After discarding the supernatant, the pellet was suspended in 3 ml of suspension buffer and 200 µl of 20%

SDS was added, mixed gently and placed on a water bath at 70°C for 15 min. After incubation, 1.5 ml ammonium acetate (7.5 M) was added, mixed well and placed on ice for half an hour. The sample was centrifuged at 14,000 rpm for 10 min at 4°C. Then supernatant was decanted in 6 ml iso-propanol to precipitate the DNA. After incubation for 30 min in ice the sample was centrifuged at 15,000 rpm for 15 min at 4°C to pellet the DNA, supernatant was discarded and the pellet was resuspended in 500 µl TE and kept in water bath at 65°C for 15 min. The DNA solution was transferred into sterilized microfuge tube using sterilized cut tips and 15 µl RNase (10 mg/ml) was added and incubated in a water bath at 37°C for 15 min. Then equal volume of choloroform:iso-amyl alcohol (24:1) was added and followed by centrifugation at 10,000 rpm for 10 min at room temperature. The aqueous phase was pipetted out and transferred into fresh microfuge tube. This was repeated two times. Then DNA was precipitated by adding 50 µl sodium acetate ($1/10^{th}$) and 1 ml of ice-cold absolute ethanol and kept at -20°C for half an hour. After this, the DNA was pelleted by centrifuging at 15000 rpm for 15 min at 4°C. The pellet was washed two times with 70% ice-cold ethanol. Finally, the pellet was dried in air and dissolved in 200 µl TE and stored at -20°C until used.

Assessment of quality and quantity of extracted DNA samples

The quantity and quality of the extracted genomic DNA were checked and normalization or adjustments to 10 ng μl^{-1} was done on agarose gel (0.8%) using lambda DNA standard (Thermo Scientific, USA). The DNA was quantified by loading the samples on 0.8% agarose gel containing 0.5 $\mu g\ ml^{-1}$ of ethidium bromide. The DNA was normalized to 10 ng μl^{-1} concentration by comparing visually the diluted DNA samples with the standard λ DNA molecular weight markers on 0.8% agarose gel by running it in 0.5xTBE (Tris-borate EDTA) buffer at a constant voltage (180 V/125 mili amp) for 45 min. The images of gels were documented under UV illumination using Uvi -Tech gel documentation system (DOL-008.XD, England).

SSR assays

Twenty two (22) SSR markers designed in laboratory of the Department of Biotechnology, Sugarcane Breeding Institute, Coimbatore, India were used (Table 2). PCR reactions were performed in 15 µl reaction mixtures in 96-well PCR plates (Thermocycler, MJ Research Inc Model: PTC-100). Each PCR reaction contained 10 ng of genomic DNA (3 µl), 10XTaq Buffer with $MgCl_2$ (1.5 µl), 1.25 mM dNTPS (2.4 µl), each 30 ng (1 µl) forward and reverse primers, 3 U/µl Taq DNA polymerase (0.3 µl) and sterile 5.8 µl milliQ water. PCR amplification was carried out at 94°C for 5 min initial denaturation, followed by 30 cycles of 94°C (denaturation) for 1 min, 50-65°C annealing temperature (depending on AT and CG content of the primers) for 1 min and 72°C extension for 1 min. After completion of all 30 cycles, a final extension of 10 min at 72°C was performed.

PAGE

Checking of the amplification of the PCR products was done on 1% agarose gel containing 0.5 ml/10 ml ethidium bromide (10 mg ml^{-1}) with 10 base-pair DNA ladder by running it at a constant voltage of 180 V/125 mili amp for 45 min. The amplification was visualized under UV illumination using Uvi Tech gel documentation system (DOL-008.XD, England). After this, PCR products were loaded on 4% PAGE gels, polyacrylamide: bis acrylamide (29:1) and electrophoresed in 1x TBE buffer at constant power of 180 V/125 milli amp) for 1 h using Sequi Gen® GT nucleic acid electrophoresis cell and the products were resolved using silver staining procedure.

Data analysis

Only clear and unambiguous bands of SSR markers were scored. No assumption on the genetic nature of the alleles was made due to the polyploid nature of sugarcane and the absence of a segregation analysis (Gillet, 1991). Hence, each allele was scored in a dominant manner and transformed into either a 0 (absent) or 1 (present) matrix. Although SSRs are classified as co-dominant type markers, they have been treated as dominant markers in this study.

Genetic diversity analysis

The software program Genalex 6.5 (Peakall and Smouse, 2006, 2012) was used to calculate parameters such as: genetic distance, number of different alleles (NA), number of effective alleles (NE), Shannon's information index, Analysis of Molecular Variance, etc. Genetic associations were determined with Darwin V. 5.0 software (Perrier et al., 2003) using neighbour-joining coefficient. Popgene program (Yeh et al., 1999) was also used to determine genetic diversity, polymorphic loci, gene flow and F-values. Finally, the basic statistics such as polymorphic information content (PIC) and gene diversity were estimated using PowerMarker v. 3.25 (Liu and Muse, 2005).

RESULTS AND DISCUSSION

Allelic diversity

The genotyping of a total of 90 introduced sugarcane varieties, 81 of them from fourteen countries around the world and the remaining 9 from unknown sources (Table 1) with 22 SSR markers were allowed to identify a total of 260 alleles (Table 2). However, variations in the total number of fragments and the number of polymorphic fragments were evident. The data for microsatellite loci diversity are summarized in Table 3. Out of the total of 260 alleles amplified, 230 were polymorphic with a mean of 10.45 alleles per SSR locus (Table 2).

The highest level of polymorphism was detected in SOMS173 (100%) followed by SOMS167 (98.89%), SOMS169 (98.89%), and SOMS29 (97.78%). The range in allele number was 4–22, with the marker SOMS147 having the highest number of alleles (22), followed by SOMS173, SOGL50, SOMS29 and SOGL38 with 21, 17, 16 and 15 alleles, respectively (Table 2). Fragments size ranged from 100 (SOGL50 and SOMS173) to 1700 bp (SOGL41) in length. The polymorphism information content (PIC) calculated as a relative measure of informativeness for each of the markers ranged from 0.231 for the markers SOGL41 to 0.375 for the marker SOMS166 with an average of 0.303. Number of different alleles was in the range of 1.09 in SOMS166 to 2.0 in SOMS173 with overall mean of 1.75. Measure of effective number of alleles on average was 1.55 across all the 22 markers evaluated. The markers with the highest levels of effective number of alleles (1.71-1.82) were SOMS29, SOMS167, SOGL11 and

Table 2. Details of SSR markers with their annealing temperature (Ta), sequences (forward and reverse), range of product size and total number of amplified bands and total number of polymorphic bands given by the primer are shown in bracket.

Primer name	Forward (5'- 3')	Reverse (3' - 5')	T_a (^{0}C)	Range of Product Size (bp)	Number of bands
SOMS167	AGCAGAGACACACGCACA	ACAAGAGGAGGTTCAGGG	54	180-900	10(10)
SOMS166	GTCTCTTCTCCAGTTCTCCTT	GTCTTCTCCACAACCACCT	50	400-950	4(2)
SOMS148	GATGACTCCTTGTGGTGG	CTTGACGACCCTGCTGCT	54	120-700	4(3)
SOMS168	ATGGCGTCTCGTCTCGTT	ACCTCAGTCTTGTCTTCCTTC	50	130-1400	10(10)
SOMS88	AGATGGATGAGGGTTTCTTT	CCTACGAGTTTATTCTTCAGT	55	260-540	10(9)
SOMS169	ACAGCACAGGCTCTCTCTT	TCCTTTCAGGCATCCATC	52	170-1200	13(13)
SOMS96	AACTTGACCCTTCTTCTTCC	GCCGATGGACACCTTGAC	55	540-780	5(3)
SOMS119	CAACATCTCACGAAACAATAC	AACACCTCCTACACTGACACA	55	350-850	10(8)
SOMS109	ATCCTTTGTCGTCTCCGT	AGTTGGGTGTGTATTTGGTG	54	280-780	9(8)
SOMS68	AACTGAAGCAGCACCAACT	TTGTCTAATACCCTGACCTGA	56	175-1200	14(12)
SOMS158	ATAATGACTGAACCTCTCCC	CTTCCTGTGCTTCCTGGT	54	260-690	9(7)
SOMS29	ACCAGTTCCTCTACGCCC	CATCCCATCCCTTGTGTC	55	165-650	16(16)
SOMS143	TGACTTGGAATAACACAAAGAA	ATGGGATGGATAATAAGCAGT	54	230-400	14(13)
SOMS147	AGCGAACCCTAATGGAGA	GGGAGACATCGTAGACCTG	55	240-820	22(20)
SOGL11	GTGCTGAATGAGAGAGTGGT	TCCAGGTCGCTGTAAGAA	55	320-600	8(8)
SOGL37	TTCTCTGACTTCCAATCCAA	ATCAAGCACGCCCGCCTC	55	290-500	7(6)
SOGL15	CATCAGTATCATTTCATCTTGG	CAGTCACAGTCGGGTAGA	56	250-450	11(10)
SOGL36	TCCTCATTACCATTTGTTCC	CCTCCTCTTGCTGGACTT	52	280-1000	14(12)
SOGL50	GCTACTATGGACAACAGGG	ATGAAGAGACGAGACGAAGA	55	100-450	18(17)
SOGL38	AAGCAAGCAAGGCAAACT	GTGGGCAACGCACTGGTC	50	180-840	16(15)
SOGL41	TGAGGACGGGATGAAGAC	CGGTTACTGTTTGAGGGAG	52	210-1700	15(9)
SOMS173	GTGGACGAGAAGTGGAAGT	ATAGGAGGGCAGGACAAG	54	100-1200	21(21)

SOMS168 whereas; the markers SOMS 96 and SOGL41 had the lowest value, 1.26 (Table 3). The highest Shannon's information index 0.63 was recorded for marker SOMS29 while the lowest value 0.26 was observed in SOMS96 with mean of 0.47 across markers. The highest gene diversity 0.440 was observed using the marker SOMS29 while the lowest value of 0.167 was observed with SOMS96 with an overall mean of 0.317.

Genetic analysis has been hindered in sugarcane due to lack of sufficiently informative markers. Less information is available about the genetic diversity within and between *Saccharum* cultivars which has been based mainly on morphological characteristic.

This study reveals considerable SSR polymorphisms with a mean of 60.51% polymorphic loci within the genotypes under study (Table 5). High levels of polymorphism were detected with an average number of 10.45 polymorphic fragments per primer pair (Tables 2). Every marker was able to amplify varying numbers of DNA fragments (bands) from all 90 clones, regardless of their geographical origins. Chen et al. (2009) reported SSR polymorphisms when studying on sugarcane cultivars from breeding programs in China and other countries. High degree of polymorphism had been also reported by Singh et al. (2010) using SSR markers on sugarcane species and commercial hybrids in India.

Genetic relatedness among accessions

Estimated genetic dissimilarities (Dice's dissimilarity) of the germplasm were visualized in radial

Table 3. Number of different alleles (N_a), number of effective alleles (N_e), Shannon index (I), gene diversity and PIC of the 22 SSR markers used in the study.

Locus	Sample size	N_a	N_e	I	Gene diversity	PIC
SOMS167	90	1.98	1.77	0.60	0.418	0.334
SOMS166	90	1.09	1.35	0.27	0.188	0.375
SOMS168	90	1.98	1.71	0.59	0.406	0.257
SOGL37	90	1.73	1.60	0.48	0.330	0.348
SOGL15	90	1.89	1.58	0.51	0.340	0.307
SOGL36	90	1.69	1.46	0.42	0.274	0.324
SOGL50	90	1.86	1.67	0.55	0.380	0.307
SOMS88	90	1.87	1.69	0.54	0.373	0.296
SOMS169	90	1.98	1.56	0.50	0.332	0.327
SOMS96	90	1.10	1.26	0.26	0.167	0.241
SOMS119	90	1.78	1.42	0.40	0.260	0.337
SOMS109	90	1.71	1.37	0.37	0.236	0.281
SOMS68	90	1.69	1.48	0.43	0.282	0.329
SOMS158	90	1.53	1.56	0.46	0.318	0.261
SOGL38	90	1.91	1.63	0.54	0.362	0.296
SOGL41	90	1.27	1.26	0.28	0.175	0.231
SOMS29	90	1.96	1.82	0.63	0.440	0.317
SOMS143	90	1.84	1.35	0.38	0.237	0.220
SOMS147	90	1.78	1.68	0.54	0.372	0.331
SOMS148	90	1.81	1.64	0.51	0.354	0.338
SOMS173	90	2.00	1.53	0.45	0.299	0.346
SOGL11	90	1.97	1.77	0.60	0.421	0.272
Mean	90	1.75	1.55	0.47	0.317	0.303

diagram (Figure 1). Analysis of the SSR data using Dice's dissimilarity indices showed that pair wise genetic dissimilarity (GD) values ranged from 6.1 (between PR1007 and Ebene 1/37) to 73.5% (between CB41-76 and H38/4443) with a mean of 38.74% (data not shown). In the dendrogram, clones CB41-76 and H38/4443 are in different clusters, diverse from each other and easily distinguishable among the genotypes tested (Figure 1). A high degree of dissimilarity was also found between CB41-76 and CP52/68 (72.3%), CB41-76 and CO740 (71.3%) and CB41-76 and F134 (71.2%). This relationship clearly shows that these genotypes were geographically diverse and evolved independently. Genetic dissimilarity values ranging from 17-48% was reported by Selvi et al. (2005) using RFLP markers in tropical and subtropical Indian sugarcane cultivars. Chen et al. (2009) reported 56 to 91.6% similarity among sugarcane cultivars of different geographic origins with SSR markers. Genetic similarity coefficient ranging from 5.6 to 77.8% has been reported by Singh et al. (2010) using SSR in a collection of sugarcane species and commercial hybrids in India.

Both neighbour joining and PCoA plot based on pairwise genetic distances (Figures 1 and 2) assigned clones into three clear distinct clusters. Moreover, the clustering pattern at the lower level of hierarchies after the differentiation of the major clusters showed sub-grouping of the clones into 11 major groups. Starting from the bottom and moving clockwise in the dendrogram (Figure1) depicting groups with different colours, group I comprised 19 clones almost from every source country except India for Bihar Orissa (BO) varieties, Louisiana, Mauritius and Demerra X Barbados crosses from Barbados. Group II included clones N50/93, L60-14 and PR980 from South Africa (Natal), Louisiana and Puerto Rico respectively.

Group III contained clones H48/4605 from USA (Hawai) and C120-78 from Cuba. Group IV had two clones from Lousiana namely L60-25 and L60-35 and one clone E188/53 from unknown source. Group V had 41 clones from every source country except Cuba, Hawaii, Demerra and Formosa (Taiwan). Group VI consisted of four clones from Hawaii, India (Coimbatore), Formosa (Taiwan) and Barbados each contributing one clone. Group VII had two clones BO3 and M53/263 from Bihar Orissa (India) and Mauritius respectively. The next group (VIII) also had two varieties C105-73 and CO331 from Cuba and India (Coimbatore) respectively. Group IX had four clones CB38-22, D42/58, DB386/60 and E188/56 from Campos Brazil, Demerra, crosses from Demerra and Barbados

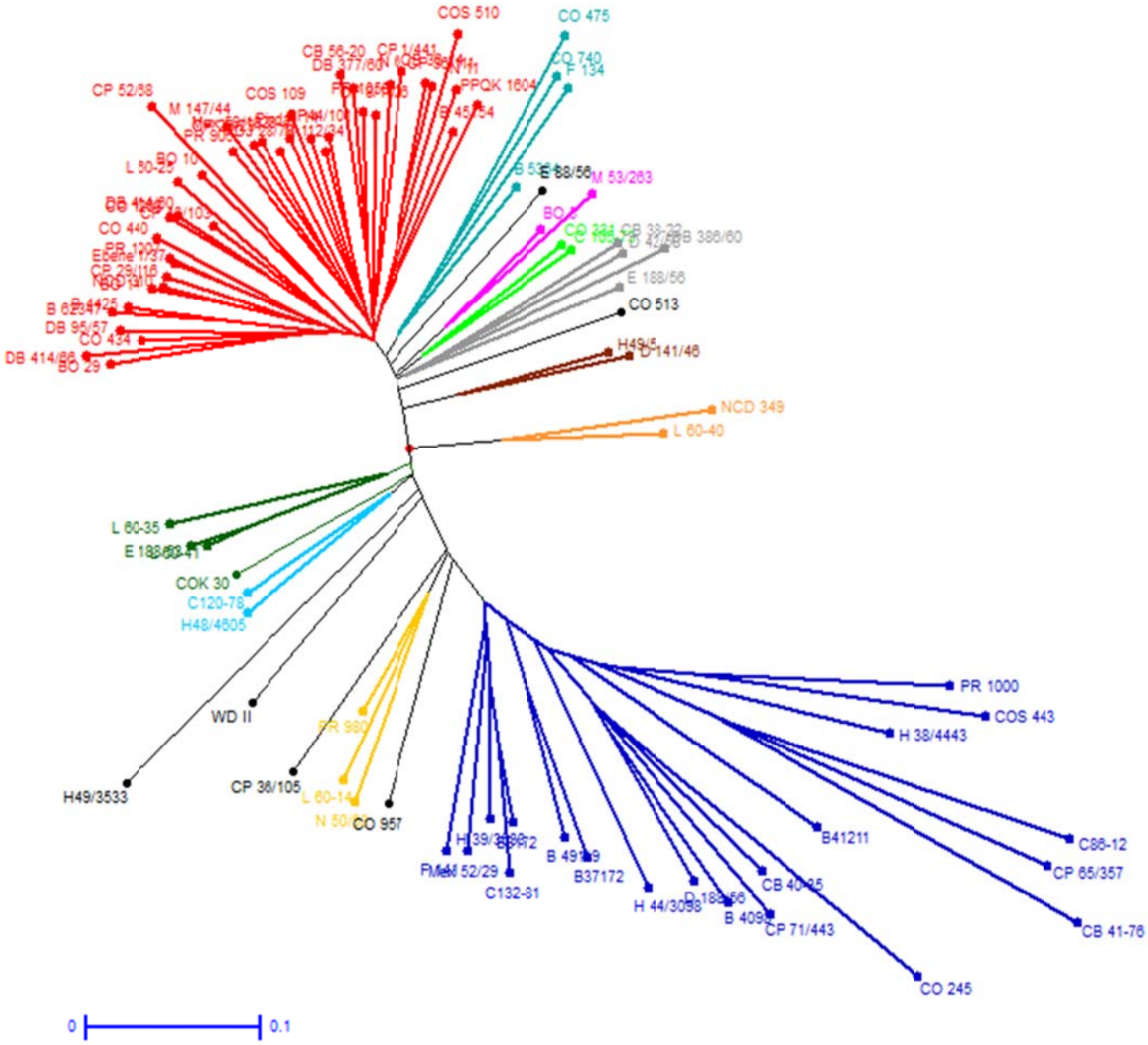

Figure 1. Unrooted UPGMA tree, calculated from SSR data (260 Loci), assembling 90 genotypes which were sugarcane species clones introduced into Ethiopia from different source countries around the world.

and unknown source. Group X consisted of two clones H49/5 (Hawaii) and D141/46 (Demerra). The last group XI contained two clones NCD349 and L60-40 from Barbados and Louisiana respectively.

Located between the different groups, seven clones namely CO957, CP36/105, WDII, H49/3533, COK30, E88/56 and CO513 did not cluster with any group. This relationship might be due to the reason that these genotypes were geographically diverse and evolved independently. Out of these seven clones COK30 and E88/56 are clones whose countries of origin were unknown. The other eight clones with unknown sources joined different clusters. Accordingly, COS443 found close to Puerto Rico variety PR1000 joined group I. E188/53 joined group IV with Louisiana varieties L60-25 and L60-35. Pindar, COS109, Ebene1/37, COS443 and COS510 clustered with accessions in group V. E188/56 joined group IX. The placement of these genotypes in the respective clusters might indicate their close similarity with the accessions with which they cluster in their evolutionary history.

Generally the results of the dendrogram and the PCoA analysis showed that regardless of their origin sugarcane cultivars from different source countries tend to cluster

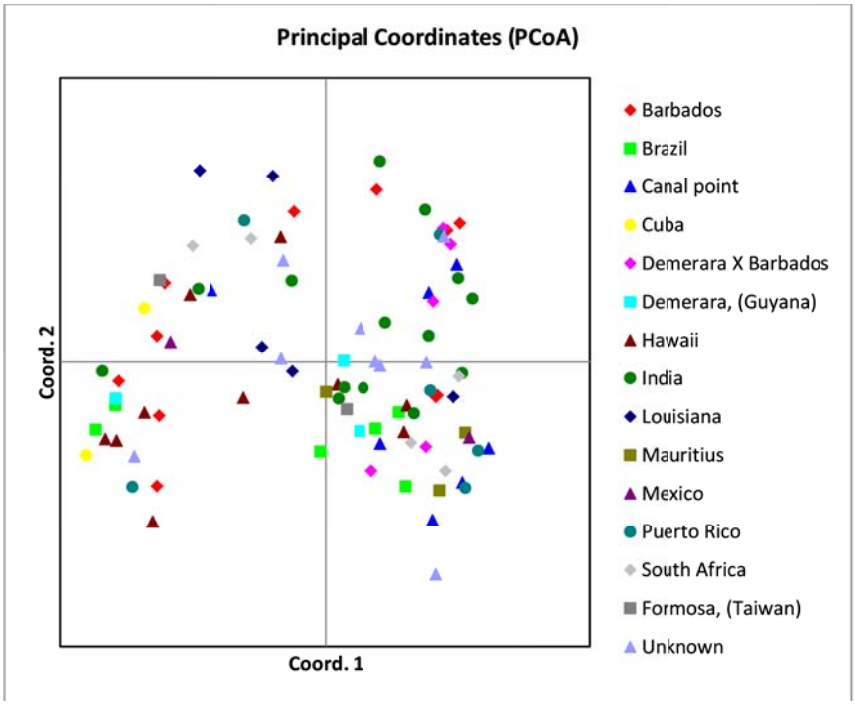

Figure 2. PCoA graph of 90 sugarcane accessions introduced into Ethiopia from 14 source countries and unknown sources around the world.

together and likewise accessions from the same country were often in different clusters (Figures 1 and 2) indicating the possibility of existence of shared alleles among the genotypes. Thus the present clustering pattern of clones also disclosed the possibility of close similarity between their evolutionary courses. No evidence was found for all genotypes with one geographical location to be single cluster in the clustering pattern of genetic diversity, thereby underscoring the importance of human-mediated gene flow in addition to artificial selection.

Principal coordinate analysis

The principal coordinate analysis (PCoA) allowed the separation of the genotypes into three gene pool groups regardless of the geographical area where the clones were introduced (Figure 2). Substantial diversity was found in the genotypes in the PCoA analysis and this was distributed both within and between gene pools. Similar to results of cluster analysis, accessions were seen clustering randomly regardless of country of origin

Results of the PCoA were in agreement with that of the neighbour-joining dendrogram, with three major clusters detected. Further differentiation into recognized clones belonging to each cluster was also observed as in the dendrogram. The overall variation explained by the PCoA was 74.96% with dimensions 1, 2 and 3 explaining 49.8, 13.69, and 11.47%, respectively.

Genetic diversity within and among accessions of country of sources

All of the microsatellite markers used in this study had highest number of polymorphic loci (Table 2). The proportions of polymorphic loci were high (86.54%) in accessions from India followed by 78.08 and 77.31% of the accessions from Barbados and Hawaii respectively (Table 5). Within populations number of different alleles was in the range of 0.523 in Cuba genotypes to 1.796 in the Indian accessions with a mean of 1.369. The average number of effective alleles per locus within the genotypes of the different countries of origin ranged from 1.144 in the genotypes of Cuba to 1.489 in those of Canal Point with a mean of 1.365 over loci and populations. Shannon's information index was highest (0.438) in Indian accessions followed by 0.411 in those of Canal Point with over all mean of 0.321 (Table 5). Gene diversity was highest (0.290) in accessions of India while the lowest (0.084) was recorded in those of Cuba with mean of 0.214 across populations (Table 5 and Figure 3). Generally, within populations, higher number of different alleles, higher number of effective alleles, high Shannon information index, higher level of gene diversity and the highest percentage of polymorphic loci were observed among Indian varieties (Table 5).

AMOVA results showed that 97% of allelic diversity was attributed to individuals within population while only 3% was distributed among populations (Table 4). This result may reflect the high degree of variation among

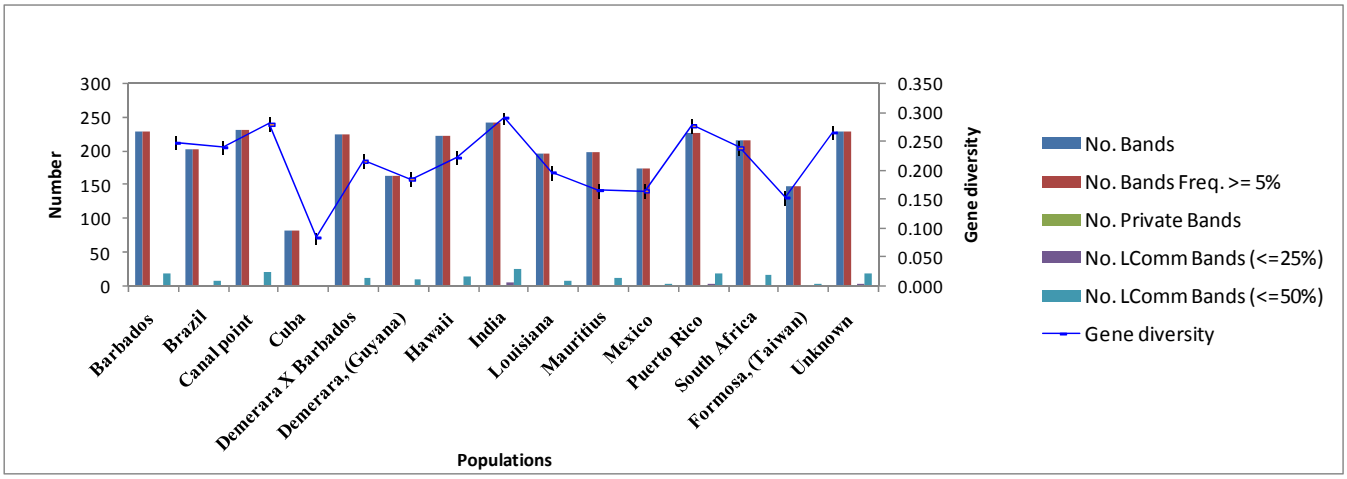

Figure 3. Band patterns across populations. No. LComm Bands<=25%)= No. of Locally Common Bands (Freq.>=5%) Found in 25% or Fewer Populations; No LComm Bands (<=50%)= No. of Locally Common Bands (Freq.>=5%) Found in 50% or Fewer Populations.

Table 4. Analysis of molecular variance (AMOVA) for 90 individual exotic sugarcane clones from different source countries based on the analysis of 260 SSR fragments generated using 22 primer pairs.

Source of variation	df	Sum of squares	Variance components	% of total variation
Among Populations	14	677.875	1.204	3
Within Populations	75	3102.880	41.372	97
Total	89	3780.756	42.576	100

Significance of comparisons; Hawaii vs Canal point, Hawaii vs Demerara X Barbados and Hawaii vs India P<0.01; Cuba vs Canal point, Demerara X Barbados vs Barbados, Demerara X Barbados vs Brazil, India vs Cuba, Louisiana vs Demerara X Barbados, Mauritius vs Demerara X Barbados, Mauritius vs Hawaii and Formosa (Taiwan) vs Demerara X Barbados P=0.05.

Table 5. SSR diversity for 22 microsatellite loci in 15 populations (genotypes from 15 respective source countries) of introduced sugarcane clones in Ethiopia.

Population	Na	Ne	I	GD	P%
Barbados	1.662	1.411	0.377	0.247	78.08
Brazil	1.481	1.402	0.361	0.239	70.00
Canal Point	1.627	1.489	0.411	0.279	74.23
Cuba	0.523	1.144	0.123	0.084	20.38
Demerara X Barbados	1.477	1.368	0.322	0.215	61.15
Demerara, (Guyana)	1.100	1.319	0.272	0.184	47.69
Hawaii	1.627	1.359	0.345	0.222	77.31
India	1.796	1.486	0.438	0.290	86.54
Louisiana	1.331	1.330	0.295	0.195	57.69
Mauritius	1.181	1.288	0.243	0.165	42.31
Mexico	1.062	1.280	0.240	0.164	39.62
Puerto Rico	1.619	1.481	0.409	0.276	74.62
South Africa	1.477	1.410	0.354	0.238	65.00
Formosa, (Taiwan)	0.935	1.261	0.223	0.153	36.92
Unknown	1.642	1.452	0.396	0.264	76.15
Mean	1.369	1.365	0.321	0.214	60.51
SE	0.013	0.006	0.005	0.015	4.94

N_a =number of different alleles; N_e=effective number of alleles; I=Shannon index; GD= genetic diversity according to Nei (1978) and %P=percentage of polymorphic loci No. LComm Bands (<=25%) = No. of Locally Common Bands (Freq. >= 5%) Found in 25% or Fewer Populations; No. LComm Bands (<=50%) = No. of Locally Common Bands (Freq. >= 5%) Found in 50% or Fewer Populations.

Table 6. Unbiased Nei's genetic distance (below diagonal) and genetic identity (above diagonal) among and between populations (genotypes from respective source countries), numbers representing populations here correspond to population numbers in Table 5.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	***	0.981	0.953	0.939	0.887	0.978	0.978	0.977	0.965	0.862	0.937	0.971	0.974	0.950	0.962
2	0.020	***	0.956	0.950	0.887	0.973	0.984	0.973	0.955	0.871	0.932	0.966	0.962	0.969	0.961
3	0.048	0.045	***	0.856	0.958	0.947	0.932	0.977	0.946	0.935	0.939	0.982	0.982	0.920	0.978
4	0.063	0.051	0.155	***	0.778	0.945	0.967	0.901	0.903	0.756	0.869	0.902	0.892	0.904	0.904
5	0.120	0.120	0.043	0.251	***	0.896	0.856	0.939	0.890	0.891	0.889	0.932	0.931	0.867	0.926
6	0.022	0.027	0.055	0.057	0.109	***	0.988	0.968	0.964	0.873	0.927	0.979	0.965	0.940	0.980
7	0.022	0.016	0.071	0.034	0.155	0.012	***	0.956	0.952	0.838	0.914	0.973	0.955	0.939	0.968
8	0.023	0.027	0.023	0.105	0.062	0.033	0.045	***	0.967	0.909	0.938	0.973	0.972	0.956	0.967
9	0.036	0.046	0.056	0.103	0.116	0.036	0.049	0.033	***	0.882	0.926	0.967	0.976	0.950	0.966
10	0.149	0.139	0.068	0.279	0.115	0.136	0.177	0.095	0.126	***	0.895	0.910	0.909	0.853	0.900
11	0.065	0.070	0.063	0.140	0.117	0.076	0.090	0.064	0.077	0.111	***	0.932	0.942	0.942	0.926
12	0.030	0.034	0.018	0.103	0.071	0.021	0.027	0.027	0.033	0.094	0.071	***	0.982	0.934	0.992
13	0.027	0.039	0.019	0.114	0.071	0.036	0.046	0.028	0.024	0.096	0.060	0.018	***	0.934	0.975
14	0.052	0.032	0.083	0.101	0.143	0.062	0.063	0.045	0.051	0.159	0.060	0.068	0.068	***	0.924
15	0.039	0.040	0.022	0.100	0.077	0.020	0.032	0.033	0.035	0.105	0.077	0.008	0.025	0.079	***

sugarcane clones. Neil et al. (2009) studying on genetic diversity among main land USA sugarcane cultivars by SSR genotyping also reported molecular variation of 3.4% between populations and 96.6% within populations.

Among accessions of different source countries highly significant variation for molecular diversity ($P<0.01$) was observed between populations of Hawaii vs Canal point, Hawaii vs Demerara X Barbados and Hawaii vs India while the variation was significant ($P<0.05$) between Cuba vs Canal point, Demerara X Barbados vs Barbados, Demerara X Barbados vs Brazil, India vs Cuba, Louisiana vs Demerara X Barbados, Mauritius vs Demerara X Barbados, Mauritius vs Hawaii and Formosa (Taiwan) vs Demerara X Barbados. The molecular variation was not significant between other pairs of populations denoting shared alleles among them. This was supported by the results of the dendrogram in which identities of cultivars with respect to country of sources did not contribute to the clustering pattern because individual clusters include cultivars form different countries. This suggests that some genetically similar cultivars may be present in the different countries. In the dendrogram, it was also observed that accessions of countries with high molecular variation mostly didn't cluster together. Future breeding efforts involving crosses between accessions of countries with high molecular variation may provide beneficial genes and alleles in new sugarcane varieties while maintaining genetic diversity.

Population differentiation

Analysis of genetic differentiation among the accessions of countries revealed F_{ST} values ranging from 0.0024 (between Demerara (Guyana) and South Africa) to 0.5134 (between Cuba and India), which was a very large differentiation with an overall average of 0.059 (Table 7). High differentiation (F_{ST}=0.4113, $p<0.05$) was also observed between Barbados and Cuba and Canal Point and Cuba (F_{ST}=0.4112, $p<0.05$). Moderate gene differentiation among many pairs of populations was also evident (Table 7). The average gene flow (Nm) among populations was 1.7213. Nei's unbiased genetic distance ranged from 0.018 (between accessions of Puerto Rico and South Africa) to 0.279 (between accessions of Cuba and Mauritius) (Table 6) with an overall average of 0.053. Genetic identity values were in the range of 0.756 to 0.992 with overall average of 0.950 (Table 6). From these results, we can therefore say that a high percentage of genetic diversity is distributed among populations. This can give a clue that some exotic genotypes have their unique genetic

Table 7. Pair-wise F_{ST} values of the 15 populations (genotypes from respective source countries) of introduced sugarcane clones in Ethiopia, numbers representing populations here correspond to population numbers in Table 5.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0.0000														
2	0.0128	0.0000													
3	0.0567	0.0526	0.0000												
4	0.4113	0.2523	0.4112	0.0000											
5	0.0988	0.0911	0.0160	0.3911	0.0000										
6	0.0716	-0.0047	0.0860	0.1104	0.0912	0.0000									
7	0.0136	-0.0041	0.1133	0.3408	0.1658	0.0218	0.0000								
8	0.0244	0.0508	0.0213	0.5134	0.0556	0.1537	0.0851	0.0000							
9	0.0439	0.0341	0.0872	0.2586	0.1013	0.0031	0.0570	0.0858	0.0000						
10	0.1701	0.0977	0.0467	0.3469	0.0869	0.0462	0.1821	0.1686	0.0890	0.0000					
11	0.1043	0.0235	0.0490	0.1237	0.0414	-0.0732	0.1013	0.1934	0.0245	-0.0296	0.0000				
12	0.0137	0.0175	-0.0192	0.3407	0.0245	0.0424	0.0391	-0.0032	0.0217	0.0480	0.0498	0.0000			
13	0.0277	0.0112	-0.0062	0.2991	0.0088	0.0024	0.0527	0.0330	0.0143	0.0448	0.0133	-0.0274	0.0000		
14	0.1090	0.0102	0.1312	0.0231	0.1092	-0.0617	0.0871	0.2095	-0.0044	0.0732	-0.0689	0.0827	0.0381	0.0000	
15	0.0288	0.0308	0.0147	0.4076	0.0603	0.0560	0.0490	0.0286	0.0484	0.1058	0.0971	-0.0192	0.0124	0.1327	0.0000

constitution apart from each other which can be exploited for improvement of the crop in the future sugarcane breeding program of the country.

Conclusion

The present genetic analysis based on the segregation at SSR loci provides genetic information on the introduced sugarcane genotypes in the country. The genetic relationship information of the cultivars will help sugarcane breeders to select the appropriate parents in their breeding programs to maximize yield as well as to maintain genetic diversity.

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Conflict of Interest

The author(s) have not declared any conflict of interest.

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