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Morphological and Microsatellite-Based Molecular Characterization of Locally Collected Sugarcane (*Saccharum officinarum* L.)

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Fifty-three sugarcane accessions from Aklan, Iloilo, Cagayan, Nueva Vizcaya, Isabela, and Bohol were characterized using forty-two morphometric parameters and forty microsatellite markers. Twenty-six morphological characters using the Shannon-Weaver diversity index showed high variability (H' = > 0.76) and were able to characterize the fifty-three sugarcane accessions. Cluster analysis of morphological traits based on sequential agglomerative hierarchical test and Euclidean distance revealed two groupings at 0.3 coefficient of dissimilarity. The clustering of some accessions was irrespective of their geographical origin indicating a high degree of phenotypic similarity in some accessions. Out of the forty microsatellite markers, twenty-five (63%) have a PIC value greater than 0.5 with Sach 53 showing the highest at 0.89. This implies that these markers are suitable for sugarcane genetic diversity studies as they can detect a high number of discernable alleles. Molecular cluster analysis generated using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and Jaccard distance showed two clusters at 0.4 coefficient of dissimilarity. In cluster I, twenty-one accessions from Aklan and Iloilo grouped indicating genetic similarity. Aklan and Iloilo provinces are both located in the same geographic island in Western Visayas, hence, the observed grouping in cluster I. On the other hand, thirty-two sugarcane accessions from Northern Luzon (Cagayan, Nueva Vizcaya, and Isabela), Iloilo, Aklan, and Bohol were grouped in cluster II. The observed clustering of accessions from different regions implies the extensive distribution of sugarcane germplasm to the different parts of the Philippines.

Key Words: sugarcane, molecular characterization, microsatellites, morphometric, polymorphism information content

Abbreviations: PIC – polymorphism information content, UPGMA - unweighted pair group method with arithmetic mean, SSR – simple sequence repeats

INTRODUCTION

Sugarcane (*Saccharum* spp.), is a perennial monocotyledonous crop that belongs to the grass (Poaceae) family. It is the main source of sugar that supplies 86% of sucrose consumption of the world (OECD -FAO 2019). Aside from being a source of sugar, the plant also produces alcohol, a key component in the production of bioethanol. Bioethanol is a renewable source of energy that serves as an alternative to fossil fuel. Moreover, sugarcane produces the highest crop tonnage due to its high sucrose level and high yielding ability (Moore et al. 2013). It is for these reasons that the expansion of sugarcane production areas throughout the world is continuously growing. The industry itself provides livelihood to millions of rural communities around the world. The diverse uses of sugarcane for table sugar and bioethanol production make it an economically important crop.

The genus *Saccharum* is composed of tall perennial grasses (Poaceae) that are highly polyploids. The founding species of this genus are thought to be

S. spontaneum and *S. robustum* (Daniels and Roach 1987) which have a basic chromosome number of X = 8 and X =10, respectively (D'Hont et al. 1998; Ha et al. 1999). Modern sugarcane cultivars are interspecific hybrids (2n = 100 to 130), of which, 70-80% is attributed to *S. officinarum*, 10-20% to *S. spontaneum*, and the remaining 10% is a product of recombination between the two species (D' Hont et al. 1996). The sugarcane ploidy level and genome size vary among hybrid cultivars which makes it a complex genome. In 2016, a study on genetic analysis of Philippine sugarcane varieties by Adajar revealed chromosome mosaicism (2n = 80-120), a result of recombination, hybridization and selection (Arruda 2001). Moreover, autosyndetic chromosome pairing was observed as the frequency of bivalent formation is very high ranging from 68-100%. Unequal chromosome contribution from the parentals to the hybrids was also observed, wherein, on average, either 47 or 49 chromosomes were transmitted from either of the parents resulting in n + n transmission.

The advent discovery of microsatellites or simple sequence repeats (SSRs) has greatly aided plant genetic studies, especially in genotyping plants for the past 20 years. Specifically, this type of molecular marker has been used for wild species for genetic diversity studies based on genetic distance, estimation of gene flow and crossing over rates and evolution (Vieira et al. 2016). Microsatellite markers allowed geneticists and plant breeders to link phenotypic and genotypic variation (Mammadov et al. 2012; Hayward et al. 2015) through the combination of genetic, physical, and sequence-based maps (Temnykh et al. 2001).

Assessing the conservation and use of plant genetic resources is of major importance for plant breeders and geneticists because it offers a wide gene pool for crop improvement and genetic studies. Having adequate knowledge of existing genetic diversity can efficiently manage these plant genetic resources.

At present, evolutionary studies and genetic relations of Philippine sugarcane germplasm have not yet been thoroughly investigated. Genetic diversity assessment will not only provide information on genotypes that can be used as parents in breeding programs (You et al. 2013) but also, classify plant collections for conservation management (Wang et al. 2009). This study aims to assess the genetic diversity of locally collected sugarcane accessions using microsatellite markers and morphometric analysis.

MATERIALS AND METHODS

Fifty-three accessions of locally collected sugarcane planted at the experimental farm of the Institute of Plant Breeding, Tranca, Bay, Laguna were used in the study. Molecular activities were done at the Molecular Plant Breeding Laboratory of the Institute of Crop Science, College of Agriculture and Food Sciences, UPLB.

Experimental Design and Field Lay-out

The study was laid out in a Randomized Completely Block Design (RCBD) with three replications. The vegetative part of the plant was cut into two budded sets and planted within two rows with five hills each row. The plot measures 5 m x 1.5 m with a 20 cm planting distance in a row.

Morphological Characterization

The IPB-Philsurin sugarcane descriptor adapted from Moore (1987) was used for the morphological characterization of the sugarcane accessions. Ten representative plants per accession were characterized for morphometric traits. Data gathered includes characteristics of the stool, leaf blade, leaf sheath, auricle, dewlap, ligule, bud and stalk. For the characterization of color and tinge, the Royal Horticultural Society Color Chart was used. Data collection was done into three parts: 5 to 6 months, 7 to 8 months and 10 to 12 months.

SSR Characterization

The modified extraction protocol of Doyle and Doyle (1990) was adapted for genomic DNA extraction. Forty pairs of SSR primers (Table 1) with different motifs developed by Sanguillosa et al. (2019) of the Institute of Plant Breeding-UPLB were used in DNA profiling for the genetic diversity assessment of the different plant samples. After optimization, primer annealing temperatures were determined.

A total of 10 µl PCR reaction was prepared to contain the following: 6.75 µl of PCR grade water, 1 µl of 10 x PCR (Vivantis®) buffer with 0.4 µl of 2 mM of $MgCl_2$, 0.40 mM of dNTP mix (Vivantis®), 0.4 µl each of 0.4 mM stock of forward and reverse SSR primers (Invitrogen®) 0.15 µl of 0.75 units per ml (5U/ µl) of *Taq* DNA polymerase (Vivantis®) and 1 µl template DNA (100 ng/ µl).

Two microliters of each of the PCR products were electrophoresed in 8% denaturing polyacrylamide gel (8 x 10 cm) at 100 voltage for 2 hours in 1x TBE using ClearPAGE™ (C. B. S. Scientific Co., Del Mar, CA) electrophoresis apparatus. Gels were stained using gel red for 1 hour (20 µl SYBR Safe stock/200 ml of 1x Tris-borate-EDTA (TBE) and were visualized under UV light using GenoSens1510 gel documentation system (Clinx Science Instruments Co., Ltd.). Gel images were captured and saved for molecular marker analysis.

Table 1. Microsatellite primers developed for sugarcane and compiled by UPLB-IPB, utilized in the assessment of genetic diversity of 53 sugarcane accessions.

CODE	MOTIF	Orientation	PRIMERS (5' TO 3')	Ta (°C)	Size (bp)
Sach 1	$(TCGA)_3$	Forward	ATCCAAATATCCAATCAATCC	54.69	170
		Reverse	TCTCGTGTCATGTGTACTTGT		
Sach 2	$(CT)_{19}$	Forward	GTACAACTTTTGCTTCCTGTG	54.95	146
		Reverse	GGTACTCAGACCCTTGAATTT		
Sach 3	$(CAAA)_3$	Forward	TCGTAATCTCGGTCTACTTTTT	56.43	150
		Reverse	AAATTTTGGGGTGATCTAAAC		
Sach 5	$(GAA)_4$	Forward	GAAACTTTCGCTCTACTGCAT	55.31	154
		Reverse	AACACCTGAGCTTCAGACAG		
Sach 6	$(AATA)_4$	Forward	GTAAAATGTTTTTCCCCCTTA	54.03	150
		Reverse	TGTGTCAAAAGATTTGATGTG		
Sach 7	$(AGAAA)_3$	Forward	CTTTCCCTGCAAGATTTTC	55.34	119
		Reverse	ATGATCACAGTCTGAGAAAGG		
Sach 8	$(CAGG)_3$	Forward	TCGTAGACTGCGTACAACCA	55.98	101
		Reverse	CGCGTAGACATAGCAACTAAC		
Sach 9	$(AAG)_4$	Forward	GAATTTCTACTCCACCCATCT	54.81	146
		Reverse	TTAGCTTTCTTTTGTCCCTTT		
Sach 11	$(TGC)_4$	Forward	CGCGGTGAGAAGAATAGATA	55.20	157
		Reverse	GGCATGCTACAGTCTACTCC		
Sach 12	$(CCAG)_3$	Forward	ATATAAAAACCACTCCCGAAC	55.26	138
		Reverse	GTTACCGGTGGTGATGAG		
Sach 13	$(CATG)_3$	Forward	AAATGTGAATTCGTAGTGACG	56.77	172
		Reverse	TCTCAAAGAGAAGTGATCAACA		
Sach 14	$(ACTG)_3$	Forward	GGTGCTGATTTGTTATGAGAG	54.93	151
		Reverse	GAACATACTCCAGCGTTCAT		
Sach 15	$(CTC)_4$	Forward	ATATGTCAACCGAGTGTTTTG	54.64	234
		Reverse	CTGAGTTGGAGAAGAAAGAGG		
Sach 16	$(AGGC)_3$	Forward	CCTCTTTCTTCTCCAACTCAG	56.47	168
		Reverse	GCTGCCATCTTCTTTAACTC		
Sach 17	$(GGAA)_3$	Forward	GTGTTGGAATTCATACAAAATG	56.69	149
		Reverse	GCATTTGACCAAGACATTTAC		
Sach 19	$(CATG)_3$	Forward	AGCCTATCTCCTATGGCTATG	54.69	157
		Reverse	TCGTAGACTGCGTACATGAG		
Sach 20	$(AATA)_3$	Forward	TAAAATGTTGTTCCAACCATC	54.95	153
		Reverse	TCTGCTCATTTTGTTTGTTCT		
Sach 21	$(GAGGAC)_3$	Forward	GGAAGTAGAGGTGGTTCTTGT	54.81	150
		Reverse	CTGTTGTCGCTATCGTAATG		
Sach 22	$(GCA)_5$	Forward	GGCGACTAACTCTATCAACAA	56.43	156
		Reverse	TAGCAGCAAAGAGATGAATGT		
Sach 23	$(TATT)_3$	Forward	ATTTCACTGAACACGTATGCT	55.31	140
		Reverse	TTGTTTAGCTTCAAAATGGAC		
Sach 24	$(TGTA)_3$	Forward	AAGATGGTCACACTGACAAAG	54.67	165
		Reverse	AGTGATTCTGCTCGTAGACTG		
Sach 25	$(ATT)_5$	Forward	TATGAAAAGGCAGGCATACTA	55.34	143
		Reverse	GTCAACTTGTCAGAACCATGT		

Statistical Analysis

Standardized Shannon-Weaver diversity index (1949) was computed using the following formula:

For qualitative data,

$$H' = \sum Pi^*\log 2Pi/\log 2k$$

For quantitative data,

$$H' = \sum Pi^*\log 2Pi/\log 2n$$

Where, k refers to the number of descriptor states, n for the number of classes, and P*i* for the relative frequency.

To determine the distance between each accession and dissimilarity coefficient based on morphometric data, the sequential agglomerative hierarchical nested cluster analysis with the specifications of the Euclidean distance and Ward clustering method was employed in the Statistical Tool for Agricultural Research (STAR) program. The formula as described by Ward (1963) and Murtagh et al. (2014) is:

$$d(i \cup j,k)=([[(w_i+ w_k)/(w_i+w_j+w_k)]\ d^2(i,k)]+ [[(w_j+ w_k)/(w_i+w_j+w_k)]\ d^2(j,k)]-[(w_k)/ (w_i+w_j+w_k)\ d^2\ (i,j)])^{1/2}$$

Where *ijk* refers to the distance between plants *i* and *j* in character *k*.

To assess the allelic diversity at a given locus for a pool of genome, the Polymorphism Information Content (PIC) was used. The formula used to determine PIC as described by Botstein et al. (1980) is,

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 - 2\left[\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} p_i^2 p_j^2\right]$$

where p_i is the frequency of the i^{th} and j^{th} allele out of the total number of alleles at a single SSR locus and n is the number of alleles.

The clustering analysis based on the results of the SSR markers was generated using the software RStudio statistical language and environment version 1.1.423 to visualize the genetic relationship among sugarcane accessions based on the similarity values of sugarcane accessions. The generated dendrogram was based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and Jaccard index.

RESULTS AND DISCUSSION

Shannon-Weaver Diversity Index (H')

Forty-two quantitative and qualitative characters of internode, stool, bud, auricle, dewlap, leaf blade, leaf sheath, and ligule were computed for the Shannon-Weaver diversity index (Shannon and Weaver 1949). As adapted from Jamago (2000), H' values < 0.45 corresponds to low diversity, whereas H'= 0.46 - 0.75 is moderately diverse and H'= >0.76 highly diverse. Table 2 shows that the diversity index values for the 42 characters ranged from 0.14 to 0.99 with a distribution of 62% (26) highly diverse, 24% (10) moderately diverse and 14% (6) low diversity.

Table 1. Continuation.

CODE	MOTIF	Orientation	PRIMERS (5' TO 3')	Ta (°C)	Size (bp)
Sach 26	$(CATG)_3$	Forward	ATCCAAATATCCAATCAATCC	55.98	148
		Reverse	TCTCGTGTCATGTGTACTTGT		
Sach 27	$(TTCTTT)_3$	Forward	GTACAACTTTTGCTTCCTGTG	54.81	151
		Reverse	GGTACTCAGACCCTTGAATTT		
Sach 28	$(TTC)_4$	Forward	TCGTAATCTCGGTCTACTTTTT	55.20	159
		Reverse	AAATTTTGGGGTGATCTAAAC		
Sach 29	$(TGCA)_3$	Forward	GAAACTTTCGCTCTACTGCAT	54.92	162
		Reverse	AACACCTGAGCTTCAGACAG		
Sach 32	$(AT)_6$	Forward	GTAAAATGTTTTTCCCCCTTA	54.64	146
		Reverse	TGTGTCAAAAGATTTGATGTG		
Sach 36	$(TTGT)_3$	Forward	CTTTCCCTGCAAGATTTTC	55.11	173
		Reverse	ATGATCACAGTCTGAGAAAGG		
Sach 38	$(AAG)_4$	Forward	TCGTAGACTGCGTACAACCA	54.98	146
		Reverse	CGCGTAGACATAGCAACTAAC		
Sach 39	$(TGT)_4$	Forward	GAATTTCTACTCCACCCATCT	54.77	118
		Reverse	TTAGCTTTCTTTTGTCCCTTT		
Sach 40	$(TTTC)_4$	Forward	CGCGGTGAGAAGAATAGATA	55.31	143
		Reverse	GGCATGCTACAGTCTACTCC		
Sach 41	$(GCCG)_3$	Forward	ATATAAAAACCACTCCCGAAC	55.72	154
		Reverse	GTTACCGGTGGTGATGAG		
Sach 43	$(AACA)_3$	Forward	AAATGTGAATTCGTAGTGACG	55.14	159
		Reverse	TCTCAAAGAGAAGTGATCAACA		
Sach 45	$(TGGA)_3$	Forward	GGTGCTGATTTGTTATGAGAG	55.17	138
		Reverse	GAACATACTCCAGCGTTCAT		
Sach 46	$(TTTC)_3$	Forward	ATATGTCAACCGAGTGTTTTG	54.77	160
		Reverse	CTGAGTTGGAGAAGAAAGAGG		
Sach 51	$(AG)_6$	Forward	CCTCTTTCTTCTCCAACTCAG	54.33	134
		Reverse	GCTGCCATCTTCTTTAACTC		
Sach 52	$(TTGC)_3$	Forward	GTGTTGGAATTCATACAAAATG	54.51	127
		Reverse	GCATTTGACCAAGACATTTAC		
Sach 53	$(CGAA)_3$	Forward	AGCCTATCTCCTATGGCTATG	55.28	152
		Reverse	TCGTAGACTGCGTACATGAG		
Sach 54	$(GTGCGC)_4$	Forward	TAAAATGTTGTTCCAACCATC	54.87	136
		Reverse	TCTGCTCATTTTGTTTGTTCT		
Sach 55	$(AAAAAG)_3$	Forward	TATGAAAAGGCAGGCATACTA	55.26	166
		Reverse	GTCAACTTGTCAGAACCATGT		

All the quantitative characters had high diversity index with leaf blade width as the highest at H' = 0.85, followed by leaf blade length and bud length at H' = 0.84 with the bud width being the lowest at H' = 0.83 is the bud width. The result indicates that descriptor frequency per character is well distributed. According to Arrey and Mih (2016), these characters are important in differentiating sugarcane landraces. On the other hand, of the 38 qualitative characters, 21 are highly diverse with dewlap margin modulation as the most diverse (H'= 0.99), followed by dewlap shape and stool corky patch (H'= 0.94), dewlap waxiness and internode tinge (H'= 0.93) and stool waxiness (H'= 0.91).

Ten qualitative characters were moderately variable which includes stool node swelling, stool trashiness, stool leaf carriage, tillering density, and root primordia rows at H'= 0.75, H'= 0.72, H'= 0.70, H'= 0.69, and H'= 0.68, respectively. The growth ring width (H'= 0.45), plant habit (H'= 0.44), presence of genetic freckles (H'= 0.31), bud prominence (H'= 0.23), corky cracks (H'= 0.23) and stripes on canes (H'= 0.14) were almost similar in the 53 accessions.

In this study, 26 characters including dewlap margin modulation, dewlap shape, stool corky patch, dewlap waxiness, and internode tinge were the most outstanding characters for the characterization of the 53 accessions.

Cluster Analysis of Morphometric Data

Based on sequential agglomerative hierarchical nested cluster analysis with the specifications of the Euclidean distance and Ward clustering method, two groups were observed at a 30% coefficient of dissimilarity (Figure 1). Cluster I grouped 29 genotypes, wherein, 17 were from Iloilo, 5 from Aklan, 4 from Isabela, 2 from Nueva Vizcaya and 1 from Bohol. Meanwhile, the 24 genotypes that grouped in cluster II were mainly from Aklan (13), followed by Isabela (8) and Iloilo (3).

The results revealed different groupings as observed in the molecular analysis. Accessions from the same geographic region did not group together and some grouped in the same cluster irrespective of their geographical origin. The group of Tahir (2013) also observed the same result. This suggests that some genotypes from different regions have a degree of similarity phenotypically.

Moreover, geographic diversity is independent of that of genetic diversity as reported by Anand and Rawat (1984). Because sugarcane is propagated asexually, as practiced in this study, the expected genetic difference accumulated through time is not that high (Moore 1987).

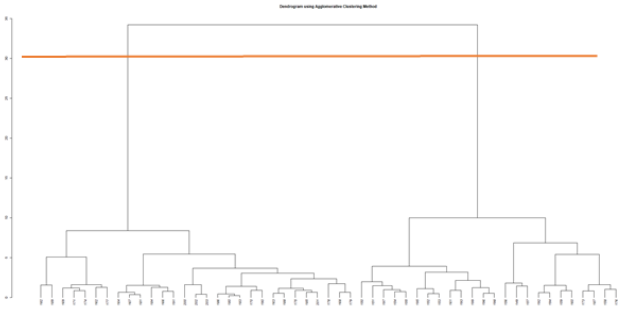

Fig. 1. Dendrogram produced by sequential agglomerative hierarchical nested (SAHN) cluster analysis of 53 sugarcane accessions using the coefficient of dissimilarity of morphometric data.

Table 2. Shannon-Weaver Diversity Index (H') on 42 quantitative and qualitative characters of 53 sugarcane accessions.

PLANT PART	TRAIT	MEAN	H'
Leaf Blade[QT]	Length	129.5 cm	0.84
	Width	43.89 cm	0.85
Bud [QT]	Length	9.46 cm	0.84
	Width	8.31 cm	0.83
Stool [QL]	Plant habit	-	0.44
	Tillering habit	-	0.77
	Tillering Density	-	0.69
	Tops	-	0.80
	Leaf Carriage	-	0.70
	Trashiness	-	0.72
	Waxiness	-	0.91
	Stripes on cane	-	0.14
	Corky cracks	-	0.23
	Corky patch	-	0.94
	Internode shape	-	0.56
	Node swelling	-	0.75
	Growth ring width	-	0.45
	root primordia rows	-	0.68
	root band shape	-	0.87
	Bud Shape	-	0.49
	Bud prominence	-	0.23
Dewlap [QL]	Waxiness	-	0.93
	Primary color	-	0.84
	Secondary color	-	0.80
	Shape	-	0.94
	Dewlap margin modulation	-	0.99
	Hair	-	0.80
Leaf Blade[QL]	Leaf Blade color	-	0.83
	Leaf Blade Texture	-	0.83
	Erectness	-	0.77
	Presence of genet-ic freckles	-	0.31
Leaf Sheath [QL]	Waxiness	-	0.85
	Primary Color	-	0.89
	Secondary Color	-	0.86
	Presence of tri-chomes	-	0.53
	Trichome quality	-	0.62
Ligule [QL]	Shape	-	0.77
	Hairness	-	0.51
Auricle [QL]	Inner auricle shape	-	0.90
	Outer Auricle shape	-	0.76
Internode [QL]	Color	-	0.88
	Tinge	-	0.93

[QT] Quantitative trait
[QL] Qualitative trait

Molecular Characterization

Plants, including sugarcane, have abundant repetitive motifs as observed by the group of Cordeiro (2000) based on genomic library screenings. They have found that the most common repeats are dinucleotide $(TG)_n/(CA)_n$ and trinucleotide $(TAC)_n/(GTA)_n$ with an average of 15 and 13 repeat motifs. Another study based on EST database generated SSR markers that were used in sugarcane molecular genotyping (Pan et al. 2001) and genetic variability assessment (Cordeiro et al. 2003). The hypervariability of microsatellites makes it a powerful marker that offers a wide range of applications in sugarcane genetics and breeding such as high-density genetic mapping, molecular tagging of genes, genotype identification, genetic diversity analysis, hybridity testing, and marker-assisted selection (Chen et al. 2009).

Characterization of Microsatellite Markers and Analysis of Banding Patterns

Forty microsatellite primers (Table 1) were screened for patterns of amplification in fifty-three sugarcane accessions based on strength, clarity of banding patterns, PCR quality and polymorphic amplification in the expected band size. Subsequently, all primers that amplified a specific band were used for the fingerprinting analysis of the sugarcane accessions. A representative gel (Figure 2) shows the differential banding pattern of 27 sugarcane accessions in microsatellite marker Sach 19. A total of 5 alleles were observed, of which, 2 alleles are polymorphic. This shows that marker Sach 19 can detect polymorphisms among sugarcane accessions.

Based on the banding pattern results in Table 3, thirty-two primer pairs (80%) amplified polymorphic and easily scorable PCR products. Four primer pairs (Sach 16, Sach 26, Sach 27, and Sach 52) showed monomorphic amplicons, while another four primers (Sach 21, Sach 24, Sach 29, and Sach 39) did not amplify at all.

Analysis of the allelic composition revealed a total of 135 alleles across genotypes with an average of 3.75 alleles per individual. The observed number of alleles per locus in individual markers ranged from 2 to 10 with band sizes ranging from 120 to 340 bp. Of the 135 alleles observed, there were 124 rare/polymorphic alleles an equivalent to 91%. On the other hand, the highest number of alleles per locus was from Sach 53.

Among the primers used, 25 (63%) have a PIC value greater than 0.5, indicating high polymorphism (Prabakan 2010; Thatikunta et al. 2016). PIC values evaluate the relative informativeness of a given marker (Botstein et al. 1980) and provide an account of the differentiation power of an allele (Smith et al. 1997). Markers that have > 0.5 PIC value are highly informative because they can detect a high number of discernable alleles which makes them suitable for genetic diversity studies (Islam et al. 2012). The markers used showed a lower number of alleles as compared to the SSR markers used by Cordeiro et al. (2003) and You et al. (2013).

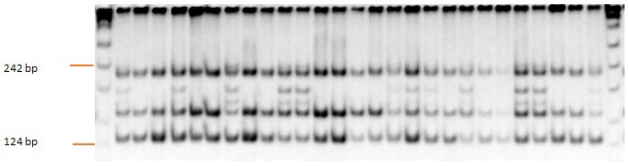

Fig. 2. Gel electrophoresis of sugarcane microsatellite marker Sach 19 across 27 sugarcane accessions.

Table 3. Allele size variation, number of alleles, number of polymorphic bands and polymorphic information content exhibited by the forty microsatellite markers.

MARKER	PSV (bp)	NA	NPB	PIC
Sach 1	130-220	8	8	0.84
Sach 2	150-190	5	5	0.76
Sach 3	130-180	4	4	0.59
Sach 5	150-220	5	4	0.65
Sach 6	230-340	6	6	0.78
Sach 7	150-180	3	3	0.65
Sach 8	150-160	2	2	0.49
Sach 9	140-180	3	3	0.51
Sach 11	170-180	2	1	0.39
Sach 12	140-220	5	4	0.75
Sach 13	150-210	4	4	0.72
Sach 14	140-230	6	6	0.76
Sach 15	120-210	9	9	0.85
Sach 16	150	1	0	0
Sach 17	150-180	3	3	0.67
Sach 19	130-230	5	2	0.75
Sach 20	120-220	6	6	0.79
Sach 21	-	-	-	-
Sach 22	160	1	1	0
Sach 23	160-280	4	4	0.62
Sach 24	-	-	-	-
Sach 25	200-300	2	2	0.46
Sach 26	150	1	0	0
Sach 27	150	1	0	0
Sach 28	130	1	1	0
Sach 29	-	-	-	-
Sach 32	130	1	1	0
Sach 36	120-160	3	3	0.34
Sach 38	150	1	1	0
Sach 39	-	-	-	-
Sach 40	140-260	5	4	0.72
Sach 41	130-190	4	4	0.73
Sach 43	140-180	5	5	0.66
Sach 45	140-190	4	4	0.71
Sach 46	150-200	3	3	0.59
Sach 51	290-350	2	2	0.50
Sach 52	120	1	0	0
Sach 53	130-250	10	10	0.89
Sach 54	120-160	4	4	0.62
Sach 55	150-210	5	5	0.79
Mean		3.75	3.44	0.58
Min		1	0	0
Max		10	10	0.89

[1]Product Size Variation
[2]Number of Allele
[3]Number of Polymorphic Allele
[4]Polymorphism Information Content

The allele banding profile of the 53 sugarcane accessions is shown in Table 4. The results revealed that the number of primers that generated a unique banding pattern varied from 25 to 31. This is in confirmation of the result in Table 9 that > 63% of the primers provided informative banding results in differentiating the accessions. The observed range of 1-4 to 1-9 bands per primer and total bands of 49 to 78 further support the result. The amplicon size ranges from 110 to 350 bp across all samples. The observed number of bands corresponds to the complexity of the sugarcane genome which is an octoploid.

Results from this study showed that the use of Microsatellites allowed the detection of high-level polymorphisms among sugarcane accessions tested. Using thirty-two pairs of primers, a highly informative allelic distribution was obtained that could be used to discriminate sugarcane genotypes that are indistinguishable by other marker types.

Cluster Analysis of Molecular Data

Jaccard distance was used to calculate genetic dissimilarity and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was used to generate a dendrogram, as shown in Figure 3. The analysis showed that the accessions can be grouped into two clusters (I-40% and II-60%) at a 0.4 dissimilarity coefficient. Cluster I, with 21 genotypes, was entirely composed of accessions coming from Aklan (62%) and Iloilo (38%). This is quite expected given that the two provinces are within the same geographic island of Western Visayas and that exchanges of planting materials are common among sugarcane farmers (Billig 1992). Cluster II, on the other hand, were predominantly composed of accessions from Northern Luzon (Cagayan, Isabela, and Nueva Vizcaya) (47%) and Iloilo (38%). The remaining 15 % genotypes were from Aklan and Bohol.

The 60% similarity of genotypes in cluster II implies that extensive distribution of sugarcane germplasm is not only limited to the Visayas region, where the sugarcane breeding institutions (Sugar Regulatory Administration and Philippine Sugar Research Institute) are located. These institutions were established in the 1980s (SRA) and 1990s (PHILSURIN) to meet the executive mandate on strengthening the Philippine sugar industry. However, before the establishment of these institutions, sugarcane has been long cultivated in the country. The extent of sugarcane germplasm distribution to northern Luzon is a manifestation of the trade of local agricultural products, especially during Spanish colonization and within that era, the exchange of planting materials is not yet restricted (Wolters 1992).

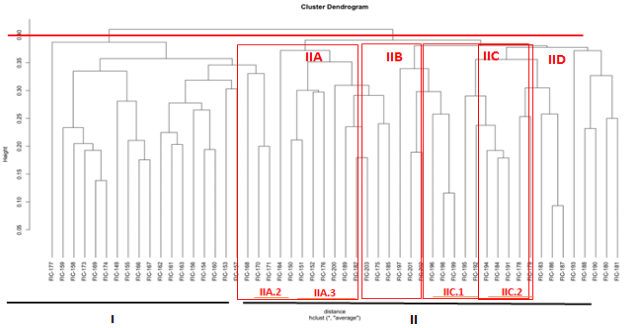

Fig. 3. Dendrogram produced by UPGMA cluster analysis using Jaccard distance based on electrophoretic separation of DNA fragments based on microsatellites.

Table 4. Allele banding profile of 53 sugarcane (*Saccharum officinarum* L.) accessions showing unique banding pattern, amplified bands per primer, total bands observed and amplicon size.

ACCESSION	NO. OF PRIMERS THAT GENERATED UNIQUE BANDING PATTERN	AMPLIFIED BANDS PER PRIMER	TOTAL BANDS OBSERVED	AMPLICON SIZE (BP)
FIC-149	27	1-4	57	120-350
FIC-150	30	1-4	63	110-350
FIC-151	29	1-5	67	120-350
FIC-152	30	1-5	69	110-350
FIC-153	27	1-5	61	120-350
FIC-154	29	1-4	67	120-350
FIC-155	30	1-5	71	110-350
FIC-156	27	1-4	61	110-350
FIC-157	28	1-5	65	110-350
FIC-158	29	1-5	65	110-350
FIC-159	27	1-5	61	110-350
FIC-160	29	1-4	62	110-350
FIC-161	28	1-4	62	120-320
FIC-162	29	1-5	64	110-350
FIC-163	30	1-5	68	110-350
FIC-164	29	1-4	69	110-350
FIC-166	30	1-5	72	110-350
FIC-167	31	1-5	69	110-350
FIC-168	29	1-5	61	120-350
FIC-169	29	1-5	69	110-350
FIC-170	25	1-5	49	120-350
FIC-171	26	1-4	57	120-350
FIC-173	27	1-5	61	110-350
FIC-174	29	1-5	68	110-350
FIC-175	28	1-5	68	110-290
FIC-176	28	1-5	73	120-350
FIC-177	29	1-5	76	110-350
FIC-178	30	1-6	76	120-350
FIC-179	30	1-5	66	120-350
FIC-180	29	1-9	64	110-350
FIC-181	28	1-6	63	110-350
FIC-182	30	1-8	73	120-350
FIC-183	29	1-5	62	110-350
FIC-184	30	1-6	71	110-350
FIC-185	31	1-6	76	110-350
FIC-186	31	1-7	76	110-350
FIC-187	30	1-8	74	110-350
FIC-188	28	1-8	59	110-350
FIC-189	28	1-8	73	110-350
FIC-190	29	1-8	69	110-350
FIC-191	27	1-8	64	120-350
FIC-192	26	1-5	61	110-350
FIC-193	27	1-5	63	110-350
FIC-194	31	1-9	76	110-350
FIC-195	31	1-4	69	110-350
FIC-196	31	1-9	78	110-350
FIC-197	29	1-6	71	120-350
FIC-198	28	1-5	65	110-350
FIC-199	31	1-5	73	120-350
FIC-200	32	1-5	78	110-350
FIC-201	27	1-5	70	110-350
FIC-202	30	1-6	73	110-350
FIC-203	30	1-7	77	110-350

Upon closer look in cluster II, 4 sub-cluster (IIA, IIB, IIC, and IID) can be observed at 0.38 dissimilarity coefficient. In sub-cluster IIA, accession FIC-164 of Aklan separated solely from the rest of the group. Sub-cluster IIA is further divided into two clusters (IIA.2 and IIA.3), wherein, FIC-150, FIC-151, and FIC-152, the remaining Aklan accessions, grouped with FIC-176 of Iloilo to form IIA.2 cluster. Genotypes from Iloilo (FIC-175, FIC-182, and FIC-185), Isabela (FIC-189), Nueva Vizcaya (FIC-200) and Bohol (FIC-203) formed cluster IIA.3.

The six accessions in sub-cluster IIB all came from the provinces of Isabela (FIC-196, FIC-197, FIC-198, FIC-199, and FIC-201) and Nueva Vizcaya (FIC-202). Meanwhile, sub-cluster IIC divided further into two clusters separating five Iloilo (IIC.2) genotypes from four Isabela (IIC.1) genotypes. Sub-cluster IID is composed of five genotypes from Iloilo (FIC-180 and FIC-181), Isabela (FIC-190 and FIC-193) and Cagayan (FIC-188).

Results of the cluster analysis based on SSR molecular makers revealed different groupings as compared to that based on morphological characters. Genotypes that appeared similar in SSR analysis were clustered in different groups implying that they had different morphological characters. Also, the range of genetic distance based on morphological data was narrow (0.05-0.33) as compared to that of SSR-based data (0.08-0.4). This showed that SSR markers provided more information in distinguishing some genotypes that are not morphologically distinguished. This may be due to the influence of environment and cultural practices on the phenotypic expression of traits (Cortese et al. 2010).

CONCLUSION

The Microsatellite markers used detected high-level polymorphisms among sugarcane accessions and can be effectively applied in the identification of sugarcane germplasm and genetic diversity studies. Morphological characterization employed in the study did not found complementary with SSR-based markers in characterizing the different sugarcane accessions. The dendrogram based on morphological characters revealed different groupings as observed from that of the molecular analysis. This suggests that some genotypes from different regions have a degree of similarity phenotypically.

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