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Analysis of genetic diversity in selected sugarcane (*Saccharum officinarum* L.) accessions using inter simple sequence repeat (ISSR) markers

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Abstract: Genetic diversity information among a population is important in exploiting heterozygosity for the improvement of crop species through breeding programmes. This study was therefore, conducted to assess genetic diversity and establish molecular relationships among 20 selected exotic sugarcane accessions from the Unilorin Sugar Research Institute germplasm using Inter Simple Sequence Repeat (ISSR) molecular markers. Genomic DNA was extracted from the sugarcane leaf. Fragments amplification was then performed by polymerase chain reaction (PCR) with ISSR markers and the data obtained were analyzed using MEGA 4 software. Analysis of the electropherogram showed a total of 39 loci consisting of 369 bands, out of which 95.8% were polymorphic. The biplot analysis showed all the markers contributed to the observed diversity with the least achieved with ISSR6. The principal co-ordinate analysis grouped the accessions into four clusters, comprising mixtures of all the six collection sites. The polymorphism obtained in the present study showed that the ISSR markers are effective for assessment of genetic diversity of the sugarcane accessions as it reveals the genetic similarity or divergence of the accessions regardless their place of origin or cultivation.

Key words: Dendrogram; genetic diversity; germplasm resources; ISSR marker; sugarcane

Analiza genetske raznolikosti izbranih akcesij sladkornega trsa (*Saccharum officinarum* L.) z uporabo označevalcev na osnovi enostavnih ponavljajočih se zaporedij (ISSR)

Izvleček: Informacija o genetski raznolikosti znotraj populacij je pomembna za uporabo heterozigotičnosti za izboljšanje gojenih rastlin v žlahtniteljskih programih. Ta raziskava je bila narejena za oceno genetske raznolikosti in vzpostavitev molekularnih povezav med 20 izbranimi ekzotičnimi akcesijami sladkornega trsa na Inštitutu za preučevanje genetskih resursov sladkornega trsa v Unilorinu (Sugar Research Institute germplasm) z uporabo molekularnih markerjev na osnovi enostavno ponavljajočih se zaporedij. Genomska DNK je bila ekstrahirana iz listov sladkornega trsa s pomočjo mini kita (DNeasy Mini Kit,Qiagen). Namnožitev fragmentov je bila izvedena s polimerazno verižno reakcijo (PCR) z ISSR označevalci, pridobljeni podatki so bili analizirani s programom MEGA 4. Analiza elektroferogramov je pokazala, da je celokupno število 39 lokusov sestavljalo 369 trakov, od katerih je bilo 95,8 % polimorfnih. Biplot analiza je pokazala, da so vsi označevalci prispevali k opaženi raznolikosti, z najmanjšim deležem označevalca ISSR6. Analiza glavnih komponent je združila akcesije v štiri skupine, ki so bile mešanica vzorcev iz vseh šestih vzorčenih mest. Polimorfizem, ugotovljen v tej raziskavi je pokazal, da so vsi ISSR označevalci učinkoviti za ugotavljanje genetske raznolikosti akcesij sladkornega trsa ne glede na njihovo mesto izvora in načina gojenja.

Ključne besede: dendrogram; genetska raznolikost; genetski viri; ISSR označevalci; sladkorni trs

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1 INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is one of the most important economic crops. In Nigeria, sugarcane is mainly grown for its sugar juice, as a raw material for manufacturing sugar (Wayagari et al., 2003a,b), molasses and bagass, and recently ethanol and renewable energy. As an industrial crop, sugarcane production has contributed to the nation's GDP and provided the opportunities for job creation. Other use of sugarcane include; fertilizer, bio-plastics, paper, sugarcane wax (Khushk and Pathan, 2006).

Commercial cultivation is mainly through planting of vegetative cutting (setts) of mature stalks. During sugarcane breeding programs, exchange and shipment of elite clones and breeding lines in the form of stalk cuttings across different test locations occur regularly for the purpose of verifying parental source or desired use of a clone in an experiment (Pan, 2010). To increase sugar yield within the Nigerian sugar industry, it is important to optimize varietal trials and breeding activities of sugarcane (NSDC, 2015).

In Sugarcane breeding, mislabeling errors could occur during the process of planting and selection, due to the use of large number of accessions in the varietal development program, which can only be revealed later in the selection program. Mislabeling could alter breeding goals by using the wrong variety for breeding activities. Molecular tools ensure that breeders have the correct clones involved in their crosses as well as varietal trials (Pan, 2010). Diversity analysis based on morphological attributes may not be sufficient or may be inflated due to environmental influences, particularly for new varieties or those that are new to a region (Animasaun et al., 2015). In these situations, a more accurate and clear means of identification is required to avoid downstream consequences such as risk of disease outbreak and/or poor productivity.

The use of molecular marker techniques such as RFLP (Restriction fragment length polymorphism), SSLP (Simple sequence length polymorphism), AFLP (Amplified fragment length polymorphism), RAPD (Random amplification of polymorphic DNA), ISSR (Inter simple sequence repeat), SSR Microsatellite polymorphism (Simple sequence repeat), SNP (Single nucleotide polymorphism), RAD markers (Restriction site associated DNA makers) etc, in the analysis of genetic variation among genetic materials, has facilitated correct determination of the nature of association among economic traits. This is because the techniques ensure development of accurate genetic maps since they are devoid of environmental influences, thereby helping to achieve set objectives within breeding programs leading to the achievement of significant yield increases in breeding programmes (Dilon et al., 2007).

The use of Inter Simple Sequence Repeat (ISSR) marker in crops plant diversity study and fingerprinting is advantageous (Animasaun et al., 2015). The markers can detect a range of loci and allelic diversity among the genetic materials (Ajibade et al., 2000, Pfeiffer et al., 2011) as well as provide information on their unique identity that deserves conservation attention and improvement programmes (Da Costa et al., 2011, Animasaun et al., 2021). Furthermore, molecular-based information obtained would be a reliable basis for developing a template and workable strategy for germplasm conservation and future improvement through the selection of appropriate parents to maximize yield, establishment of proper identity of the genotypes and maintain genetic diversity. The approach also has the capacity to provide useful information on the extent of genetic diversity among the germplasm accessions, and prevent possible misidentification, which may render the work previously done during selection unreliable.

The ultimate goal in sugarcane breeding is to develop genetically improved varieties with high sugar yield (cane yield and sucrose content) that is economically sustained over several ratoon crops. Therefore, germplasm materials are usually assessed for their breeding behaviour with the objective of utilizing them either for direct cultivation on the sugar estates or as parents in hybridization for evolving new and superior progenies intended as replacement to the existing cultivars (Kwajaffa & Olaoye, 2014). In an effort to identify productive sugarcane varieties for the rainforest and savanna ecologies of Nigeria, a detailed evaluation of 40 selected sugarcane varieties from six (6) breeding stations was conducted under the auspices of the West Africa sugarcane Development (WASD) Project (Olaoye et al. 2017), which provided some information on morphology, cane yield and yield components.

However, in order to have a proper diagnostic assessment of the genetic attributes of these varieties, twenty accessions were further selected for genetic diversity and allelic polymorphism assessment using the molecular approach. The objectives were to characterize the selected sugarcane accessions using ISSR marker and provide detailed information on the nature of genetic diversity among them for further varietal improvement activities.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

Twenty (20) exotic sugarcane accessions were selected from the pool of germplasm materials from six sugarcane breeding stations currently being maintained at the Unilorin Sugar Research Institute (USRI) Farm. The accessions were selected base on the morphological superiority reported elsewhere (Olaoye et al., 2017). Details of their origin, parentage (where available) and yield attributes are contained in Table 1.

2.2 DNA ISOLATION

Genomic DNA (gDNA) was isolated from young unfolding leaf tissues. About 1 g of fresh leaf tissue was grounded into a fine powder in prechilled mortar and genomic DNA from individual accession was extracted using, DNAeasy Plant Mini Kit (QIAGEN, USA). The DNA extraction was performed in accordance with the manufacturer's instruction. DNA concentration were determined comparatively by electrophoresis at current of 100 amps, 80 volts, for 40 minutes using agarose gel (0.8 %) electrophoresis, by applying 5 µl gDNA loaded after mixing with 3 µl 6X loading dye (Promega, USA) to check the quality of the DNA by comparing the intensity of the bands with a 1kb standard (Thermo Scientific, USA). The gel was visualized under a UV transilluminator and the gel was imaged with a gel documentation system (Ingenius-3, Syngene, USA) to confirm the quality of the genomic DNA.

Table 1: List of selected 20 Sugarcane varieties and their attributes

					Parental identification	
S/N	Variety	Point of collection	°Brix content (°Bx):	Cane yield (t ha^{-1})	Female	Male
1	B97375	Barbados	NA	NA		
2	B96812	Barbados	NA	NA		
3	B96723	Barbados	NA	NA		
4	B93757	Barbados	23.71	58.52		
5	B47419+	Barbados	20.13	72.89		
6	DB8134	Demarara	NA	NA		
7	M1176/77	Mauritius	22.31	81.91	N55805	CP5530
8	M1246/84	Mauritius	21.2	93.40	M555/60	R570
9	M1334/84	Mauritius	19.58	95.55	M555/60	
10	M1954/91	Mauritius	21.89	69.38	M2077/78	M1030/71
11	RB72/454	Brazil	21.26	58.89	CP53-76	
12	RB86/3129	Brazil	21.63	608.09	RB763411	
13	SP81-3250	Brazil	20.53	72.09	CP70-1547	SP71-1279
14	RB94/2991	Brazil	22.89	66.42		
15	Co88025	Coimbatore	20.92	59.00		
16	Co91017	Coimbatore	21.27	64.64		
17	Co997+	Coimbatore	21.48	79.29		
18	CoC671	Coimbatore	22.63	72.80		
19	KNB9288	Sudan	NA	NA	B871245	POLY
20	KNB9253	Sudan	NA	NA		

Brix content (°Bx): This is the proportion of sucrose in a solution, therefore its correlates with density of liquid. One degree brix is 1 g of sucrose in 100 g of solution
Sources: G. Olaoye, Y. A Abayomi and F.O .Takim (2017). NSDC 2015
NA: Information not available

2.3 ISSR PRIMER SELECTION AND PCR CONDITION

Eight reproducible and informative ISSR primers of 14-19 bp were selected from a total of 10 tested ISSR primers. The primers were selected based on published experimental results on sugarcane and related *Saccharum* species (Da Costa *et al.*, 2011). The selected primers synthesized by a commercial molecular biology company (Inqaba Biotec West Africa Ltd. Ibadan, Nigeria) were used for the Polymerase Chain Reaction (PCR) procedure. The primers were optimized and PCR conditions for experiment were set-up. For an efficient molecular characterization of sugarcane genotypes, initially six individuals were randomly selected to screen the primers for their polymorphism and reproducibility at 52, 54, 57, 61, 62 and 65 °C annealing temperature (Table 2). Band intensity and reproducibility of all conditions were compared and optimized. The selection includes a spectrum of primers with different repeat motifs.

The PCR reaction was carried out with 20 µl final reaction volume in 200 µl thin wall PCR tube in a Thermocycler (Applied Biosystems, Foster city, USA), containing 10.5 µl reaction mixture, 1.5 µl of 10 pmole primer 1 µl of 50 ng genomic DNA and 7 µl of ultra-pure nuclease free water (Ambion, USA). The PCR condition was an initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 1 sec, annealing at 59 °C (annealing temperature was different for each primers) for 30 sec and extension at 72 °C for 1 min followed by a single cycle of final extension at 72 °C for 10 min. The reaction was put on hold at 4 °C. The PCR products were visualized to confirm amplification by the method of Animasaun et al., 2018.

The targeted PCR amplification was confirmed by mixing 5 µl of PCR product with 2 µl of 6X gel 6X gel loading dye (Promega, USA) and electrophoresed on 1.5 % agarose gel stained with 0.75 µl EN-Vision blue eye DNA dye for 40 min at 100 volts in 1X TBE buffer. Fragment sizes of the amplicons were determined from the gel by comparison with standard molecular weight marker ladder-low range Generuler1 Kb DNA Ladder (Thermofisher, USA). The amplified loci were visualized and photographed in a gel documentation system (Ingenius-3, Syngene, USA).

2.4 BAND SCORING AND DATA ANALYSIS

The PCR fragments were scored for the presence (1) or absence (0) of equally sized bands and two matrices of the different ISSR phenotypes were assembled and used in the statistical analysis. The fragments were only considered on ability to detect clearly resolved and polymorphic amplified loci among the populations studied for the eight ISSR primers selected for analysis. The data were entered in to binary matrix for analysis. Module analysis was performed with NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System) and Cluster Analysis was performed using the unweighted pair group method with arithmetic averages (UPGMA), genetic differentiation and Shannon's index (I) was determined using PAST software.

In addition, to compare genotypes and evaluate patterns of genotype clustering, neighbour-joining (NJ) was used with Free Tree 0.9.1.50 (Saitou & Nei, 1987; Pavliček et al., 1999). To further examine patterns of genetic relationship among individual genotypes principal coordinate analysis (PCoA) was performed using agglomerative technique using the Un-weighted Pair Group Method with Arithmetic Mean (UPGMA) Method and dendrogram was constructed as the output for the genetic relationship.

Table 2: List and properties of ISSR primers selected for the molecular study of the molecular characterization of 20 exotic sugarcane varieties (Ilorin, Nigeria)

S/N	Oligo ID	Sequence (5' – 3')	*Tm (°C)	No of base pairs	GC- contents (%)
1	ISSR 1	GAGAGAGAGAGACC	52.61	14	57.14
2	ISSR 2	CTCTCTCTCTCTCTCTAC	57.62	18	50.00
3	ISSR 3	CACACACACACAAG	49.69	14	50.00
4	ISSR 4	CAGCACACACACACACA	60.16	19	52.63
5	ISSR 5	GTGTGTGTGTGTCC	52.61	14	57.14
6	ISSR 6	CTCTCTCTCTCTCTCTGC	59.9	18	55.56
7	ISSR 8	AGCACGAGCAGCAGCGG	64.43	17	70.59
8	ISSR 10	AGCACGAGCAGCAGCGT	62.02	17	64.71

*Tm: melting temperature of the primers

3 RESULT AND DISCUSSION

Genetic diversity in sugarcane provides breeders with the necessary materials and opportunity to develop improved and new varieties possessing desirable characteristics (Govindaraj et al., 2015). Molecular analysis is important to protect genetic identity/purity because, morphological traits could be environmentally influenced (Animasaun et al., 2018). This is also necessary for authentication of accession prior to multiplication of setts for planting. Data obtained from molecular techniques can be analysed prior to application in diversity studies by analyzing the genetic relationship among samples (Govindaraj et al., 2015).

3.1 LEVEL OF POLYMORPHISM

The ISSR analysis, carried out on 20 varieties produced 369 bands/alleles with an average of 46.13 alleles per primer. Eight primers produced distinct and reproducible bands among the primers tested and the amplified PCR products showed arrays of monomorphic and polymorphic bands, 349 were polymorphic alleles and 20 monomorphic alleles with an average of 43.25 ISSR polymorphic alleles per primer**.** In other words, the ISSR markers detected high level of polymorphism. A total of 39 loci were amplified by eight ISSR primers, out of which 38 (91.66 %) were polymorphic. Loci amplification per primer ranged from 3 to 7 with an average of 4.88 loci per primer, and a mean allelic richness of 46.1 alleles/primers (Table 3), number of polymorphic alleles ranged from 2 (ISSR 6) to 7 (ISSR8). The code, sequence and other properties of the primers used are presented in Table 2. Though, seven out of eight of the primers were highly polymorphic, ISSR6 resulted in the least polymorphic loci with 33.3 % polymorphism, ability of primer ISSR 8 and ISSR 10 to produce higher allele frequencies and polymorphic loci in this study indicated that the two primers are most informative and suitable for diversity study in sugarcane accessions.

ISSR markers have been showed to possess high resolution ability in sugarcane fingerprinting and diversity analysis (Da Costa et al., 2011), they are effective and efficient in the identification of polymorphisms within and among populations and/or species. This current study is the first investigation to assess molecular genetic diversity within and among introduced sugarcane accessions in the USRI germplasm. Again, since polymorphic information is related to expected heterozygosity and is usually determined from allele frequency (Animasaun et al., 2015), the existing variation in the studied accession could be selected for the crop improvement.

The present study reveals the existence of high level of genetic diversity and relatedness among and within the investigated sugarcane accessions, which were introduced into Nigeria as part of University of Ilorin Sugar Research Institute (USRI) germplasm. Similar findings were reported for some sugarcane accessions by Srivastava and Guota (2008) who recorded 78.48 % polymorphism in a diversity screening among sugarcane varieties in India using ISSR markers. Smiullah et al. (2013) also detected 85.25 % polymorphism with ISSR markers on Sugarcane accessions from Pakistan. Thus, the studied genotypes showed considerable heterologous amplification of the alleles, whereby 91.66 % were polymorphic and only 8.34 % were monomorphic. High polymorphism and higher number of alleles are very important for correct estimation of genetic diversity of a germplasm (Animasaun et al., 2015). The degree of polymorphism

Table 3: Amplification information of 8 ISSR markers used in the diversity study of the 20 exotic sugarcane accessions in the germplasm of USRI

S/N	Marker Code	TNA	TNL	NML	NPL	P %	M %
1	ISSR1	32	5	0	5	100	-
2	ISSR 2	34	5	0	5	100	-
3	ISSR 3	16	4	0	4	100	-
4	ISSR4	50	5	0	5	100	-
5	ISSR 5	49	4	0	4	100	-
6	ISSR 6	30	3	1	2	33.3	66.6
7	ISSR 8	81	7	0	7	100	-
8	ISSR 10	77	6	0	6	100	-
	TOTAL	369	39	1	38	91.66 %	8.3%
	Average	46.1	4.88				

TNA: Total number of alleles; TNL: total number of loci; NML: Number of monomorphic loci; NPL: Number of polymorphic loci; P %: percentage polymorphism; M %: Percentage monomorphism

showed the extent of diversity and effectiveness of the markers (Pfeifer et al., 2011) and allele phenotype are use as reference to interpret microsatellite profile in diversity studies (Esselink et al., 2004).

3.2 GENETIC SIMILARITY AND DISTANCE

Estimate of similarity coefficient was determined using Jaccard similarity coefficient-based pairwise comparisons, based on the DNA amplification of the 20 accessions of sugarcane (supplementary table 1), similarity ranged from 0.78 to 0.13 with mean of 0.455. Accessions DB8134 and M1246/84 showed the highest genetic similarity having similarity coefficient of 0.78 and were adjudged to be closely related. However, the maximum genetic distance was observed between accessions DB8134 and SP81-3250 with 0.13, M1246/84 and SP81-3250, B74541 and SP81-3250 had a genetic similarity coefficient of 0.136 each, followed by M1334/4 and SP81-3250 with genetic distance of 0.148.

3.3 BIPLOT ANALYSIS

The distribution of the accessions into different spatial plane and co-ordinates by biplot analysis (Fig 1) showed the involvement of markers in separating accessions into quadrants. Co-occurrence of B74541 and M1246/84 in quadrant three and B80689 and Co88025 in quadrant four on the same plane suggested a common ancestor. The overlapping of B85266 and B93757, which are close to RB72/454, indicated the accessions are genetically similar. Samples in quadrant I, III and IV are more closely related, except for M1954/91, while those in quadrant II are diffused i.e. they are relations separated by geographical isolation for a long time (Animasaun et al., 2015).

The spatial closeness of the accessions in biplot analysis indicate their genetic similarity. In addition, the dispersion of the markers from the centroid reflects their effectiveness in delimiting the accessions (Animasaun et al., 2015). However, the distant location of M1954/91, SP81-3250 and DB8134 in quadrant I, II and IV implies the existence of genetic distance. This may be due to accumulation of some genes through selection resulting from domestication of the accessions by local farmers (Animasaun et al., 2015). Meanwhile the obtained marker efficiency as revealed by biplot analysis supported ISSR makers as a useful tool for the initial assessment of intraspecific genetic variation (Devarumath et al., 2012).

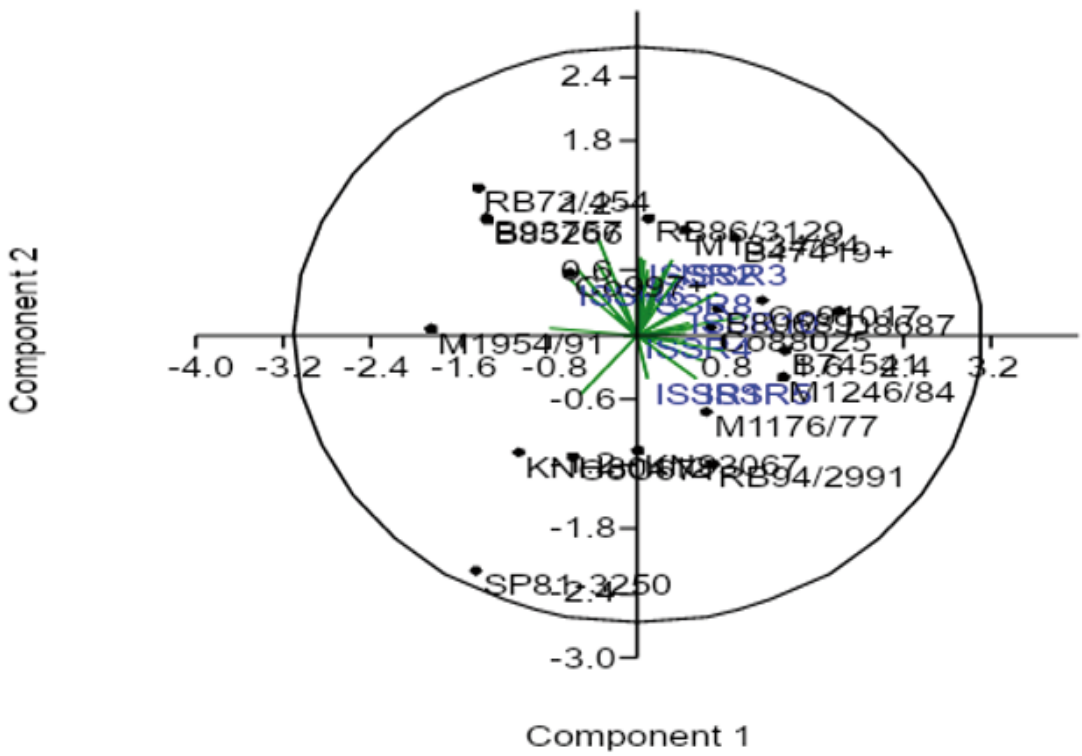

Figure 1: Bootstrapped Biplot of twenty accessions of sugarcane accessions characterized by eight ISSR primers for diversity and genotyping analysis (at $p < 0.05$)

3.4 CLUSTER ANALYSIS

Neighbor Joining-based and UPGMA tree construction methods on the basis of Jaccard's similarity coefficient was used to construct dendrogram to examine the relationship among sugarcane accessions based on 369 ISSR bands amplified by eight primers. The dendrogram derived from UPGMA-based cluster analysis of the whole ISSR data with 20 sugarcane accessions shows all the accessions in the first cluster were clustered closely together which shows high genetic similarity consisting of nine (9) accessions (group 2). Cluster analysis by dendrogram depicting the genetic relationship classified the accessions into to group (1 and 2). On the other hand, the second group (group 2) sub-divided into 2a and 2b (Fig 3). The 2b group split into two clusters, 2b(i) and 2b(ii) (Fig 3). Cluster 2a is comprised of four genotypes, cluster 2b(i) and 2b(ii) is made up of three and four accessions respectively. The different accessions formed clusters irrespective of their different geographical origins. The neighbor joining (Fig 2) showed that B97375 is a distance neighbors from the other accessions. In addition, RB86/3129 and Co997+ were joined together as neighbours, M134/84 was a close neighbor to Co88025. Also, B96723 was the closest neighbour to RB72/454.

Clustering parameters such as, Principal coordinate analysis, UPGMA, Neighbour joining showed close clustering of individuals and intermixing in clusters irrespective of their origin. Interestingly, according to Ullah et al. (2013) accessions grouped in same cluster are more similar to each other but less similar to the accessions in other clusters. This means that accessions in the same cluster are genetically similar or related. This knowledge is important because, based on their genetic relationship, recognizing and classifying different individuals in homogeneous groups will help breeders select parents and improve efficiency in preparing crosses for breeding programs (Zeni Neto et al., 2020).

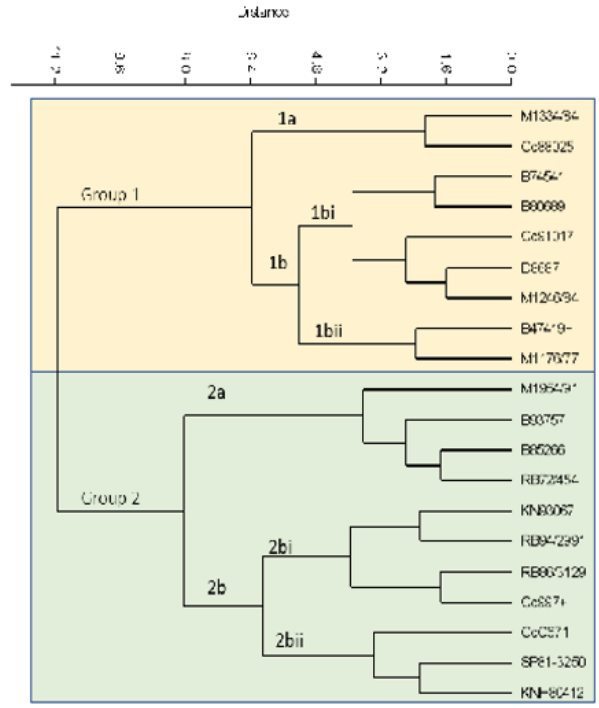

Figure 3: Genetic relatedness of the sugarcane genotypes based on UPGMA cluster analysis

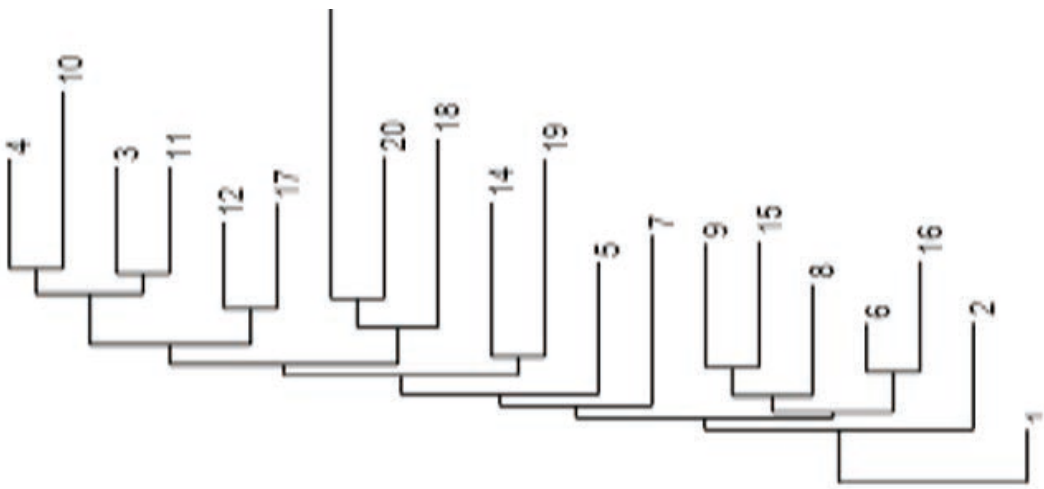

Figure 2: Neighbor joining diagram of genotypes based on ISSR marker analysis. 1 = B97375, 2 = B96812, 3 = B96723, 4 = B93757, 5 = B47419+, 6 = DB8134, 7 = M1176/77, 8 = M1246/84, 9 = M1334/84, 10 = M1954/91, 11 = RB72/454, 12 = RB86/3129, 13 = SP81-3250, 14 = RB94/2991 15 = Co88025, 16 = Co91017, 17 = Co997+, 18 = CoC671, 19 = KNB9288, 20 = KNB9253

3.5 PRINCIPAL COORDINATE ANALYSIS

Pattern of variation among the sugarcane accessions was also observed using Principal coordinate analysis based on Jaccard's similarity coefficient using PAST Statistical software package. The sugarcane accessions clustered in the four quadrants irrespective of their place of origin. The ordination of the accessions on principal component axes PCo1 versus PCo2 based on cluster analysis of ISSR allelic data (Fig 4). It provided distinct, groupings that established sub-groups within the quadrants, which illustrate the degree of relatedness and diversity within the quadrants. In quadrant 1 accessions B96723, B93757, RB72/454 appeared to be closely related, but formed a sub group with accessions Co997+ and M1954/91 located a far distance away from the other accessions in quadrant 1. The occurrence of accessions B96723 and B93757 and the closeness of RB72/454 in quadrant 1 showed that they are closely related and indicates a common ancestor. In quadrant II, accessions SP81-3250, KNB9253, CoC671 formed a sub group while KNB9288 stands alone located close to the centroid. Also clustering of accessions SP81-3250, CoC671, KNB-9288, SP81-3250, KNB-9253 are far apart in quadrant II suggesting different origin. The diffuse pattern shows genetic divergence among the populations with accession SP81-3250 being at the farther end of the quadrant revealing utmost genetic variation and distance from other populations. In quadrant III, two subgroups were formed one with accessions M1176/77 and RB94/2991 and another with B97375 and M1246/84 showing similarity. Quadrant IV had the highest number of accessions (7) among other quadrants. Accessions RB86/3129, M1334/84, B47419+ formed a sub-group within the quadrant. The clustering of accessions B97375, B96812 and Co88025 in quadrant IV indicates a common ancestor, while accession DB8134 is far away from other accessions in quadrant IV.

Principal coordinate analysis revealed the important components contributing to the observed variation among the 20 sugarcane accessions. This form of admixture may result from the participation of sugarcane genotypes in breeding programs to enhance some of the characteristics of commercially exploited varieties, so that parental breeding lines may be exchanged across the world's sugarcane growing regions to achieve these goals (Tazeb et al., 2017). Another explanation for the high levels of similarity between subgroups and groups is

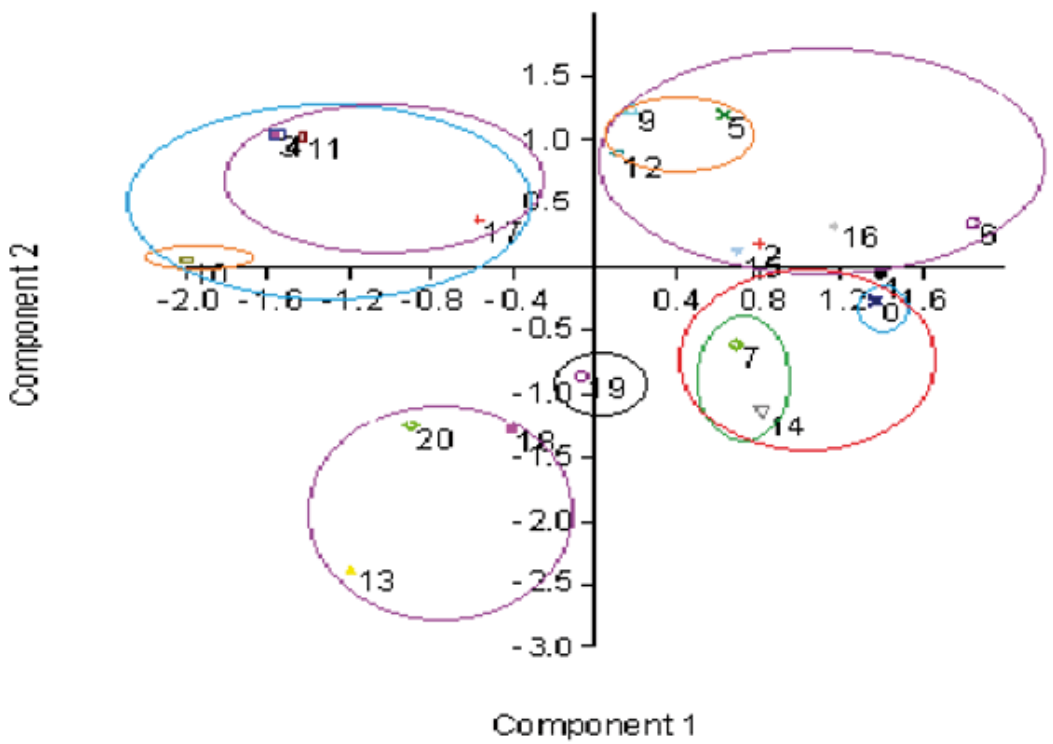

Figure 4: The ordination of twenty Sugarcane (*Saccharum officinarum*) accessions on principal component axes PCo 1 versus PCo 2 based on cluster analysis of ISSR allelic data. 1 = B97375, 2 = B96812, 3 = B96723, 4 = B93757, 5 = B47419+, 6 = DB8134, 7 = M1176/77, 8 = M1246/84, 9 = M1334/84, 10 = M1954/91, 11 = RB72/454, 12 = RB86/3129, 13 = SP81-3250, 14 = RB94/2991, 15 = Co88025, 16 = Co91017, 17 = Co997+, 18 = CoC671, 19 = KNB9288, 20 = KNB9253

that during the sugarcane breeding program, the lineages were exposed to a higher degree of intercultivar gene flow (Rodriguez et al., 2005). Comprehensively, it is possible for geographically different population to form a cluster with other population, because the majority of commercial sugar cane cultivars bred after the turn of the 20th century are interspecific hybrids between *Saccharrum officinarum* and *Saccharrum spontaneum* L. (D'Hont et al., 1996*)*. Thus, the cross progeny may have clustered from other regions with their progenitors or parents or the parents may have clustered from distantly related populations with their cross progeny *(*Tazeb et al., 2017*)*. Clustering of all sugarcane accessions together irrespective of their sources showed their remarkable genetic similarity and reinforced the postulate of a common progenitor for the accessions (Jauhar & Hanna, 1998).

4 CONCLUSION

Adequate genetic information is prerequisite to identify potential parental combinations required in hybridization programme aimed to create segregating progenies with maximum genetic variability for further selection. The information from this study could provide accurate information to sugarcane breeding in Nigeria for strategic conservation of the germplasm resources and future improvement work of the sugarcane by selecting suitable parents for breeding programs aimed at optimizing sugar yield, establishing the proper identity of the accessions and preventing duplication of accessions.

In addition, ISSR markers showed reliability and efficiency in detecting polymorphisms within and among the sugarcane accessions studied. It has also been shown that ISSR markers have high-resolution ability in sugarcane fingerprinting and diversity analysis and are therefore effective and efficient in the identification of polymorphisms within and among populations and/or species. However, seven out of the eight primers were polymorphic, ability of primer ISSR 8 and ISSR 10 to produce higher allele frequencies and polymorphic loci in this study indicated that the two primers are the most informative and suitable for diversity study in sugarcane. It is, therefore recommended that molecular marker approach be deployed in investigating the remaining germplasm for diversity and breeding programs.

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