International Journal of Agricultural Technology 2018 Vol. 14(5): 717-730
Available online http://www.ijat-aatsea.com
ISSN: 2630-0613 (Print) 2630-0192 (Online)

Fluorescent Inter Simple Sequence Repeat (F-ISSR) Markers and Capillary Electrophoresis to Assess Genetic Diversity and Relatedness within Commercial Sugarcane Varieties

Mondal, A., Pal, T. and De, K. K.*

Department of Botany, Hooghly Mohsin College (Estd. 1836), University of Burdwan, Chinsurah, Hooghly, West Bengal, India.

Mondal, A., Pal, T. and De, K. K. (2018). Fluorescent Inter Simple Sequence Repeat (F-ISSR) markers and capillary electrophoresis to assess genetic diversity and relatedness within commercial sugarcane varieties. International Journal of Agricultural Technology 14(5):717-730.

Abstract The proper selection of genetically rich diverse parents is a very useful strategy for the success of sugarcane breeding program. One of the crucial factors in sugarcane breeding and sugar production is the selection of the suitable variety because different varieties have different yield potentials, pest and disease resistance property and is bred for different ecological and economic conditions. The level and patterns of molecular genetic diversity and establishing relationships among 19 varieties of sugarcane using fluorescent Inter Simple Sequence Repeat (F-ISSR) markers and capillary electrophoresis was evaluated. Out of nine primers, 3 primers were found to give clear and polymorphic patterns. F-ISSR primers were labeled with 6-carboxyfluorescein and consists of di-nucleotide motifs. Total countable bands were 1142 among which 1099 comprised of polymorphic markers, with an average of 366 polymorphic bands per primers. Dendrogram showed that there are 5 clear clusters. The size range of the amplification products varied from 50.14 to 500bp. A total number of 246 alleles were detected per primer and the average number of allele per locus was 12.95.Only two monomorphic bands were detected with two primers at a particular locus. 41 unique bands were identified. The study showed that the F-ISSR markers detected high level of polymorphism (96.42 %) or genetic diversity among nineteen varieties of sugarcane. The present information recoded in this study would definitely give a useful guideline in planning hybridization for the maintenance and improvement of genetic diversity in sugarcane varieties.

Keywords: allele, dendrogram, genetic diversity, sugarcane, 6-carboxyfluorescein.

Introduction

DNA markers have proved valuable in crop breeding, especially in studies on genetic diversity and relatedness among the varieties/species on which the majority of success of crop improvement depends. The commonly used DNA marker systems are random amplified polymorphic DNA (RAPD),

* **Corresponding Author:** De, K. K.; **E-mail:** kalyannet2003@yahoo.co.in

amplified fragment length polymorphism (AFLP) and more recently simple sequence repeats (SSRs) or microsatellites (Staub *et al*., 1996; Gupta and and Varshney, 2000). Low reproducibility of RAPD, high cost of AFLP and the need to know the flanking sequences to develop species specific primers for SSR polymorphism are the major disadvantages. However, Inter Simple Sequence Repeat **(**ISSR) marker is a technique that overcomes most of these limitations (Zietkiewicz *et al*., 1994; Gupta *et al*., 1994; Wu *et al*., 1994; Meyer *et al*., 1993). So it is now widely being used in various fields of crop improvement (Godwin *et al*., 1997). ISSR is a PCR based technique by which DNA segments present at an amplifiable distance in between two identical microsatellite repeat or SSR regions oriented in opposite direction are amplified precisely (Figure 1). In this method SSRs are used as primers to amplify mainly the Inter Simple Sequence Repeat regions. Fluorescent ISSR marker is a new efficient and better sensitivity PCR based techniques and has been proven to be superior to traditional or non- fluorescent ISSR for its good data reproducibility and high resolution power (Obručová *et al*., 2016). In these techniques, PCR products are tagged with fluorescent dye. Number of bands obtained in traditional ISSR gel is very few (Kawar *et al*., 2009), whereas in fluorescent ISSR, total number of bands is 20-30 times more which are resolved clearly only by peaks with different heights depending on DNA fragment length but bands are not accurately appeared visually in the gel by naked eye or photographically due to appearance of huge number of bands in gel.

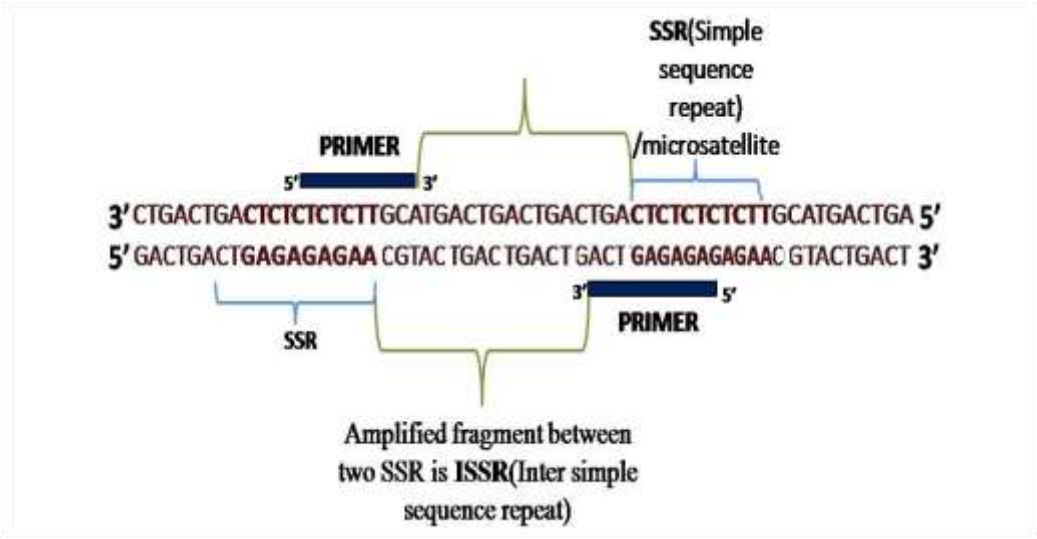


Figure 1. Two identical microsatellite repeat or SSR regions oriented in opposite direction.

Sugarcane (*Saccharum* spp., Poaceae), is a large perennial crop plant mostly grown in the tropical and subtropical environments globally for its sugar-rich culms and accounts for 80% of the world's raw sugar. Sugarcane is a biofuel crop used for ethanol production as an alternative source of energy (Lam *et al*., 2009). The main objective of this study was to determine the level of genetic diversity and relatedness among 19 Indian sugarcane varieties using fluorescent Inter Simple Sequence Repeat (F-ISSR) markers. The application of ISSR marker is found to be quick and cost-effective based on polymerase chain reaction (PCR) amplification of inter-microsatellite sequences to target multiple loci in the genome (Kassahun *et al*., 2014) and in this technique; no DNA sequence information for primer designing is required (Zietkiewicz *et al*., 1994). The molecular assessment of genetic variability and relatedness within and between the sugarcane varieties being used as presumed parents in crossing programmes is valuable to increase the efficiency of genetic improvement of sugarcane. Moreover, the varietal differentiation plays an important role in DUS testing strategies (DUS stands for distinctness, uniformity and stability of new varieties of plants for the purpose of granting the Breeders' Right) in the light of International Union for the Protection of New Varieties of Plants (UPOV) initiatives and Plant variety protection (PVP) act (Srivastava and Rai, 2012**)**. Therefore, this study was initiated with the aim of evaluating the level and patterns of molecular genetic diversity and establishing relationships among 19 varieties of sugarcane using fluorescent Inter Simple Sequence Repeat (ISSR) markers and capillary electrophoresis.

Materials and methods

Nineteen commercial varieties of *Saccharum* were collected from the National Hybridization Garden (NHG) Sugarcane Breeding Institute, Coimbatore (11°1′6″N 76°58′21″E, elevation = 411.2 m (1,349.1 ft above mean sea level), Tamil Nadu, India. Name of the commercial varieties are: Co B 94164 (Madhuri), Co 775, Bo 91, Co Se 92423 (Rajbhog),Co Se 01424, Co 453, Co Se 96436 (Jalpari), Co 62198, Co 658, Co 62175, Co 740, Co 7527, Co 449, Co A 88081, Co C 671, Co 8371 (Bhima), Co 6304, Co 6806 and Co 7219 (Sanjeevani).

DNA was extracted from the young leaves using a hexadecyl trimethyl ammonium bromide (CTAB) protocol adapted from Doyle and Doyle (1990). Briefly, 250mg of tissue was ground with mortar and pestle and subsequently incubated at 65^0C for 60 minutes with 600µl of Isolation Buffer. The composition of Isolation buffer is CTAB (2w/v), NaCl (1.4M), Tris-HCl buffer (pH 8.0) (100mM), Polyvinylpyrrolidone (PVP 40) (1%w/v) 2-mercaptoethanol

(1%w/v).The mixture was allowed to cool to room temperature and a 1:1 chloroform-isoamyl alcohol (24:1) extraction was performed. The preparation was mixed by inversion to form an emulsion and then centrifuged at room temperature for 10 minutes (15000g) to separate the phases. A RNase digestion was performed (10µg/ml RNase A at 37^{o}C during 60 minutes) followed by a second chloroform-isoamyl alcohol extraction. Subsequently, DNA was precipitated from the aqueous phase by adding 2/3 volume of cold Isopropanol. The pellet was washed with 0.2M sodium acetate and 76% (v/v) ethanol. The DNA was redissolved in 50µl of Buffer containing 10mM Tris-HCl and 1mM of EDTA (TE) pH 8.0. The DNA preparation was diluted in TE pH 8.0 at 50ng/µl for ISSR analysis. The concentration and purity of DNA was assessed spectrophotometrically (UV vis spectrophotometer 1700DC. Simarju, Japan). The DNA sample had OD_{280} / OD_{280} ~1.7 and OD_{260} / OD_{230} ~ 1.8 – 2.8.

Setting up fluorescence ISSR was done in the following steps

DNA isolation →PCR amplification →PCR Product Purification →Capillary Electrophoresis →Data Analysis

Primer Details of F-ISSR

ISSR primers were labeled with 6-carboxyfluorescein (6-FAM) from Applied Biosystems, USA, which gives blue color in Genescan analysis. Nine fluorescent labeled ISSR primers consisting of dinucleotide motifs were used for amplification for initial screening with 19 verities. Out of these, 6 primers gave no amplification at all, while only 3 primers were found to give clear and polymorphic patterns and were subsequently used to analyze the entire set of 19 verities for repetition. Such 3 primers are marked by bold letters. The choice of primers (motif, repeat length etc.) used in ISSR amplification is critical to obtain high levels of polymorphism.

Primer 1: 5’ - $(AG)_{10}$ T-3' Primer 2: 5'- $(CT)_{10}$ T-3' Primer 3: 5'- $(GA)_{6}$ CC-3' **Primer 4: 5’- $(CA)_{7}$ AG - 3’ Primer 5: 5’- $(GA)_{8}$ CG - 3’ Primer 6: 5’- $(AC)_{8}$ T – 3'** Primer 7:5'- $(GT)_{6}$ CC-3' Primer 8:5'- $(CA)_{6}$ AC -3' Primer 9:5'- $(CA)_{7}$ G -3'

All primers are 3’-anchored (primer consists of a repeated motif with one or several non-motif nucleotides at the 3’-end. For the study aimed to evaluate genetic variability, either the 3’ or 5’-anchored ISSR primers are generally used for better reproducibility of results. Otherwise, unanchored ISSR primers may slip along the length of the complementary microsatellite region, producing inconsistent amplification in every cycle, and thus affecting the

reproducibility of results. PCR amplification with ISSR primers (ISSR-PCR) is slightly different from the usual PCR reaction that involves a pair of different primers, ISSR-PCR involves only one primer in each reaction, e.g. single-primer PCR amplification because this single primer actually acts as both the 'forward' and 'reverse' primers which are essential for an amplification to take place. DNA amplifications were done in 25µl of reaction mixture containing 50ng template DNA, 2.5 µl of 10X PCR buffer, 2.5mM $MgCl_2$, 2mM of dNTPs, 15ng of degenerate primer and 1 unit of AmpliTaq-Gold polymerase (Life technologies, Grand Island, NY). DNA amplification was done in BIO-RAD thermo cycler programmed for 35 cycles as follows: 5min at 94℃, followed by 30sec at 94℃, 90sec at 56℃ and 90sec at 72℃ followed by final extension cycle of 5min at 72℃. PCR products were purified using MinElute PCR Purification Kit (Qiagen, Valencia, CA).

Fragment analysis of PCR products by capillary electrophoresis

PCR products were processed for fragment separation by capillary electrophoresis on ABI 3500 Genetic Analyzer using POP-7 polymer. The ROX 500 size standard (Life technologies, Grand Island, NY) was run as an internal size marker by adding 10 µl of 1∶25 mix of ROX 500 and HiDi-formamide to 1.5 µl of PCR product. Samples were denatured at 95℃ for 5 minutes and run on the Genetic Analyzer. Data were analyzed for allele sizes and peak heights using the software of Gene Mapper version 4.1 packages (Life technologies, Grand Island, NY).

ISSR amplifications were performed at least three times and only the reproducible PCR products were scored. The number of polymorphic band was the number of ISSR bands that showed variation, i.e. the bands are presented for some samples and absent for the others; and "percentage of polymorphic bands" is represented by the formula:

$$\% \text{ of polymorphic bands} = \frac{\text{No. of polymorphic bands}}{\text{Total no. of bands}} \text{ X } 100$$

Results

Genomic DNA samples were checked by running on 1% agarose gel and then amplified. Nine primers were scored initially but only 3 primers resulted in good banding patterns. Bands were counted and the presence and absence of bands were scored as 1 and 0 (binary data), respectively. Genetic

profiling studies of nineteen sugarcane varieties were carried out by using three effective and informative sets of ISSR primers having di-nucleotide repeat motifs of (CA), (GA) and (AC). A grand total of 1142 bands were scored with 1099 polymorphic, 2 monomorphic and 41 unique bands among the 19 sugarcane varieties. The average percentage of polymorphism was 96.42 %. The size of the 1142 amplification products produced ranged from 50.14 to 500 bp. Three main primers revealed intra-varietal variations. The three primers generated high percentage of polymorphic loci, which means that genetic polymorphism in the population, is generally high. A total number of 246 alleles were detected per primer among 19 sugarcane varieties. The average number of allele per locus was 12.95.

Pair wise comparison between varieties based on the proportion of shared bands produced by the primers used, were calculated using Jaccard's similarity (Ansorge *et al*., 1987). The similarity matrix showed the extent of similarity among 19 sugarcane varieties. Distance matrix was calculated by Nei and Li/Dice and Tree Construction using Neighbor joining method and presented in Table 1. This shows the similarity index/distance matrix among 19 varieties of Sugarcane. The range of genetic distance among sugarcane varieties were 0.42308 – 0.87597. Maximum genetic distance was seen between Co Se 92423 (Rajbhog) and Co C 671 (0. 87597). The least distance was 0. 42308 found in between Co 658 and Co 62175.

Table 1. Similarity index/Distance Matrix table of the 19 varieties of Sugarcane.

	CoC 671	CoA 88081	Co 775	Co 6806	Co 453	Co7219	Co 6304	Co 8371	Co 449	CoSe 01434	CoSe 92423	Bo 91	Co 62175	Co 658	Co 7527	Co 740	CoSe 96436	Co 62399	CoB 94064
CoC 671		0.78205	0.82609	0.82258	0.83607	0.84848	0.84828	0.80328	0.82906	0.82759	0.87597	0.85307	0.85827	0.78151	0.76786	0.80513	0.80000	0.81818	0.82456
CoA 88081	0.78205		0.76744	0.65217	0.77941	0.69863	0.63522	0.79412	0.81679	0.76923	0.62238	0.83420	0.59865	0.63910	0.73016	0.77381	0.71429	0.76296	0.82813
Co 775	0.82609	0.76744		0.50515	0.55789	0.63810	0.66102	0.64211	0.53333	0.55056	0.72549	0.82895	0.64000	0.54348	0.48235	0.60630	0.61062	0.44681	0.63218
Co 6806	0.82258	0.65217	0.50515		0.55769	0.52632	0.54331	0.57592	0.69697	0.59184	0.59369	0.87578	0.57798	0.54455	0.63830	0.66176	0.67213	0.61165	0.64583
Co 453	0.83607	0.77941	0.55789	0.55769		0.55357	0.64800	0.56863	0.77320	0.60417	0.77982	0.83648	0.57009	0.59596	0.59656	0.62687	0.61667	0.54455	0.59574
Co 7219	0.84848	0.69863	0.63810	0.52632	0.55357		0.51111	0.67857	0.79439	0.58491	0.73109	0.85799	0.52137	0.43119	0.64706	0.65278	0.58462	0.54955	0.48077
Co 6304	0.84828	0.63522	0.66102	0.54331	0.64800	0.51111		0.61600	0.71667	0.57983	0.75758	0.84615	0.55385	0.55738	0.60000	0.70701	0.56643	0.58065	0.57265
Co 8371	0.80328	0.79412	0.64211	0.57692	0.56863	0.67857	0.61600		0.67010	0.62500	0.74312	0.82390	0.64486	0.63636	0.63043	0.68657	0.68333	0.58416	0.63830
Co 449	0.82906	0.81679	0.53333	0.69697	0.77320	0.79439	0.71667	0.67010		0.67033	0.82692	0.76623	0.76471	0.72340	0.70115	0.67442	0.63478	0.56250	0.73034
CoSe 01434	0.82759	0.76923	0.55056	0.59184	0.60417	0.58491	0.57983	0.62500	0.67033		0.74757	0.84314	0.70297	0.61290	0.51163	0.57813	0.45614	0.53684	0.40909
CoSe 92423	0.87597	0.62238	0.72549	0.69369	0.77982	0.73109	0.75758	0.74312	0.82692	0.74757		0.85542	0.70175	0.66038	0.77778	0.75887	0.73228	0.64815	0.72277
Bo 91	0.85307	0.83420	0.82895	0.87578	0.83648	0.85799	0.84615	0.82390	0.76623	0.84314	0.85542		0.81707	0.79487	0.75839	0.74869	0.76271	0.83544	0.84106
Co 62175	0.85827	0.59865	0.64000	0.57798	0.57009	0.52137	0.55385	0.64486	0.76471	0.70297	0.70175	0.81707		0.42308	0.62887	0.71223	0.66406	0.59491	0.59596
Co 658	0.78151	0.63910	0.54348	0.54455	0.59596	0.43119	0.55738	0.63636	0.72340	0.61290	0.66038	0.79487	0.42308		0.48315	0.58779	0.64103	0.53061	0.51648
Co 7527	0.76786	0.73016	0.48235	0.63830	0.59656	0.64706	0.60000	0.63043	0.70115	0.51163	0.77778	0.75839	0.62887	0.48315		0.56452	0.54545	0.56242	0.54762
Co 740	0.80519	0.77381	0.60630	0.66176	0.62687	0.65278	0.70701	0.68657	0.67442	0.57813	0.75887	0.74869	0.71223	0.58779	0.56452		0.51316	0.62406	0.60317
CoSe 96436	0.80000	0.71429	0.61062	0.67213	0.61667	0.58462	0.56643	0.68333	0.63478	0.45614	0.73228	0.76271	0.66406	0.64103	0.54545	0.51316		0.51261	0.53571
Co 62399	0.81818	0.76296	0.44681	0.61165	0.54455	0.54955	0.58065	0.58416	0.56250	0.53684	0.64815	0.83544	0.59491	0.53061	0.58242	0.62406	0.51261		0.46237
CoB 94064	0.82456	0.82813	0.63218	0.64583	0.59574	0.48077	0.57265	0.63830	0.73034	0.40909	0.72277	0.84106	0.59596	0.51648	0.54762	0.60317	0.53571	0.46237	

The Phylogenetic Tree (Dendrogram) was constructed to measure the resulting phenotypic groups of the 19 sugarcane varieties based on the genetic distance generated by three fluorescent labeled ISSR primers (Figure 2). It shows that there are 5 clear clusters. Cluster-I includes five varieties such as Co6806, Co6304, Co7219 (Sanjeevani), Co62175 and Co658 and Cluster II consists of Co453 and Co8371 (Bhima). Varieties Co C 671, Bo 91, Co Se 92423 (Rajbhog) and Co A 88081 are grouped in cluster III. Co7527, Co Se 01424, Co B 94164 (Madhuri), Co 740 and Co Se 96436 (Jalpari) are includ3ed in cluster IV. Cluster V comprises of 3 varieties such as Co 62198, Co 775 and Co 449.

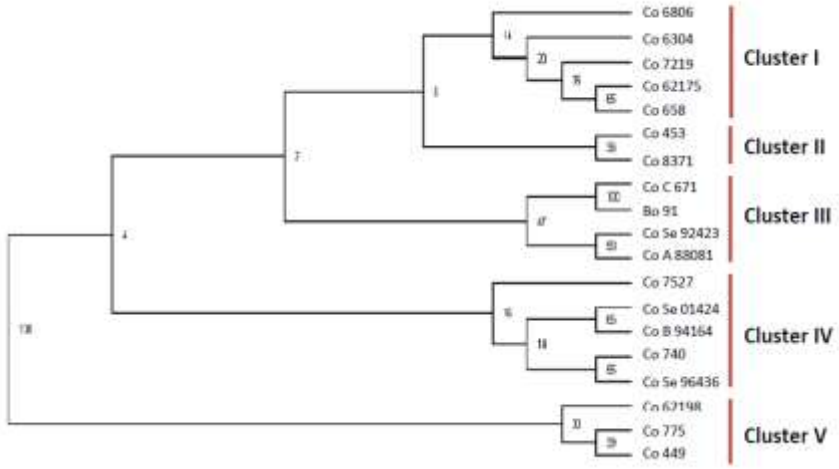


Figure 2. Phylogenetic Tree (Dendrogram) of 19 varieties of Sugarcane based on genetic distance generated by three fluorescent (FAM) labeled ISSR primers.

Further, the Reference Tree (Figure 3), which was checked on the basis of the distance matrices obtained, reflects a 65 % similarity between Co 62175 and Co658 which also reflects the results obtained with the Jaccard's Coefficient. There is a similarity of 35% between Co7219 (Sanjeevani) and (Co 62175 and Co 658) and the similarity between the Co 6304 and [Co 62175, Co 658 and Co 7219 (Sanjeevani)] is 20% in cluster I. However, the similarity between Co 6806 and [Co62175, Co658, Co 7219 (Sanjeevani) and Co6304] is only 14%. The similarity between Co 453 and Co 8371 (Bhima) in cluster II is 36%. In cluster III, there are two sub-clusters. In first sub-cluster, the similarity between Co C 671 and Bo 91 is 100%, whereas, Co Se 92423(Rajbhog) and Co A 88081 of second sub-cluster show similarity by 90%. First and second sub-clusters of the cluster III are related with each other by 47%. In cluster IV, again there are two sub-clusters. In first sub-cluster, the similarity value of Co

740 and Co Se 96436 is 55%, whereas, Co B 94164(Madhuri) and Co Se 01424 show 65% similarity value. First and second sub-clusters of the cluster IV are related with each other by 14%. The similarity value of Co 7527 with First and second sub-clusters of the cluster IV are only 16%. In the cluster V, the similarity value between Co 775 and Co 449 is 39%. Co 62198 shows similarity with Co 775 and Co 449 by 33%.

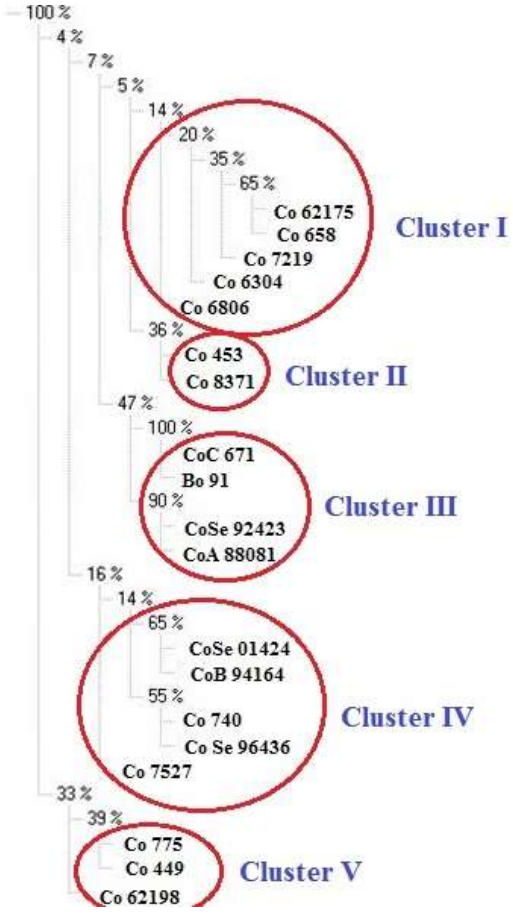
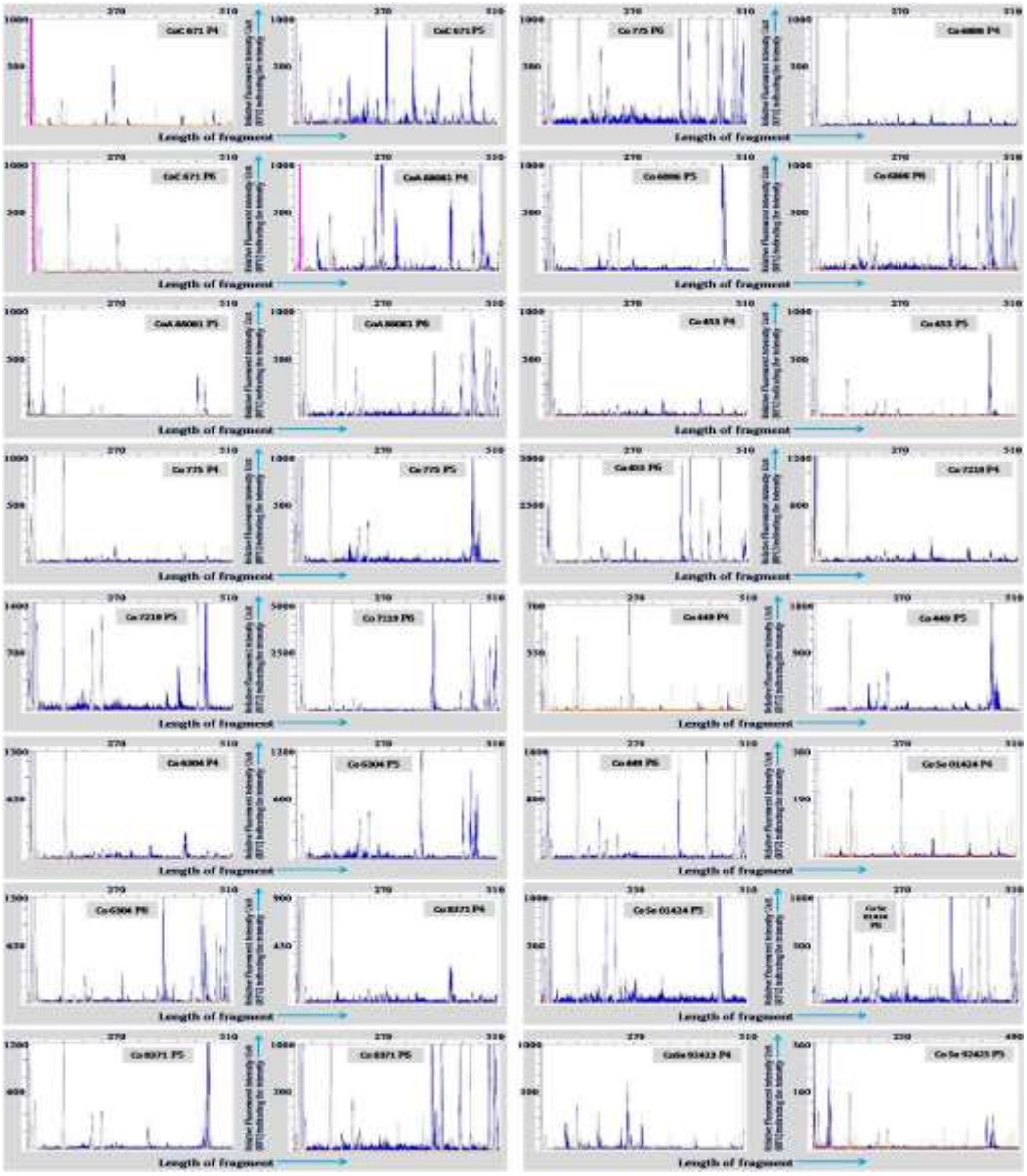


Figure 3. Reference Tree of 19 varieties of Sugarcane based on genetic distance generated by three fluorescent (FAM) labeled ISSR primers.

Among the 3 primers amplified in 19 sugarcane varieties, 14+2 unique bands at different loci were obtained by amplification with two different primers P_5 and P_6 respectively that differentiate the Co C 671 variety from the rest of varieties. However, in case of Co A 88081, Co 6304, Co 6304, Co Se 01424, Co Se 92423(Rajbhog), Bo 91, Co 740 and Co Se 96436 (Jalpari)

varieties different number of unique bands at different loci were obtained with all three primers. Only one specific unique band observed in case of Co 6806 and Co 62198 with P_5 primer by which they are separated from all varieties. Co 4536 and Co 8371(Bhima) showed 6(1+5) and 7(3+4) unique bands with P_4 and P_6 primers respectively. 5(1+4) unique bands observed in case of Co 62175 with P_4 and P_5 primers. However, only 2 unique bands noted in Co B 94164(Madhuri) with P_6 primer.



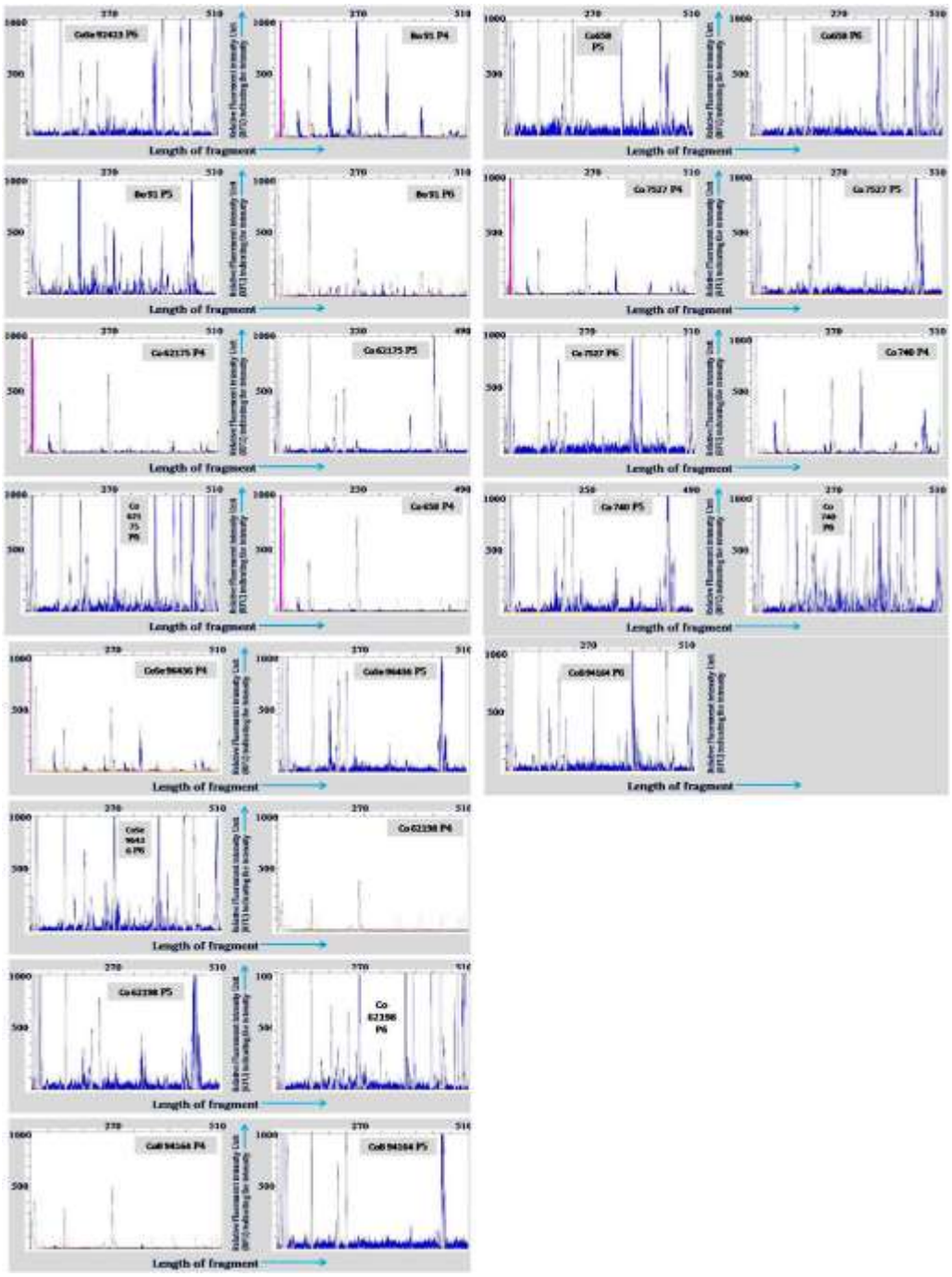


Figure 4. Capillary gel electropherogram showing the separation of the PCR products of DNA fragments of different sizes in the form of different heights of peaks against the relative fluorescent intensity.

The presence of bands on each and all the loci of all varieties indicates monomorphism while the presence of bands in some and not all the loci indicates polymorphism or genetic diversity among the attributes under investigation. Nineteen varieties of sugarcane showed one monomorphic band each with P_4 and P_5 primers at a particular locus. No monomophic band was found with P_6 primer. Besides, unique and monomorphic bands, remaining bands at different loci are obviously polymorphic. The variation of peak profiles obtained from capillary electrophoresis also demonstrated the genetic variability (Figure 4).

Discussion

ISSR markers have high resolution power in fingerprinting and diversity analysis of sugarcane. ISSRs have been successfully used to estimate the extent of genetic diversity in a wide range of crop species which include sugarcane (Srivastava and Gupta, 2008; Da Costa *et al*., 2011). In addition to the advantages (inexpensive, easy to generate), F- ISSR markers are more powerful and efficient in detecting polymorphisms within and among populations and/or species. The present study also utilized the F-ISSR markers to investigate genetic diversity and relationships within and among the nineteen groups of sugarcane varieties of India. Molecular marker-based analysis of genetic diversity in plant species have become an important tool in crop improvement and conservation purpose (Weising *et al*., 2005).

In general, the present study showed that the ISSR markers detected high level of polymorphism (96.42%) or genetic diversity among nineteen varieties of sugarcane. These variations can happen due to various reasons such as modifications in DNA methylation, gene amplification, chromosomal abnormality and point mutation (Saker *et al*., 2000). We anticipated that the results of ISSR-based studies would play a major role in the management, conservation and improvement of sugarcane because the ISSRs indicate high polymorphism. Avise (1994) and Dunham (2002) concluded that the high value of polymorphism is important for a population to maintain its long-term survival and also to ensure fitness to enable the population to adapt to environmental changes. Presence of only one ISSR monomorphic band each with onlyP_4 and P_5 primers at a particular locus confirms their apparent lack of genetic variability. The unique banding pattern resulted from varieties can be used as DNA fingerprint for variety identification and it maintains a variety-specific gene pool. Unique allele may be originated from point mutation (Reddy *et al*., 2002). For an example, in case of Co 6806 and Co 62198 with P_5

primer showed only one specific unique band at a particular locus by which they are separated from all other varieties.

The small value of the genetic distance between Co 658 and Co 62175 showed that the populations can maintain their allele which is indicated by the number of the common bands, they have. On the other hand, the high value of genetic distance between Co Se 92423(Rajbhog) and Co C 671population exists presumably because the two populations are not able to maintain their alleles as indicated by the number of specific bands they possessed.

The high level of polymorphism detected in nineteen varieties of sugarcane by ISSR markers was expected due to use of external varieties during breeding programme and also considering the segmental allopolyploid nature of sugarcane (Da Costa *et al*., 2011). The justification of the higher level of polymorphism could be correlated with the nature of ISSR, targeting region especially rich in microsatellites because those regions are known to accumulate a large number of mutations due to DNA polymerase slippage during replication and unequal crossing over (Scl ötterer and Tautz, 1992).

Conclusion

Thus, it can be concluded that the success of any crop improvement programme depends on the variability present in a population. Recent studies on genetic diversity of 19 varieties of sugarcane through molecular analysis by using fluorescent ISSR and capillary electrophoresis technique reveal that variation exists to a great extent. This variation has to be exploited for its effective utilization in plant breeding programmes. Thus, varietal evaluation and screening are major areas where molecular markers can be utilized through diversity studies, fingerprinting etc.

Acknowledgments

The authors gratefully acknowledge the assistance and support of the National Hybridization Garden (NHG) Sugarcane Breeding Institute, Coimbatore, India. We also gratefully appreciate the cooperation of the Post Graduate Department of Botany, Hooghly Mohsin College (Estd. 1836) in conducting this research work.

References

Ansorge, W., Sproat, B., Stegemann, J., Schwager, C. and Zenke, M. (1987). Automated DNA sequencing: ultrasensitive detection of fluorescent bands during electrophoresis. Nucleic Acids Research 15:4593-4602.

Avise, J. C. (1994). Molecular Markers, Natural History, and Evolution. Chapman & Hall, New York. 511 p.

Da Costa, M. L. M., Amorim, B. L. L., Onofre, C. V. A., de Melo, T. O. J. L., de Oliveira, M. B. M., de Carvalho, R. and Benko-Iseppon, M. A. (2011). Assessment of genetic diversity in contrasting sugarcane varieties using Inter-Simple Sequence Repeat (ISSR) markers. American Journal of Plant Sciences 2:425-432.

Dunham, R. A. (2002). Aquaculture and Fisheries Biotechnology, Genetic Approach. CABI Publishing, Cambridge, USA. pp.85-99.

Godwin, I. D., Aitken, E. A. B. and Smith, L. W. (1997). Application of inter simple sequence repeat (ISSR) markers to plant genetics. Electrophoresis 18:1524–1528

Gupta, M., Chyi, Y-S., Romero-Severson, J. and Owen, J. L. (1994). Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. Theoretical and Applied Genetics 89:998-1006.

Gupta, P. K. and Varshney, R. K. (2000). The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica 113:163-185.

Kassahun, T., Govers, K., Endashaw, B. and Borsch, T. (2014). ISSR fingerprinting of *Coffea arabica* throughout Ethiopia reveals high variability in wild populations and distinguishes them from landraces. Plant Systematics and Evolution 300:881-897.

Kawar, P. G., Devarumath, R. M. and Nerkar, Y. (2009). Use of RAPD marker for assessment of genetic diversity in sugarcane cultivars. Indian Journal of Biotechnology 8:67-71.

Meyer, W., Mitchell, T. G., Freedman, E. Z. and Vilgalys, R. (1993). Hybridization probes for conventional DNA fingerprinting used as single primers in the polymerase chain reaction to distinguish strains of *Cryptococcus neoformans.* Journal of Clinical Microbiology 31:2274-2280.

Obručová, H., Tihelková, R., Kotásková, I., Růžička, F., Holá, V., Němcová, E. and Freiberger, T. (2016). Evaluation of Fluorescent Capillary Electrophoresis for Rapid Identification of *Candida* Fungal Infections. Journal of Clinical Microbiology 54:1295-1303.

Reddy, M. P., Sarla, N. and Siddiq, E. A. (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica 128:9-17.

Saker, M. M., Bekheet, S. A., Taha, H. S., Fahmy, A. S. and Moursy, H. A. (2000). Detection of somaclonal variations in tissue culture-derived date palm plants using isoenzyme analysis and RAPD fingerprints. Biologia Plantarum 43:347-351.

Schlötterer, C. and Tautz, D. (1992). Slippage Synthesis of Simple Sequence DNA. Nucleic Acids Research 20:211-215.

Srivastava, S. and Gupta, P. S. (2008). Inter Simple Sequence repeat profile as a genetic marker system in sugarcane. Sugar Tech 10:48-52.

Srivastava, A. K. and Rai, M. K. (2012). Sugarcane production: Impact of climate change and its mitigation. Biodiversitas 13:214-227.

Staub, J. E., Serquen, F. C. and Gupta, M. (1996). Genetic markers, map construction, and their application in plant breeding. HortScience 31:729-739.

Weising, K., Nybom, H., Wolf, K. and Kahl, G. (2005). DNA fingerprinting in plants: Principles, Methods and Applications (2nd ed). CRC Press, Boca Raton, Florida, USA.

Wu, K., Jones, R., Dannaeberger, L. and Scolnik, P. A. (1994). Detection of microsatellite polymorphisms without cloning. Nucleic Acids Research 22:3257-3258.

Zietkiewicz, E., Rafalski, A. and Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR) -anchored polymerase chain reaction amplification. Genomics 20:176-183.

(Received: 26 May 2018, accepted: 8 August 2018)