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Molecular Characterization of Early Maturing Sugarcane Clones Using Microsatellite Markers

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Authors' contributions

This work was carried out in collaboration among all authors. Authors RS and DNK designed, executed the experiment, performed the molecular analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PK and SKS managed the analysis of the study. Authors DS and RS managed the literature search. All authors read and approved the final manuscript.

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ABSTRACT

Sugarcane is an important cash crop of the entire world including India. It alone contributes to 75% of the worldwide sugar trade. Molecular markers are powerful tools and provide the basis for the estimation of genetic variability to start reasonable breeding program. Microsatellite markers have unique ability to determine the extent of genetic divergence among sugarcane genotypes. The objective of this study was to evaluate the genetic divergence of 12 early maturing sugarcane clones using 11 SSR markers. A total 55 alleles were found during the amplification of the primers out of which 21 alleles were found unique and 34 alleles were shared. The number of shared alleles per locus ranged from two out of five alleles in the case of primer NKS 1 and nine out of ten alleles in NKS 34. Similarly no. of unique alleles per locus ranged from one out of ten alleles in NKS 34, three out of six alleles in NKS 57. The primer pairs NKS 1 and NKS 8 generated considerably greater percentage of unique alleles. The PIC values revealing allelic diversity and frequency among the genotypes varied from 0.034 in case of NKS 48 to 0.778 in case of NKS 9 with an average of 0.549. Pair-wise combinations of CoSe15451 and CoSe15452 showed the highest similarity with the value of

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similarity coefficient (0.890). The lowest value of similarity coefficient was found to be 0.490 in the pair CoBln15501 and CoSe01421. The dendogram based on SSR marker analysis grouped the 12 sugarcane clones into four clusters which shows the CoSe15451 and CoSe15452 clones had maximum similarity and CoBln15501 and CoSe15452 clones had maximum diversity between each other.

Keywords: DNA; molecular; microsatellite marker; SSR; sugarcane.

1. INTRODUCTION

Sugarcane breeding is advancing in a broad direction with product diversification including sugar, energy, and fuel from the crop as comprehensive goals. *Saccharum* species are one of the most genetically complex plants due to polyploidy and relatively large genome size. Saccharum is a complex genus characterized by high ploidy levels and composed of at least six distinct species - *Saccharum officinarum*, *Saccharum barberi*, *Saccharum sinensi*, *Saccharum spontaneum*, *Saccharum robustum* and *Saccharum edule* (Daniels and Roach, 1987). These features, together with the high heterozygous nature, make planned genetic improvement programs guided by the principles of genetics a difficult exercise. Due to the genomic complexities of this crop, sufficient researches on agronomically important traits have not yet been conducted so far. However, significant improvements in sugarcane cultivars have been achieved because of successful research in the field of genetics and breeding both at classical and molecular levels [1].

Molecular markers are powerful tools for the estimation of genetic variability. These include random amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSR), amplified fragment length polymorphism (AFLP), and simple sequence repeats (SSR), also known as microsatellites. Among these ISSR markers have proved to be the most powerful tool for diversity analysis in molecular breeding due to their abundance, high reproducibility, multi-allelic nature, co-dominant inheritance, and cross transferability. The hypervariability of SSRs among related organisms makes them an informative and excellent choice of markers for a wide range of applications in sugarcane, which include assessment of genetic diversity is an essential prerequisite for identifying potential parents for hybridization Suman et al. [2], high-density genetic mapping [3], molecular tagging of genes [4], genotype identification [5].

The present study was undertaken to investigate the genetic diversity and establish the relationship between different sugarcane genotypes using SSR markers. Obtaining accurate estimates of the genetic diversity among germplasm sources may increase the efficiency of plant breeding. Knowledge of genetic diversity and relationships among breeding genome, their polymorphic nature, codominance and materials has a significant impact on crop improvement.

2. MATERIALS AND METHODS

2.1 Plant Material

A total of 12 early maturing sugarcane clones *viz*. CoBln15501, CoLk15466, CoLk15467, CoP15436, CoP15437, CoSe15451, CoSe15452, CoSe15455, CoSe15456, CoSe01421, CoLk94184 and CoSe95422 were used for present study. The experiment was conducted in the nursery block of the research farm of DRPCAU, Pusa, Samastipur, Bihar. Fresh leaf samples of each genotype were taken from three randomly selected plants in the sugarcane plots. Leaf samples were immediately put into an isotherm bucket containing ice gel pads and brought to the molecular laboratory of Plant Breeding and Genetics, Faculty of Agriculture, and samples were utilized for extraction of DNA [6].

2.2 DNA Extraction and Purification

DNA extraction is a basic procedure in scientific laboratories and is important to the study of heredity as well as to determining evolutionary relatedness. In this experiment, DNA was extracted from fresh leaf tissues using the CTAB method suggested by Suman et al. [7] without 2-Mercaptoethanol. Young and healthy leaves of 14-15 day old seedlings were used for DNA extraction. 0.2 g of fresh leaves were collected and washed with distilled water. They were cut into fine pieces and crushed with 1.5 ml CTAB buffer (0.2M Tris-HCl, 0.02M EDTA, 2% (w/v) CTAB, and 1.4M NaCl) using a mortar and pestle. Crushed samples were transferred to a 2 ml centrifuge tube and incubated at 65ºC for 1 hour in the water bath for effective lysis. After

incubation, 400 µl of Chloroform: isoamyl alcohol (24:1) was added to each tube and mixed well by back and forth movement several times till it reached milky form. Then tubes were centrifuged at 8000 rpm for 10 minutes. Repeat this step till the upper aqueous layer became transparent. If the supernatant appear transparent then it was transferred to another fresh 1.5 ml centrifuge tube using a pipette. Add 400 µl of pre-chilled isopropyl alcohol was added to each tube and the contents were mixed gently by inverting the tubes to precipitate the DNA. Centrifugation was done for 10 minutes at 10000 rpm, and DNA appeared as a pellet at the bottom of the centrifuge tube. Wash the DNA pellet with 76% ethanol containing 0.2M CH_3COONa followed by 76% ethanol containing 10mM $CH3COONH_4$ to remove contamination. Pellet was air dried for approximately 40 minutes (till the smell of ethanol disappear), by inverting the centrifuge tube on autoclaved tissue paper. Then dissolve the pellet into 50 µl of 1X TE buffer (10 mM Tris-HCl, 1 mM EDTA) along with 2 µl RNase A (10mg/ml) and incubated at 37ºC for 1 hr to digest the RNA. The dissolved DNA was stored at -20 °C until the DNA was utilized.

2.3 PCR Amplification

PCR-based simple sequence repeat (SSR) markers were used in order to examine the genetic variability among all collected samples. Optimization of PCR was done at a different temperatures depending upon the annealing temperature of the primer pairs. PCR amplification was performed on 20µl reaction volume containing 0.5 µl template DNA, including 0.2 µl Taq polymerase (5U/µl, Thermo Scientific), 2.0 µl green PCR buffer containing 20 mM $MgCl_2$ (10X buffer, Thermo Scientific), 2.0 µl dNTPs (2 mM dNTPs, Thermo Scientific), 0.5 µl of 5 mM of primer pair. The PCR condition was: initial denaturation at 94°C for 3 minutes, denaturation for 30 sec at 94°C, annealing for 60 seconds at 55°C & 58°C (depending upon the annealing temperature of the primer pairs) followed by 60 sec at 73°C. The final elongation step was at 72°C for 7 min. Storage of PCR products was done at 4°C before loading. Amplification was done using thermal cycler Benchtop, USA model number K960.

2.4 Gel Electrophoresis

Amplified fragments were resolved on 2% agarose (GeNei™) gel containing 2 µl, EtBr (10mg/ml, G-Biosciences) for 100 ml gel in 1X TBE buffer. An electrophoretic system (BIORAD) was used to run amplified products along with 50bp ladder (Thermo Scientific). The banding pattern was observed using a gel documentation system (Chemi Imager™ Ready).

2.5 Statistical Analysis

The present study was carried out to study the genetic diversity of 12 early maturing sugarcane clones using 11 SSR primers. For each SSR marker and each sample, fragment sizes were calculated by comparison with a 50 bp ladder (Thermo Scientific), and genotype scoring was carried out using software (1D image viewer). Genotypic data in the form of digits of the different band sizes were fed into an MS-Excel sheet for further analysis. The observed bands in Gel Documentation System were in decreasing order of the molecular weights for each primer. The following parameters were calculated.

Table 1. Details of SSR markers used to examine genetic variability among the Cross/self-populations with their sequences and annealing temperature (T_a)

Sl. no	SSR primer	Primer sequence		T_a (˚C)
		Forward(F)	Reverse (R)	
1	NKS 1	TGGCATGTGTCATAGCCAAT	CCCCAACTGGGACTTTTACA	58
2	NKS3	CGTGTTCCTCTTCAACAACG	TGCTTCGCTATATATGGGTTCA	58
3	NKS 8	GTGACAGCGGCTTGTTCAG	TTAAACACGCAGCCATTCAG	58
4	NKS 9	CTTTCAGTGGCCATCTCCAT	GAATGCGCAGGGATAGGATA	58
5	NKS 31	AACCACCACTCATCGTCCTC	CACCGAGTTCCCATTGTTCT	58
6	NKS 34	CGTCTTGTGGATTGGATTGG	TGGATTGCTCAGGTGTTTCA	58
7	NKS 38	TGAACTCGGCAACAGTTTTT	CCCACCAAGTCGTTCTGAAT	55
8	NKS 48	ACAATAACCCCGCAGACATC	TAATGCGTCATTTGGAGCAG	55
9	NKS 49	CTCACGTCCTGTTGGTGCTA	TACATGGGACACATGCTTGC	55
10	NKS 57	CGAGCCTCCCTCCATAGATT	ACCACCACCAACCTCATCTC	55
11	NKS 61	TTGGACATGGCAAGTCTTTG	AGGAACCTCCCAAGAACACA	55

2.6 Polymorphism Information Content (PIC) is Obtained by the Formula

PIC values were estimated by using the formula established by Anderson et al. [8].

$$PIC = 1-\sum_{j-1}^{k} P^2ij$$

Where, k is the number of marker alleles for marker i and Pij is the frequency of the jth allele for marker i.

Although, SSR markers considered as co-dominant markers, in this study they were found to be dominant in nature, because in highly polyploid genomes like sugarcane microsatellites have difficulty in differentiating the alleles of homologous chromosomes, thereby making it difficult to identify the homozygosity or heterozygosity at any specific locus [9]. From the above analysis, all possible alleles detected in the progenies were scored. For each genotype, clear and distinct alleles were transformed into a binary character matrix ("1" for the presence and "0" for the absence of a band at a particular position), which was used for designing genetic symmetry matrix of pairwise similarities (SM coefficients) between genotypes. The genetic similarity matrices were subjected to UPGMA (Unweighted Pair Group Method with Arithmetic Mean clustering) cluster analysis following the SAHN (Sequential Agglomerative Hierarchical Nested) cluster analysis module by NTSYS-pc v.2.1 software.

2.7 Computation of Similarity Coefficient

Molecular polymorphism detected by the microsatellite marker was recorded on the basis of the presence or absence of the microsatellite bands in different entries. All the entries were scored for the presence and absence of the microsatellite bands. Using the binary matrix as discrete variables, genetic similarities among the entries were calculated on the basis of pair-wise comparisons based on the proportions of shared bands (Dice, 1945) as under:

$$\text{Similarity coefficient} = 2a/a+b+c$$

Where, a, b and c represent the number of bands between j^{th} and k^{th} genotypes, a number of bands presented in j^{th} genotypes but absent in kth genotype and number of bands absent in j^{th} genotype but present in k^{th} genotype, respectively.

3. RESULTS

Molecular characterization of different progenies of sugarcane was done using genomic DNA amplification through SSR markers for assessment of genetic variability. For molecular characterization, 12 genotypes of early sugarcane were subjected to PCR using 11 SSR primer pairs for amplification. The data obtained from SSR loci could be used for distinguishing genotypes for the study of genetic relationships and assessment of genetic variability in the present experiment. After performing agarose gel electrophoresis, only clear and unambiguous bands were taken for scoring. Bands were scored for the presence (1) or absence (0) among all 12 genotypes. All pairs of SSR primers (11 pairs) showed polymorphism in terms of banding patterns whereas monomorphism among the given sugarcane clones is absent. The range of product size (bp) and the total number of bands generated.

Similarity coefficients for the pair-wise combinations of twelve genotypes under evaluation ranged from 0.491 to 0.891. Pair-wise combinations of CoSe15451 and CoSe15452 showed the highest similarity with the value similarity coefficient (0.891). The lowest value of the similarity coefficient was found to be 0.491 in the pair CoBln15501 and CoSe01421. Genetic divergence at the molecular level amongst the twelve entries under evaluation in relation to twenty-one characters was revealed by the range of similarity coefficients registered for the pair-wise combinations of entries (Table 2). Polymorphic information content PIC value ranged from 0.034 to 0.778 in NKS-48 and NKS-9 respectively with an average of 0.549 per primer. Considerably greater magnitude of PIC value was obtained in cases of primers NKS-9 (0.778) followed by NKS-61 (0.777), NKS-8 (0.667), NKS-3 (0.597), NKS-49 (0.583), NKS-34 (0.577), NKS-57 (0.569), NKS-31 (0.514), NKS-38 (0.479), NKS-1 (0.465) and NKS- 48 (0.034).

According to the SAHN (Sequential Agglomerative Hierarchical Nested) cluster analysis module by NTSYS-pc v.2.1 software (Fig. 2), the strains were divided into four clusters at appropriate phenon level, *viz*. cluster A, B, C & D. Cluster A consist of one genotype *i.e*. CoBln15501. Similarly, cluster B consist of two genotypes *i.e*. CoLk15466 and CoLk15467. Cluster C consist of five genotypes *i.e*. CoSe15455 followed by CoSe15456, CoSe95422(C), CoSe01421 and CoLk94184(C).

Cluster D consist of four genotypes which is CoP15436 followed by CoP15437, CoSe15451 and CoSe15452.

Cluster C can be sub-divided into two groups C_1 and C_2. C_1 contains genotypes CoSe15455, CoSe15456, CoSe95422 and CoSe01421 while C_2 contains CoLk94184 genotype.

C_1 group was further sub-clustered into two groups *i.e*. C_1a and C_1b. Cluster D was also sub-clustered into two groups D_1 and D_2. D_1 consist of one genotype *i.e*. CoP15436 whereas D_2 again sub-clustered into two groups having D_2a and D_2b. D_2a consist CoP15437 and D_2b contains CoSe15451 and CoSe15452 genotypes with maximum similarity.

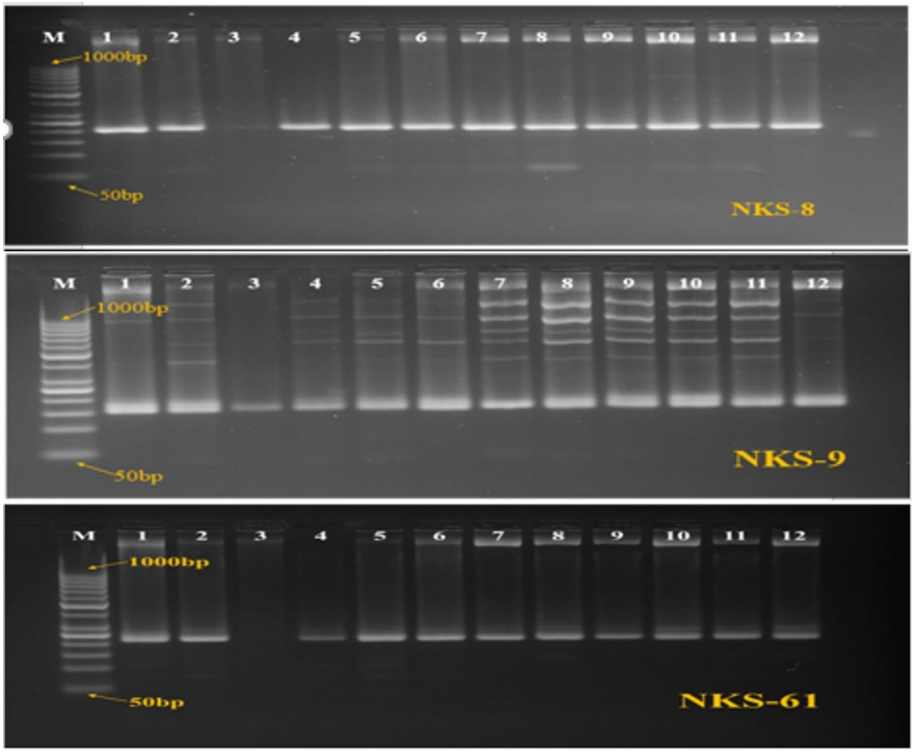

Fig. 1. Amplification pattern of a targeted genomic region in twelve genotypes of early maturing sugarcane clones obtained with the primers NKS-8, NKS-9, and NKS-61

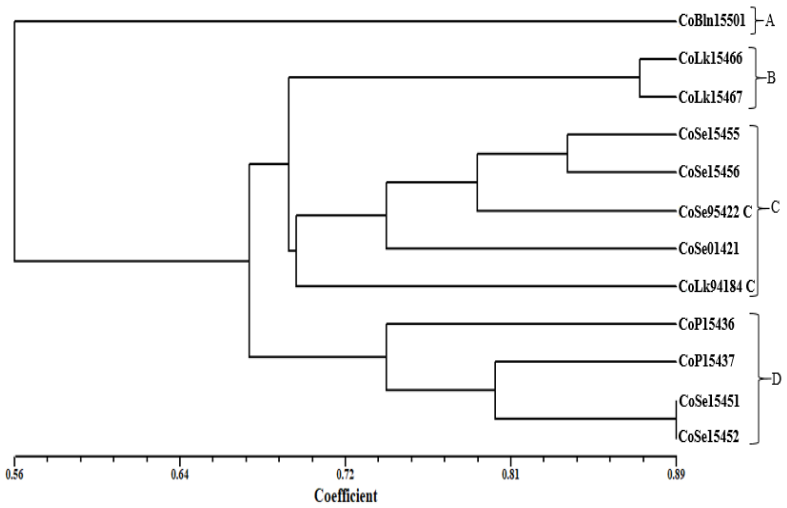

Fig. 2. Dendogram based on molecular characterization showing 12 early maturing sugarcane clones with 11 SSR markers

Table 2. Dice similarity coefficient matrices of 12 early maturing sugarcane clones

Clones	CoLk15466	CoLk15467	CoP15436	CoP15437	CoSe15451	CoSe15452	CoP15455	CoP15456	CoSe01421	CoLk94184 (C)	CoSe95422 (C)
CoBln15501	0.673	0.655	0.636	0.527	0.564	0.527	0.527	0.527	0.491	0.527	0.509
CoLk15466		0.873	0.745	0.600	0.673	0.673	0.673	0.709	0.673	0.673	0.691
CoLk15467			0.727	0.618	0.727	0.691	0.727	0.764	0.655	0.727	0.709
CoP15436				0.745	0.745	0.745	0.673	0.673	0.564	0.564	0.618
CoP15437					0.818	0.782	0.636	0.673	0.636	0.564	0.618
CoSe15451						0.891	0.782	0.818	0.709	0.636	0.727
CoSe15452							0.782	0.782	0.673	0.636	0.764
CoP15455								0.855	0.709	0.673	0.800
CoP15456									0.782	0.673	0.800
CoSe01421										0.709	0.764
CoLk94184 (C)											0.764

Table 3. Analysis of primer pairs used for the amplification of genomic DNA extracted from twelve early maturing sugarcane clones

Primers	No. of locus	Size of alleles	Number of alleles	Number of unique alleles	Percentage of unique alleles	Number of shared alleles	PIC	Number of entries having null alleles
NKS-1	2	255-314	5	3	60.00	2	0.465	0
NKS-3	1	200-214	4	1	25.00	3	0.597	0
NKS-8	1	208-235	5	3	60.00	2	0.667	0
NKS-9	1	170-200	5	1	20.00	4	0.778	0
NKS-31	1	208-223	4	2	50.00	2	0.514	0
NKS-34	3	129-230	10	1	10.00	9	0.577	0
NKS-38	2	149-200	4	2	50.00	2	0.479	0
NKS-48	2	123-225	4	2	50.00	2	0.034	0
NKS-49	2	130-163	4	2	50.00	2	0.583	1
NKS-57	2	108-293	6	3	50.00	3	0.569	0
NKS-61	1	234-251	4	0	00.00	4	0.777	1

4. DISCUSSION

4.1 Molecular Marker-Based Characterization of Entries

The analysis of variations in SSR fragments provides an effective tool for examining diversity to improve plant breeding strategies. The estimates of genetic similarity based on molecular markers may provide more accurate information to plant breeder. This data will support the exploitation of sugarcane germplasm on molecular basis Khan et al. [10].

4.2 Scoring of Alleles Based on Molecular Markers

The total alleles identified in the twelve clones were classified into two categories; (a) shared alleles and (b) unique alleles. A total of 55 alleles were found during amplification out of which 34 shared and 21 unique allelic variants were generated in the form of amplified product by polymerase chain reaction using eleven primer pairs. The number of shared alleles per locus ranged from two out of five alleles in the case of primer NKS 1 and nine out of ten alleles in NKS 34. Similarly number of unique alleles per locus ranged from one out of ten alleles in NKS 34 and three out of six alleles in NKS 57. The primer pairs NKS 1 and NKS 8 generated considerably greater percentage of unique alleles. Similar pattern were also studied by Liu et al. [11], Santosh et al. [12], Khan et al. [10], Padmanabhan and Hemaprabha [13], Ali et al. [14] and Suman et al. [7].

4.3 Evaluation of Allelic Diversity

In present investigation the level of polymorphism exhibited among the entries under evaluation by using eleven primer pairs were assessed by calculating polymorphism information content (PIC) of each of the primer pairs. The PIC values revealing allelic diversity and frequency among the genotypes varied from 0.034 in case of NKS 48 to 0.778 in case of NKS 9 with an average of 0.549 (Table 3). The work of several scientists indicated that PIC values varied for SSR markers used in sugarcane [15, 4]. Polymorphic information content (PIC) is a measure of the relative information content of a marker that indicates whether the marker is useful in determining polymorphism in germplasm [15]. PIC measures the extent of a marker system to differentiate among genotypes. Similar pattern were also studied by Liu et al. [11], Santosh et al. [12], Khan et al. [10], Padmanabhan and Hemaprabha [13], Ali et al. [14], Suman et al. [7], Khan et al. [16] and Huang et al. [17].

4.4 Evaluation of Genetic Similarity

The similarity coefficient values were computed amongst twelve sugarcane genotypes on the basis of the presence and absence of amplified product generated by using eleven primer pairs. Statistical measures of the similarity coefficients revealing genetic similarity with respect to the size of the amplified products generated from targeted regions of the genome varied from 0.491 to 0.891 for the pair-wise combinations among twelve entries under evaluation (Table 2). Genotypes CoBln15501 and CoSe01421 showed the lowest level of similarity *i.e.* 0.491 and genotypes CoSe15451 and CoSe15452 showed the highest level of similarity *i.e*. 0.891 as shown in Table 2. A similar pattern was also studied by Singh et al. [18], who suggested that the classification based on microsatellite markers will be useful for sugarcane breeders to plan crosses for agronomic traits. Padmanabhan and Hemaprabha [13] suggested that less/moderate genetic similarity indicates the availability of sufficient genetic diversity in the experimental material and hence their value in the genetic improvement of sugarcane. Khan et al. [10] concluded that SSRs markers are the best tool for the investigation of genetic diversity in sugarcane.

4.5 Clustering of Genotypes Based on Markers

A measure of genetic similarity was utilized for analysis of the nature and extent of differentiation and divergence amongst the entries. A dendrogram was constructed from the binary data deduced from the microsatellite-based molecular profiles of the samples analyzed where the genotype that was genetically more similar appeared to be clustered together. The clusters were identified at the appropriate phenon level. By drawing the phenon line taking into consideration 42 similarity units as cut off point, for drawing a phenon line in order to allow the genotypes with comparatively more similar patterns for markers to be clustered together. Therefore, four cluster were obtained when phenon line was drawn at 42 similarity units. Cluster I consist of one genotype, cluster II consists of two genotypes, cluster III consists of five genotypes and cluster IV consists of four

genotypes. At fifty similarity coefficient unit cut-off points, when phenon line was drawn to discriminate the entries, cluster III was further divided into sub-cluster. Clusters showed that the genotypes CoBln15501 and CoSe15452 had maximum dissimilarity and genotypes CoSe15451 and CoSe15452 had maximum similarity between each other. Similar patterns and high genetic diversity were also reported by Cardeiro et al. (2003) in sugarcane species.

A perusal of the dendrogram clearly indicated that the genetic polymorphism revealed at the molecular level on the basis of variation in the length of simple sequence repeats was an efficient tool for discrimination of entries and analysis of differentiation and divergence. The ample diversity exhibited by the markers seemed to be unbiased and not due to chance since the markers were chosen from all the chromosomes of sugarcane. So, these markers can be efficiently utilized for discrimination and unambiguous identification of different entries.

5. CONCLUSION

Using a panel of 11 SSR primer pairs, reproducible amplification was successfully achieved in a set of 12 purposefully selected sugarcane clones. The PIC values revealing allelic diversity and frequency among the genotypes ranged from 0.034 to 0.778 with an average value of 0.549. A total of 55 alleles were found during the amplification of the primers out of which 21 alleles were found unique and 34 alleles were shared. Ample genetic differentiation and divergence were revealed at the molecular level amongst the early maturing sugarcane clones using the SSR primer pairs. Hierarchical cluster analysis using SSR markers enabled differentiation amongst the selected genotype. Several of these highly polymorphic SSR markers have proven useful in sugarcane germplasm evaluation, variety identity tests, cross-fidelity assessment and poly cross paternity analysis. Hence, these markers can be effectively and efficiently utilized in a breeding program for the selection of better parental combinations to get a better hybrid. From the present study, it may be concluded that SSRs markers are best tool for investigation of genetic diversity in sugarcane.

SIGNIFICANE OF STUDY

This manuscript reveals the genetic diversity and similarity between the early maturing sugarcane clones for further breeding programs.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Swapna M, Srivastava S. Molecular marker applications for improving sugar content in sugarcane. In Molecular Marker Applications for Improving Sugar Content in Sugarcane Springer, Boston, MA. 2012;1-49.
Available:https://link.springer.com/chapter/10.1007/978-1-4614-2257-0_1
2. Suman SK, Kumar M, Kumar R, Kumar A, Singh D, Kumar A. Assessment of genetic diversity in inbred lines of maize (*Zea mays* L.) and its relationship with heterosis. International journal of Chemical Studies. 2020a;8(4):2917-2920.
Available:https://doi.org/10.22271/chemi.2020.v8.i4ai.10089
3. Chen C, Yu Q, Hou S, Li Y, Eustice M, Skelton RL, Feng Y. Construction of a sequence-tagged high-density genetic map of papaya for comparative structural and evolutionary genomics in brassicales. Genetics. 2007;177(4):2481-2491.
Available:https://academic.oup.com/genetics/article/177/4/2481/6064480
4. Singh RK, Singh P, Misra P, Singh SP, Singh SB. STMS marker for tagging high sugar genes in sugarcane. Sugar Tech. 2005;7(2-3):74-76.
Available:https://link.springer.com/article/10.1007/BF02942534
5. Pan YB. Highly polymorphic microsatellite DNA markers for sugarcane germplasm evaluation and variety identity testing. Sugar Tech. 2006;8(4):246-256.
Available:https://link.springer.com/article/10.1007/BF02943564
6. Hoisington D. Laboratory protocol. Mexico, DF: CIMMYT applied molecular genetics laboratory; 1992.
Available:https://scholar.google.com/scholar

7. Suman SK, Kumar M, Sharma VK, Kumar R. A modified and efficient CTAB genomic DNA extraction method from maize leaf for PCR analysis. Journal of Pharmacognosy and Phytochemistry. 2020;9(4):3418-3420.
Available:https://www.phytojournal.com/archives/2020/vol9issue4/PartAI/9-4-427338.pdf
8. Anderson JA, Churchil GA, Autrique JE, Tanksley SO, Sorrels ME. Optimizing parent selection for genetic linkage maps. Genome. 1993;36:181-186.
Available:https://cdnsciencepub.com/doi/abs/10.1139/g93-024
9. Oliveira KM, Pinto LR, Marconi TG, Mollinari M, Ulian EC, Chabregas SM, Souza AP. Characterization of new polymorphic functional markers for sugarcane. Genome. 2009;52(2): 191-209.
Available:https://cdnsciencepub.com/doi/abs/10.1139/G08-105
10. Khan FA, Afzal A, Ijaz A, Ijaz U. Diversity analysis of sugarcane genotypes by microsatellite (SSR) markers. International Journal of Biotechnology and Molecular Biology Research. 2013;4(7):105-110.
Available:https://academicjournals.org/journal/IJBMBR/article-full-text-pdf/CEDB85541726
11. Liu YQ, Yong B, Pan P. Highly polymorphic microsatellite DNA markers for sugarcane germplasm evaluation and variety identity testing. Sugar Tech. 2011;13(2):129-136.
Available:https://link.springer.com/article/10.1007/s12355-011-0077-1
12. Santosh M, Liu SJ, Tsunogae T, Li JH. Paleoproterozoic ultrahigh-temperature granulites in the North China Craton: implications for tectonic models on extreme crustal metamorphism. Precambrian Research. 2012;222:77-106.
Available:https://www.sciencedirect.com/science/article/abs/pii/S0301926811001045
13. Sarath Padmanabhan TS, Hemaprabha G. Genetic diversity and population structure among 133 elite genotypes of sugarcane (*Saccharum* spp.) for use as parents in sugarcane varietal improvement. 3 Biotech. 2018;8(8):1-12.
Available:https://link.springer.com/article/10.1007/s13205-018-1364-2
14. Ali A, Pan YB, Wang QN, Wang JD, Chen JL, Gao SJ. Genetic diversity and population structure analysis of Saccharum and Erianthus genera using microsatellite (SSR) markers. Scientific Reports. 2019;9(1):1-10.
Available:https://www.nature.com/articles/s41598-018-36630-7
15. Cordeiro GM, Pan YB, Henry RJ. Sugarcane microsatellites for the assessment of genetic diversity in sugarcane germplasm. J. of Plant Sci. 2003;165:181-189.
Available:https://www.sciencedirect.com/science/article/pii/S0168945203001572
16. Khan Q, Qin Y, Guo DJ, Zeng XP, Chen JY, Huang YY, Li YR. Morphological, agronomical, physiological and molecular characterization of a high sugar mutant of sugarcane in comparison to mother variety. Plos one. 2022;17(3): e0264990.
Available:https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0264990
17. Huang HR, Gao YJ, Malviya MK, Verma KK, Solanki MK, Huang YX, Xu L. Genetic diversity analysis of sugarcane (*Saccharum* spp. hybrids) among high-sucrose clones of GT series and commonly used parents by using microsatellite markers in Guangxi, China. Sugar Tech. 2022;24(2):397-407.
Available:https://link.springer.com/article/10.1007/s12355-021-01033-y
18. Singh RK, Singh RB, Singh SP, Sharma ML. Identification of sugarcane microsatellites associated to sugar content in sugarcane and transferability to other cereal genomes. Euphytica. 2011;182(1): 335–354.
Available:https://link.springer.com/article/10.1007/s10681-011-0484-0

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