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# Molecular Characterization of Mid-late Maturing Sugarcane Clones by Using Microsatellite Markers

**Pooja Kumari [a*], Balwant Kumar [a], D. N. Kamat [a], Rajvinder Singh [a], Digvijay Singh [a,b] and Sandeep Kumar Suman [c]**

*[a] Department of Plant Breeding and Genetics, Dr Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar-848125, India.*
*[b] Narayan Institute of Agricultural Sciences, GNS University, Jamuhar, Sasaram, Bihar-821305, India.*
*[c] Department of Agricultural Biotechnology and Molecular Biology, Dr Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar-848125, India.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

A total of 56 alleles were obtained, of which 41 shared and 15 unique allelic variants were generated as the amplified product by polymerase chain reaction using eleven primer pairs. The PIC values revealing allelic diversity and frequency among the genotypes varied from 0.36 in the case of NKS 57 to 0.90 in the case of NKS 1 with an average of 0.62. The similarity coefficients revealing genetic similarity with respect to the size of the amplified products generated from targeted regions of the genome varied from 0.518 to 0.857 for the pairwise combinations amongst the thirteen entries under evaluation. Two Clusters were obtained when the phenol line was drawn at twenty-five similarity units. Cluster I consist of ten genotypes. The tri-genotypic cluster II consists

**Corresponding author: E-mail: poojaudaysah11@gmail.com;*

of three genotypes. When phenol level drew at fifty similarity unit clusters I and II were further divided into sub-clusters. It is concluded that using an SSR marker is a very reliable approach for identifying diverse genotype(s) where phenotypic similarity of the cultivars leads to difficulty while selecting parents for hybridization.

*Keywords: Sugarcane; molecular; shared alleles; unique alleles; microsatellite marker; and similarity coefficient.*

## 1. INTRODUCTION

*Saccharum officinarum* L. most probably originated in the East Indonesian/New Guinea area, *S. barberi* in India, and *S. sinenses* in China. The present-day sugarcane is considered to be the outcome of polyploidization and hybridization consisting predominantly of *S. officinarum* L. (2n = 80), known as 'noble cane' [1] and 'wild cane' *S. spontaneum* (2n = 40–120), along with minor contributions from *S. robustum, S. sinense, S. barberi* and related genera such as *Miscanthus, Narenga and Erianthus* [2]. The genus Saccharum is complex and consists of six species viz. *S. officinarum, S*. *barberi*, *S*. *sinenses*, *S*. *edule*, S. *spontaneum,* and *S*. *robustum*, out of this S. spontaneum and S. *robustum* is the wild in nature while the other four are cultivated species [3-8]. The sugarcane varieties are man-made hybrid clones involving *Saccharum officinarum* and *S. spontaneum* with a few genes incorporated from, *S. sinense*, *S*. *barberi,* and to a limited extent *S*. *robustum* [9]. "Sugarcane crop is the main feedstock for the sugar industry which contributes about 70–80% of global sugar production" [10]. "Many years ago, sugar industry operated only for sugar production, and their leftovers were discarded as unserviceable wastes, while presently this cellulosic biomass is reprocessed for electricity cogeneration, and other industrially useful products" [11]. The mid-late clones are those clones that achieve 16% sucrose and 85% purity for maturity during the 10 to 12months stage [12-15]. The majority of sugarcane areas of Bihar are covered by mid-late varieties. It is a well-known fact that for the smooth running of sugar factories scheduling of varieties as early and mid-late maturity groups must be 40:60 ratios, respectively [16,17].

Screening and evaluating the available genetic variability with molecular markers will help facilitate molecular-based genetic relationships for the exploitation of new gene resources of sugarcane to help broaden the genetic base of sugarcane in subtropical India [18]. Morphological markers are routinely used for genetic diversity analysis, but recently many molecular marker techniques have been developed as powerful tools to analyze sugarcane genotypes for their commercial exploitation and selecting specific genetically diverse parents for use in introgressive breeding [19,20]. "The SSR markers have proved to be the most powerful tool for diversity analysis in molecular breeding due to their abundant genetic distribution, high reproducibility, multi-allelic nature codominant inheritance, and cross-transferability to closely related genera" [21], Gupta and Prashad, [22], Yu et al. [23].

In the present study, the SSR (Simple Sequence Repeats) marker system was used to detect genetic variation and relatedness among the different genotypes of sugarcane (*Saccharum* spp.). DNA-based marker systems offer a more reliable and environmentally neutral alternative to detect genetic polymorphism useful for genotype identification. The genotypes were subjected to SSR (Simple Sequence Repeat) molecular markers. Experimental strategies were developed by adopting various standards with some modifications as per the need of the experiments.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

The material of this investigation comprises 13 promising mid-late maturing sugarcane clones *viz*, CoP15438, CoP15439, CoP15440, CoP15441, CoSe15453, CoSe15454, CoSe15457, CoLk15468, CoLk15469, CoBln15502 CoP2061, BO91(C) and CoP9301(C). "The experiment was conducted in the nursery block of the research farm of DRPCAU, Pusa, Samastipur, Bihar (2018). Fresh leaf samples of each genotype were taken and put into zip lock bags then put immediately 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". [24].

## 2.2 Genomic DNA Isolation and Purification

DNA isolation 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 will be extracted from fresh sugarcane leaf tissue as described by Srivastava and Gupta [25]. CTAB procedure was used for the isolation of DNA. CTAB (Cetyl tri methyl ammonium bromide) is a cationic detergent that solutes membranes and forms a complex with DNA. This method could remove polysaccharides, which inhibit the activity of endonuclease enzymes. Before starting the DNA isolation, the water bath was turned on and the temperature was set to 65oC and pre-heat 2X CTAB with 0.2% 2-mercaptoethanol in the water bath. Autoclaved pestle and mortar with liquid nitrogen were pre-cooled. Young and healthy leaves of 14-15 days old seedlings were used for DNA extraction. Then after taking 0.2 g of leaves and cut them into pieces and crushed them with CTAB buffer using a mortar and pestle. Crushed samples are transferred to a 2 ml centrifuge al tube. The samples are then incubated for 1hr at 65ºC. After incubation, samples are centrifuged for 5 min at 8000 rpm. The supernatant is filtered through a 1 ml pipette into another fresh 2 ml tube. 700 µl of chloroform/isoamyl alcohol (24:1) is added to each tube. The tubes are centrifuged for 20 min at 4000 rpm. The supernatant is filtered through a pipette into another 1.5 ml centrifuge tube. 400µl of pre-chilled isopropanol alcohol was added to each tube and the contents were mixed gently by inverting the tubes several times till they reached to milky state the tubes were kept overnight in the refrigerator. Although DNA precipitation began as soon as isopropanol was added, it was kept overnight for better and complete precipitation. Then, samples were centrifuged for 15 min at 14,000 rpm. The solution was poured without disturbing the DNA pellet at the bottom of the tubes. Wash the DNA pellet with 70% ethanol. Add 1.5 ml 70% ethanol to all tubes and then centrifuged at 5,000 rpm for 2 min. The supernatant was decanted. Pellet was air-dried for 2-3 hrs then add TE buffer or PCR was carried out after which measured the concentration of the DNA was by loading it in agarose gel and then quantified in a Spectrophotometer.

## 2.3 PCR Amplification

"PCR amplification was performed on 20 µl 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 nM MgCl2 (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 a thermal cycler Benchtop, USA model number K960" [24].

## 2.4 Agarose 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 a 50bp ladder (Thermo Scientific). The banding pattern was observed using a gel documentation system (Chemi Imager™ Ready)" [24].

## 2.5 Molecular Analysis of Banding Pattern and Statistical Analysis

"The present study was carried out to study the genetic diversity of 13 mid-late 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" [24]. The amplification products were viewed under UV light and photographs were saved for the experimental evaluation. The amplification products were scored separately for each primer. The bands were scored for the presence or absence by binary coding i.e., assigning a value of 1 for presence and 0 for absence in a lane. The molecular size (bp) of the amplified DNA fragment was determined by the DNA ladder marker which was used in two wells of agarose gel and for the clustering method using NTSYS software.

**Table 1. List of eleven SSR primer pairs used for DNA amplification**

| S.no. | Primer | Forward sequence | Reverse sequence | Annealing temperature |
|---|---|---|---|---|
| 1. | NKS 1 | TGGCATGTGTCATAGCCAAT | CCCCAACTGGGACTTTTACA | 55ºC |
| 2. | NKS 3 | CGTGTTCCTCTTCAACAACG | TGCTTCGCTATATATGGGTTCA | 55ºC |
| 3. | NKS 8 | GTGACAGCGGCTTGTTCAG | TTAAACACGCAGCCATTCAG | 55ºC |
| 4. | NKS 9 | CTTTCAGTGGCCATCTCCAT | GAATGCGCAGGGATAGGATA | 55ºC |
| 5. | NKS 31 | AACCACCACTCATCGTCCTC | CACCGAGTTCCCATTGTTCT | 55ºC |
| 6. | NKS 34 | CGTCTTGTGGATTGGATTGG | TGGATTGCTCAGGTGTTCA | 55ºC |
| 7. | NKS 38 | TGAACTCGGCAACAGTTTTT | CCCACCAAGTCGTTCTGAAT | 58ºC |
| 8. | NKS 48 | ACAATAACCCCGCAGACATC | TAATGCGTCATTTGGAGCAG | 55ºC |
| 9. | NKS 49 | CTCACGTCCTGTTGGTGCTA | TACATGGGACACATGCTTGC | 55ºC |
| 10. | NKS 57 | CGAGCCTCCCTCCATAGATT | ACCACCACCAACCTCATCTC | 55ºC |
| 11. | NKS 61 | TTGGACATGGCAAGTCTTTG | AGGAACCTCCCAAGAACACA | 55ºC |

## 2.6 Computation of Polymorphic Information Content (PIC)

"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" [26]. Polymorphic and informative markers were identified on the basis of a comparison of polymorphism information content (PIC) of the microsatellite primer pairs. The polymorphism information content (PIC) of the microsatellite primer pairs was calculated according to the formula (Anderson *et al.,* 1993) [27] as follows:

Polymorphic information content (PIC) = $1-\sum X_i^2$

Where $X_i$ is the relative frequency of the $i^{th}$ allele of the SSR loci.

## 2.7 Computation of Similarity Coefficient

Molecular polymorphism detected by 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:

Similarity coefficient = $2a/a+b+c$

Where,

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

## 3. RESULTS AND DISCUSSION

A total of 11 SSR primer pairs were used for molecular characterization. Molecular markers are powerful tools to estimate genetic variability in vivo and in vitro as they are accurate, abundant, and not affected by the environment. In the present study, the SSR (Simple Sequence Repeats) marker system was used to detect genetic variation and relatedness among the different genotypes of the sugarcane (*Saccharum* spp) hybrid complex.

## 3.1 Scoring of Alleles Based on Molecular Markers

Recognizable polymorphism in the form of the presence or absence of bands, in addition to variation in respect of the number and position of bands, was observed among the thirteen clones of sugarcane under evaluation in the present study. Differential ability was exhibited by the primer pairs to determine variability among the clones. It was observed that some of the primers generated several allelic variants but some generated only a few. The total alleles identified in the thirteen clones were classified into two categories; (a) shared alleles and (b) unique alleles (Table 2). A total of 56 alleles were obtained of which 41 shared and 15 unique allelic variants were generated in the form of the amplified product by polymerase chain reaction using eleven primer pairs. The number of shared alleles per locus ranged from two out of four alleles in the case of primer NKS 8 and six out of seven alleles in NKS 34. Similarly, the number of unique alleles per locus ranged from one out of seven alleles in NKS 34, three out of seven alleles in NKS1, and two in the case of four alleles in NKS8. The primer pairs NKS 1 and NKS 31 generated a considerably greater percentage of unique alleles. The number of null

alleles is absent. Null alleles are generally generated due to the failure of amplification for a particular repeat locus specific to the unique flanking sequence of the microsatellite.

Remarkably higher polymorphism percent was exhibited by the primer pairs NKS 1, NKS 31, NKS 8, NKS 9, NKS 61, NKS 49, NKS 34, and NKS 3 with a greater percentage of unique alleles in descending order of magnitude. The polymorphism percent revealed in the form of the percentage of unique alleles was recorded to be the maximum in the case of NKS 1 and NKS 31 (23.07) and the minimum in the case of NKS 3 and NKS 34 (7.69) with an average value of 40.29 percent. The level of polymorphism exhibited among the entries under evaluation in the present study using eleven primer pairs was assessed by calculating the polymorphism information content (PIC) of each of the primer pairs (Table 2). The PIC values revealing allelic diversity and frequency among the genotypes varied from 0.36 in the case of NKS 57 to 0.90 in the case of NKS 1 with an average of 0.62. Similar patterns were also studied by Yon Ban Pan [21], Liu et al. [28], Santos et al. [29], Simullah et al. [30], Ahmed and Gardezi [31], Padmanabhan and Hemaprabha [32], Ali et al. [33].

## 3.2 Evaluation of Allelic Diversity

"In the present investigation, the level of polymorphism exhibited among the entries under evaluation by using eleven primer pairs was assessed by calculating the polymorphism information content (PIC) of each of the primer pairs (Table 2). The PIC values revealing allelic diversity and frequency among the genotypes varied from 0.36 in the case of NKS 57 to 0.90 in the case of NKS 1 with an average of 0.62. The work of several scientists indicated that PIC values varied for SSR markers used in sugarcane" [34], and Singh et al. [24]. "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" [26]. "PIC measures the extent of a marker system to differentiate among genotypes" [35]. Similar patterns were also studied by Yon Ban Pan [21], Liu et al. [28], Santos et al. [29], Simullah et al. [30], Ahmed and Gardezi [31], Padmanabhan and Hemaprabha [32] and Ali et al. [33].

## 3.3 Evaluation of Genetic Similarity

The similarity coefficients were computed amongst thirteen sugarcane genotypes on the basis of the presence and absence of amplified product generated by using eleven primer pairs (Table 3). 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 0.518 to 0.857 for the pair-wise combinations among thirteen entries under evaluation. The value of the similarity coefficient between BO91 and CoSe15454 (0.857) also CoP9301 and CoLk15468 (0.857) were found to be the maximum among the pair-wise combinations of all the entries (Table 3). This was followed by the remarkably higher magnitude of similarity coefficients between CoP15453 and CoP15438 (0.839), CoSe15454 and CoBln15502 (0.839), BO91 and CoBln (0.839), CoP2061 and BO91 (0.821), CoP15439 and CoP15440 (0.821) in descending order of magnitude. The minimum value of similarity

**Table 2. Analysis of primer pairs used for the amplification of genomic DNA extracted from thirteen sugarcane genotypes**

| Primers | 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 | 274 – 320 | 7 | 3 | 23.07 | 4 | 0.90 | 0 |
| NKS 3 | 195 – 226 | 5 | 1 | 7.69 | 4 | 0.76 | 0 |
| NKS 8 | 210 – 221 | 4 | 2 | 15.38 | 2 | 0.62 | 0 |
| NKS 9 | 166 – 216 | 5 | 2 | 15.38 | 3 | 0.62 | 0 |
| NKS 31 | 222 – 245 | 6 | 3 | 23.07 | 3 | 0.78 | 0 |
| NKS 34 | 126 – 184 | 7 | 1 | 7.69 | 6 | 0.61 | 0 |
| NKS 38 | 181 – 192 | 3 | 0 | 0 | 3 | 0.57 | 0 |
| NKS 47 | 116 – 200 | 3 | 0 | 0 | 3 | 0.44 | 0 |
| NKS 49 | 156 – 174 | 5 | 1 | 7.89 | 4 | 0.70 | 0 |
| NKS 57 | 109 – 305 | 6 | 0 | 0 | 6 | 0.36 | 0 |
| NKS 61 | 220 – 278 | 5 | 2 | 15.38 | 3 | 0.42 | 0 |

**Table 3. Dice similarity coefficient matrix between thirteen mid-late sugarcane clones**

| | CoP15440 | CoLk15469 | CoP9301 | CoLk15468 | CoSe15453 | CoP15438 | CoP2061 | BO91 | CoSe15454 | CoBln15502 | CoP15441 | CoSe15457 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CoP15439 | 0.821 | 0.786 | 0.643 | 0.679 | 0.607 | 0.661 | 0.589 | 0.554 | 0.589 | 0.607 | 0.554 | 0.589 |
| CoP15440 | | 0.750 | 0.643 | 0.643 | 0.607 | 0.625 | 0.554 | 0.518 | 0.554 | 0.571 | 0.554 | 0.554 |
| CoLk15469 | | | 0.679 | 0.714 | 0.643 | 0.661 | 0.554 | 0.554 | 0.589 | 0.607 | 0.554 | 0.589 |
| CoP9301 | | | | 0.857 | 0.714 | 0.768 | 0.661 | 0.696 | 0.732 | 0.714 | 0.589 | 0.589 |
| CoLk15468 | | | | | 0.714 | 0.696 | 0.625 | 0.625 | 0.696 | 0.714 | 0.696 | 0.625 |
| CoSe15453 | | | | | | 0.839 | 0.696 | 0.732 | 0.696 | 0.714 | 0.589 | 0.589 |
| CoP15438 | | | | | | | 0.786 | 0.821 | 0.786 | 0.768 | 0.571 | 0.607 |
| CoP2061 | | | | | | | | 0.821 | 0.750 | 0.768 | 0.679 | 0.786 |
| BO91 | | | | | | | | | 0.857 | 0.839 | 0.607 | 0.679 |
| CoSe15454 | | | | | | | | | | 0.839 | 0.643 | 0.714 |
| CoBln15502 | | | | | | | | | | | 0.732 | 0.768 |
| CoP15441 | | | | | | | | | | | | 0.786 |

coefficient between CoP15440 and BO91 (0.518), CoP15439 and CoP15441 (0.554), CoP15440 and CoP15441, CoP15440 and CoSe15454 (0.554). The results revealed that ample diversity at the molecular level among the thirteen entries under evaluation in the present analysis. The similarity coefficients were computed amongst thirteen 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 0.518 to 0.857 for the pair-wise combinations among the thirteen entries under evaluation. A similar pattern was also studied [36] who suggested that "the classification based on microsatellite markers will be useful for sugarcane breeders to plan crosses for agronomic traits", Padmanabhan and Hemaprabha [32] also suggested that "less/moderate genetic similarity indicating the availability of sufficient genetic diversity in the experimental material and hence their value in the genetic improvement of sugarcane". Simullah et al. [30] concluded that "SSR markers are the best tool for the investigation of genetic diversity in sugarcane".

### 3.4 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 obtained 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 appropriate phenon levels. (Table 4). By drawing the phenon line taking into consideration 25 similarity units as cut-off points, drawing a phenon line in order to to allow the genotypes with a comparatively more similar pattern for markers to be clustered together. Therefore, two clusters were obtained when phenon line was drawn at twenty-five similarity units. Cluster I consist of ten genotypes CoP9301, CoLk15468, CoSe15453, CoP 15438, BO91, CoSe15454, CoBln15502, CoP2061, CoSe15457 and CoP15441. The tri-genotypic cluster II consist of three clones CoP15439, CoP15440 and CoLk15469. Keeping fifty similarity coefficient unit cut-off point, when phenon line was drawn to discriminate the entries, the cluster I and II were further divided into sub-cluster. Cluster I was divided into three sub cluster IA, IB and IC, IA sub cluster divided into two genotypes CoP9301 and CoLk15468 while sub-cluster IB is divided into five genotypes CoSe15453, CoP15438, B091, CoSe15454 and CoBln15502 lastly sub- cluster IC was divided into three genotypes CoP2061, CoSe15457 and CoP 15441. Similarly, cluster II were further not divided into sub cluster.

At seventy five similarity coefficient units cut-off point, when phenol line was drawn to discriminate the entries, the sub-cluster IA, IB, IC and II were divided into sub sub-clusters. Cluster IA were not further divided and has two entries CoP9301, and CoLk15468, while sub-cluster IB is further divided into three more sub-sub-cluster IBa and IBb. The sub-sub-cluster IBa has two clones CoSe15453 and CoP15438, and sub-sub-cluster IBb has one genotype BO91, CoSe15454 and CoBln15502. Similarly, sub-cluster IC is divided into three sub-sub-cluster ICa, ICb and ICc. Sub-sub-cluster ICa having onegenotypes CoP2061, while mono genotypic sub-sub-cluster ICb have CoSe15457 at lastly sub-sub-cluster ICc also contains one genotype CoP15441. Cluster II further divided into two sub-cluster IIA and IIB. The di-genotypic Cluster IIA contains CoP15439 and CoP15440 similarly monogenotypic sub-cluster IIB contains CoLk15469.

"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 the discrimination of entries and analysis of differentiation and divergence. A measure of genetic similarity was utilized for analysis of the nature and extent of differentiation and divergence amongst the entries. A dendrogram was obtained 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 25 similarity units as the cut-off point, drawing a phenon line in order to allow the genotypes with a comparatively more similar pattern for markers to be clustered together. Therefore, two clusters were obtained when the phenon line was drawn at twenty-five similarity units. Cluster I consist of ten genotypes and Cluster II consists of three genotypes" [24]. At

fifty similarity coefficient unit cut-off points, when phenon line was drawn to discriminate the entries, cluster I and II were further divided into sub-clusters. Similar patterns were also studied by Cardeiro et al. [26] and Silva et al. [29] who reported high genetic diversity in sugarcane species. 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.

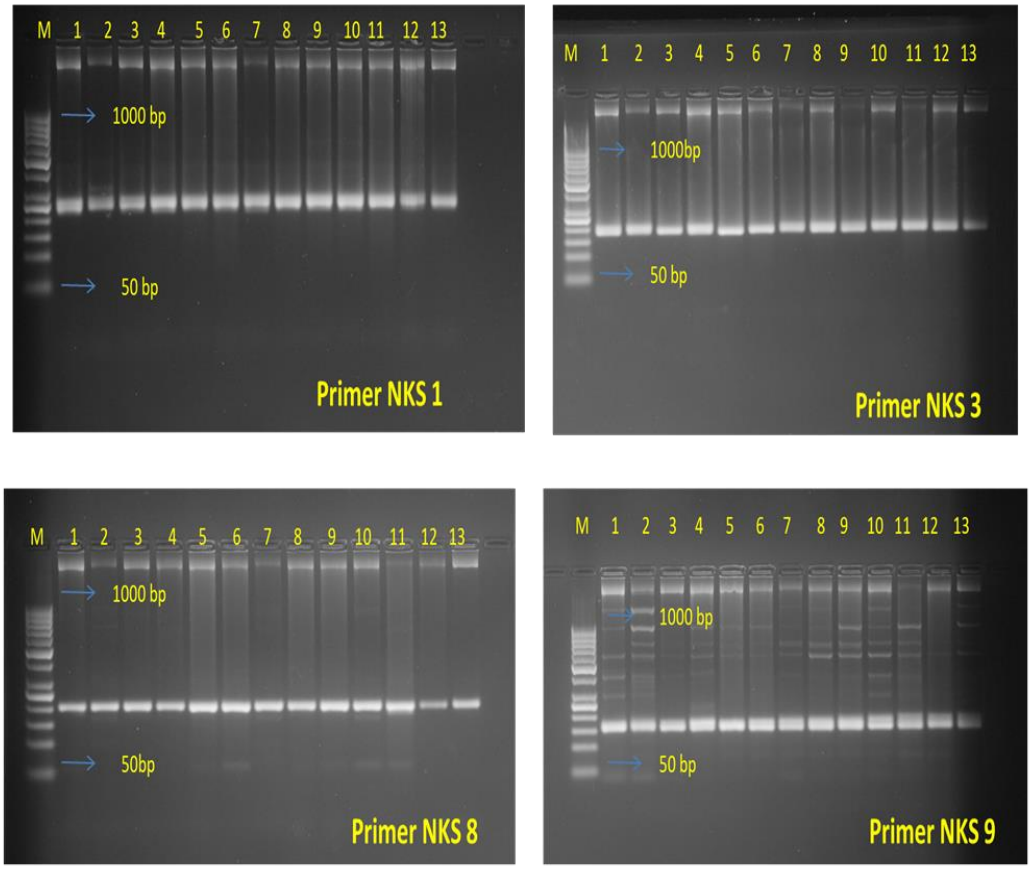


Fig. 1: Amplification region of genomic DNA extracted from leaf at seedling stage in sugarcane by primer NKS 1, NKS 3, NKS 8 and NKS 9

| | | | | | | |
|---|---|---|---|---|---|---|
| 1.CoP15439 | 2.CoP15440 | 3.CoLk15469 | 4.CoP9301 | 5.CoLk 15468 | 6.CoSe15453 | 7.CoP15438 |
| 8.CoP 2061 | 9.BO91 | 10.CoSe15454 | 11.CoBln 15502 | 12.CoP15441 | 13.CoSe15457 | |

**Table 4. Grouping of clones into various clusters at different phenon level among thirteen genotypes of mid late maturing sugarcane clones**

| No. of clusters identified at different phenon levels * | | | Genotypes included in each cluster |
|---|---|---|---|
| 25 | 50 | 75 | |
| I (10) | IA (2) | IA (2) | CoP9301 and CoLk15468 |
| | IB (5) | IBa (2) | CoSe15453 and CoP15438 |
| | IC (3) | IBb (3) | BO91, CoSe15454 and CoBln15502 |
| | | ICa (1) | CoP2061 |
| | | ICb (1) | CoSe15457 |
| | | ICc (1) | CoP15441 |
| II (2) | II (3) | IIA (2) | CoP15439 and CoP15440 |
| | | IIB (1) | CoLk15469 |

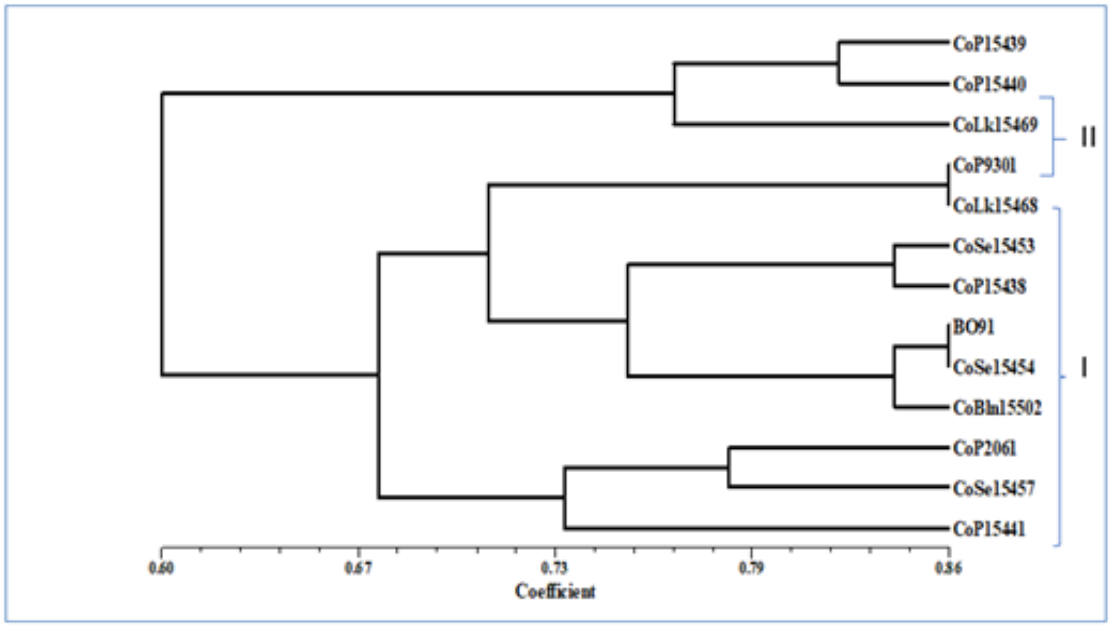


**Fig. 2. Dendogram depicting the classification of thirteen mid-late maturing sugarcane clones constructed on the basis of UPGMA methods and based on SSR markers**

## 4. CONCLUSION

Microsatellite marker-based analysis revealed unique or variety-specific alleles which could be useful as DNA fingerprints of sugarcane genotypes. The use of eleven microsatellite markers in the analysis of sugarcane clones exhibited a remarkably higher level of genetic polymorphism, which allowed unique genotyping of thirteen entries included in the analysis. Altogether 56 allelic variants were detected among the thirteen sugarcane clones with an average of 4.30 alleles per primer. A total of 41 shared and 15 unique allelic variants were generated in the form of amplified products by using 11 primer pairs. Considerably a greater number of alleles had been detected in primer pairs NKS 1, NKS 34, NKS 31, and NKS 5. The PIC values revealing allelic diversity and frequency among the genotypes varied from 0.36 in the case of NKS 57 to 0.90 in the case of NKS 1 with an average of 0.62. The similarity coefficients revealing genetic similarity with respect to the size of the amplified products generated from targeted regions of the genome varied from 0.518 to 0.857 for the pairwise combinations amongst the thirteen entries under evaluation. The similarity coefficient value between BO91 and CoSe15454 (0.857) and CoP9301 and CoLk15468 (0.857) was found to be the maximum amongst the pair-wise combinations of all the entries. The minimum value of the similarity coefficient between CoP15440 and BO91 is 0.518. 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.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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