IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Assessment Of Genetic Diversity Analysis In Selected Varieties Of *Saccharum Officinarum* Using RAPD

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ABSTRACT: Sugarcane is a vital tropical and subtropical cash crop with significant economic and ecological importance. Understanding its genetic diversity is essential for effective conservation and breeding strategies, as it provides a critical reservoir of genetic variation for trait selection and crop improvement. In this study, leaf samples from five different sugarcane varieties representing their prevalent genomic variations were characterized using molecular markers to assess the extent of genetic variation and population structure. The results revealed a continuous variation in morphology and associated traits across the sampled breeds, indicating extensive genetic diversity within the species. Molecular markers proved instrumental in identifying genotypes and mapping single genes linked to commercially important traits. Additionally, these markers facilitated investigations into genetic introgression, population structure, and phylogenetic relationships. The five different samples used were sourced from co96011, co99006, co86032, co62175, and co13010. Five random primers; RP11, RP12, RP13, RP14, and RP15, were used in the analysis. The number of bands per primer product ranged from 1-19. The size of the amplified product ranged from 100 bp - 3 kb. Four primers reveal 100% polymorphism between cultivars. Out of 72 scored, 65 bands were found to be polymorphic. Maximum number of polymorphic bands (19) was obtained with primer 12. The average number of polymorphic bands per primer is 16.25. Based on the bands obtained, we can say that the level of genetic diversity among the culture varieties is high.

INTRODUCTION

Sugarcane (*Saccharum officinarum*) is a species of tall perennial true grasses of the genus *Saccharum*, tribe *Andropogoneae*, and sub tribe *Saccharininea*, used for sugar production. Sugarcane is the main source of sugar (80%) globally and holds a prominent position as a cash crop. It is one of the main crops for earning foreign exchange. The estimation of genetic diversity between different genotypes is the first process in any plant breeding program. DNA-based techniques introduced over the past two decades have the potential to identify polymorphism represented by differences in DNA sequences. Random Amplified Polymorphic

DNA (RAPD) detects nucleotide sequence polymorphisms in DNA by using a single primer of arbitrary nucleotide sequence (Welsh and McClelland, 1990). In this reaction, a single species of primer anneals to the genomic DNA at two different sites in the complementary strands of the DNA template. If these priming sites are within an amplifiable range of each other, a discrete DNA product is formed through thermocyclic amplification.

RAPD is a widely accepted characterization of the DNA of plants and other organisms. In the early 1990s, a new genetic assay was developed independently by two different laboratories (Eelsh and McClelland, 1990; Williams *et al*., 1990). RAPD technique provides an unlimited number of rapid markers which can be used for genetics and breeding purposes (Williams *et al*., 1990). Because RAPD polymorphisms are the result of either a nucleotide base change that alters the primer binding site or an insertion or deletion within the amplified region (Williams *et al*., 1990), polymorphisms are usually noted by the presence or absence of an amplification product from a single locus. The area of research that has shown the most growth concerning the use of RAPD technology is that of population genetics.

Shahid Afghan *et al*. (2005) used a Polymerase Chain Reaction (PCR) based marker assay to detect genetic diversity at the molecular level among local sugarcane genotypes, which were difficult to discriminate otherwise based on morphological and physiological characters. P.G Kawar *et al*. (2008) analysed RAPD in 17 cultivars of sugarcane. Selected 40 primers generated 325 bands, 134 of which were found to be polymorphic. The number of amplification products ranged from 3 to 15 for different primers. The genetic similarity among sugarcane cultivars ranged from 0.77 to 0.99. Forough J. Farsangi *et al*. (2017) used RAPD and ISSR markers to analyse the molecular assortment within 55 sugarcane cultivars/clones, including associated genera. The molecular diversity study with 15 RAPD markers generated a total of 153 alleles, of which 146 (95.4%) were found polymorphic. The PA varied from 7-14 amplified with an average of 0.46 found and Rp varied from 2.55-10.29 with an average of 6.42. Similarly, 15 ISSR markers generated a total of 138 alleles, of which 123 (89.1%) were found polymorphic. Pratap Singh *et al*. (2017) utilized twelve decamer oligonucleotide random amplified polymorphic DNA (RAPD) markers to investigate the genetic potential among 24 sugarcane cultivars. The 12 RAPD primers yielded a total of 120 fragments. An average number of fragments was obtained as 11.42 fragments per cultivar, which ranged from 4 to 21 fragments. The genetic similarity among 24 sugarcane cultivars ranged from 0.236 to 0.944, with the mean similarity value of 0.508

MATERIALS AND METHODS

SAMPLE COLLECTION

The present study selected leaf samples of 5 *Saccharum officinarum* varieties (co96011, co99006, co86032, co62175, and co13010) that were collected from Sugarcane Breeding Research Centre, Kannur, Kerala, India, which may represent the wide variation prevalent in the genome. The recently matured leaves were collected and used for DNA extraction.

\DNA ISOLATION

Total cellular genomic DNA was isolated and purified from young leaves by the CTAB extraction procedure (Doyle and Doyle 1990). Young leaves were ground to a very fine powder in liquid nitrogen using a mortar and pestle. CTAB buffer was added and centrifuged at 10,000 g for 10 min. Following extraction with an equal volume of Phenol: chloroform: isoamyl alcohol (25:24:1), the aqueous phase was transferred to a new centrifuge tube, where RNA was eliminated by treatment with RNase. The aqueous phase was re-extracted twice with chloroform, and DNA was precipitated by the addition of 0.6 volumes of ice-cold isopropanol (100%).

AGAROSE GEL ELECTROPHORESIS

The quality of the isolated DNA was checked by agarose gel electrophoresis. A total of 2µl of loading buffer was mixed with 8µl of DNA sample, and the resulting mixture was loaded onto a 0.8% agarose gel prepared in 0.5X TBE buffer. Electrophoresis was carried out at 70 Volts for nearly 2 hours until the bromophenol dye front had migrated to the bottom of the gel. Staining was carried out with 0.5 µg/ml of ethidium bromide. The gel was visualized using a gel documentation system and was photographed under UV light.

PCR AMPLIFICATION

A reaction mixture containing 8µl of template DNA, 4µl of RAPD primer, 25µl of PCR master mix, and 13 µl of nuclease-free water was assembled in a microfuge tube. The solution was mixed gently, and amplification was carried out using the following reaction conditions **(Table 1**)

Temperature	94°C	94°C	35°C	72°C	94°C	38°C	72°C	72°C
Time	5 min	45 s	1 min	1.5 min	45 s	1 min	1 min	10 min
Steps	Initial denaturation	Denaturation	Annealing	Extension	Denaturation	Annealing	Extension	Final extension
Cycles		8 cycles			35 cycles			

Table 1. PCR conditions for the RAPD profile of *Saccharum officinarum*

RESULTS AND DISCUSSION

Characterization and quantification of genetic diversity have been a major goal in evolutionary biology and plant breeding. Investigating genetic polymorphisms offers a scientific foundation for the strategic utilization of germplasm resources in crop improvement programs. Sugarcane is the world's largest crop by production quantity. About 70% of the sugar produced globally comes from a sugarcane species called *Saccharum officinarum.*

Molecular markers are powerful techniques that reflect variation at the DNA sequence level. The molecular markers are present in unlimited numbers compared to phenotypic markers; hence, they detect diversity at very high resolution. Molecular markers play a pivotal role in crop research by facilitating germplasm screening to uncover novel sources of genetic variation, identifying genotypes associated with single genes governing economically significant traits, analyzing the genetic basis of quantitative trait loss, studying genetic introgression, and supporting investigations in population genetics and anatomical studies. Molecular markers, in contrast to phenotypic markers, are independent of the environment and reflect variations at the DNA sequence level. Germplasm knowledge is quite important in breeding programs, since it is a source of variation that can be used for selection.

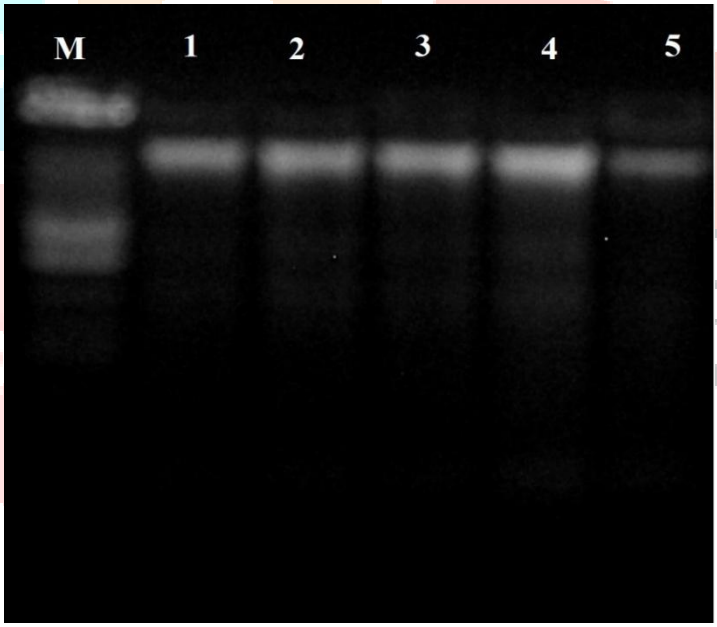

Figure 3. Genomic DNA isolated from different varieties of *Saccharum officinarum.* M=Mol wt. marker, Lane1= co96011, Lane 2= co99006, Lane 3= co86032, Lane 4= co62175, Lane 5= co13010

Characterization and evaluation of sugarcane population, based on morphological and agronomical traits, is time-consuming, labour-intensive, and subject to environmental effects. Therefore, sensitive and reliable techniques for assessing genetic diversity and screening agronomic traits are necessary to establish effective collecting and gene banking strategies. RAPD is useful for the characterization of DNA from plants and other organisms. This technique involves short oligonucleotide primers that are arbitrarily selected to amplify a set of DNA fragments randomly distributed throughout the genome.

RAPD markers provide an effective tool for the detection and evaluation of genetic variation existing among promising species of sugarcane. It demonstrated the utility of using RAPD markers to characterize genetic diversity among promising species of sugarcane. Differential polymorphism was noted in 2 species of sugarcane, showing variation in the percentage of polymorphic bands. Among various molecular markers, Random Amplified Polymorphic DNA (RAPD) has been extensively employed over recent decades for species identification and assessment of genetic diversity across taxa. Its widespread application stems from its cost-effectiveness and methodological simplicity, notably without the need for prior sequence information of the target species (Williams *et al*., 1990).

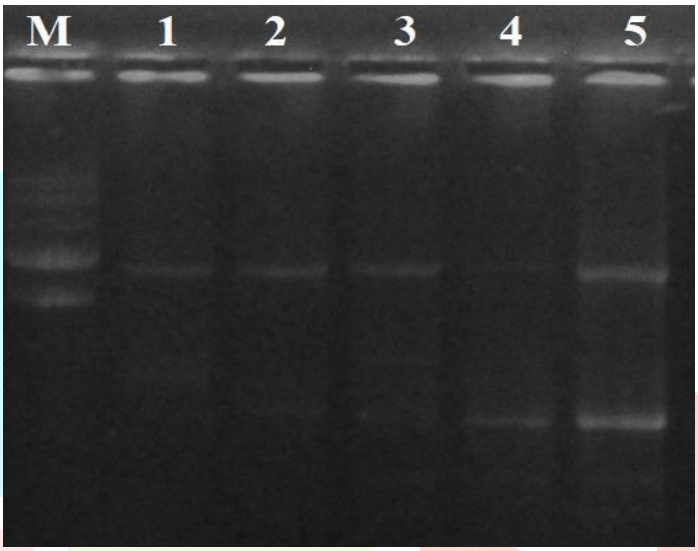

Figure 4. RAPD profile of *Saccharum officinarum* using random primer 11. M= Mol wt. marker, Lane 1= co96011, Lane 2= co99006, Lane 3= 86032, Lane 4= co62175, Lane 5= co13010.

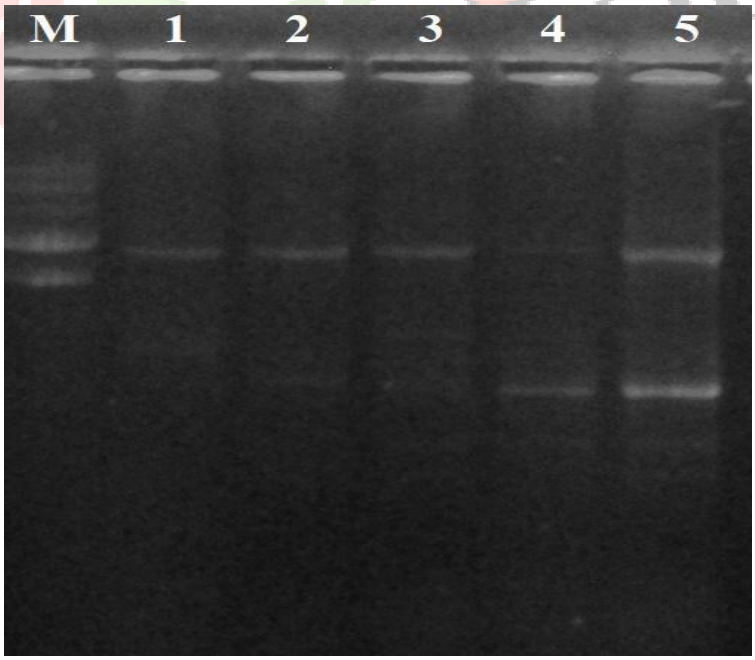

Figure 5. RAPD profile of *Saccharum officinarum* using random primer 12. M= Mol wt. marker, Lane 1= co96011, Lane 2= co 99006, Lane 3= co86032, Lane 4= co62175, Lane 5= co13010

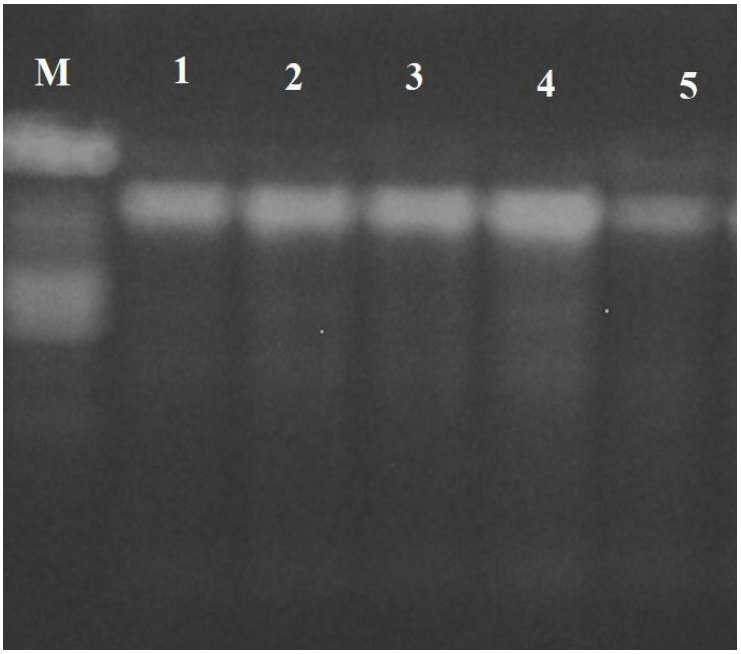

Figure 6. RAPD profile of *Saccharum officinarum* using random primer 13. M = Mol wt. primer, Lane1 = co96011, Lane 2 = co99006, Lane 3 = co86032, Lane 4 = co62175, Lane 5 = co13010

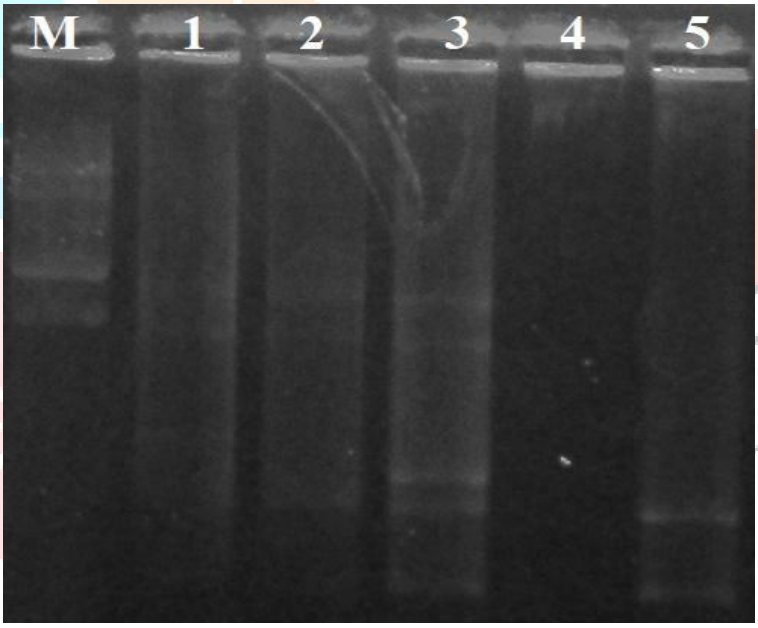

Figure 7. RAPD profile of *Saccharum officinarum* using random primer 14. M = Mol wt. marker, Lane 1 = co96011, Lane 2 = co99006, Lane 3 = co86032, Lane 4 = co62175, Lane 5 = co13010

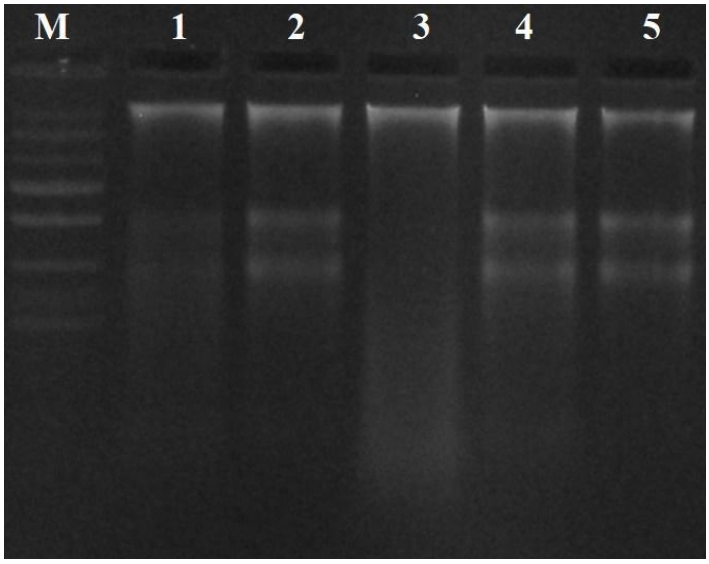

Figure 8. RAPD profile of *Saccharum officinarum* using random primer 15. M = Mol wt. primer. Lane 1 = co96011, Lane 2 = co99006, Lane3 = co86032, Lane 4 = co62175, Lane 5 = co13010

In the current study, the number of bands per primer product ranged from 1-19 (**Figs. 4-8**). The size of the amplified product ranged from 100 bp – 3 kb. Out of 72 scored, 65 bands were found to be polymorphic. Therefore, a high contrast is expected when crossing these with any other progenitors. The maximum number of bands (19) was obtained with the primer 12. The average number of polymorphic bands per primer is 16.25. **(Table 3)**

In a previous study, ten sugarcane genotypes were studied using the RAPD technique. 38 decamer primers generated a total of 258 discrete markers ranging from 0.2 to 2.5 kilo base pairs. (Shahid Afghan *et al*., 2005). The Polymorphism rate of the markers for the genotypes examined was 28.58%. Average genetic similarity amongst the genotypes was 78.96%. Sugarcane genotypes SPSG-26, CP77-400, CSSG-668, CSSG-676, and NSG-311 showed the highest genetic diversity. Another interesting observation made in this study was that the RAPD technique could be used to detect somatic tissue culture-induced genetic variation. RAPD markers seemed to be an effective tool for germplasm identification and breeding.

In comparison with the previous studies, the current study reveals a higher level of polymorphism and genetic variations. This may be since only a limited number of clones were used for the study. Further studies are needed to detect the exact level of genetic variations among large varieties of sugarcane genotypes. The current study may be useful for detecting more genetic variations and similarities among the different genotypes of sugarcane.

P.G Kawar *et al*. (2008) analysed RAPD in 17 cultivars of sugarcane. Selected 40 primers generated 325 bands, 134 of which were found to be polymorphic. The number of amplification products ranged from 3 to 15 for different primers. The genetic similarity among sugarcane cultivars ranged from 0.77 to 0.99. The average genetic similarity was 0.87. The studies reveal the limited genetic base of the current Indian commercial varieties and the need to diversify the genetic base by using new sources from the germplasm. RAPD is useful for the characterization of DNA from plants and other organisms. The current RAPD profile can be used to develop an effective molecular marker system for marker-assisted selection and breeding for

an effective crop improvement programme. The RAPD bands show that sugarcane plants are genetically heterogeneous. This genetic variability may be due to the different geographical locations from which the isolates were obtained, or it could indicate that the isolates may have originated from the same species with a wide genetic base or from closely related species.

Sl. NO	PRIMER	NATURE OF BANDS		TOTAL NO. OF BANDS	SIZE RANGE OF BANDS
		MONOMORPHIC	POLYMORPHIC		
1	RP11	0	18	18	100bp-3kb
2	RP12	0	19	19	100bp-3kb
3	RP13	5	0	5	100bp-3kb
4	RP14	1	16	17	100bp-3kb
5	RP15	1	12	13	100kb-3kb
		7	65	72	

Table 3. Analysis of banding patterns of RAPD profile of *Saccharum officinarum* using random primers

Polymorphic DNA uses only one short primer with an arbitrary sequence and binds at many complementary sites in the genomic DNA, amplifying a variety of differentially sized fragments that can be separated by electrophoresis to give specific banding patterns. Being a simple and non-radioactive technique, it is quite sensitive and used to detect genetic variation in several living things. Germplasm characterization using RAPD will be useful in assessing genetic diversity among natural populations. The RAPD technique is highly protocol-sensitive, which restricts the direct comparison of data generated using this method with results obtained through other approaches. Effectiveness of present RAPD analysis in revealing the genetic diversity is evident from the amplification product.

CONCLUSION

Present RAPD study was done using five sugarcane varieties i.e. co96011, co99006, co86032, co62175, co13010. Five random primers RP11, RP12, RP13, RP14 and RP15 were used in RAPD analysis. The number of bands per primer product ranged from 1-19. The size of the amplified product ranged from 100 bp – 3 kb. Four primers reveal 100 % polymorphism between cultivars. Out of 72 scored, 65 bands found to be polymorphic. Maximum number of polymorphic bands (19) was obtained with the primer 12. The average number of polymorphic bands per primer is 16.25.

Based on the bands obtained we can say that the level of genetic diversity among the culture varieties is high. The present study reveals the high genetic diversity among the sugarcane populations of co96011, co99006, co86032, co62175, co13010. These findings may facilitate more comprehensive genetic diversity analysis of sugarcane varieties across diverse geographic regions. The RAPD bands show that sugarcane plants are genetically heterogeneous. This genetic variability may be due to the different geographical locations from which the isolates were obtained or it could indicate that the isolates may have originated from the same species with a wide genetic base or from closely related species.

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