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RESEARCH ARTICLE

Molecular profiling and genetic diversity in sugarcane (*Saccharum officinarum* L) hybrid cultivars by RAPD markers

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Abstract

Genetic diversity is fundamental to crop improvement, especially in genetically complex crops like sugarcane (Saccharum officinarum L.). The objective of this study was to assess the genetic diversity and relationships among 22 elite sugarcane hybrid cultivars usingRandom Amplified Polymorphic DNA (RAPD) markers, in order to identify genetically diverse parental lines for effective breeding strategies. Fifteen RAPD primers produced a total of 129 bands, out of which 108 (83.32%) were polymorphic, indicating a high level of variability. The band sizes ranged from 325 to 2858 base pairs, and the number of bands per primer varied from four to twelve. Genetic similarity coefficients amongcultivars ranged from 0.27 to 0.98, suggesting a broad genetic spectrum. Cluster analysis using the UPGMA method revealed that some cultivarswith similar parentages grouped together, while others with different parentages were clustered in the same group, demonstrating that pedigree alone may not reflect true genetic diversity. Co 6907 was identified as a genetically distinct cultivar. The study emphasizes the value of molecular profiling for identifying genetically diverse parents, broadening the genetic base, and optimizing sugarcane breeding programs for enhanced productivity.

Keywords: Molecular Profiling, RAPD, Sugarcane hybrids, Genetic Diversity, cluster analysis, breeding strategy

Abbreviation: RAPD- Random Amplified Polymorphic DNA, CTAB- Cetyltrimethylammonium Bromide, PCR- Polymerase Chain Reaction, TBE-Tris-Borate-EDTA, UPGMA- Unweighted Pair Group Method with Arithmetic Mean, SAHN- Sequential Agglomerative Hierarchical Nested, JS-Jaccard's Similarity

Introduction

Sugarcane (*Saccharum spp*. hybrids) is a genetically intricate crop of significant economic relevance in tropical and subtropical regions. It is grown in diverse agroclimatic conditions across India, where specific locations vary significantly in terms of climatic, nutritional, and stress factors. Therefore, the breeding and selection of cane hybrids are highly location-specific, encompassing the selection of parents through to the final evaluation and selection stages. This approach has resulted in the development of several improved cultivars tailored for regional adaptation. In 2025, India is projected to produce approximately 35 million metric tons of sugar from over 5 million hectares under cultivation, reaffirming its position as the second -largest sugarcane producer globally after Brazil. Uttar Pradesh, Maharashtra, Karnataka, and Tamil Nadu are the key sugarcane-growing states, with Tamil Nadu often reporting the highest productivity per hectare, despite lower total production. Given this national importance, maintaining a high level of genetic diversity among commercial hybrids and breeding populations is critical for sustaining yield improvement and resilience in the face of biotic and abiotic stresses.

Researchers have used molecular marker approaches to study genetic variation in numerous crops. Here, powerful tools such as RFLPs, RAPDs, AFLPs, and microsatellites have been developed for the purpose of analyzing sugarcane hybrid cultivars for commercial exploitation, selecting parents with diverse genetic makeups for introgressive breeding to resolve species-specific relationships, and identifying elite sugarcane hybrid cultivars from hybrid populations (1-7). RAPDs and other PCR-based methods are becoming more popular for studying genetic diversity in crop plants. This popularity is due to their many advantages, including being easy to use, inexpensive, fast, and not requiring sequence information of template DNA. Additionally, they only require a small amount of DNA for analysis, and previous knowledge about the genome is not necessary (8-17). A larger number of these informative markers are needed for a variety of uses in genetics and molecular breeding (18, 19) to achieve desirable agronomic traits in Saccharum sp., a species with a complex polyploid genome, and that was the impetus for their development. To choose elite hybrids for breeding programs and increase variability, plant breeders could use the genetic similarity data produced by the study to choose parents from varied backgrounds. This paper

reports the genetic diversity among 22 sugarcane hybrid cultivars, as demonstrated by RAPD studies (20).

Materials and Methods

Plant Material used in the study

Twenty-two elite sugarcane (Saccharum spp.) hybrid cultivars maintained at the Regional Agricultural Research Station (RARS), Anakapalle, Andhra Pradesh, India, were selected for molecular characterization (Table 1). The experimental site is situated at 17.68 ° N latitude and 83.00 ° E longitude, with an elevation of 29.6 meters above sea level. The region has a tropical wet and dry climate, with an average annual rainfall of approximately 1,100 mm and temperatures ranging between 24 °C and 36 °C.

Plant DNA extraction

Healthy, young leaf tissues were collected from field-grown plants established under standard agronomic practices, including recommended doses of fertilizers, irrigation and pest management, to minimize external variation. The cultivars were grown in a completely randomized design (CRD) with three replications to ensure uniformity in growth conditions. In addition to molecular sampling, observations were recorded on morphological uniformity, plant vigor and tissue health to ensure consistent and reliable sample selection for DNA extraction. Total genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method (21). DNA quality was assessed using 0.8% agarose gel electrophoresis and quantification was performed using a UV spectrophotometer. The DNA was then diluted in TE buffer to a final working concentration of 20 ng/µL for downstream RAPD analysis.

PCR Amplification and Gel Electrophoresis

The PCR amplification of DNA extracted from plants was conducted using a total of 15 arbitrary decamer primers (Operon Technologies, USA) (Table 2). A 25 µL mixture was prepared for the reaction, which included 50 ng of DNA, 2.5 µL of 10 x PCR buffer, 2.5 mM $MgCl_2$, 2 mM dNTPs, 15 ng of primer and 1 unit of Taq polymerase (Genei, Bangalore) to carry out the amplifications. The BIO-RAD thermocycler was programmed for 42 cycles to perform DNA amplification, as described below: Two minutes at 940 °C, followed by 40 cycles of 1 minute at 940 °C, 1 minute at 370°C and 2 minutes at 720 °C. In 1.4 %, the amplification products were separated by size using gel electrophoresis. Agarose (Sigma) was dissolved in 1 X TBE buffer and stained with 0.2 ml ethidium bromide (10 mg/ml) at 120 V for 1 hour and 30 minutes. The gel is gradually transferred to the UV transilluminator following electrophoresis, and an image is captured. The only bands that were consistently reproducible were considered, and all reactions were repeated twice.

Statistical analysis

Data on clearly resolved bands obtained in 22 sugarcane hybrid cultivars using 27 primers were examined. Clearly resolved bands were manually graded for presence [1] or absence [0] throughout the hybrid cultivars and arranged as binary data in a matrix table. This binary data was utilised to quantify genetic similarity [JS] among hybrid cultivars using the Jaccard's coefficient. The genetic distance between each pair of lines was determined using D=1-JS. This matrix was examined using UPGMA [Unweighted Pair Group Method with Arithmetic Mean Clustering] and SAHN [Sequential Agglomerative Hierarchical Nested] cluster analysis modules to produce a dendrogram. All of these calculations were performed using the NTSYS-PC version 2.1 software (22).

Table 1. The sugarcane promising and pre-release hybrid cultivars used for the study of genetic diversity.

S.No.	Hybrid cultivar	Parents		Origin	Maturity Group
		Female	Male		
1	CoA92081	Co 7704	Co C 671	Coimbatore, Anakapalle	Early
2	CoA99082	Co T 8201	B 38192	Coimbatore, Anakapalle	Early
3	CoA07321	87 A 298	HR 83-65	Coimbatore, Anakapalle	Early
4	CoA11323	79 A 28	Co A 7602	Coimbatore, Anakapalle	Early
5	CoA05321	Co 740 PC	-	Coimbatore, Anakapalle	Early
6	2001 A 6	86 A 146 GC	-	Coimbatore, Anakapalle	Early
7	CoA08323	Co 8371	Co T 8201	Coimbatore, Anakapalle	Early
8	CoA09321	Co 86002	Co 92008	Coimbatore, Anakapalle	Early
9	CoA11322	Co A 7602 PC	-	Coimbatore, Anakapalle	Early
10	CoA11321	80 R 41 GC	-	Coimbatore, Anakapalle	Early
11	Co 6907	Co 740	Co 1287	Coimbatore, Anakapalle	Early
12	CoV92102	Co C 671	Co 6806	Coimbatore, Anakapalle	Midlate
13	CoA02082	85 A 261	Co A 7602	Coimbatore, Anakapalle	Midlate
14	CoA05322	Co 7706	Co 6904	Coimbatore, Anakapalle	Midlate
15	CoA05323	Co 85002 PC	-	Coimbatore, Anakapalle	Midlate
16	2000 A 226	Co 85002 PC	-	Coimbatore, Anakapalle	Midlate
17	CoA07322	79 A 28	Co A 7602	Coimbatore, Anakapalle	Midlate
18	CoA10321	87 A 380 PC	-	Coimbatore, Anakapalle	Midlate
19	CoA10322	Co V 92101	Co T 8201	Coimbatore, Anakapalle	Midlate
20	CoA10323	80 R 41 GC	-	Coimbatore, Anakapalle	Midlate
21	CoA11324	80 R 41 GC	-	Coimbatore, Anakapalle	Midlate
22	Co 7219	Co 449	Co 658	Coimbatore, Anakapalle	Midlate

Results and Discussion

Fifteen primers were employed to resolve a total of 129 bands, with 108 of those bands exhibiting polymorphism (Table 2). The result was an average of 8.60 bands per primer. From 325 to 2858 base pairs, the amplification products varied in size. Primers OPA-02 (12 bands) and OPC-19 (4 bands) produced the most and least bands, respectively. No particular genotype was distinguished by a single band. It is possible that the primers that were tested may not have diagnostic value for varietal identification because they produced polymorphic bands that were exclusive to a set of hybrid cultivars (Table 3). RAPD markers like OPA-08 (85.71 %), OPD-18 (90 %), OPC-08 (85.71 %), OPD-03 (100%), OPD-19 (100 %), OPE-04 (100 %) and OPN-11 (85.71 %) showed the highest variety among the fifteen markers tested (Fig.1).

The NTSYSpc program produced the UPGMA-based dendrogram of 22 sugarcane hybrid cultivars, as illustrated in Fig. 2. It was noted that two main clusters were formed, each with two subclusters. The first sub-cluster contains a single genotype, Co 6907, while the second sub-cluster contains three hybrid cultivars, CoA 92081, CoA 07321, and CoA 06321. Five groups comprised the second main cluster. Three hybrid cultivars, namely CoA 99082, CoA 11323, and CoA 11322, are included in Group I. 5 hybrid cultivars, namely CoA 05321, CoA 08323, CoA 09321, CoA 11321, and CoV 92102, are included in Group II. Five hybrid cultivars, namely CoA 02082, CoA 07322, CoA 07323, 2000 A 226, and CoA 10323, are included in Group III. Two hybrid cultivars, CoA 11324 and 2005 A 122, are included in Group IV. Two hybrid cultivars, 98 A 163 and 2004 A 104, are included in Group V. The hybrid cultivars CoA 10323 and CoA 11324 were on the same trajectory, as their parentages were identical (80 R 41 GC). The hybrid cultivars CoA 05323 and 2000 A 226 are similar in that their parentages are identical (Co 85002 PC). The hybrid cultivars CoA 02082 and CoA 07322 are nearly identical, as one of their parentages is common (CoA 7602). Hybrid cultivars CoA 99082, CoA 08323, and CoA 10322, which share the same parentage as CoT 8201, were not classified together. Instead, they were organized into distinct clusters. Conversely, CoA 05321, CoA 08323, CoA 09321, CoA 11323, and CoV 92102 comprised a single group, despite having distinct parentages. This was also the case for CoA02082 and CoA 10321. The dendrogram classified the variety Co 6907 as an outgroup, as it did not form a group with any other hybrids. Consequently, the dendrogram indicates that the clustering is influenced by the parentage and the genetic similarity between these hybrid cultivars was also observed through DNA polymorphism.

Most hybrids lacked a variety-specific band. Some primers produced hybrid cultivar-specific polymorphism bands. Genetic distances should be estimated using at least 50 loci, according to Nei (23). Fernandez et al. (24) found 77 polymorphic bands from 106 Cuban sugarcane hybrids using 18 primers. There were reports stating 998 RAPD bands in 42 hybrid cultivars indicate 77.5 % polymorphism. Kawar et al. (25) found 44.9 % polymorphism in 134 bands. A total of 162 RAPD bands were generated using 20 primers, revealing 87.18 % polymorphism among 10 sugarcane hybrid cultivars. In the present study, 129 RAPD bands were obtained, showing 83.32 % polymorphism.

Table 2. Molecular polymorphism detected by 15 primers in 22 sugarcane hybrid cultivars

S.No	Random primer	Sequence (5'-3')	Total bands (TB)	Polymorphic bands (PB)	Monomorphic Bands (MB)	Percent polymorphism	Size of loci (bp)
1	OPA -02	TGCCGAGCTG	12	10	2	83.33	605-1191
2	OPA – 08	GTGACGTAGG	7	6	1	85.71	325-1503
3	OPA – 12	TCGGCGATAG	10	8	2	80.00	499-2858
4	OPA – 18	AGGTGACCGT	9	6	3	66.70	553-2586
5	OPA – 19	CAAACGTCGG	11	8	3	72.72	582-2283
6	OPB – 18	AGGTGACCGT	10	9	1	90.00	525-2190
7	OPC – 06	GGTCCCTGAC	8	6	2	75.00	761-2198
8	OPC – 08	GTGACGTAGG	7	6	1	85.71	920-2660
9	OPC – 13	CAGCACCCAC	8	6	2	75.00	331-1678
10	OPC – 17	TTCCCCCCAG	8	6	2	75.00	325-2180
11	OPC – 19	GTTGCCAGCC	4	3	1	75.00	586-1534
12	OPD – 03	AGTCAGCCAC	11	11	0	100.00	894-2209
13	OPD – 19	GTTGCCAGCC	10	10	0	100.00	455-2361
14	OPE – 04	AATCGGGCTG	7	7	0	100.00	523-2325
15	OPN – 11	TCGCCGCAAA	7	6	1	85.71	398-1498
			129	108	21	83.32	

Table 3. Primers that produced specific bands with respect to a set of hybrids

S.No.	Random primer	Sequence (5'-3')	Band size (bp)	Specific to
1	OPA -02	TGCCGAGCTG	605	87 A 298, 2000 A 56
2	OPC – 08	GTGACGTAGG	1130	2000 A 213
3	OPA – 12	TCGGCGATAG	980	2005 A 128, 2005 A 108, 2005 A 122
4	OPA – 18	AGGTGACCGT	1550	2000 A 240, 2000 A 241
5	OPC – 06	GGTCCCTGAC	1020	93 A 145
6	OPC – 13	CAGCACCCAC	1350	2001 A 6
7	OPC – 17	TTCCCCCCAG	980	2003 A 255, 2004 A 55
8	OPC – 19	GTTGCCAGCC	1120	2004 A 107
9	OPN – 11	GTTGCCAGCC	1390	2000 A 225, 2000 A 226

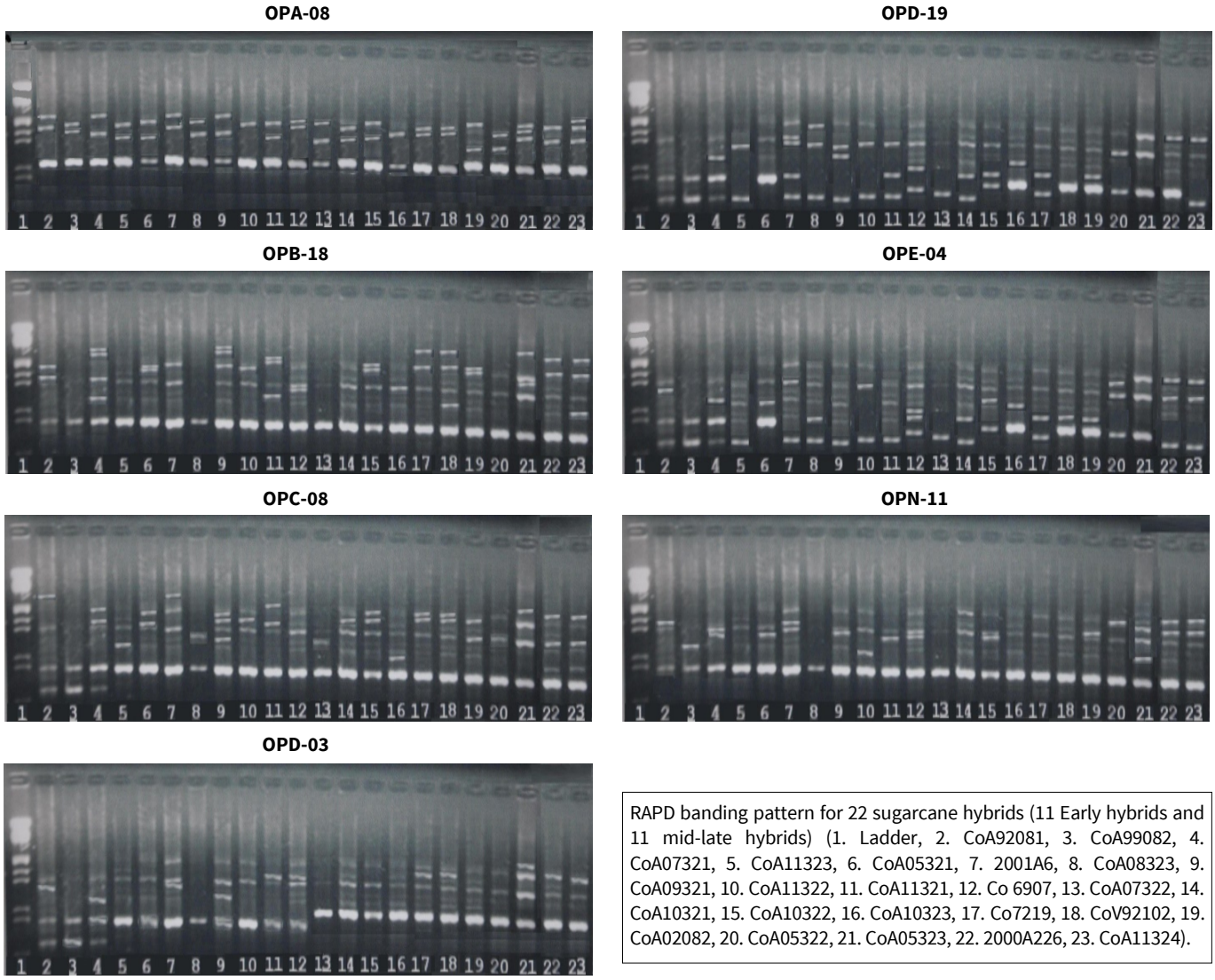

Fig 1: RAPD banding pattern for 22 sugarcane hybrids for molecular markers depicting maximum polymorphism

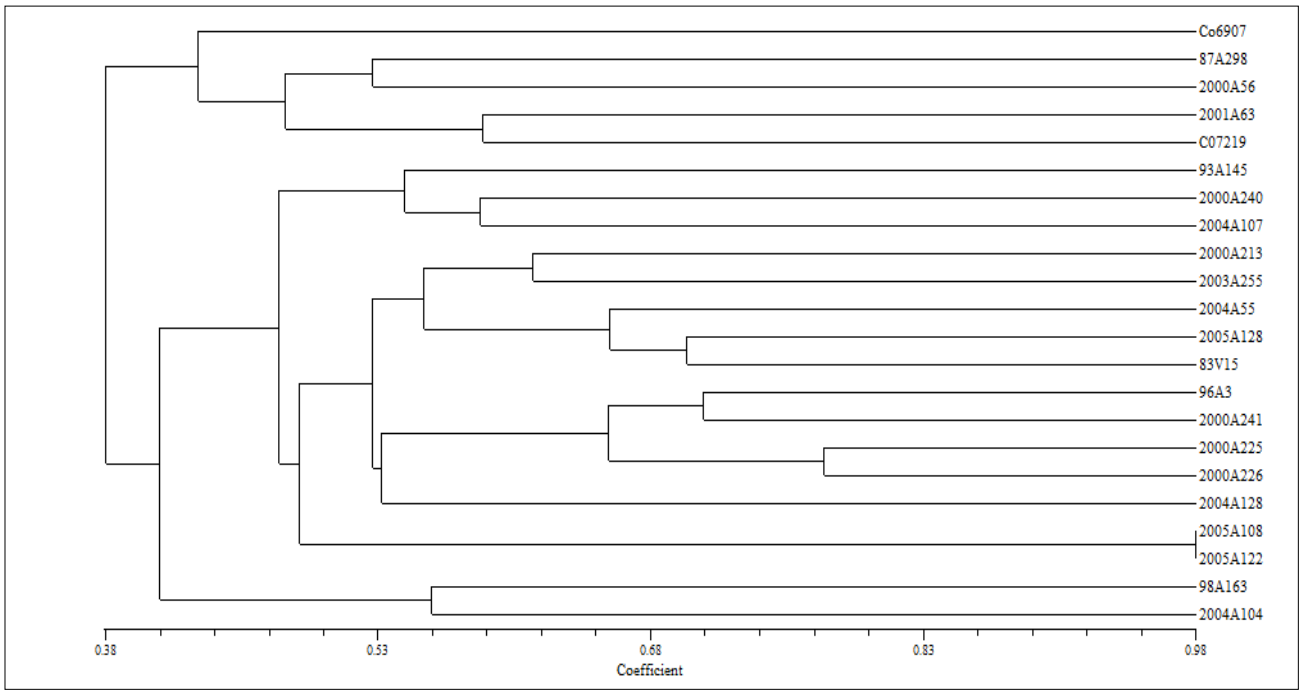

Fig. 2. Consensus tree showing clustering of 22 sugarcane hybrid cultivars using RAPD data.

The information is effective for the identification of hybrids, the evaluation of the genetic variability of the reproductive population, and the enhancement of cultivars. Similar findings have been reported in earlier studies using RAPD markers, which also observed high genetic similarity among sugarcane hybrids (26). The genetic similarity values in the current study ranged from 0.27 to 0.98, indicating a high degree of genetic relatedness among the sugarcane hybrid cultivars. It has been observed that the clustering pattern of sugarcane hybrid cultivars is not substantially influenced by their parentage (27). In several studies, cultivars with common parentage were grouped into distinct clusters, while those with diverse parentages were sometimes clustered together, indicating that genetic background alone does not always determine clustering patterns when using RAPD markers. The hybrid cultivars CoA 05323 and 2000 A 226 are similar in the present investigation due to their identical parentages (Co 85002 PC). The hybrid cultivars CoA 02082 and CoA 07322 are nearly identical, as one of their parentages is common (CoA 7602). Some of the hybrid cultivars that share common parentage were not grouped together, such as hybrid cultivars CoA 99082, CoA 08323, and CoA 10322, which have CoT 8201. Rather, they were classified into separate clusters. The hybrids CoA 05321, CoA 8323, CoA 09321, CoA 11323, and CoV 92102 were grouped together, but their parentage was distinct. Co 6907 was identified as entirely distinct from the other hybrids and did not form any association with any other genotype. Genetic diversity is a critical component of crop improvement programs, particularly for enhancing yield potential and tolerance to environmental stresses (28). The limited genetic base identified in this study underscores the necessity of incorporating novel alleles into breeding populations. Genetically divergent cultivars, such as Co 6907, represent valuable resources for developing heterotic crosses with superior agronomic performance. Molecular-level insights into genetic relationships among cultivars enable the strategic selection of complementary parents, thereby increasing the likelihood of producing high-performing progenies and sustaining genetic gains over time.

Conclusion

Genetic diversity is essential for the development of high-yielding and stress-resilient sugarcane cultivars. This study revealed a relatively narrow genetic base among the hybrid cultivars, likely due to the repeated use of closely related parental lines over time. The identification of genetically distinct hybrids, such as Co 6907, highlights the potential to introduce novel alleles into breeding programs, thereby expanding the genetic pool. RAPD marker-based clustering provided a more accurate depiction of genetic relationships than pedigree data alone, allowing for informed selection of parents. These insights can guide both immediate hybridization strategies and long-term breeding plans aimed at enhancing productivity and maintaining genetic diversity in sugarcane improvement programs.

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Authors' contributions

MC carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. AVSDP carried out the immunoassays and participated in the sequence alignment and design of the study and performed the statistical analysis. PKV conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: **"**None".

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