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DNA Fingerprinting of Sugarcane Genotypes/Varieties for Molecular Evidence and Protection

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ABSTRACT: DNA fingerprint databases are vital and imperative tools for plant molecular research because they provide potent information for crop breeding, variety quality control, variety right protection, and molecular marker-assisted breeding. Morphological descriptors may also use for physical fingerprinting but the reduced number of descriptors makes it difficult to identify the large number of varieties. DNA fingerprinting of seventeen sugarcane (*Saccharum* spp.) genotypes were constructed with three SSR primers were found polymorphic and gave clear bands. Sugarcane genotypes of both commercial hybrids and genetic stocks commonly used as parents in breeding programme were included in the study. A total of 157 markers were amplified, of which 68.0% were polymorphic with an average of ~23% polymorphic bands per primer. Number of bands produced by various primers was ranged from 4 to 12 with an average of 5.3 fragments per primer. DNA fragments size were ranged between 147 to 1474 bp. Analyses of molecular profiles generated by SSR markers revealed the unique bands specific for genotypes. The all used sugarcane genotypes/varieties were identified by either presence or absence of specific DNA fragments. Primers used in this experiment were able to distinguish all genotypes of sugarcane, demonstrating the ability of the methodology to determine unique genetic profiles. The identified unique markers could be useful for identification, utilization and management of the genotypes.

Keywords: Sugarcane, DNA Fingerprinting, Genotypes, Varieties, SSR Markers.

INTRODUCTION

Sugarcane (*Saccharum* spp.) is a farmer liking C4 cash crops, is mainly distributed in tropics and subtropics areas (Fickett *et al.,* 2020). Sugarcane production can improve with the improvement of soil health (Bairwa *et al.,* 2022) but the protection of varieties are also more important for farmers and researchers. Many types of challenges i.e. blood grid, narrow genetic basis etc, occurred in sugarcane for genotype/varieties identification and protection from other similar varieties (Medeiros *et al.,* 2020). For example, the same varieties, at a same place may be known by different names, or different varieties known by the same name. thats why the genetic relationship among genotypes/varieties is not clear (Hui *et al*., 2022). Morphological descriptors are protected new varieties of sugarcane as well as other crops, which have some limitations in identifications of morphologically similar cultivars (Silva *et al.,* 2012). Sometimes such descriptors are presented an inaccurate identification due to environmental influence on character expression. DNA based profiling system make reliable fingerprints for the identification of sugarcane varieties. DNA sequences are not influenced by genotype and environmental interaction, that's why it is permitting accurate molecular identification of any sugarcane varieties across the environments. The molecular identification of any newly originated sugarcane clone is required for the real and accurate identification along with their specific characteristics for varietal protection. DNA fingerprinting of any sugarcane clone is necessity for the review of varietal release committee. The aim of this present investigation was to develop strong fingerprints among seventeen sugarcane genotypes based on microsatellite (SSR) markers.

MATERIALS AND METHODS

Sugarcane genotypes/varieties. Seventeen newly developed sugarcane genotypes/varieties such as CoS 17233, CoS 17231, CoLk 15206, CoS 15232, CoS 15233, CoLk 15205, CoLk 14201, CoS 16232, CoLk 16204, CoLk 16203, CoS 16233, CoS 10239, CoS 16231, CoLk 14203, CoLk 14204, CoS 17239 and CoS 17234 which were high yielding, high sucrose content and also resistant to red rot, were collected from

experimental field of breeding department, UP Council of sugarcane research, Shahjahanpur, UP, India.

DNA extraction. Leaf samples of these genotypes/varieties were directly collected from experimental field. Sample was freeze-dried and then stored at -80°C. Approximately 500 mg leaves were separately ground to fine powder in liquid nitrogen using pre-chilled mortar pestle. Genomic DNA was extracted from young leaf tissues of each genotype using modified CTAB method (Shailendra *et al*., 2011; Hoisington, 1992). Genomic DNA isolation and purification were extracted using standard protocol. The DNA was diluted to a final concentration of 25ng/µl as determined by agarose-gel electrophoresis using known concentration of standard DNA. Genomic DNA was then run on a 0.8% agarose gel for confirmation of quality and concentration, final adjustments were made in 10 mM Tris HCl buffer to obtain the working concentration of 25ng/µl.

SSR markers and PCR amplification. Agarose gel based DNA fingerprinting was conducted with 10 polymorphic Simple Sequence Repeats (SSR) markers. Highly polymorphic SSR UGSM 354, UGSM 358, UGSM 351, UGSM 359, UGSM 375, UGSM 385, UGSM 393, UGSM 394, UGSM 407 and UGSM 432 (Parida *et al*., 2006) was used for the fingerprinting of seventeen clones. PCR reactions were performed in a 10µl reaction mixture containing 1.0µl template DNA, 1.0µl taq buffer containing $MgCl_2$, 0.2µl dNTPs, 1.0µl forward and 1.0µl reverse primer, 0.07µl taq polymerase and maintain 10µl reaction mixture volume with 5.73µl dd H_2O. The PCR cycle for SSR markers were performed at 94°C for 5 min followed by 30 cycles consisting of denaturation at 94°C for 1 min, primer annealing at optimised temperature were varies for each primer pair (53-55°C) for 1 min, and primer extension were performed at 72°C for 2 min followed by a final extension step at 72°C for 7 min. The amplified fragments were run by 2.0 percent high resolution agarose gel in 0.5X TBE buffer. The size of the amplified fragments was estimating using 100bp DNA ladder (Fermentas, Inc. USA). Amplified bands were visualized after staining with 0.5 µg/ml ethidium bromide (Singh *et al.*, 2011). Gel photographs were taken under UV light in GelDoc system (Alpha Innotech).

RESULTS AND DISCUSSIONS

DNA fingerprints, which were developed in 1986 by A. Jefferys. DNA finger printing is a potent tool to identify genotypes/varieties and it is also suitable for identifying plant germplasm resources. 10 SSR polymorphic markers were used in this experiment for identification and protection of seventeen sugarcane genotypes/varieties. Earlier Ali (2018) was also used and developed the SSR marker-based molecular identification database for sugarcane. The DNA profile of different genotypes/verities has huge importance in breeding and permitting for the identification of duplicates and mixture in the germplasm bank to a better breeding result (Ruiz *et al*., 2011). A total of 157 fragments were amplified which 68% were sowing polymorphic. Number of bands produced by various primers ranged from 4 to 12 with an average of 5.3 fragments per primer. DNA fragments size ranged between 147 to 1474bp. Analyses of molecular profiles generated by SSR markers revealed the unique bands specific for seventeen genotypes. The result of present study is also confirmed by Ibrar *et al.* (2022). The sugarcane clones *viz*., CoS 17233, CoS 17231, CoLk 15206, CoS 15232, CoS 15233, CoLk 15205, CoLk 14201, CoS 16232, CoLk 16204, CoLk 16203, CoS 16233 (UGSM 354), CoS 10239 (UGSM 358), CoS 16231, CoLk 14203, CoLk 14204, CoS 17239, CoS 17234 (UGSM 351) were identified by either presence or absence of specific DNA fragments. Earlier many researchers have performed DNA profiling with other molecular marker tool to establishment of criteria for the protection of varieties (Govindaraj *et al.,* 2012; Hemaprabha *et al.,* 2006). The identification of varieties based on molecular markers is important to establish distinctness, uniformity and stability of protected cultivars (Swapna *et al*., 2010). The microsatellites analysis in this study were found highly polymorphic and allowing for the identification of individual clones and the generation of unique genetic profiles of sugarcane. The results of that study provide a reference for establishing a SSR standard fingerprint database. The identified unique markers could be useful for identification, utilization and management of the sugarcane varieties.

Table 1: DNA Fingerprinting of seventeen sugarcane genotypes/varieties and size of amplified bands with three SSR primers.

UGSM 354											UGSM 358	UGSM 351				
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
CoS 17233	**CoS 17231**	**CoLk 15206**	**CoS 15232**	**CoS 15233**	**CoLk 15205**	**CoLk 14201**	**CoS 16232**	**CoLk 16204**	**CoLk 16203**	**CoS 16233**	**CoS 10239**	**CoS 16231**	**CoLk 14203**	**CoLk 14204**	**CoS 17239**	**CoS 17234**
-	-	-	-	-	1250	-	892	1250	-	-	-	-	-	-	-	-
-	-	717	-	-	1036	-	764	1071	808	-	-	-	-	-	-	1474
-	-	636	867	-	900	-	671	800	700	-	-	1450	-	-	-	1053
764	-	513	671	791	791	-	587	539	591	-	-	1050	1472	-	1075	958
671	664	421	583	664	671	-	530	438	548	-	-	973	1056	1139	957	903
583	504	389	522	565	587	-	438	400	488	840	-	824	969	984	814	819
535	454	369	479	438	517	-	378	381	438	727	-	716	688	839	714	694
429	417	331	429	389	446	446	342	331	386	450	-	568	562	694	657	583
375	325	291	378	331	381	389	324	292	333	386	449	487	516	613	543	528
333	292	223	336	292	331	344	294	275	291	336	380	411	479	548	482	476
287	283	209	298	208	285	308	226	223	211	225	344	367	395	486	415	369
260	192	192	189	189	192	294	193	192	188	194	147	329	300	295	342	339

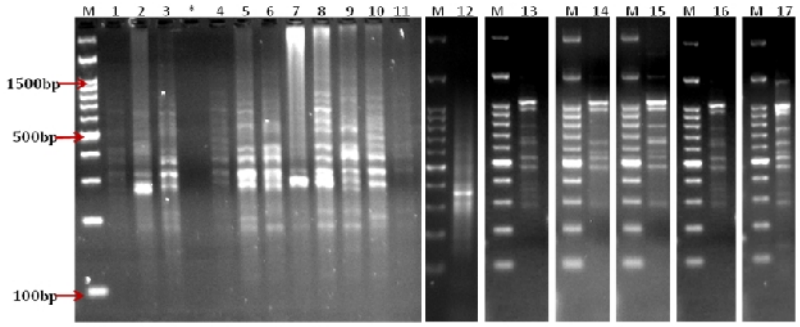

Fig. 1. PCR amplification of seventeen genotypes/vatieties with three SSR primers. (1) CoS 17233, (2) CoS 17231, (3) CoLk 15206, (4) CoS 15232, (5) CoS 15233, (6) CoLk 15205, (7) CoLk 14201, (8) CoS 16232, (9) CoLk 16204, (10) CoLk 16203, (11) CoS 16233 (UGSM 354), (12) CoS 10239 (UGSM 358), (13) CoS 16231, (14) CoLk 14203, (15) CoLk 14204, (16) CoS 17239, (17) CoS 17234 (UGSM 351).

CONCLUSIONS

SSR based DNA fingerprinting was used to develop 157 markers for genotypes/varieties identification and fingerprint construction. The conclusion of this study provides a useful approach for genotyping, classifying, and identifying genotypes/varieties and resources in sugarcane breeding. The identified unique markers could be useful for identification, utilization and management of the genotypes.

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Conflict of Interest. None.

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