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Assessment of Genetic Diversity in Promising Sugarcane Mutants Developed Through Chemical Mutagenesis

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Abstract

Sugarcane is an important cash crop gaining importance as ideal raw material for bioenergy production. Conventional sugarcane improvement is carried out through intensive crossing and selection breeding within the Saccharum complex. Being highly polyploidy crop the mutation breeding is also integrated for specifically improving the desired genetic trait in sugarcane varieties developed through conventional breeding. In the present work RAPD markers based assessment of genetic diversity and kinship relationships of six promising mutants developed using CoC 671 has been reported. The banding pattern of the PCR amplified products showed polymorphism with the RAPD profile in all six mutants. Mutants CoC 671, TC 2813, TC 2819, TC 2826, TC 2875 formed a separate cluster, and TC 906, TC 922, and TC 906 B formed a separate cluster distinct from the earlier one with the parent (CoC 671) and the standard Check (Co 86023). The genetic distance between the groups was found to be only marginally higher than the respective group distances and the overall mean genetic distance. The similarity indices indicated that there was 15 % genetic dissimilarity between CoC 671 and Co 86032. This may be because CoC 671 was one of the parents for Co 86032. In the present investigation, the genetic similarity value ranged from 0.97 to 0.78 among the mutants. A total of 34 band positions were scored, out of which 15 RAPD bands were polymorphic. From the genetic similarity coefficient based on RAPD band data sharing, it was found that the majority of the clones were almost more than 78% similar to the mother plant. Thus the level of similarity between varieties seemed to be not very high but the mutants could be distinguished from each other easily. RAPD analysis indicated EMS-induced point mutations resulting in specific rectifications without much change in the genetic backbone of genotype CoC 671. Since this analysis was carried out on mutants after multiplying in field for three successive generations, we presume that these mutants/genotypes are stable and express the stable genetic variations.

Keywords: Sugarcane; EMS; Mutagenesis; RAPD; Genetic variability

Introduction

Sugarcane, (*Saccharum offcinarum*) is a perennial grass, is primarily an important high biomass producing crop, nowadays targeted for its bio-energy potential apart from the sugar. Sugarcane is the raw material for many by-products for the generation of paper pulp, plywood boards, animal feed, wax, biofertilizers, alcohols, and many other useful products. Sugarcane is long duration crop during its crop cycle in the field various biotic and abiotic stress affects its productivity and sugar recovery causing huge losses to farmers and the sugar industry. Therefore developing better sugarcane varieties is a continuous quest. The development of superior sugarcane varieties through conventional hybridization programs is time-consuming and has the problem of the transfer of undesirable characters/traits into the newly developed hybrids/variety. Therefore, attempts were made to introduce genetic variability through mutagenesis. Due to the merits of CoC 671 *in-vitro* mutagenesis and somaclonal variation was attempted by various researchers to rectify the defective agronomic traits in it [1-7] and two varieties as CoM 94012 and VSI 434 have been released by proving CoC 671 by somaclonal variation but none was having smut resistance in them. Dalvi et al. [1,2] reported EMS-based mutagenesis in CoC 671 for smut-resistant genotypes by field evaluation. In the present work screening of six genotypes developed through *in vitro* improvement have been screened with RAPD to find the genetic variability between them.

RAPD is a widely used technique for screening somaclonal variation, mutants with different agronomic traits (Table 1). RAPD employs single short primers with arbitrary sequences to genome-specific fingerprints of multiple amplification fragments. RAPD is a simple and common technique frequently used for screening variability in sugarcane genotypes as well as the agronomic traits like resistance to diseases viz. Resistance to SCMV [8]. Resistance and susceptibility to red rot [9]. In the present work RAPD technique was used to find out the variability in seven genotypes developed aiming smut resistance with improved agronomic traits in CoC 671.

Material and Methods

The planting material CoC 671 and Co 86032 was provided by the Breeding section of Vasantdada Sugar Institute, Pune. The method of somaclonal variation and mutant development has been reported by Dalvi et al. [1] and [2]. The genotypes were maintained

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in the field following standard agronomic practices. The leaf samples for extraction of DNA were collected during the harvest at the 12th month period.

DNA isolation and quantification

Genomic DNA was extracted by using the CTAB method. DNA from leaves of sugarcane mutants was quantified by analyzing the samples in 0.8% Agarose gel (dissolved in 1 x TBE). Quantification was done visually by comparing the DNA with another standard gel loaded with different known concentrations of DNA (12.5 to 200 ng). For confirmation quantification was carried out using Nano Photometer (IMPLEN).

Primer selection

RAPD analysis was carried out with sugarcane mutants using 60 decamer oligonucleotide primers (OPA, OPB, and OPC) from Operon Technology Inc., USA. Among the primers screened five primers (OPA-01, OPA-05, OPB-02, OPC-01, and OPC-19) producing the distinct scorable bands has been reported in this study. The primer sequence details are as follows (Table 1).

PCR amplification

Amplification reactions were performed in M. J. Research, USA (PTC100) thermocycler. The reaction conditions were initial denaturation at 95^0C for 5 minutes followed by 45 cycles of denaturation at 940C for 1min, primer annealing at 37^0C for 1min, primer extension at 72^0C for 2min, and final extension at 72^0C for 10min. The amplified products were subjected to agarose gel electrophoresis using 1.5% agarose and the gel was analyzed using the BIORAD gel documentation unit (USA). The sizes of amplification products were determined by comparison with DNA digested with 100+ bp marker from Fermentas.

PCR optimization

The different components of PCR were optimized to get appropriate amplification products from the sugarcane genomic DNA. Genomic DNA (50ng per 20μl reaction mix), MgC12 (2mM), and Taq DNA polymerase (1U) were used. For primer annealing, temperatures (35, 36, 37, 38, and 39^0C) were tested.

RAPD data analysis

Amplified products that were reproducible and consistent were used for scoring. RAPD fragments were scored as '1' for the presence and '0' for the absence of a band generating the 0 and 1 matrix. Bands were analyzed using NTSYSpc Ver.2.1. Similarity matrices were generated according to the coefficient of Jaccard and used to perform cluster analysis using Unweighted Pair Group Method with Arithmetic Average (UPGMA). The coefficient of similarity among cultivars was calculated according to Nei & Li.

Analysis of Mutants Using Molecular Markers

Optimization of PCR conditions for RAPD analysis

MgCl2 at 2.5mM concentration produced a scorable banding pattern whereas the concentrations below 2.5mM produced faint bands or no bands. Of the different concentrations of genomic DNA tried (50, 100, 150, and 200 ng per 20ml reaction mix), a reaction mixture containing DNA 50 ng/ml found optimum. The lower DNA quantity yielded less intense bands, whereas the higher concentrations added background effect. Taq DNA polymerase (1U) has resulted in a good amplification profile compared to 0.5U. Among the primer annealing temperatures (35, 36, 37, 38, 39, and 40^0C), tested, 37^0C was found to be optimum. Band number decreased above the annealing temperature of 37^0C and no bands were observed above 39^0C. The molecular characterization of sugarcane varieties CoC 671, Co 86032, and mutants (TC 906, TC 922, TC 2813, TC 2819, TC 2826, and TC 2875) of CoC 671 was assessed by using RAPD primers (Figure 1 and Table 1 and 2). The genetic similarity between mutants was assessed based on the Jaccard's similarity coefficient and complemented with UPGMA-based cluster analysis. Scoring of morphological characters and molecular marker data support the distinctness of mutants from the parent CoC 671. RAPD analysis of DNA isolated from sugarcane mutants was carried out using 60 decamer oligonucleotide primers (OPA, OPB, and OPC) from Operon Technology Inc., USA. Among the primers screened, five primers, OPA-01, OPA-05, OPB-02, OPC-01, and OPC-19 showed distinct and good banding patterns (Figure 1 and Table 2).

Primer selection

Five primers produced clear polymorphic bands in all the mutants on preliminary analysis and were selected for further analysis. Only clear and unambiguous bands were taken for scoring. It has been observed that the RAPD profile showed a total of 34 bands from which 13 were polymorphic and there was 38.23 % polymorphism (Figure 1 and Table 2). The number of bands for each primer varied from 2 to 10 with an average of 6.8 bands per primer. The size of amplicons generated by five primers ranged from 300 to 1300 bp.

Genetic similarity between genotypes was assessed based on the Jaccard's similarity coefficient and complemented with UPGMA-based cluster analysis. The scoring of morphological characters and molecular marker data supported the distinctness of the genotypes from the parent CoC 671. A cophenetic correlation coefficient r = 0.82476 was obtained from two way Mantel test which indicates a good fit between the original similarity matrix and the resulting clustering analysis. Pair wise comparisons of RAPD profiles resulted in a similarity matrix used to develop a consensus tree and estimate

Table 1: Details of RAPD Primer Sequence and Band Polymorphism.

Sr. No.	Primer Code	Sequence	Total no. of bands	No. of Polymorphic Bands	% Polymorphism
1	OPA-01	CAGGCCCTTC	8	2	25
2	OPA-05	AGGGGTCTTG	10	6	60
3	OPB-02	TGATCCCTGG	3	2	66.66
4	OPC-01	GTTGCCAGCC	8	1	12.5
5	OPC-19	GTCCCGACGA	5	2	40
		Total	34	15	38.23

Table 2: Partial similarity matrix, showing the similarity indices between sugarcane mutants developed from variety CoC 671.

Genotype	Co 86032	CoC 671	TC 906	TC 922	TC 2813	TC 2819	TC 2826	TC 2875
Co 86032	1							
CoC 671	0.85	1						
TC 906	0.76	0.78	1					
TC 922	0.79	0.88	0.9	1				
TC 2813	0.85	1	0.78	0.88	1			
TC 2819	0.76	0.9	0.8	0.9	0.9	1		
TC 2826	0.76	0.9	0.8	0.9	0.9	0.93	1	
TC 2875	0.74	0.87	0.77	0.87	0.87	0.9	0.96	1

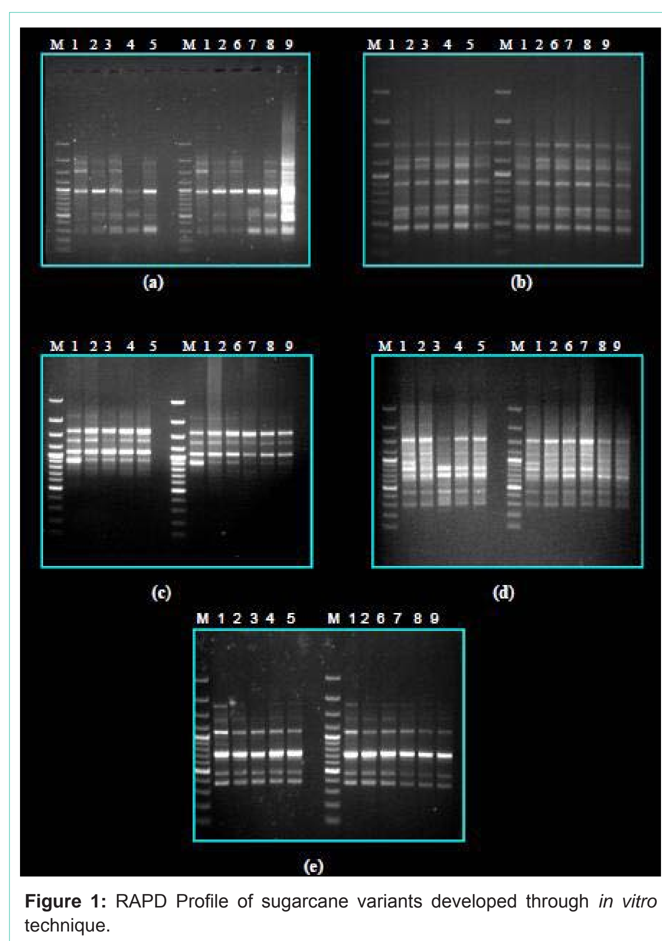

Figure 1: RAPD Profile of sugarcane variants developed through *in vitro* technique.

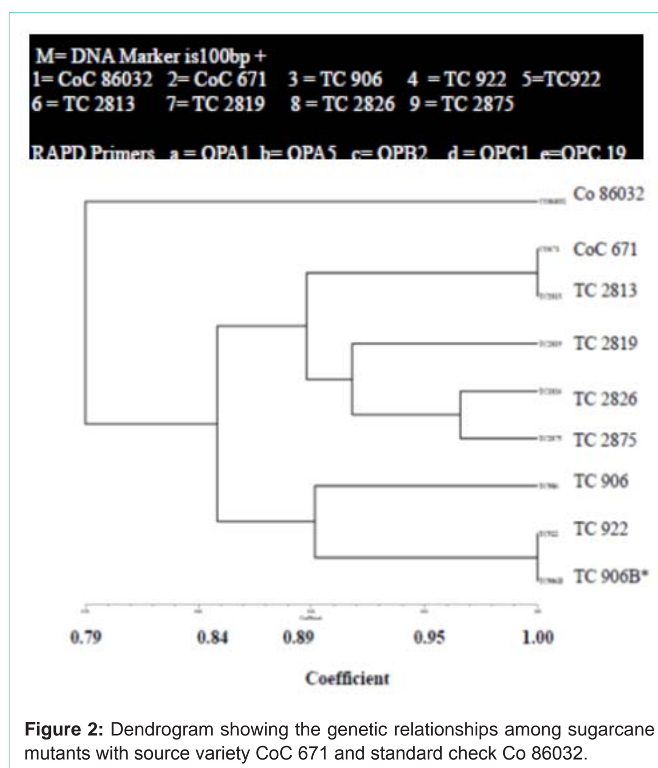

Figure 2: Dendrogram showing the genetic relationships among sugarcane mutants with source variety CoC 671 and standard check Co 86032.

their similarity indices for these mutants (Figure 2) Genotypes CoC 671, TC 2813, TC 2819, TC 2826, TC 2875 formed a separate cluster, and TC 906, TC 922 and TC 906 B* formed a separate cluster distinct from the earlier one with parent (CoC 671) and standard Check (Co 86023). TC 906B sugarcane mutant included in RAPD analysis as smut susceptible variant for looking variability of susceptible and resistant genes. CoC 671 and TC 2813 formed a tertiary sub-cluster making them genetically separate from the remaining genotypes. Among the remaining genotypes, TC 2819 was distinct from the parent CoC 671, TC 2826, and TC 2875. Sugarcane mutants TC 2826 and TC 2875 were closely associated with forming a separate cluster group. It was notable that mutants and some clones considered all showed variations.

The mean distance of individual varieties with the rest was computed from the distance matrix for comparison. Mean genetic distance among the varieties within a particular cluster, between standard check, CoC 671, Co 86032, and between mutants developed is calculated. The genetic distance between the groups was found to be only marginally higher than the respective within-group distances and the overall mean genetic distance. The similarity indices indicated that there was 15 % genetic dissimilarity between CoC 671 and Co 86032. This may be because CoC 671 was one of the parents for Co 86032.

TC 906 (22%), TC 922 (12%), TC 2819 (10%), TC 2826 (10%) and TC 2875 (13%) showed genetic dissimilarities with the parent CoC671. TC 2813 showed 100% genetic similarity with CoC 671 while TC 922 and TC 906B showed 100% genetic similarity between themselves even though they are morphologically distinct (Table 2). Nair et al., [10] reported that despite the sexual reproduction, the mean genetic distance among 28 sugarcane varieties was only 29.31%, implying that a large part of the sugarcane genome is similar

among the varieties. This probably arises from little parental diversity among the clones used in hybridization. RAPD analysis indicates that EMS induces point mutations resulting in specific rectifications without much change in the genetic backbone of genotype CoC 671. However, studies on radiation-induced mutants reveal 37% genetic dissimilarity [6]. In the present investigation, the genetic similarity value ranged from 0.97 to 0.78 among the mutants (Table 2). Since this analysis was carried out on mutants after three successive generations, we presume that these mutants/genotypes are stable and express the variations minimizing the possibility of epigenecity. Thus the application of the molecular marker technique will be of great help to establish an efficient system for the selection of clones through *in vitro* mutagenesis. The results obtained in the present studies confirm the efficiency of the RAPD technique for the determination and estimation of genetic distances and relatedness among different sugarcane mutants.

Sundar et al. have reported the significance of molecular markers for the resistance mechanism of plants. The study also highlighted their relevance in marking genetic variability but also the resistance and susceptibility in sugarcane linked to red rot disease. Barnes and Botha [11] had identified 2 RAPD markers linked to rust resistance in sugarcane variety to-376 based on reproducible polymorphism of loci in rust susceptible clones, while we had ten markers showing polymorphism in rust-resistant genotypes and only one marker in rust susceptible genotypes. Oropeza, et al. [8] has reported the identification of the somaclonal SCMV resistant variants from the maternal line and the nonresistant somaclones, using the RAPD technique. In sugarcane, two major clusters separating the resistant and moderately resistant sugarcane cultivars from the highly susceptible cultivars were reported using RAPD based clustering. Further Selvi et al., have shown RAPD marker OPV17 917 is associated with top borer resistance and susceptibility. Khan et al. [12] reported RAPD based SCAR markers for smut resistance in sugarcane. SCAR marker-based linkages with drought tolerance and rust resistance have been also reported by Wang et al., [13], Srivastav et al. [14] suggested that four primers (K07, H02, K10, and F01) produced multiple genotype-specific bands that aided in identifying red-rot resistant genotypes in sugarcane. Muhammad et al. attempted to analyze the rust-resistant (R) and rust susceptible (S) of six commercially available sugarcane elite genotypes in Pakistan with RAPD markers. Ali et al. [15] reported RAPD markers for characterization of rust resistance in sugarcane. Vishalakshi et al. [16] showed RAPD assisted Black gram germplasm selection for multiple disease resistance. Nair et al. [10,17] and Ardiel et al. [17] have shown the utility of RAPD analysis in identifying DNA sequence variations with resistance and susceptibility by converting them into SCAR markers. Araujo et al 2002 has also found a tightly linked marker for disease resistance gene in Rice. Abou-Taleb [19] showed the use of RAPD markers for studying the genetic diversity of potatoes for late blight resistance and susceptibility. Esmaiel et al. [20] showed RAPD marker utility for the determination of Fusarium resistance in Carnation. Srivastava et al., [21] identified RAPD marker linked to powdery mildew resistant gene(s) in black gram. The mutants in present study has been evaluated for smut resistance in filed for two years artificial inoculation trial and TC 906 and TC 2826 were reported to be resistant for smut [1,2]. Thus the distinct molecular fingerprinting patterns with these mutants open up new ways providing stable genetic differences for analyzing the resistance and susceptibility of sugarcane genotypes with the RAPD technique. These studies suggest the application and potential of the RAPD marker's utility for the facilitation of breeding programs and marker-assisted selection in sugarcane. The characteristic fingerprints of present mutants will provide a tool to distinguish disease resistance in sugarcane variety CoC671.

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References

1. Dalvi SG, Vasekar VC, Yadav A, Tawar PN, Dixit GB, Prasad DT et al. Screening of the promising sugarcane somaclones for agronomic traits, and smut resistance using PCR amplification of Inter Transcribed Region (ITS) of *Sporisorium scitaminae*. Sugar Tech. 2012; 14: 68-75.
2. Dalvi SG, Tawar PN, Suprasanna P, Dixit GB, Teerthprasad D. EMS-Based *In Vitro* Mutagenesis and Mutant Screening for Smut Resistance with Agronomic Traits in Sugarcane. Sugar Tech. 2021.
3. Dhumale DB, Ingole GI, Durge DV. Variations for morphological and quality attributes in clones of callus regenerants in sugarcane CV. CoC 671. Indian J. of Pt. Breeding. 1994; 54: 317-320.
4. Doule RB, Kawar PG, Devarumath RM and Nerkar YS. Field performance and RAPD analysis for assessment of genetic variation in sugarcane somaclones. Indian J. Genet. 2008; 68: 301-306.
5. Jalaja NC, Sreenivasan TV, Pawar SM, Bhoi PG and Garker RM. Co 94012 - a new sugarcane variety through somaclonal variation. Sugar Tech. 2006; 8: 132-136.
6. Patade VY, Suprasanna P. Radiation-induced *in vitro* mutagenesis for sugarcane improvement. Sugar Tech. 2008; 10: 14-19.
7. Tawar PN, Sawant RA, Sushir KV, Devarumath RM, Hapse RS, Meti NT. VSI 434: New Sugarcane Variety Obtained Through Somaclonal Variation. Agric Res. 2016; 5: 127-136.
8. Oropeza M, Guevara P, Garcia E and Ramirez JL. Identification of sugarcane (*Saccharum spp.*) somaclonal variants resistant to sugarcane mosaic virus *via* RAPD markers. Plant Mol. Biol. Rep. 1995; 13: 182-191.
9. Alvi AK, Iqbal J, Shah AH and Pan YB. DNA-based genetic variation for red rot resistance in sugarcane. Pakistan J. Bot. 2008; 40: 1419-1425.
10. Nair NV, Selvi A, Sreenivasan TV and Pushpalatha KN. Molecular diversity in Indian sugarcane cultivars as revealed by randomly amplified DNA polymorphisms. Euphytica. 2002; 127: 219-225.
11. Barnes JM and Botha FC. Progress towards identifying a marker for rust resistance in sugarcane variety NCo376. Proc. S. Afi. Sug. Technol. Ass. 1998; 72: 149-152.
12. Khan M, Yong B-P, Javed I. Development of an RAPD-based SCAR marker for smut disease resistance in commercial sugarcane cultivars of Pakistan. Crop Protection. 2017; 94: 166-172.
13. Wang XH, Yang QH, Li FS, He LL, He SC. Molecular identification of S*accharum sppX Erianthus fulvus* hybrids using SCAR. Crop Sci. 2009; 49: 864-870.
14. Srivastav MK, Li CN, Li YR. Development of sequence characterized amplified region marker for identifying drought-tolerant genotypes in sugarcane. Australian J. of Crop Sci. 2012; 6: 763-767.
15. Ali W, Muhammad K, Muhammad SN, Imanullah Ahmad H, Iqbal J. Use of RAPD markers to characterize commercially grown rust-resistant cultivars of sugarcane. Int. J. Biosci. 2013; 3: 115-121.

16. Vishalakshi B, Umakanth B, Anirudh P Shanbhag, Ghatak A, Sathyanarayanan N, Madhav MS, et al. RAPD assisted the selection of black gram (*Vigna mungo L.* Hepper) towards the development of multiple disease-resistant germplasm. 3Biotech. 2017; 7: 1-6.

17. Nair NV, Nair S, Sreenivasan TV and Mohan M. Analysis of genetic diversity and phylogeny in Saccharum and related genera using RAPD markers. Gen. Res. Crop Evaluation. 1999; 46: 73-79.

18. Ardiel GS, Grewal TS, Deberdt P, Rossnagel BG, Sciles GJ. Inheritance of resistance to covered smut in barley and development of a tightly linked scar marker. Springer-Ver. 2002; 104: 454-457.

19. Abou-Taleb EM, Aboshosha SM, El-Sherif EM, El-Komy MH. Genetic diversity among late blight resistant and susceptible potato genotypes. Saudi J. Biol. Sciences. 2010; 17: 133-138.

20. Esmaiel NM, Al-Doss AA and Barakat MN. *In vitro* selection for resistance to *Fusarium oxysporum f. sp.* dianthi and detection of genetic polymorphism *via* RAPD analysis in carnation. J. of Medicinal Plants Res. 2012; 6: 3997-4004.

21. Srivastava P, Pandey A and Singh M. Identification of the RAPD Marker linked to Powdery Mildew Resistant Gene(s) in Black Gram by using Bulk Segregant Analysis. Research J. of Biotechnology. 2013; 8: 9-15.