

ASSESSMENT OF GENETIC VARIABILITY IN SOMACLONAL POPULATION OF SUGARCANE

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Abstract

In the present study plant tissue culture technique was used to create the genetic variability in three sugarcane clones NIA98, BL4 and AEC82-1026. Callus induced in these clones in media containing MS + 2, 4 D (2mg lit⁻¹) and Dicamba (1mg lit⁻¹). The embryogenic calli then regenerated in media containing MS basal media + Kinetin (2mg lit⁻¹) + IBA (2mg lit⁻¹) + IAA (2mg lit⁻¹). After shooting and rooting, plants were exposed to green house and acclimatization of the somaclones in the field condition. RAPD markers were used to evaluate the genetic variation at DNA level between parents and somaclones of NIA98, BL4 and AEC82-1026 developed through callus culture. Fourteen RAPD primer chosen randomly were used to amplify DNA from plant material to assess the genetic variation between parents and regenerated somaclones. The highest similarity was obtained between BL4 parent and BL4 somaclone (96%). While minimum similarity found between NIA-98 parent and AEC82-1026 somaclone (69%). In this study, we used RAPD to investigate the somaclonal variation in sugarcane clones derived from callus cultures.

Key words: Genetic variability, Somaclone, Sugarcane.

Introduction

Sugarcane (*Saccharum* spp.) is an important industrial crop, ranking among the ten most planted crops in the world. The *Saccharum* complex includes the agronomically and industrially important sugarcane genotypes obtained from *S. officinarum*, *S. spontaneum* and *S. robustum* crosses (Menossi, 2008). Although conventional breeding has contributed to the development of agronomically improved cultivars, limitations such as narrow gene pool, complex genome, poor fertility and the long breeding selection cycle make it difficult to undertake further improvement. In addition, modern cultivars have a variable chromosome number (2n=100-120) and rarely flower. To sustain sugarcane production, improve the productivity, tolerance to biotic and a biotic stresses, nutrient management and improve sugar recovery are some are concerns of sugarcane improvement programmes (Suprasanna, 2010). Both the conventional and biotechnology method have greatly contributed in solving some of these constraints. Plant tissue culture one of the biotechnological technique that has been developed as a breeding tool for improving the quality and production of vegetatively propagated crops such as sugarcane. Somaclones regenerated from this technique show variation for different parameters such as yield, sugar recovery, disease resistance, drought tolerance and maturity. Assessment of genetic variability in tissue culture-derived plants would be helpful for plant breeders to select appropriate material for their breeding program (Smiullah *et al.*, 2011).

Technological advances in molecular biology have contributed greatly to understanding the genetic diversity of plants. These new techniques are not intended to replace conventional breeding methods, but rather to facilitate and supplement crop improvement (Ahemed & Khaled, 2009). Morphological markers are routinely used for genetic diversity analysis, but recently many

molecular marker techniques have developed in to powerful tools to analyze sugarcane genotypes for their commercial exploitation and selecting genetically diverse parents for use in introgressive breeding (Shinwari, 2000). As compared to the earlier techniques, random amplified polymorphic DNA (RAPD) analysis employs single short primers with arbitrary sequence to generate genome specific fingerprints of multiple amplification products. (Kawar *et al.*, 2009). This technique has been used in an array of field crops, like rice (Ravi *et al.*, 2003; Pervaiz *et al.*, 2010) and sorghum (Ayana *et al.*, 2004). In sugarcane RAPD are used to assess levels and patterns of variation among varieties, species as well as members of *Saccharum* complex and in to identify putative markers linked to phenotypic traits (Srivastava & Gupta, 2006).

Molecular markers are widely used to detect and characterize somaclonal variation at the DNA level (Edme *et al.*, 2005; Swain *et al.*, 2006). Molecular markers, however, do allow the separation of sugarcane varieties and offer the potential to examine differences in genetic variation. Several different molecular markers have been used in previous studies that have examined diversity between sugarcane cultivars from different world regions. The objective of the present research work to select desirable clone through molecular markers.

Methodology

Plant material: Ten selected 6 month old plants of each parent/somaclone (NIA-98, AEC82-1026 and BL4) were used for RAPD analysis.

Isolation of genomic DNA: Total genomic DNA was isolated from fresh leaves by modified Genra Kit method DNA sample were quantified by spectrophotometer (BIOMATE 3) and also in 8% agarose gels. The DNA was diluted in TE buffer to working concentration of 10ng/ul.

PCR amplification and gel electrophoresis: DNA amplification, fourteen primers from Gene Link (New York, U.S.A), each ten bases in length, were used to amplify the DNA. PCR reaction was carried out in 25µl reaction mixture containing 13ng of template (genomic DNA), 2.5mM MgCl₂ (Eppendorf, Hamburg, Germany), 0.33mM of each dNTPs (Eppendorf, Hamburg, Germany), 2.5U of Taq polymerase (Eppendorf, Hamburg, Germany) and 1µM of primer in a 1xPCR reaction buffer (Eppendorf, Hamburg, Germany). The amplification reaction was performed in the Eppendorf Master cycler with an initial denaturation for 5 min at 94°C, then 32 cycles: 1 min denaturation at 94°C; 1 min annealing at 52°C; 2min extension at 72°C. Final extension was carried out at 72°C for 10 min. Amplified products were analyzed through electrophoresis on 1.5% agarose gel containing 0.5X TBE (Tris Borate EDTA) at 72 Volts for 2 hours, the gel contained 0.5µg/ml ethidium bromide to stain the DNA and photograph was taken under UV light using gel documentation system (Vilber Lourmat, France).

Data analysis: Somaclones regenerated from each clone were compared with each other using amplification profiles and band of DNA fragments were scored as presence of bands as (1) and absence of band as (0) from random amplified polymorphic DNA (RAPD). Coefficient of similarity among cultivars was calculated according to Nei & Li (1979). A dendrogram based on these similarity coefficients was constructed by using Unweighted Pair Group Method of Arithmetic means (UPGAM).

Sequence of the primers (RAPD):

Primer	Sequence	Primer	Sequence
A-01	CAGGCCCTTC	B-06	TGCTCTGCCC
A-02	TGCCGAGCTG	B-12	AGGGAACGAG
A-04	AATCGGGCTG	C-02	GTGAGGCGTC
A-13	CAGCACCCAC	C-05	GATGACCGCC
A-18	AGGTGACCGT	C-07	GTCCCGACGA
A-20	GTTGCGATCC	C-08	TGGACCGGTG
B-10	CTGCTGGGAC	C-09	CTCACCGTCC

Results

Amplification products in somaclones and their parent with fourteen primers yielded a total of 86 scorable bands, out of which 52 (60.47%) were polymorphic and only 34 (39.53%) were monomorphic (Table 1). Fragments ranged in size from 196bp-3.2kbp. The number of fragments produced by various primers ranged from 1-12 with an average of 6.14 fragments per primer. The highest number of bands (12) was obtained with Primer A-02, while the lowest numbers (1) were obtained with primers B-10 and C-08 (Figs. 1&2).

Some specific bands were also identified thus, reflecting the RAPDs application for the identification of sugarcane mutants. Results revealed that somaclone AEC82-1026 and NIA-98 somaclone contains a specific band of 451bp amplified with primer A-19.

Table 1. Primer name and amplified product.

Primer	Sequence	Range of amplified loci (bp-kbp)	Polymorphic	Monomorphic	Total no of loci
A-01	CAGGCCCTTC	396-1.5	2	2	4
A-02	TGCCGAGCTG	201-2.5	8	4	12
A-04	AATCGGGCTG	583-3.2	4	2	6
A-13	CAGCACCCAC	344-1.5	6	4	10
A-18	AGGTGACCGT	203-2.9	4	4	8
A-20	GTTGCGATCC	386-1.2	4	4	8
B-10	CTGCTGGGAC	451-1.1	Nil	1	1
B-06	TGCTCTGCCC	196-1.9	3	5	8
B-12	AGGGAACGAG	765-2.2	2	3	5
C-02	GTGAGGCGTC	331-2.5	2	1	3
C-05	GATGACCGCC	272-1.6	9	1	10
C-07	GTCCCGACGA	443-1.2	2	1	3
C-08	TGGACCGGTG	309-363	Nil	1	1
C-09	CTCACCGTCC	220-2.7	6	1	7
			52(60.47%)	34(39.53%)	86

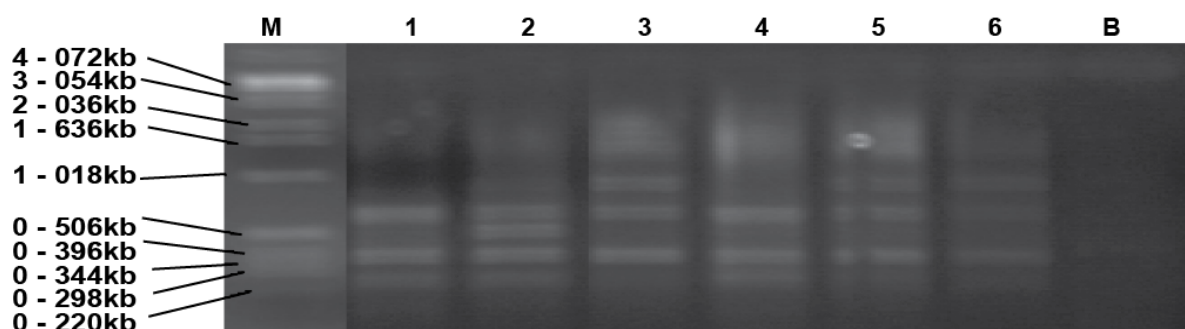


Fig. 1. RAPD-PCR with primer A-04, M=DNA marker, 1=AEC82-1026P, 2=AEC82-1026SC, 3=NIA-98P, 4=NIA-98SC, 5=BL4P, 6=BL4SC, B=Control.

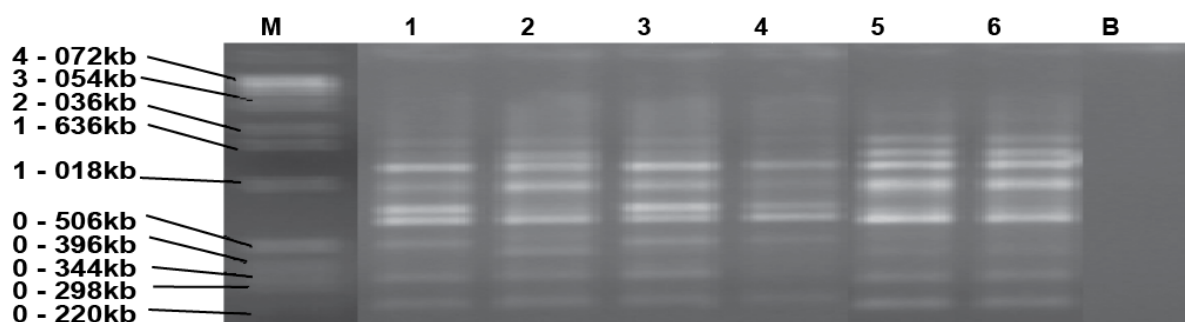


Fig. 2. RAPD-PCR with primer A-13, M=DNA marker, 1=AEC82-1026P, 2=AEC82-1026SC, 3=NIA-98P, 4=NIA-98SC, 5=BL4P, 6=BL4SC, B=Control.

Table 2. Similarity coefficient among the sugarcane somaclones with parent calculated according to Ne'i and Li's coefficient.

	NIA-8P	BL4P	BL4 SC	AEC82-1026P	NIA-98SC	AEC82-1026SC
NIA-98 Parent	1					
BL4 Parent	0.89	1				
BL4 Somaclone	0.78	0.96	1			
AEC82-1026 Parent	0.79	0.84	0.84	1		
NIA-98 Somaclone	0.7	0.74	0.74	0.7	1	
AEC82-1026 Somaclone	0.69	0.72	0.74	0.7	0.61	1

The RAPD amplification data were used to obtain a similarity matrix and for the generation dendrogram. Similarity matrix reflects the genetic relationship between the sugarcane somaclones (Table 2). The highest similarity was obtained between BL4 parent and BL4 somaclone (96%). While minimum similarity found between NIA-98 parent and AEC82-1026 somaclone (69%). In sugarcane, RAPDs have been used to detect polymorphisms in a quick and reproducible manner to determine genetic variability successfully in 20 commercial sugarcane hybrids as well as between member of the *Saccharum* complex and in resolving taxonomical group in cluster analyses (Harvey and Botha, 1996; Nair *et al.*, 1999).

The dendrogram constructed on the basis of the similarity matrix showed that cluster 'A' and 'B' formed group 'One'. Cluster 'A' with the highest boot strap values of 84%, indicating that BL4 parent was genetically

closer to BL4 somaclone and a additional line include AEC82-1026 parent in this cluster. Cluster 'B' comprises of NIA-98 parent and NIA-98 somaclone showing genetically similar to each other (82%). A distinct somaclones AEC82-1026 somaclone was falling in group one showing 71% similarity.

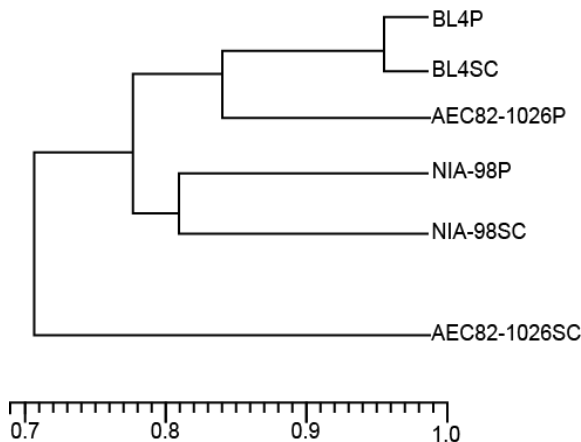
The genetic variability created in sugarcane through callus culture was efficiently screened out with molecular marker technique (RAPD). However, RAPD is a dominant marker therefore minor changes which may cause by the receive gene could not be identified during the screening processes.

Discussion

The RAPD amplification data were used to obtain a similarity matrix and for the generation dendrogram. Similarity matrix reflects the genetic relationship between

the sugarcane somaclones and parent. The highest similarity was obtained between BL4P and BL4SC (96%). While minimum similarity found between NIA-98P and AEC82-1026SC (69%). Nair *et al.*, (2002) also reported high genetic similarity among sugarcane varieties using RAPD marker.

Molecular markers have been proved to be powerful tools in the characterization and evaluation of genetic diversity within the species and population (Lal *et al.*, 2008; Suprasanna *et al.*, 2006, 2007; Devarumath *et al.*, 2007; Sultan *et al.*, 2013). RAPD markers have proven to be a reliable marker system for genetic fingerprinting (Khan *et al.*, 2013) and also for detecting genetic diversity (Saini *et al.*, 2004; Jain *et al.*, 2005; Jan *et al.*, 2011). Huckett and Botha (1995) reported that some RAPD bands were inherited in sugarcane families, indicating that RAPD bands could successfully be used as genetic markers. While RAPD-PCR fingerprints have been widely used in diversity studies, the utility of RAPD-based bands as markers is confounded as they may not be locus-specific (Besse *et al.*, 1998) and products of different sequences or concentrations can co-migrate with other amplification products (Pillay & Kenny 1995 and Nair *et al.*, 2002). It is suggested that RAPD bands possibly represent mainly repetitive DNA (Grattapaglia & Sederoff, 1994). Polymorphism in repetitive DNA sequences has frequently been observed during plant propagation by tissue culture (Ahmed & Khaled, 2009) and undergoes more alterations than the coding sequences. *In vitro* stress may provoke changes at preferential sites, such as repetitive DNA, thereby activating transposable elements.



Dendrogram of somaclones with parent developed from RAPD data using unweighted pair group method of arithmetic means (UPGMA).

Tissue culture was thus responsible for the generation of new variability therefore high rate of molecular polymorphism was observed. Heinz and Mee (1971) while working on callus-derived cultures from sugarcane variety H50-7209, detected clones with chromosomal numbers ranging from $2n = 94$ to 120. In contrast, chromosome stability was described for varieties NA56-79 ($2n = 114$) and Co419 ($2n = 213$) Shahid *et al.*, 2012, who developed a technique to obtain intact somatic

metaphase sugarcane cells. Together, these observations suggest either that some genotypes are more susceptible to somaclonal variation, or that the *In vitro* instability is actually a consequence of a genotype versus culture medium interaction.

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