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Short communication

# Analysis of genetic fidelity in micropropagated plants of sugarcane using SSR-SSCP assay

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Effective utilization of biotechnological approaches relies on efficient and reliable regeneration systems *in vitro*. Sugarcane micropropagation from shoot meristems is very useful in sugarcane breeding programmes, as it saves time in multiplying the promising varieties and clones. Despite the advantages of *in vitro* propagation, phenotypic instability has been observed in micropropagated plants, including sugarcane [1]. Parameters employed for assessing the effect of *in vitro* culture include morphological, physiological and cytogenetical studies, field assessment, and molecular studies [2-5]. Peroxidase isozymes detected gross genetic changes in tissue culture raised cultivars of sugarcane [6] Isozyme markers have limitations because DNA regions coding for soluble proteins can only be sampled which describe the genetic changes only partially. Several PCR based markers have been developed which are generally highly species-specific because recognition of specific sequences by the PCR primers allows for very little sequence redundancy [7]. Among these, RAPD has been used successfully to assess genetic stability [8,9]. Alternatively Simple Sequence Repeats (SSRs) or microsatellites have become a method of choice to determine the clonal fidelity and somaclonal variation, as they are more stable abundant, reproducibile and co-dominant [10-12]. The SSR amplified products when visualized for their single strand conformations, are expected to unravel the variations if any due to the changes in nucleotide sequences because single-strand conformation polymorphisms (SSCP) depends on differential mobilities of single stranded DNA molecules due to conformational differences [13]. SSCP-PCR analysis of simple sequence repeats has proved useful to differentiate closely related genotypes of sugarcane [14]. Hence it is presumed that by subjecting the sugarcane plantlets derived *in vitro* to SSR-SSCP assay, it would be possible to detect genetical changes more efficiently. The present study was therefore undertaken to assess the utility of SSR-SSCP markers in determining the genetic fidelity of micropropagated plantlets of sugarcane derived from shoot tip cultures.

Apical portions of sugarcane (*Saccharum* spp. hybrid) varieties; CoLk 9617 and Co 1148 were cultured *in vitro* using standard protocol with some modifications [15]. Micrpropagated plantlets with roots were transferred to sterilized polyethylene pots filled with soil, sand and organic compost (1:1:1) and kept at 25-30°C with 85% relative humidity for hardening. Approximately 250 mg of fresh tissue from six micropropagated plantlets of each genotype was used for DNA extraction by the modified CTAB method [16]. DNA from plants established in the B.O.D. Incubator was taken as control. Five SSR markers derived from genomic libraries were used as flanking primers (Table 1). Amplification reactions were carried out in PTC 200 MJ thermocycler in medium containing IX PCR buffer, 1.25mM d NTPs, 20 pmoles of primer reverse and forward each, 4mM $MgCl_2$, 1 Unit Taq Polymerase and 10 ng template DNA. The PCR amplification of each reaction sample involved 30 cycles of the following reaction profile: 3 min preamplification denaturation at 94°C for 3 minutes, denaturation for 45 sec at 94°C, annealing for 30 sec at (Tm-5)°C followed by 30 sec at 73°C. As a final step, products were fully elongated for 3 min at 73°C. Amplified products were denatured; snap chilled to convert them in single strands, separated on 10% (w/v) non-denaturing PAGE using TBE buffer and silver stained to detect conformational polymorphism if any. A 100bp DNA ladder (Bangalore Genei, India) was used as molecular weight marker. The gels were photographed in Alphalmager™1220 Gel Documentation System. The reproducible bands from the PAGE gels were designated as conformers as they showed conformation of single stranded DNA on the gel matrix.

All the primer pairs amplified DNA fragments for SSR primers in both the genotypes; parent clones as well as regenerants and provided a distinct multiple band profile of conformers (Figs. 1 and 2). The resulting patterns showed four to thirteen band ladders for these primers amounting to 77 amplicons of product size ranging from 117-1011 bp (Table 1) with an average amplification rate of 6.49 fragments per primer and 9.625 fragments per genotype. The pattern of the amplicons was reproducible. Maximum number of amplicons of SSR amplified products was obtained with

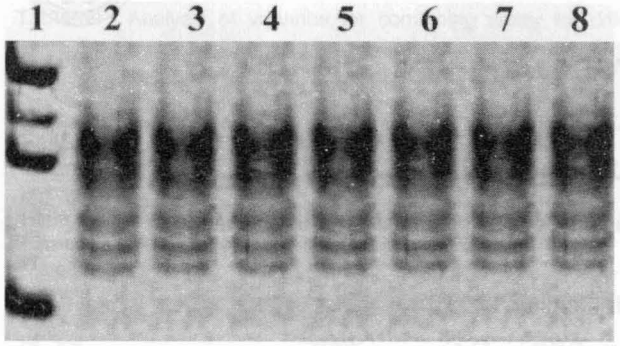


**Fig. 1. SSR-SSCP profile of conformers of amplicons using SSR primer SMC 477CG in CoLk 9617. Lane 1. 100 bp DNA ladder of Bangalore Genei; Lane 2. Control; Lane 3-8 micropropagated plantlets**

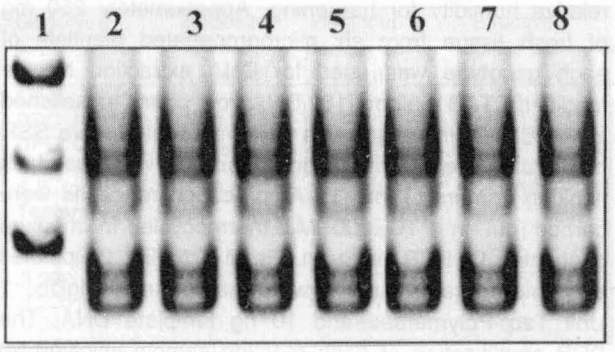


**Fig. 2. SSR-SSCP profile of conformers of amplicons using SSR primer mSSCIR 36 in CoLk 1148. Lane 1. 100 bp DNA ladder of Bangalore Genei; Lane 2. Control; Lane 3-8 micropropagated plantlets**

**Table 1.** SSR primers used, number of conformers and amplified product size obtained in two sugarcane genotypes CoLk 9617 and Co 1148

| SSR Primers | Expected size (bp) | Product Size range (bp) | | No. of Conformers | |
|---|---|---|---|---|---|
| | | Co 1148 | CoLk 9617 | Co 1148 | CoLk 9617 |
| mSSCIR1 | 227 | 202-1011 | 120-980 | 9 | 11 |
| mSSCIR36 | 166 | 138-367 | 121-361 | 8 | 13 |
| mSSCIR57 | 226 | 230-324 | 222-324 | 6 | 7 |
| SMC248CG | 142 | 142-252 | 142-252 | 5 | 4 |
| SMC477CG | 168 | 118-201 | 117-290 | 6 | 8 |

primer mSSCIR36. The amplified products exhibited monomorphism among all the *in vitro* raised sugarcane plants and were similar to those from control plants. Absence of polymorphism in DNA samples from regenerants as well as their respective controls after SSR-SSCP indicated genetic stability of sugarcane plantlets derived *in vitro*. The SSR-SSCP approach can thus be used to assess genetic fidelity of tissue at several stages of *in vitro* culture. This technique might also be useful for monitoring the stability of a large population of micropropagated plantlets, *in vitro* germplasm collections and cryopreserved material as the method is simple and reproducible.

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