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Early assessment of genetic fidelity in sugarcane (*Saccharum officinarum*) plantlets regenerated through direct organogenesis with RAPD and SSR markers

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Abstract

Early assessment of genetic fidelity of micropropagated plants aids in fine tuning protocol parameters and gauge suitability of regeneration protocol for large scale applications. Induction of direct organogenesis leading to reduced duration *in vitro* can lead to production of genetically stable plantlets. The present work describes early assessment of clonal fidelity in Sugarcane plants regenerated through direct organogenesis using RAPD and SSR markers. Analysis of RAPD banding patterns generated by PCR amplification using 20 random primers gave no evidences for somaclonal variation and the percent of polymorphic bands in a total of 110 amplicons was 0.02%. RAPD patterns of the plantlets were identical with the original mother plant, indicating that direct adventitious organogenesis did not induce somaclonal variation that can be detected by RAPD. Mean while SSR banding pattern analysis generated with 15 primers (112 amplicons) also gave no evidences for somaclonal variation. The genetic fidelity testing of micro-shoots, based on a RAPD and SSR analysis indicated a strong genetic purity like the parent genotype. Lack of variation confirms the genetic purity of tissue culture plants of sugarcane raised through direct organogenesis in young whorl leaf roll explants and confirms to the suitability of overall regeneration protocol.

Keywords: Genetic stability; RAPD & SSRs markers; Sugarcane Cultivar; Young whorl leaf roll.
Abbreviations: NAA- α- Naphthaleneacetic acid; BA- Benzylamino purine; 2,4D- 2,4 Dichlorophenoxyacetic acid; MS- Murashige & Skoog 1962; CTAB- Cetyl trimethylammonium bromide, RAPD- Random Amplified Polymorphic DNA, SSRs- Simple Sequence Repeats.

Introduction

The modern *Saccharum* spp. hybrids (cultivated sugarcane) is originated from complex hybridization events between *Saccharum officinarum*, *S. Spontaneum*, *S barberi*, *S sinense* and the related species (Sreenivasan et al., 1987). It is propagated vegetatively for commercial planting by stem cuttings called 'setts'. Seed production of an elite sugarcane cultivar in sufficient quantity for planting in a vast area generally takes 10-15 years if multiplied through conventional methods of seed multiplication. There are also chances of perpetuation of sett-borne diseases. *In vitro* micropropogation technique is emerging as a powerful tool for fast multiplication at larger scale and production of disease free planting material in a number of crops (Hussain et al., 2008; Tripathi and Kumari, 2010). Several agro-industries and research organizations are now engaged in *in vitro* micropropogation for quick multiplication of elite commercial cultivars of sugarcane (Yadav et al., 2004). Tissue culture induced somaclonal variation limits the applications of tissue culture in sugarcane which owing to its high ploidy level is very unstable *in vitro*. Assessment of Genetic fidelity of sugarcane plantlets raised through Protoplast, suspension and callus cultures and indirect embryogenesis (Heinz and Mee, 1971; Chowdhary and Vasil, 1993; Taylor et al., 1995; Suprasanna et al., 2007), Shoot tip culture (Lal et al., 2008) Rhizome and Meristem culture (Zucchi et al., 2002) has found varying levels of somaclonal variation in the micropropagated population of sugarcane through RAPD technique. *In Vitro* multiplication techniques were proved fruitful earlier in leguminous trees (Hussain et al., 2008), conservation of genetic resources (Kumari et al., 2009) and also in micropropagation of fruit trees (Tripathi and Kumari, 2010) Thus, the use of modern *in vitro* techniques on industrially important crop like sugarcane has opened new possibilities for rapid mass multiplication of the newly developed elite sugarcane commercial cultivars to meet out the seed cane demands of the sugarcane growers. Besides affording multiplication in limited time and space, the technique of tissue culture circumvents the limitations posed by the somaclonal variation during the micropropagation of sugarcane due to long subculturing passage. Contrary to this direct adventitious organogenesis provides genetically pure plantlets of sugarcane. Early assessment of genetic fidelity of regenerated plantlets through direct adventitious organogenesis with PCR based RAPD and SSR markers would be helpful to prove the purity of micro-shoots. Regeneration through direct embryogenesis in sugarcane (Snyman et al., 2006) utilizes immature inflorescence explants (limited availability in time and space) and thus falls short of expectations to be used for routine experimentation. The Direct organogenesis pathway developed by Lakshmanan et al., 2006; Gill et al., 2006; and Franklin et al., 2006 can be very useful for large scale applications as it eliminates / minimizes callus formation in culture and minimizes culture duration leading to fast multiplication from widely available leaf explants. Moreover the method is also genotype independent (Franklin et al.,

2006). The organogenic mode of regeneration is more prone to somaclonal variation as compared to axillary bud proliferation and somatic embryogenesis (Vasil, 1987). It is also desirable to test genetic fidelity of micropropagated plants irrespective of regeneration pathway they are derived from, to optimize culture conditions for minimal variation with efficient production for large scale applications (Rani and Raina, 2000). Given all the advantages of direct organogenesis as a method for routine application in sugarcane, the fear of somaclonal variation needs to be ruled out before it could be used for further applications. Molecular markers have come up as the most desirable tool for establishing genetic uniformity of the micropropagated plantlets. This is apparent in studies conducted to screen somaclonal variations produced in tissue cultured plants such as in turmeric (Salvi et al., 2001), tea (Singh et al., 2004) and soyabean (Hofmann et al., 2004), *Swertia chirayta* (Joshi and Dhawan, 2007), Date palms (Saker et al., 2006), Sugarcane (Chowdhary and Vasil, 1993; Taylor et al., 1995; Lal et al., 2008), leguminous trees (Hussain et al., 2008) and *Spondias mangifera* (Tripathi and Kumari, 2010) PCR based molecular markers such as RAPD, SSR, ISSR and AFLP have several advantages over the other methods and have been used for genotyping and detection of polymorphism/variability in plants. A better analysis of genetic stability of plantlets can be made by using a combination of two types of markers that amplify different regions of the genome (Martins et al., 2004). Hence, in the present study, two PCR-based molecular markers, Randomly Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeats (SSRs), were adopted for evaluation of clonal fidelity in Sugarcane plantlets. The microsatellite markers developed from the genomic sequences are reported to be more polymorphic than ESTs-derived markers and are thus suitable for various genotyping applications in Sugarcane (Singh et al., 2011). Direct regeneration through young whorl leaf roll disc would be advantageous in *Agrobacterium* mediated genetic transformation in sugarcane due to having high regeneration potential in pre-treated leaf discs tissues and quick recovery of complete plantlets. Further there is no callus phase involved thus, minimum chances of somaclonal variation. Our objectives were to assess the genetic fidelity of sugarcane plantlets regenerated through direct organogenesis with RAPD and SSR markers. So far, there are no reports on the use of two marker systems (RAPD and SSRs) for the assessment of genetic fidelity in Sugarcane. The findings reported herein could be used for large scale co-cultivation experiments with *Agrobacterium* and also for micro propagation at large to reduce cost of plant
Production (Pandey et al., 2011).

Results and Discussions

The present study was conducted to screen tissue culture (TC) - induced genetic variations (if any) in sugarcane plantlets regenerated through direct organogenesis in young whorl leaf roll explants of commercial sugarcane cultivar CoS96268 by PCR based RAPD and SSR analysis of the DNA samples from 17 ex agar plantlets (lane 1-17) and donor plant as a control (lane 18). A total of 222 loci were scored using both RAPD (110) and SSR primers (112) which were uniformly present in all the samples tested (except two polymorphic bands, one produced by RAPD primer OPA13 and OPB2 each) (Table 2).

Genetic purity through SSRs

The sensitivity, reproducibility and strong discriminatory power of microsatellite simple sequence repeat (SSR) markers (Parida et al., 2009) make them particularly suitable for detecting somaclonal variation, but their application in the study of somaclonal variation has been rather quite limited. Recently Castillo et al., 2007 and Wanmei et al., 2009 have described the suitability of SSR markers for evaluation of clonal fidelity and genetic variation in *Rubus* and grapes, respectively. So far, there are no reports on the use of SSR markers for evaluation of genetic fidelity in tissue culture raised micro-shoots of sugarcane. However, SSRs have been used for discriminating all hybrid varieties viz; *Saccharum* species and related genera from major germplasm collections (Maccheroni et al., 2009; Singh et al., 2011). The 15 SSR primers used in the present study generated 112 amplicons. Thus, on an average, 7.46 loci were amplified per SSR primer. SSR primer UGSM 351 and UGSM 364 amplified the maximum number of 12 loci each and primer UGSM 358 amplified the lowest number of two loci (Table 2). Amplification pattern of direct organogenesis raised Sugarcane plantlets using SSR primer UGSM 351 is shown in Fig 1b.The total number of amplicons (112) generated in our study using SSR markers is sufficient to reveal somatic variations. This is evident by comparable numbers of amplified loci scored (65, 56 and 64) in various plant taxa by employing SSR-based marker assay (Leroy et al., 2000; Palombi and Damiano, 2002; Martins et al., 2004). So far there are no studies on early assessment of genetic fidelity among micropropagated sugarcane using SSR-PCR analysis.

Genetic stability through RAPD

RAPD technique has been frequently used for analysis of somaclonal variation and assessment of genetic fidelity in Sugarcane (Suprasanna et al., 2006, 2007; Lal et al., 2008). The 20 RAPD primers used in our study produced 110 amplicons with an average of 5.5 bands per primer. Amplification pattern of direct organogenesis raised Sugarcane plantlets using RAPD primer OPA 13 is shown in Fig 1b. The total number of RAPD fragments (110) obtained in the present study is sufficient to reveal genetic variation in sugarcane. This is evident by the fact that (Suprasanna et al., 2007) reported more than 46 polymorphic bands (29.1%) among a total of 158 RAPD amplicons generated for 68 regenerants of somatic embryogenesis from inflorescence tissues using 17 RAPD primers where as almost no polymorphic band (only 0.02%) was detected in the present study using 20 RAPD primers. The level of genetic fidelity based on molecular analysis of regenerated plantlets in our study is more than all the reports on Sugarcane, i.e 100% as against those reported by Lal et al., 2008 (97%) and Saini et al., 2004 (90%). Studies of Jain et al., 2005 indicated that isozyme and RAPD analysis showed no variation in meristem derived sugarcane clones. The results described herein appear to be more reliable as they are based on molecular analysis using two different marker systems (RAPD and SSR) which amplify different regions of the genome. Moreover the use of SSR markers further enhances the confidence in present findings as SSR's or microsatellites are arranged in tandem repeats of one to twenty nucleotide long DNA motifs dispersed throughout the genome and have strong discriminatory power to detect molecular genetic diversity in sugarcane (Parida et al., 2009).

Table 1. Composition of media used at various stages of regeneration of Sugarcane (CoS96268) through direct organogenesis.

Medium for	Composition with growth regulators	Period of incubation	Remarks
Pre treatment of Leaf roll discs	Full strength MS medium gellified with Agar (0.7%)with 3 mg/l 2,4 D	8 days	Swelling of leaf tissue and formation of globular stage
Regeneration of shoots	Full strength MS medium gellified with Agar (0.7%)with 0.1 mg/l NAA and 0.2 mg/l BAP	15-21 days	Formation of Shoots (20-25 per explants)
Regeneration of complete plantlets	Same as above	40 days	Formation of complete plantlets, each having 3-4 shoots and 5-10 roots. Each explant gives rise to 4-5 healthy plantlets

Thus, these results suggest that direct organogenesis in leaf roll explants could be adopted as a method of regeneration for transformation studies and rapid multiplication as it keeps intact the integrity of the genotype under regeneration. The regeneration of complete plantlets on the same medium within 40 days makes this protocol even more desirable for extensive application. The factors affecting somaclonal variations can be many including culture method and environment, explants source, ploidy level and *in vitro* culture age (Rani and Raina, 2000). *In vitro* conditions and rapid multiplication of a tissue may affect genetic stability and lead to somaclonal variation (Martinez et al., 1998). This may be attributed to both (1) increased mutation rate per cell-generation over time, and (2) accumulation of mutations over a period of time (Rodrigues et al., 1998). The feature of protocol used in the present study that might have contributed to zero variation in the regenerated population is direct regeneration without an intermittent callus phase. The earlier reports describing high degree of variation in micropropagated population of sugarcane may be due to an intermittent callus phase and long durations of tissues in culture (Saini et al., 2004; Suprasanna et al., 2007). In the protocol used for the present study each explant gave rise to 20-25 shoots and thus the method overcomes need for subsequent culturing of shoots and yet produces large number of plants. The uniform banding patterns (Fig 1a and 1b) confirms that direct regeneration methods present effective strategy to reduce culture induced somaclonal variations by reducing *in vitro* culture durations and avoiding callus phase.

The study also ascertained that organogenic mode of regeneration is not necessarily detrimental to *in vitro* cultures vis-à-vis clonal fidelity. This is in line with genetically uniform plants raised though organogenesis in leaf explants of grapes (Wanmei et al., 2009) which was confirmed by using SSR markers. Organogenic differentiation which is more prone to somaclonal variation (Vasil, 1987) could lead to genetically uniform plants if it is manifested by explant cells directly without an intervening callus phase. Genetically uniform plantlets obtained in the present study, suggest that the overall protocol is suitable for clonal multiplication of at least the genotype under study (CoS96268) and should be extended to other important genotypes. The primary event that possibly triggers tissue culture-induced variability is cell-cycle disturbance caused by exogenously applied hormones (Peschke and Phillips, 1992). In the present study on sugarcane, regeneration was obtained fairly rapidly on a medium supplemented with 0.1 mg/l NAA and 0.2 mg/l BAP which is less than that used in sugarcane micropropagation through shoot tips (Lal et al., 2008), axillary buds (Wagih et al., 2009) and callus culture methods (Suprasanna et al., 2007) used 1-3 mg/l 2, 4-D to produce sugarcane plantlets through somatic embryogenesis which led to more than 29% variation in micropropgated population. High concentrations of 6-benzylaminopurine (BA; 22.2, 44.44, and 66.6mM) have

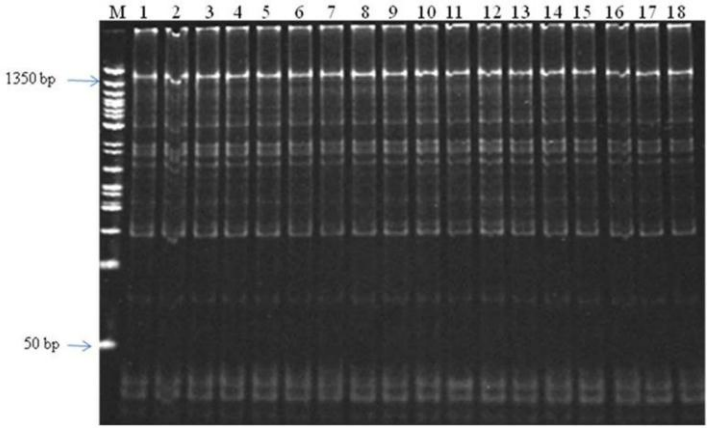

Fig1 a. SSR Amplification patterns of sugarcane plantlets produced through direct organogenesis using UGSM 351 primer pairs on 7.5 % PAGE. Lane M 50bp ladder, Lane 1 to 17 represent samples from regenerated plantlets of variety CoS 96268 and lane numbered 18 represents the sample from mother plant (Control). Arrow indicates the size of the marker (bp).

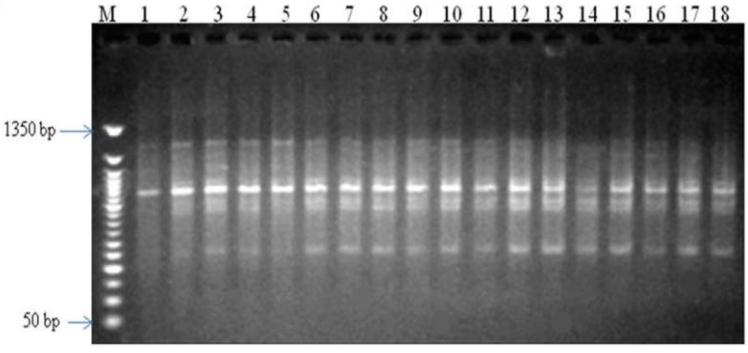

Fig 1b. RAPD profile of control and micropropagated plantlets of sugarcane produced through direct organogenesis using primer OPA-13 on 1.2% Agarose Gel. Lane M 50bp ladder, Lane 1 to 17 represent samples from regenerated plantlets of variety CoS 96268 and lane numbered 18 represents the sample from mother plant (Control). Arrow indicates the size of the marker (bp).

Table 2. Details of SSR and RAPD Primers used for detecting genetic fidelity in sugarcane plantlets regenerated through direct organogenesis.

Sl. No.	Primers	Primer sequences F- Forward primer sequences (5'-3') R- Reverse primer sequences (5'-3')	No. of loci amplified	No. of polymorphic loci	Range of product size (bp)
SSR Primers					
1	UGSM 351 Ta= 53^0C	F5'AAGAAGAGCCGTAGAAACAAC3' R5'ATTGAGCGAGGGATGAAC3'	12	0	76-1114
2	UGSM 358 Ta= 53^0C	F5'ACCCTTCCCATTCCCATC3' R5'CTCCAGGTTCGCCACCAC3'	2	0	278-396
3	UGSM 361 Ta= 55^0C	F5'GTTCTTAGTCCAGCCGTAGTT3' R5'ATCGTTGTTGTCGGTGTC3'	8	0	176-1169
4	UGSM 363 Ta= 55^0C	F5'GTTTAAGACAAGATGGTGTAGATG3' R5'TACATATTTACATTGTTACTCCGC3'	6	0	272-1197
5	UGSM 364 Ta= 53^0C	F5'GCGTCTTCATCATCTGCAAC3' R5'TAGAGAGACATGGGGTGCAT3'	12	0	145-1150
6	UGSM 367 Ta= 53^0C	F5'GTTGTCGAGATGATACAGAAGTAA3' R5'GTACAATATTACACACACAAAGGG3'	6	0	69-610
7	SEGMS 9 Ta= 55^0C	F5'CTTCAGTGGCGTGCTCGGAAT3' R5'TTGTGTGCTCTTGCTTAGTATC3'	8	0	80-619
8	SEGMS 45 Ta= 55^0C	F5'TTCTCTTGCTTAGATCTGGACT3' R5'TAACTAATTAAGCAAGCCACCT3'	5	0	50-600
9	SEGMS 48 Ta= 55^0C	F5'GTATAGACTCACTATAGGGCCG3' R5'ACAACAAGAGCGACAACAAG3'	5	0	94-385
10	SGM 31 Ta= 55^0C	F5'CTCTCGCTTGCTTGTGTC3' R5'TAGTGGAAGGGTTCTGTTTG3'	9	0	56-649
11	SGM 118 Ta= 55^0C	F5'GAGGAAGCCAAGAAGGTG3' R5'TAGAGCGAGGAGCGAAGG3'	8	0	76-940
12	SGM 136 Ta= 52^0C	F5'ACATCCTTCCCACGCCAG3' R5'CCTACTCCTCCTCCTCCTC3'	7	0	186-820
13	SCM 4 Ta= 52^0C	F5'CATTGTTCTGTGCCTGCT3' R5'CCGTTTCCCTTCCTTCCC3'	7	0	134-705
14	SCM 16 Ta= 50^0C	F5'GTGCGAGAGGAACTGTGT3' R5'AGCCCTGCCTAACAAGGA3'	8	0	123-792
15	SCM 18 Ta= 55^0C	F5'CATCAGTATCATTTCATCTTGG3' R5'CAGTCACAGTCGGGTAGA3'	9	0	82-1018
		Total	112	0	_
RAPD Primers					
16	OPA 5	5'AGGGGTCTTG3'	7	0	157-959
17	OPA 13	5'CAGCACCCAC3'	6	1	254-1324
18	OPAA 7	5'CTACGCTCAC3'	4	0	168-455
19	OPAA 10	5'TGGTCGGGTG3'	4	0	268-945
20	OPAA 12	5'GGACCTCTTG3'	5	0	269-956
21	OPB 2	5'TGATCCCTGG3'	3	1	505-911
22	OPC 1	5'TTCGAGCCAG3'	7	0	244-1316
23	OPE 1	5'CCCAAGGTCC3'	3	0	166-345
24	OPF 12	5'ACGGTACCAG3'	5	0	236-869
25	OPP 10	5'TCCCGCCTAC3'	6	0	212-956
26	OPT 2	5'GGAGAGACTC3'	4	0	156-902
27	OPW 6	5'AGGCCCGATG3'	7	0	244-1344
28	OPW 9	5'GTGACCGAGT3'	8	0	188-1332
28	OPW 12	5'TGGGCAGAAG3'	6	0	268-1296
30	OPX 1	5'CTGGGCACGA3'	6	0	163-813
31	OPX 2	5'TTCCGCCACC3'	5	0	242-1056
32	OPX 3	5'TGGCGCAGTG3'	6	0	216-1120
33	OPX 7	5'GAGCGAGGCT3'	5	0	165-1123
34	OPX 12	5'TCGCCAGCCA3'	6	0	168-865
35	OPX 18	5'GACTAGGTGG3'	7	0	312-1302
Total			110	02	
Grand total			220	02	

been shown to induce genetic variability in banana (Trujillo and Garcia, 1996). High concentrations of a combination of BA and adenine also induced chromosome number aberrations in banana (Zhenxun and Hongxian, 1997). Our assessment indicates that the level of hormones used in our study is good enough to produce efficient multiplication without disturbing genetic integrity of the regenerated plants. Inclusion of both Auxin and cytokinin may also have a positive impact on plants as any kind of extreme treatment may be deleterious to plant genetic constitution. 2,4-D is a strong auxin and thought to induce genetic instability in tissue cultures by inducing rapid multiplication of cells (Gill et al. 2006). The protocol used for the present study were pre treated the explants on 2, 4-D supplemented medium for short exposure (eight days) so that the genetic integrity of regenerated plants remained intact, indicating that moderate levels of a strong auxin such as 2, 4-D may not be deleterious to genetic integrity of sugarcane cultures provided callus formation is avoided. Same findings were also reported by Franklin et al., 2006. In our study, no variability was detected among the tissue culture raised plantlets by SSRs assay. Although EST-SSRs markers have the ability to detect the highest levels of polymorphism. We can conclude that the tissue culture raised plantlets through young whorl leaf roll disc developed in the existing study did not exhibit any somaclonal variation despite being raised through organogenesis (direct pathway). Considering that sugarcane is quite unstable *in vitro* the results obtained in the present study become significant. Lack of genetic variation confirms the genetic purity of tissue culture plantlets of sugarcane raised through direct organogenesis in leaf roll explants and confirms to the suitability of overall regeneration protocol.

Materials and methods

Plant materials

Young whorl leaf roll disc explants from shoot tops of field grown plants of Sugarcane (CoS96268) were prepared as described by (Snyman et al., 2006). These explants were pretreated for 8 days on MS medium (Murashige & Skoog, 1962) supplemented with 3 mg/l 2-4, D for shoot induction on regeneration medium as described by (Table 1) to achieve successful shoot induction and plantlet production in CoS96268, an elite early maturing elite cultivar Cultivated widely in northern India. The culture conditions were same as those described by Franklin et al., 2006). The chemicals used (Sucrose, agar, Plant growth regulators) in the study were of Duchefa Biochemie, Netherlands. The media composition and passage duration at different stages of plantlet regeneration is presented in Table 1.

DNA extraction and PCR amplification

Eighteen samples were collected at random from regenerated plantlets being transferred for hardening and form field grown mother plant. Total DNA was isolated from young leaves by CTAB method described by Dellaporta et al. (1983). Working DNA was quantified on 0.8% Agarose gel with uncut λ DNA. Each sample was diluted to 25 ng μl^{-1} in Tris-Cl buffer and stored at -20^{0}C. Twenty arbitrary, 10-mer RAPD primers (Operon Technologies, USA) from kits A, AA, B, C, E, F, P, T, W, X were used for the RAPD analysis following the method of Williams et al., 1990. PCR was performed in volume of 25 µl containing 1 µl of genomic DNA (25 ng μl^{-1}) as template, 2.5 µl of 10X PCR buffer, 0.25 µl each dNTPs (10 mM), 1 µl of decamer primer (15 ng μl^{-1}), 1 U of Taq DNA polymerase (Fermentas, Germany) and rest 20 µl milliQ water. The optimized PCR conditions for RAPD analyses consisted of an initial denaturation at 94^{0}C for 5 min followed by 44 cycles of 60s at 94^{0}C, 30s at 37^{0}C, and 60s at 72^{0}C and finally terminated with an extension of 7 min at 72^{0}C. For SSR analysis 15 microsatellite markers including, Six unigene derived microsatellite markers (UGSM), three cDNA derived microsatellite marker (SCM), three Sugarcane genomic microsatellite markers (SGM), three sugarcane enriched genomic microsatellite markers (SEGM) were randomly selected from previously screened polymorphic primers (Singh et al., 2008, 2011; Parida et al., 2009). PCR amplifications were performed in a volume of 10 µl containing 1 µl of genomic DNA (25 ng μl^{-1}) as template, 1.0 µl of each forward and reverse primers (10 ng μl^{-1}), 0.25 µl each dNTPs (10 mM), 0.5 unit of taq DNA polymerase (Fermentas, Germany), 1.0 µl of 10X PCR buffer and rest milliQ water. The amplification reaction consisted of an initial denaturation at 94^{0}C for 5 min followed by 25 cycles of 60s at 94^{0}C, 60s at appropriate annealing temperatures (50-55^{0}C), and 60s at 72^{0}C and finally terminated with an extension of 5 min at 72^{0}C. The detailed description of the RAPD and SSR primers used in the present study are given in the table 2. Amplifications were carried out in Gene Amp PCR system 9700 (Applied Biosystems USA). Amplification products in both the cases were mixed with DNA gel loading dye. The amplification products for RAPD analysis were size separated by horizontal electrophoresis on a1.5% (w/v) Agarose gel using 1X TBE buffer (pH 8.0). PAGE for SSR analysis was carried on in 0.5X TBE buffer under 100 volt current for 4 h in 7.5% Acrylamide (29): Bis Acrylamide (1) gel matrix.The gels were stained with ethedium bromide and analyzed with gel Documentation System (Gel Doc Applied Biosystem USA). All the reactions were repeated thrice to ensure reproducibility and reliability of the results.

Conclusion

Early assessment of genetic fidelity of micropropagated plants aids in fine tuning protocol parameters and gauge suitability of regeneration protocol for large scale applications. The genetic stability of young whorl leaf disc tissue raised plantlets was tested by RAPD and SSR markers. The banding pattern of PCR amplified products from direct regeneration through young whorl leaf roll disc plantlets was monomorphic across all the micropropagated plantlets based on SSR and RAPD profile. SSR and RAPD marker analysis using 35 primers showed that clonal fidelity was >99% of young whorl leaf disc tissue raised plants. SSR profiling and RAPD analysis confirmed the genetic purity, of sugarcane plantlets derived in vitro. Direct regeneration through young whorl leaf roll disc would be also advantageous in *Agrobacterium* mediated genetic transformation in sugarcane. The findings reported herein could be used for large scale co-cultivation experiments with *Agrobacterium* and also for micro propagation at large to reduce cost of plant Production by saving the time, space and energy.

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