Journal of Medicinal Plants Research Vol. 5(23), pp. 5636-5641, 23 October, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Evaluation of clonal fidelity of *in vitro* raised plants of *Guadua angustifolia* Kunth using DNA-based markers

Harleen Kaur Nadha[2], Rahul Kumar[2], Ram Kumar Sharma[2], Manju Anand[1] and Anil Sood[2]*

[1]Department of Biotechnology and Environmental Sciences, Thapar University, Patiala (Punjab) - 147004, India.
[2]CSIR-Institute of Himalayan Bioresource Technology, Palampur (HP) - 176061, India.

Accepted 22 August, 2011

Guadua angustifolia **Kunth a large and multipurpose bamboo has extensive utility in pharmaceutical, paper, charcoal, and construction industries. Therefore, it is pertinent to scale up their production through micropropagation technique. This would enable us to meet the growing demand for quality planting material. Although, the common practice to use axillary bud method allows large-scale production, there are always possibilities of somaclonal variations which appear in *in vitro* cultures due to its rapid multiplication. Therefore, we utilized random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers in *in vitro* raised *Guadua* clones to assure their genetic fidelity. We screened 30 RAPD and 27 ISSR primers, and found 15 RAPD and 17 ISSR markers to produce clear, reproducible and scorable bands. We found 15 RAPD primers which produced 84 distinct bands with an average of 5.6 bands per primer. In addition, we found 17 ISSR primers which produced 61 distinct bands in the size range of 300 to 2500 bp. All banding profiles from micropropagated plants were monomorphic and similar to those of the mother plant, thus ascertaining the true nature of the *in vitro* raised plants.**

Key words: *In vitro* propagation, *Guadua* clones, DNA markers.

INTRODUCTION

Bamboos, the giant grasses with woody culms growing uprightly, are widely grown plant source for medicinal products, food, fiber, shelter, and building materials. Traditionally, the bamboos have been used for treating infectious diseases and wound healing. The bamboo leaves are also used for counteracting spasmodic disorders. Inefficient and labour intensive conventional methods for propagating bamboos, gregarious flowering habit, poor seed set and low viability, and human population pressure disrupting the natural cycle of reforestation present an urgent need for developing efficient methods for large scale propagation of bamboos. Overexploitation of bamboo has led to the rapid depletion of natural strands hence, generating a grave concern about conservation as well as to develop propagation methodologies for new plantations and reestablishment of cleared strands. Limitations in traditional propagation methods, such as the use of offsets, branch cuttings together with unpredictable and long flowering cycle (about 35 years), warrants an urgent need for an alternative approach for developing efficient and reproducible protocols for its mass propagation. Given the difficulties of conventional propagation techniques, *in vitro* propagation provides a promising alternative.

Among the various methods of *in vitro* propagation, the axillary shoot proliferation is a least susceptible to genetic modification (Shenoy and Vasil, 1992). However, the possibility of somaclonal variations cannot be ruled out even with this method as reported in *Populus deltoids* (Rani et al., 1995), *Robinia pseudoacacia* (Bindiya and Kanwar, 2003), *Hagenia abyssinica* (Feyissa et al., 2007), *Olea europaea* (Peyvandi et al., 2009). Plant tissue culture is regarded as a major area of biotechnology because of its potentiality to regenerate elite genetic resources but scaling up of any

*Corresponding author. E-mail: asood.ihbt@gmail.com. Tel: 01894-233337, +919418042984. Fax: 91-1894-230433/230428.

micropropagation protocol is severely hindered due to incidences of somaclonal variations (Larkin and Scowcroft, 1981). Somaclonal variations are induced due to the stress imposed on the plant during propagation and is incorporated in the form of DNA methylation, chromosome rearrangements and point mutations (Phillips et al., 1994). The occurrence of somaclonal variation is a potential drawback when propagation of an elite plant species is intended. Here, the clonal fidelity is essential to maintain the advantages of desired elite genotype (Rahman and Rajora, 2001). Several techniques are available to assess tissue culture induced variations in plants such as morphological descriptions, physiological supervisions, cytological studies, isozymes (Gupta and Varshney, 1999) and molecular markers. However, the molecular markers are regarded as rapid, sensitive and more reliable alternative approach (Sharma et al., 2008).

Several DNA markers viz. inter simple sequence repeat (ISSR), random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) have been employed to assess the genomic stability in regenerated plants (Mehta et al., 2010). RAPD and ISSR markers are simple, fast, cost effective, highly discriminative and reliable. ISSR circumvents the requirement for flanking sequence information and thus, has wide applicability in a variety of plants (Srivastava and Gupta, 2008). The ISSR markers have also been useful in establishing genetic stability of several micropropagated plants, such as cauliflower (Leroy et al*.,* 2000), *Populus tremuloides* (Rahman and Rajora, 2001), *Swertia chirayita* (Joshi and Dhawan, 2007) and *Dictyospermum ovalifolium* (Chandrika et al., 2008), *Ochreinauclea missionis* (Chandrika and Rai, 2009), *Bambusa balcooa* (Negi and Saxena, 2010). Similarly, RAPD markers have been applied for characterisation of micropropagated forest trees viz. *Picea mariana* (Isabel et al., 1993), *Populus deltoids* (Rani et al., 1995), Oak (Barrett et al., 1997), *Populus tremuloides* (Rahman and Rajora, 2001).

Here, we show a method to assess the clonal fidelity of *in vitro* raised *Guadua angustifolia* plants using RAPD and ISSR markers. This will provide us with a useful tool for establishing a unique micropropagation system for the production of genetically identical and stable plants before they are released for large scale plantations or other commercial purposes.

MATERIALS AND METHODS

Nodal segments of 2 to 4 cm in length obtained from 4 year old potted plant were used for initiating aseptic cultures. Murashige and Skoog's (1962) (MS) medium supplemented with BAP (6-Benzylaminopurine) (2 mg/L) was used for inducing bud break. The *in vitro* derived shoots were cultured on MS medium supplemented with BAP (2 mg/L) and adenine sulphate (10 mg/L) for proliferation and multiplication. The rooting was also obtained on the same multiplication medium. The cultures were maintained at 25 ± 2°C under fluorescent white light (70 µmol/m^2/s) during a photoperiod of 16:8 h light and dark cycle. The rooted plantlets were hardened on sand and soil mixture (1:1) under greenhouse conditions.

DNA extraction and PCR amplification

To test the clonal fidelity, the *in vitro* raised plants at various stages of subculture along with the hardened plants were chosen randomly. These plants were compared with the mother plant from which explants were taken. Total genomic DNA of the mother plant and *in vitro* raised plants was extracted from young leaf tissue by using modified Cetyl Trimethyl Ammonium Bromide (CTAB) method (Doyle and Doyle, 1990). Thirty RAPD primers and twenty seven ISSR primers (Sigma-Aldirch, Bangalore, India) were used for initial screening. PCR amplifications were carried out in total volume of 25 µl containing 2 µl (20 to 25 ng) of genomic DNA. The reaction buffer for ISSR consisted of 2.5 µl Taq buffer, 1 µl $MgCl_2$, 0.15 µl dNTPs (10 mM each of dATP, dGTP, dTTP and dCTP), 1.5 µl primer, 0.17 µl Taq polymerase (Bangalore Genei Pvt. Ltd, Bangalore, India) and 17.68 µl water. PCR amplification was performed in a DNA thermal cycler, which was programmed for initial DNA denaturation at 94°C for 4 min, followed by 44 cycles of 1 min denaturation at 94°C, 1 min annealing (temperature specific to the primer) and 1 min extension at 72°C, with a final extension at 72°C for 7 min. For RAPD, reaction buffer consisted of 2.5 µl Taq buffer, 0.5 µl $MgCl_2$, 0.2 µl dNTPs, 1.5 µl primer, 0.17 µl Taq polymerase and 18.13 µl water. PCR amplification consisted of initial denaturation at 94°C for 5 min, followed by 45 cycles of 1 min denaturation at 94°C, 1 min annealing at 37°C and 2 min extension at 72°C, with a final extension at 72°C for 7 min. The amplified products were resolved by electrophoresis on 1.8% agarose gel in tris-borate EDTA (TBE) buffer stained with ethidium bromide. The fragment sizes were estimated with 100 and 500 bp DNA ladders (Bangalore Genei Pvt. Ltd, Bangalore, India)

RESULTS

We used 30 RAPD primers for initial screening with mother plant of *Guadua.* However, only 15 primers generated clear and reproducible bands. The number of scorable bands for each primer varied from 2 (OPO-14) to 11 (OPT-17) (Table 1). The 15 RAPD primers produced 84 distinct and scorable bands in the size range of 200 to 2500 bp, with an average of 5.6 bands per primer. We did not observe any polymorphism during the RAPD analysis of *in vitro* raised clones (Figure 1). Out of 27 ISSR primers used in initial screening, only 17 primers produced clear and reproducible bands. These 17 ISSR primers produced 61 distinct and scorable bands in the size range of 300 to 2500 bp. The number of scorable bands for each primer varied from 1 to 7 (Table 2). We found that all banding profiles from micropropagated plants were monomorphic and similar to those of mother plant (Figure 2).

DISCUSSION

Although, the *in vitro* germplasm conservation depends on

Table 1. The random amplified polymorphic DNA (RAPD) primers utilized to verify *Guadua* clones.

S/N	Primers	5'-3' motif	No. of scorable bands	Range of amplification (bp)
1	OPO 02	ACGTAGCGTC	3	600-1200
2	OPO 03	CTGTTGCTAC	8	400-2000
3	OPO 04	AAGTCCGCTC	6	650-1700
4	OPO 05	CCCAGTCACT	4	450-900
5	OPO 06	CCACGGGAAG	5	550-2000
6	OPO 10	TCAGAGCGCC	8	600-2000
7	OPO 14	AGCATGGCTC	2	900-1100
8	OPO 18	CTCGCTATCC	4	400-1300
9	OPO 19	GGTGCACGTT	5	550-1600
10	OPT 01	GGGCCACTCA	4	900-1800
11	OPT 03	TCCACTCCTG	2	1500-2000
12	OPT 07	GGCAGGCTGT	9	400-2100
13	OPT 13	AGGACTGCCA	7	450-2100
14	OPT 16	GGTGAACGCT	6	700-2500
15	OPT 17	CCAACGTCGT	11	200-2000

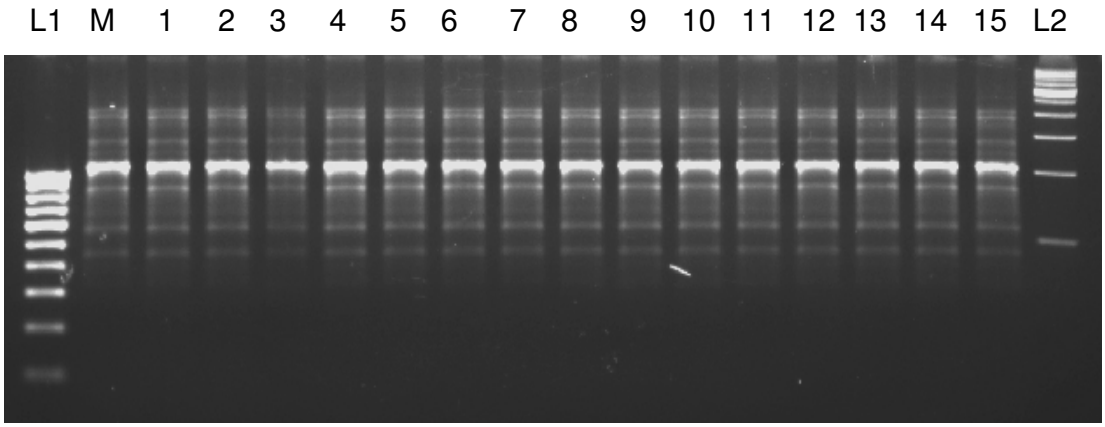

Figure 1. Polymerase chain reaction (PCR) amplification products obtained with a random amplified polymorphic DNA (RAPD) primer (OPT-13). Lane L1 represents 100 bp ladder, Lane M represents mother plant, Lane 1 to 15 represent *in vitro* raised clones of *Guadua angustifolia* and Lane L2 represents 500 bp ladder.

micropropagation techniques, the phenotypic and genetic variations may occur during *in vitro* propagation and subsequently may give rise to somaclonal variants (Kaeppler et al., 2000). Therefore, it is essential to assess the genetic stability of *in vitro* derived clones for micropropagation of true-to-type clones (Eshraghi et al., 2005, Chandrika and Rai, 2009). Many factors are responsible for inducing variability during tissue culture, such as explants source, time of culture, number of subcultures, phytoharmone, genotype, media composition, the level of ploidy and genetic mosaicism (Silvarolla, 1992). The activation of transposable elements (Hirochika et al., 1996), DNA hypomethylation (Jaligot et al., 2000; Keyte et al., 2000; Lukens and Zhan, 2007), genome adaptation to different regulatory microelements (Bogani et al., 1996) and the presence of hot spots (Linacero et al., 2000) are major mechanisms expected to induce the previous variations (Peyvandi et al., 2009). There are very few reports which can confirm the clonal fidelity of bamboo plantlets derived from axillary bud proliferation. The scarcity of reports on ascertaining the genetic fidelity of tissue culture raised plantlets can jeopardise the quality of micropropogated plants, especially in perennials like bamboo where any undesirable variant would last for several years (Negi and Saxena, 2010). Therefore, it is pertinent to screen the regenerants at regular intervals for the occurrence of any somaclonal variation.

Table 2. The inter-simple sequence repeat (ISSR) primers utilized to verify *Guadua* clones.

S/N	Primers	5'-3' motif	Annealing Temperature (°C)	No. of scorable bands	Range of amplification (bp)
1	UBC 807	AGAGAGAGAGAGAGAGT	37.5	6	300-700
2	UBC 808	AGAGAGAGAGAGAGAGC	41.8	7	750-1600
3	UBC 810	GAGAGAGAGAGAGAGAT	37.9	5	700-2500
4	UBC 811	GAGAGAGAGAGAGAGAC	38.3	4	750-2000
5	UBC 812	GAGAGAGAGAGAGAGAA	39.3	4	700-2000
6	UBC 815	CTCTCTCTCTCTCTCTG	39.9	1	1500
7	UBC 818	CACACACACACACACAG	47.1	3	1400-2500
8	UBC 830	TGTGTGTGTGTGTGTGG	51.1	2	800-1500
9	UBC 834	AGAGAGAGAGAGAGAGYT	40.4	2	800-1100
10	UBC 835	AGAGAGAGAGAGAGAGC	41.8	4	850-1500
11	UBC 840	GAGAGAGAGAGAGAGAYT	40.8	3	1100-1500
12	UBC 841	GAGAGAGAGAGAGAGAYC	41	6	700-1700
13	UBC 844	CTCTCTCTCTCTCTCTRC	41.5	1	1400
14	UBC 848	CACACACACACACACARG	50.5	2	900-1450
15	UBC 850	GTGTGTGTGTGTGTGTYC	48	1	1100
16	UBC 873	GACAGACAGACAGACA	40	6	700-2500
17	UBC 888	BDBCACACACACACACA	47.3	4	1000-1600

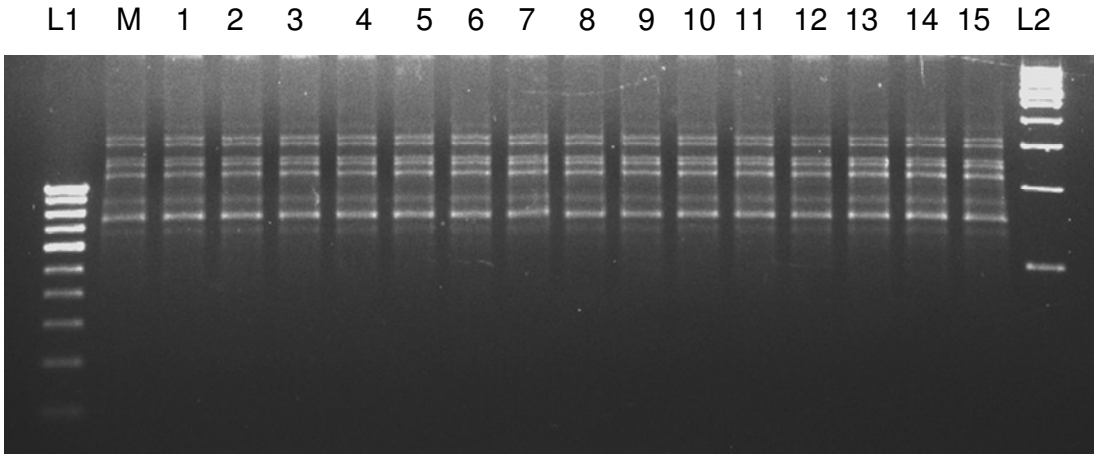

Figure 2. ISSR products generated from 15 *in vitro* regenerated plants and mother plants of *Guadua angustifolia* amplified with primer UBC 808 showing monomorphic pattern. Lane L1 represents 100-bp ladder, Lane M represents mother plant, Lane 1 to 15 represent *in vitro* raised clones of *G. angustifolia* and Lane L2 represents 500-bp ladder.

Earlier, Das and Pal (2005) established the clonal fidelity of regenerants of *Bambusa tulda* and *Bambusa balcooa* using only four markers to assess their genetic uniformity among the regenerants. Later, Negi and Saxena (2010) employed 15 ISSR markers to validate the clonal fidelity of *in vitro* raised *B. balcooa* plantlets through the axillary bud proliferation. However, there is no report available on the comparative genetic stability of regenerants and mother plant of *G. angustifolia* by using RAPD and ISSR markers. In the present study, we did not find any polymorphism during the RAPD analysis of *in vitro* raised clones (Figure 1). This is consistent with the absence of genetic variations observed during micropropagation of *Pinus thunbergii* (Goto et al., 1998), turmeric (Salvi et al., 2001), Lilium (Varshney et al., 2001), *B. balcooa* and *B. tulda* (Das and Pal, 2005), *Dendrocalamus hamiltonii* (Agnihotri et al., 2009) and Gerbera (Bhatia et al., 2011), analyzed using RAPD markers. We screened 27 ISSR primers, however only 17 ISSR primers produced 61 distinct and scorable bands in the size range of 300 to

2500 bp. In addition, the banding profiles from micropropagated plants were monomorphic and similar to those of mother plant (Figure 2). Similar results have been reported in almond (Martin et al., 2004), banana (Lakshmanan et al., 2007), *Swertia chirayita* (Joshi and Dhawan, 2007), *Crataeva magna* (Bopana and Saxena, 2009), *B. balcooa* (Negi and Saxena, 2010), *Bambusa nutans* (Negi and Saxena, 2011), gerbera (Bhatia et al., 2011). Moreover, the common practice to use AFLP markers to identify clonal fidelity of plants (Mehta et al., 2010) requires state-of-art set up and expensive reagents. In our experience, AFLP method requires 10 times more investment for instruments and 5 times more disbursement for reagents compared to RAPD or ISSR method. Thus, the RAPD and ISSR primers allow simple and cost-effective method to (1) test clonal fidelity and (2) amplify different regions of genomes. This will provide us with better chances to identify the genetic variations in *in vitro* raised *Guadua* clones.

Conclusion

In summary, we have confirmed the true nature of the *in vitro* raised clones of *G. angustifolia* Kunth using DNA based RAPD and ISSR markers as we did not detect any variability in the tissue culture raised plantlets. Thus, this will allow us to employ the axillary bud proliferation method for the commercial multiplication of *Guadua* without any risk of genetic instability.

ACKNOWLEDGEMENTS

The authors are grateful to the Council of Scientific and Industrial Research, New Delhi, for providing the Senior Research Fellowship (to Harleen Kaur Nadha) and also to Dr. PS Ahuja, Director, CSIR- IHBT, Palampur for providing all the facilities used for carrying out this research work.

REFERENCES

Agnihotri RK, Mishra J, Nandi SK (2009). Improved *in vitro* shoot multiplication and rooting of *D. hamiltonii* Nees et Arn. Ex Munro:production of genetically uniform plants and field evaluation. Acta Physiol. Plant, 31: 961-967.

Barrett C, Lefort F, Douglas GC (1997). Genetic characterization of oak seedlings, epicormic, crown and micropropagated shoots from mature trees by RAPD and microsatellite PCR. Scientia Horti., 70: 319-330.

Bhatia R, Singh KP, Sharma TR, Jhang T (2011). Evaluation of the genetic fidelity of *in vitro* propagated gerbera (*Gerbera jamesonii* Bolus) using DNA-based markers. Plant Cell Tissue Organ Cult., 104: 131-135.

Bindiya K, Kanwar K (2003). Random amplified polymorphic DNA (RAPDs) markers for genetic analysis in micropropagated plants of *Robinia pseudoacacia* L. Euphytica, 132: 41-47.

Bogani P, Simoni A, Lio P, Salpi A, Buiatti M (1996). Genome flux in tomato cell clones cultured *in vitro* in different physiological equilibria.II. A RAPD analysis of variability. Genome, 39: 846-853.

Bopana N, Saxena S (2009). *In vitro* regeneration of clonally uniform plants of *Crataeva magna*: A high value medicinal tree by axillary branching method. New For., 38(1): 53-65.

Chandrika M, Thoyajaksha, Rai VR, Ramachandra K (2008). Assessment of genetic stability of *in vitro* grown *Dictyospermum ovalifolium*. Biol. Plant, 52(4): 735-739.

Chandrika M, Rai VR (2009). Genetic fidelity in micropropagated plantlets of *Ochreinauclea missionis* an endemic, threatened and medicinal tree using ISSR markers. African J. Biotechnol. 8(13): 2933-2938.

Eshraghi P, Zarghami R, Ofoghi H (2005). Genetic stability of micropropagated plantlets in Date Palm. J. Sci., 16(4): 311-315.

Feyissa T, Welander M, Negash L (2007). Genetic stability, *ex vitro* rooting and gene expression studies in *Hagenia abyssinica*. Biol. Plant, 51(1): 15-21.

Gupta PK, Varshney RK (1999). Molecular markers for genetic fidelity during micropropagtion and germplasm conservation. Curr. Sci., 76: 1308-1310.

Goto S, Thakur RC, Ishii K (1998). Detemination of genetic stability in long-tem micropropagated shoots of *Pinus thunbegii* Parl. Using RAPD markers. Plant Cell Rep., 18: 193-197.

Hirochika H, Sugimoto K, Otsuki Y, Tsugawa H, Kanda M (1996). Retrotransposon of rice involved in mutations induced by tissue culture. Proc. Natl. Acad. Sci., 97: 7783-7788.

Isabel NL, Tremblay M, Michaud F, Tremblay M, Bousquet J (1993). RAPDs as an aid to evaluate the genetic integrity of somatic embryogenesis derived population of *Picea mariana* (Mill.) B.S.P. Theor. Appl. Genet., 86: 81-87.

Jaligot E, Rival A, Beule T, Dussert S, Verdeil J (2000). Somaclonal variation in oil palm (*Elaeis guineensis Jacq*). The DNA methylation hypothesis. Plant Cell Rep., 19: 684-690.

Joshi P, Dhawan V (2007). Assessment of genetic fidelity of micropropagated *Swertia chirayita* plantlets by ISSR marker assay. Biol. Plant, 51(1): 22-26.

Kaeppler SM, Kaeppler HF, Rhee Y (2000). Epigenetic aspects of somaclonal variation in plants. Plant Mol. Biol., 43: 179-188.

Keyte AL, Percifield R, Liu B, Wendel JF (2006). Intraspecific DNA methylation polymorphism in cotton (*Gossypium hirsutum*). J. Heredity, 97: 444-450.

Lakshmanan V, Venkataramareddy SR, Neelwarne B (2007). Molecular analysis of genetic stability in long-term micropropagated shoots of banana using RAPD and ISSR markers. Electron. J. Biotech., 10: 1-8.

Larkin PJ, Scowcroft W (1981). Somaclonal variation- a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet., 60: 197-214.

Leroy XJ, Leon K, Charles G, Branchard M (2000). Cauliflower somatic embryogenesis and analysis of regenerant stability by ISSRs. Plant Cell Rep., 19(11): 1102-1107.

Linacero R, Freitas-Alves E, Vasquez AM (2000). Hot spots of DNA instability revealed through the study of somaclonal variations in rye. Theor. Appl. Genet., 100: 506-511.

Lukens LN, Zhan S (2007). The plant genome's methylation status and response to stress. Implication for plant improvement. Curr. Opinion. Plant. Biol., 10: 317-322.

Martin M, Sarmento D, Oliveira MM (2004). Genetic stability of micropropagated almond plantlets, as assessed by RAPD and ISSR markers. Plant Cell Rep., 23: 492-496.

Mehta R, Sharma V, Sood A, Sharma M, Sharma RK (2010). Induction of somatic embryogenesis and analysis of genetic fidelity of *in vitro*-derived plantlets of *B. nutans* Wall, using AFLP markers. Eur. J. For. Res., (in press).

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassaya with tobacco tissue cultures. Physiol. Plant, 15: 473-497.

Negi D, Saxena S (2010). Ascertaining clonal fidelity of tissue culture raised plants of *Bambusa balcooa* Roxb. Using inter simple sequence repeat markers. New For., 40: 1-8.

Negi D, Saxena S (2011). *In vitro* propagation of *B. nutans* Wall. exMunro through axillary shoot proliferation. Plant Biotechnol. Rep.,

5: 35-43.
Peyvandi M, Noormohammadi Z, Banihashemi O, Farahani F, Majd A, Hosseini-Mazinani M, Sheidai M (2009). Molecular analysis of genetic stability in long-term micropropagated shoots of *Olea euroaea* L. (ev. Dezful). Asian J. Plant Sci., 8: 146-152.
Phillips RL, Kaeppler SM, Olhoft P (1994). Genetic instability of plant tissue cultures: Breakdown of normal controls. Proc. Nat. Acad. Sci. USA, 91: 5222-5226.
Rahman MH, Rajora OP (2001). Microsatellite DNA somaclonal variation in micropropagated trembling aspen (*Populus tremuloides*). Plant Cell Rep., 20: 531-536.
Rani V, Parida A, Raina SN (1995). Random amplified polymorphic DNA (RAPD) markers for genetic analysis in micropropagated plants of *Populus deltoides* Marsh. Plant Cell Rep., 14: 459-462.
Salvi ND, George L, Eapen S (2001). Plant regeneration from leaf base callus of turmeric and random amplified polymorphic DNA analysis of regenerated plants. Plant Cell Tiss. Organ Cult., 66: 113-119.
Sharma RK, Gupta P, Sharma V, Sood A, Mohapatra T, Ahuja PS (2008). Evaluation of rice and sugarcane SSR markers for phylogenetic and genetic diversity analysis in bamboo. Genome, 51: 91-103.
Sharma V, Bhardwaj P, Kumar R, Sharma RK, Sood A, Ahuja PS (2008). Identification and cross-species amplification of EST derived SSR markers in different bamboo species. Conserv. Genet., 10: 721-724.
Silvarolla MB (1992). Plant genomic alterations due to tissue culture. J. Br. Assoc. Adv. Sci., 44: 329-335.
Shenoy VB, Vasil IK (1992). Biochemical and molecular analysis of plants derived from embryogenic tissue cultures of napier grass (*Pannisetum purpureum* K. Schum). Theor. Appl. Genet., 83: 947-955.
Srivastava S, Gupta PS (2008). Inter simple sequence repeat profile as a genetic marker system in sugarcane. Sugar Tech., 10(1): 48-52.
Varshney A, Lakshmikumaran M, Shrivastava PS, Dhawan V (2001). Establishment of genetic fidelity of *in vitro*-raised Lilium Bulblets through RAPD markers. *In vitro* Cell Dev. Biol. Plant, 227: 23-32.