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Indian Forester, 142 (6) : 558-562, 2016
http://www.indianforester.co.in

ISSN No. 0019-4816 (Print)
ISSN No. 2321-094X (Online)

GENETIC FIDELITY TESTING IN MICROPROPAGATED PLANTLETS OF *ALBIZIA PROCERA* (ROXB.) USING RAPD AND ISSR MARKERS

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ABSTRACT

Random amplified polymorphic DNA (RAPD) and Inter simple sequence repeat (ISSR) markers were employed to check the genetic fidelity of *Albizia procera* plantlets multiplied *in vitro* by using nodal explant. A total of 33 distinct and scorable bands were produced by five RAPD primers with an average of 6.6 bands per primer while the number of bands for ISSR primers varied from two (UBC-840) to six (UBC-812), with an average of four bands per primer. All bands were monomorphic across all the micropropagated plants studied. Amplification of monomorphic bands with both marker systems authenticated the true to type nature of the *in vitro* raised plantlets of *Albizia procera.* These results suggest that the micropropagation protocol followed in this study is appropriate and applicable for mass clonal propagation of true-to-type superior *Albizia procera* trees.

Key words: Molecular markers, Genetic fidelity, Somaclonal variation, *Albizia procera.*

Introduction

In vitro propagation of forest trees hold an attractive promise for obtaining the mass multiplication of elite genotypes due to high multiplication rates obtained. Cloning of mature trees is generally preferred over seedling because it is often not possible to determine whether these seedlings have the genetic potential to develop the desired qualities later in their life cycle (Nanda *et al.,* 2004). Since the propagation is carried out under aseptic conditions, regenerants are free of diseases. However, for use of tissue culture as continuous source of planting material for commercial utilization, periodic monitoring of the degree of genetic stability among *in vitro* grown plantlets is of outmost importance. When plant tissue is passaged through *in vitro* culture many of the regenerated plantlets appear to be no longer clonal copies of their donor genotype, probably due to somaclonal variations. *Somaclonal* variations occur mostly in response to the stresses imposed on the plant under *in vitro* conditions (Venkatachalam *et al.,* 2007, Peschke and Phillips, 1992) and are manifested in the form of DNA methylations, chromosome rearrangements and point mutations (Phillips *et al.,* 1994), alteration of cell's ability to repair damaged and mutated DNA (Leroy *et al.,* 2000). *Somaclonal* variation is of special relevance in perennial long-generation forest trees since occasional mutations can sometime only be noticed at very late developmental stages, or even in their offsprings. The occurrence of somaclonal variation is a potential drawback when the propagation of an elite tree is intended, where clonal fidelity is required to maintain the advantages of desired elite genotypes (e.g. superior growth, wood properties, disease resistance, and other quality traits). Dunstan and Thorpe (1986) rightly cautioned that commercial application of tissue culture to perennial crops must await adequate quality checks and field testing with proper controls.

Several techniques have been developed to assess the genetic purity of tissue culture raised plants such as morphological descriptions, physiological traits, cytological studies, isozymes (Gupta and Varshney, 1999; Devarumath *et al*., 2007; Agnihotri *et al.,* 2009; Singh *et al.,* 2012a, 2012b). However, these morphological and physiological traits require extensive observations until maturity and are highly sensitive to environmental fluctuations (Singh *et al.,* 2013). In addition to this initially uniform looking plants may behave differently during flowering/fruiting stages due to genetic aberrations. Also, some changes induced during *in vitro* culture may not be apparent under *ex vitro* conditions (Palombi and Damiano, 2002). Therefore, more efficient detection tools like DNA markers must be used to ascertain the genetic fidelity of *in vitro* raised plants. The inherent characteristics of molecular markers such as abundantness, insensitivity to environmental conditions render them much more useful than morphological and physiological traits in establishing the identity of

Production of monomorphic bands by RAPD and ISSR markers confers the genetic uniformity of micro propagated plantlets.

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particular tree/clone or testing the genetic purity or tracing its genetic relationship. Several DNA markers have been successfully employed to assess the genomic stability in regenerated plants. Among these, inter simple sequence repeat (ISSR) (Zietkiewicz *et al.,* 1994) and random amplified polymorphic DNA (RAPD) (Williams *et al.,* 1990) have been mostly favored.

The present study was undertaken to assess the genetic fidelity of *in vitro* raised plantlets of *Albizia procera*, a multipurpose forest tree speciessss using a combination of RAPD and ISSR markers.

Material and Methods

Plant material and culture conditions

The plant material consisted of nine *in vitro* raised plantlets and one mother plant. These plantlets were regenerated clonally via enhanced axillary bud culture of nodal explants collected from a mature tree of *Albizia procera* having aged about 24 years. The micropropagation procedure consisted of culturing the nodal explants on MS medium supplemented with 5µM BAP and 1µM IAA, rooting of shoots on B5 medium supplemented with 3µM IBA and finally hardening and acclimatization of *in vitro* raised plants in the green house (Rai, 2013).

DNA extraction and PCR amplification conditions

Total DNA was extracted from leaf material of 09 micropropagated plants and a mother plant (control), following the method described by Deshmukh *et al.* (2007). Purified total DNA was quantified and its quality was verified by UV spectrophotometer and each sample was diluted to 40 ng/ 3 l with TE buffer and stored at 4 °C.

RAPD amplification was performed in a volume of 25 l containing 40 ng of genomic DNA, 0.2 M primer (Integrated DNA Technologies, USA), 2 mM $MgCl_2$ and 0.5 unit of Taq polymerase. The other assey condition included 50 mM KCl, 10Mm Tris HCl and 100 uM dNTPs. The amplification reaction consisted of an initial denaturation step at 94°C for 5 min, 45 cycles comprising denaturation at 94°C for 1 min, annealing at 38°C for 1 min and extension at 72°C for 2 min and a final extension step at 72°C for 5 min.

ISSR amplifications were performed in a volume of 10 l containing 40 ng of genomic DNA, 1X Taq polymerase buffer, 0.1 mM of each dNTPs, 2.5 mM $MgCl_2$, 1U Taq polymerase and 0.8 M of ISSR primer (Table 2). The amplification reaction consisted of an initial denaturation step at 94°C for 3 min, followed by 35 cycles of 30 seconds at 94°C (denaturation), 30 seconds at a specific annealing temperatures (Table 2) and 1 min at 72°C (extension) followed by a final extension step at 72°C for 10 min.

Amplifications were performed in a Thermocycler (Bio-Rad, Germany) for both RAPD and ISSR. DNA amplification fragments were separated in 1.8 % agarose gel using 1× TBE buffer and stained with ethidium bromide. Gels were visualized using a gel documentation system (Alfa Innotech, USA). The scoring of bands was done on the basis of their presence ('1') or absence ('0') in the gel and missing data was denoted by '9'. The genetic associations were evaluated by calculating the Jaccard's similarity coefficient for pair-wise comparisons based on the proportion of shared bands produced by the primers using NTSYS-pc version 2.1 software (Rohlf, 2000).

Table 1 : Random amplified polymorphic DNA (RAPD) primers used for testing the genetic fidelity of *Albizia procera* plantlets through tissue culture

Sr. no	Primer code	Primer sequence (5'-3')	Melting temperature (Tm) °C	Number of amplified/ monomorphic bands	Size range amplified products (bp)
1	OPAW-01	5'- ACC TAG GGG A -3'	32.5	6/6	400-3000
2	OPAW-09	5'- ACT GGG TCG G -3'	38.5	8/8	300-3000
3	OPP-01	5'- GTA GCA CTC C -3'	30.8	6/6	300-3000
4	OPP-02	5'- TCG GCA CGC A -3'	44.5	5/5	300-2000
5	OPY-10	5'- CAA ACG TGG G -3'	33.7	8/8	300-1500

Table 2 : Inter-simple sequence repeats (ISSR) primers used for testing the genetic fidelity of *Albizia procera* plantlets through tissue culture

Sr. no.	Primer code	Primer sequence (5'-3')	Melting temperature (Tm) °C	Number of amplified/ Monomorphic bands	Size range amplified products (bp)
1	UBC-810	5'-GAG AGA GAG AGA GAG AT-3'	45.4	5/5	300-2000
2	UBC-812	5'-GAG AGA GAG AGA GAG AA-3'	45.7	6/6	300-2500
3	UBC-830	5'-TGT GTG TGT GTG TGT GG -3'	52.7	3/3	500-1000
4	UBC-840	5'-GAG AGA GAG AGA GAG AAT-3'	46.3	2/2	500-1000
5	UBC-859	5'-TGT GTG TGT GTG TGT GGC -3'	56.1	4/4	300-1000

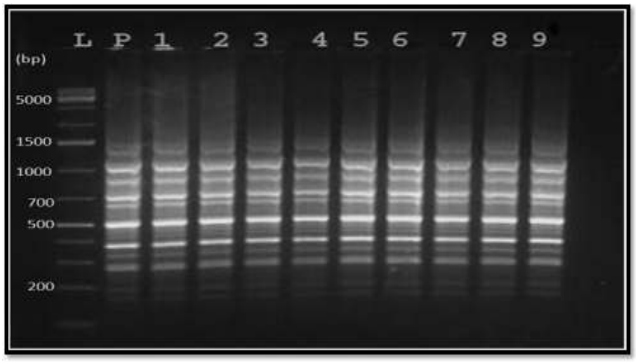

Fig. 1 : DNA Amplification obtained with primer: RAPD (OPY-10), Lanes L-100 bp plus DNA ladder, P- mother plant, 1–09 in vitro raised plantlets of *A. procera*

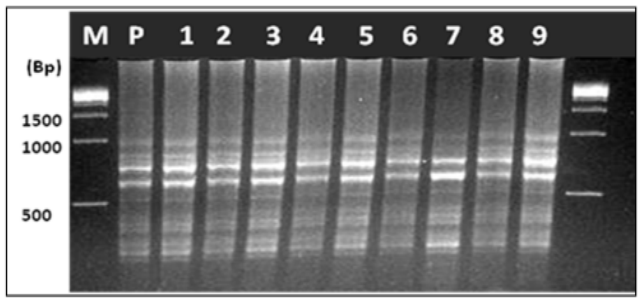

Fig. 2 : DNA Amplification obtained with primer: ISSR (UBC-840), Lanes M-500 bp DNA ladder, P- mother plant, 1–09 in vitro raised plantlets of *A. procera*

Results and Discussion

Micropropagation protocol for *Albizia procera* standardized by Rai (2013) was used for *in vitro* propagation of *A. procera* plantlets using nodal explants. There are many published research papers reporting about the tissue culture induced genetic variations (Larkin and Scowcroft, 1981; Earle and Demarly, 1982). In most cases these somaclonal variations were in undesirable direction. Mutated plants may even have lost the desired character that formed the basis of selection of the elite genotype, thereby resulting in considerable economic losses. Therefore testing of genetic fidelity becomes very much essential especially in forest trees having long rotation cycles. Many approaches ranging from morpho-metric-physiologic to biochemical features were tried to assess the genetic fidelity of the tissue culture raised plants. However, these traits are found not reliable as it got affected by the environment and expression is stage dependent.

DNA-based molecular markers have emerged as a powerful technique for the purpose and therefore are being used in many crops and trees (Cuesta *et al.,* 2010; Negi and Saxena, 2011; Pandey *et al.,* 2012). Use of more than one marker system has been suggested for better analysis of genetic stability of plants, as they will target different regions of the genome (Palombi and Damiano 2002; Lakshmanan *et al*., 2007). Therefore, two marker systems were employed for the present investigation.

The amplification profiles of the *in vitro* raised plantlets and the mother plant generated using the above markers are shown in Fig. 1 and 2 and their scoring data is presented in Tables 1 and 2. Five random decamer oligonucleotides were used as single primers for the amplification of RAPD fragments. Total 33 bands were amplified out of which all were monomorphic (Table 1). Similarly, five ISSR primers used for the genetic fidelity testing amplified 20 scorable bands and all were monomorphic, indicating the genetic uniformity of the *in vitro* raised plantlets. No polymorphic bands were observed. The number of bands for each primer varied from five to eight, with an average of 6.6 bands per RAPD primer. Whereas for ISSR primers, it ranged from three to six bands with an average of 4.0 bands per primer. These large numbers of amplicons being produced by two different markers are reasonably adequate for detecting somaclonal variations.

Figure 1 and 2 provides the representative example of monomorphic bands obtained with RAPD and ISSR primers. More fragments were amplified by RAPD primers compared to ISSR, which is in agreement to the results of Cuesta *et al*. (2010) wherein ISSR markers amplified fewer bands compared to RAPD markers. Devarumath *et al*. (2002) also observed that ISSR detected more polymorphic loci (12.8%) than RAPD fingerprinting (4.28%) in micropropagated tea clones. However, Singh *et al.* (2013) reported fewer RAPD

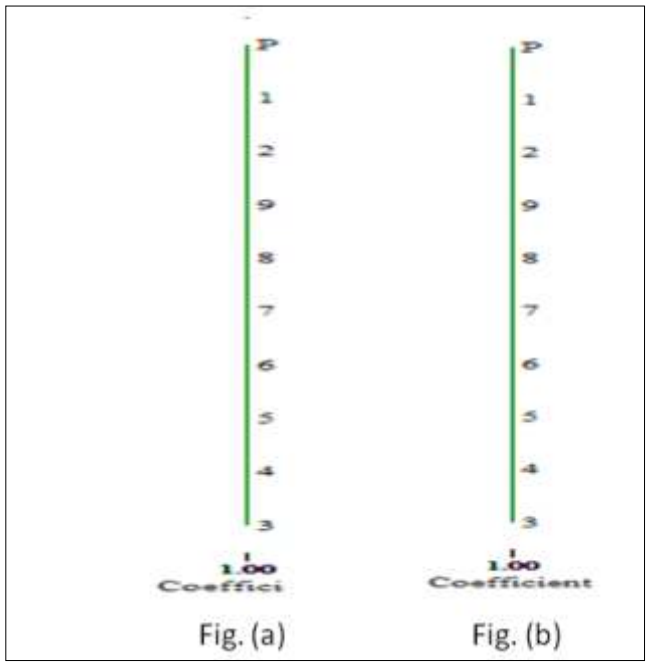

Fig. 3 : Dendrogramme based on the jaccards similarity matrix generated using (a) RAPD and (b) ISSR primers

amplified bands compared to ISSR while evaluating the genetic fidelity in bamboo species.

The scoring data of well-resolved bands of RAPD and ISSR, markers was subjected to calculation of similarity matrix based on Jaccard's similarity coefficient (1908). Dendrogram was constructed using the Jaccard's similarity matrices (Fig. 3). The pair-wise value of the *in vitro* raised plantlets and the mother plant was 1, indicating 100 % similarity. This confirmed the true-to-type nature of the *in vitro* raised plantlets. This establishes the usefulness of these marker systems in ascertaining the genetic purity of clones. Earlier also RAPD and ISSR markers were successfully employed in bamboo species (Agnihotri *et al.,* 2009; Singh *et al.,* 2013), apple (Gupta *et al*., 2009), Jalamdasa (Chandrika and Rai, 2009), *Acacia mangium* (Nanda *et al*., 2004), *Stevia rebaudiana* (Lata *et al*., 2013) and many more. The use of two types of markers, which amplify different regions of the genome, allows better chances for identification of genetic variation in the tissue culture raised plants. Hence, we employed both marker systems for the investigation.

Conclusions

Production of monomorphic bands by *in vitro* raised plantlets and mother plant of *A. procera* against RAPD and ISSR markers confirmed the genetic uniformity of micropropagated plantlets studied. Since no variations were observed in the banding patterns in micropropagated plants as compared to the mother plant, we conclude that, micropropagation protocol developed by Rai (2013) through nodal explant can be effectively used without risk of genetic instability in *A. procera.*

Acknowledgement

The authors are grateful to Dr. U. Prakasham, Director, Tropical Forest Research Institute, Jabalpur for providing necessary facilities. Authors are also thankful to the Madhya Pradesh Council of Science and Technology, Bhopal for providing the financial assistance for the investigation under the project 141/TFRI/2010/Gen-1(MPCST) (18).

vkj , ih Mh vkj vkbZ ,l ,l vkj fpgudk dk mi;kx djds *एल्बिजिया प्रोसेरा* (jkDlc) dh l{eiof/r iknfidkvk e vkuof'kd lR;rk dh tkp

u'khj ekgEen] food o".ko] t;idk'k feJk] 'k'kkd eg'k] iekn dekj vkj 'kehe v[rj vlkjh

lkjk'k

xfUFky dUrkrd dk mi;kx djd ik=k lof/r *एल्बिजिया प्रोसेरा* iknfidkvk dh vkuof'kd lR;rk dh tkp dju d fy, crjrhc ifjof/r cg:ih Mh ,u , (vkj , ih Mh) vkj vUr% lk/kj.k vuØe vkofRr (vkbZ ,l ,l vkj) fpgudk dk i;Dr fd;kA ifr ikbej 6-6 c.Mk d ,d vkSlr d lkFk ikp vkj , ih Mh ikbejk }kjk dy 33 fof'k"V vkj Ldkjcy c.Mk dk mRifnr fd;k x;k tcfd ifr ikbej pkj c.Mk d ,d vkSlr d lkFk vkbZ ,l ,l vkj ikbejk d fy, c.Mk dh l[;k nk (;chlh&840) l N% (;chlh&812) rd FkhA vè;;u fd, x, lHkh l{e iof/r iknik e lHkh c.M ,d:ih FkA nkuk fpgud i.kkfy;k d lkFk ,d:ih c.Mk d ifjo/u u *एल्बिजिया प्रोसेरा* dh ik=k mxh iknfidkvk dh [kjh iÑfr dk vf/ekf.kr fd;kA ; ifj.kke l>ko nr g fd bl vè;;u e viuk, x, l{e io/u ikVkdky mi;Dr g vkj [kj mRÑ"V *एल्बिजिया प्रोसेरा* o{kk d cgek=k Dykuh; io/u d fy, 0;ogk; gA

References

Agnihotri R.K., Mishra J. and Nandi S.K. (2009). Improved in vitro shoot multiplication and rooting of *Dendrocalamus hamiltonii* Nees et Arn. ex Munro: production of genetically uniform plants and field evaluation. *Acta Physiol Plant,* 31:961–967

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

Cuesta C., Ordas R.J., Rodriguez A. and Fernandez B. (2010). PCR-based molecular markers for assessment of somaclonal variation in *Pinus pinea* clones micropropagated *in vitro*. *Biol. Pl,* 54: 435–442.

Deshmukh V.P., Thakare P.V., Chaudhari U.S. and Gawande P.A. (2007). A simple method for isolation of genomic DNA from fresh and dry leaves of *Terminalia arjuna* (Roxb.) Wight and Arnot. *Electronic J. Biotechnology*, 10(3): 468-472.

Devarumath R.M., Nandy S., Rani V., Marimuthu S., Muraleedharan N. and Raina S.N. (2002). RAPD, ISSR and AFLP fingerprints as useful markers to evaluate genetic integrity of micropropagated plants of three diploid and triploid elite tea clones representing *Camellia sinensis* (China type) and *C. assamica* spp assamica (Assam-India type). *Plant Cell Rep.*, 21: 166-173.

Dunstan D.I. and Thorpe T.A. (1986). Regeneration in forest trees. *In*: Cell culture and somatic cell genetics of plants, volume 3 (IK Vasil ed), Academic Press, Orlando, pp 223–241.

Earle E.D. and Demarly Y. (1982). Variability in plants regenerated from tissue culture. Praeger, New York: 1-392pp.

Gupta P.K. and Varshney R.K. (1999). Molecular markers for genetic fidelity during micropropagation and germplasm conservation. *Current Science*, 76: 1308-1310.

Gupta R., Modgil M. and Chakrabarti S.K. (2009). Assessment of genetic fidelity of micropropagated apple rootstock plants, EMLA-111 using RAPD markers. *Indian J. Experimental Biology*, 47: 925-928

Jaccard P. (1908). Nouvelles recherches sur la distribution florale.- *Bulletin Societe Vaudoise des Sciences Naturelles*, 44: 223-270.

Lakshmanan V., Venkataramareddy S.R. and Neelwarne B. (2007). Molecular analysis of genetic stability in long-term micropropagated shoots of banana using RAPD and ISSR markers. *Electron J. Biotechnology,* 10:106–113.

Larkin P.J. and Scowcroft W.R. (1982). Somaclonal Variation- A Novel Source of Variability from Cell Cultures for Plant Improvement. *Theoretical and Applied Genetics*, 60 (4): 197-214.

Lata H., Chandra S., Techen N., Wang Y.H. and Khan I.A. (2013). Molecular Analysis of Genetic Fidelity in Micropropagated Plants of *Stevia rebaudiana* Bert. using ISSR Marker. *American J. Plant Sciences*, 4 (5): 964-971.

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

Nanda R.M., Premananda D.P. and Rout G.R. (2004). *In vitro* clonal propagation of *Acacia mangium* Willd. and its evaluation of genetic stability through RAPD marker. *Annals of Forest Science*, 61(4): 381-386.

Negi D. and Saxena S. (2011). *In vitro* propagation of *B. nutans* Wall. Ex Munro through axillary shoot proliferation. *Plant Biotechnol Rep*., 5: 35–43.

Palombi M.A. and Damiano C. (2002). Comparison between RAPD and SSR molecular markers in detecting genetic variation in kiwifruit (*Actinidia deliciosa* A. Chev.). *Plant Cell Rep.,* 20: 1061–1066

Pandey R.N., Singh S.P., Rastogi J., Sharma M.L. and Singh R.K. (2012). Early assessment of genetic fidelity in sugarcane (*Saccharum officinarum*) plantlets regenerated through direct organogenesis with RAPD and SSR markers. *Aust J. Crop Sci.,* 6: 618–624.

Peschke V.M. and Phillips R.L. (1992). Genetic implications of somaclonal variation in plants. *Adv. Genet.,* 30: 41–75.

Phillips R.L., Kaeppler S.M. and Olhoft P. (1994). Genetic stability of plant tissue cultures: breakdown of normal controls. *Proc. Natl. Acad. Sci. USA,* 91: 5222–5226.

Rai E. (2013). Studies on development of *in vitro* regeneration system for *Albizia procera* (Roxb.) Benth. Thesis, Ph.D. Forest Research Institute Deemed University, Dehradun.

Rohlf F.J. (2000). NTSYS-pc: numerical taxonomy and multivariate analysis system. Version 2.0 Exeter Software, Setauket, New York, USA

Singh S.R., Dalal S., Singh R., Dhawan A.K. and Kalia R.K. (2013). Evaluation of genetic fidelity of in vitro raised plants of *Dendrocalamus asper* (Schult. & Schult. F.) Backer ex K. Heyne using DNA-based markers. *Acta. Physiol. Plant*, 35:419-430.

Singh S.R., Dalal S., Singh R., Dhawan A.K. and Kalia R.K. (2012a). Micropropagation of *Dendrocalamus asper* {Schult. & Schult. F.}Backer ex K. Heyne): an exotic edible bamboo. *J. Plant Biochem. Biotechnol.,* 21: 220–228.

Singh S.R., Dalal S., Singh R., Dhawan A.K. and Kalia R.K. (2012b). Seasonal influences on in vitro bud break in *Dendrocalamus hamiltonii* Arn. ex Munro nodal explants and effect of culture microenvironment on large scale shoot multiplication and plantlet regeneration. *Indian J. Plant Physiol.,* 17: 9–21.

Venkatachalam L., Sreedhar R.V. and Bhagyalakshmi N. (2007). Micropropagation in banana using high levels of cytokinins does not involve any genetic changes as revealed by RAPD and ISSR markers. *Plant Growth Regul.,* 51: 193–205.

Williams J.G.K., Kubelik A.R., Livak K.J., Rafalski J.A. and Tingey S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*., 18: 6531-6535.

Zietkiewicz E., Rafalski A. and Labuda D. (1994). Genome Fingerprinting by Simple Sequence Repeat (SSR)-Anchored Polymerase Chain Reaction Amplification. *Genomics*, 20 (2): 176-183.