

Micropropagation and assessment of genetic fidelity of *Dendrocalamus strictus* (Roxb.) nees using RAPD and ISSR markers

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Abstract *Dendrocalamus strictus* popularly known as ‘Male bamboo’ is a multipurpose bamboo which is extensively utilized in pharmaceutical, paper, agricultural and other industrial implements. In this study, in vitro regeneration of *D. strictus* through nodal culture has been attempted. Murashige and Skoog’s medium supplemented with 4 mg/l BAP was found to be most effective in shoot regeneration with 3.68 ± 0.37 shoots per explant. The effect of Kn was found to be moderate. These hormones also had considerable effect on the shoot length. The highest shoot length after 6 weeks (3.11 ± 0.41 cm) was noted with 5 mg/l BAP followed by 3.07 ± 0.28 cm with 5 mg/l Kn, while decrease in the shoot length was noted with other treatments. The effect of IBA and NAA individually or in combination at different concentrations on rooting was evaluated. The highest number of root (1.36 ± 0.04) was regenerated on full-strength MS medium supplemented with 3 mg/l NAA, while maximum length of 1.64 ± 0.03 cm of roots was recorded with combination of 1 mg/l IBA and 3 mg/l NAA. Tissue-cultured plants thus obtained were successfully transferred to the soil. The clonal fidelity among the in vitro-regenerated plantlets was assessed by RAPD and ISSR markers. The

ten RAPD decamers produced 58 amplicons, while nine ISSR primers generated a total of 66 bands. All the bands generated were monomorphic. These results confirmed the clonal fidelity of the tissue culture-raised *D. strictus* plantlets and corroborated the fact that nodal culture is perhaps the safest mode for multiplication of true to type plants.

Keywords *Dendrocalamus strictus* · Micropropagation · RAPD · ISSR · Clonal fidelity · Somaclonal variation

Abbreviations

BAP	6-Benzyl amino purine
Kn	Kinetin
NAA	Naphthalene acetic acid
RAPD	Random amplified polymorphic DNA
ISSR	Inter-simple sequence repeats
MS	Murashige and Skoog medium
IBA	Indole-3-butyric acid

Introduction

Popularly known as ‘Male bamboo’, *Dendrocalamus strictus* (Roxb.) Nees belongs to the subfamily Bambusoideae of the family Poaceae. It is deciduous in nature and is one of the most important and commonly found bamboo species, native to India (Saxena and Dhawan 1999). The culms attain a height of 18.5 m and thickness of 12.7 cm and are densely tufted and often solid at the basal portion (Goyal et al. 2010a). Traditionally, *D. strictus* has both medicinal and industrial uses. The pulp is extensively used in paper industries. The culms are

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used for making shaft, walking sticks, axe handle apart from other agricultural and industrial implements (Reddy 2006). Because of the high nutritive values, the young shoots are consumed in different part of North-east India (Goyal et al. 2010a). The leaf decoction of *D. strictus* is used as abortifacient (Sharma and Borthakur 2008) and in combination with *Curcuma longa* powder it is used to treat cold, cough and fever (Kamble et al. 2010). In addition, leaf powder is a rich source of natural antioxidants (Goyal et al. 2011) and also possesses cut and wound healing property (Mohapatra et al. 2008). Though this plant is versatile having multifarious uses, but its production is quiet less compared to that required to meet the global demand. The main problem that hinders the propagation is the erratic flowering behavior (30–45 years), poor seed sets with very short viability period. These along with various other factors limit the production of this species using conventional means (Saxena and Dhawan 1999). Thus, tissue culture offers reliable and efficient alternative for mass production to meet the global demand. In the recent years, in vitro culture methods have been widely used as a tool for mass multiplication and germplasm conservation of bamboo like other plants. The main aim of tissue culture is to obtain true to type plants to maintain the germplasm, but, however, during tissue culture there is a chance of genetic aberration which is commonly known as ‘somaclonal variations’. Thus, the plantlets obtained from micropropagation must be screened for its clonal fidelity. Tissue culture of different species of bamboo including *D. strictus* has been attempted using different explants by several researchers (Saxena and Dhawan 1999; Chaturvedi et al. 1993; Ravikumar et al. 1998; Mehta et al. 2011; Pandey and Singh 2012; Bejoy et al. 2012; Devi et al. 2012), but, however, so far there are only few reports on bamboo where clonal fidelity of the micropropagated plantlets has been tested which includes *Bambusa tulda* and *B. balcooa* using RAPD markers (Das and Pal 2005), *B. balcooa* using ISSR (Negi and Saxena 2010), *B. nutans* employing AFLP (Mehta et al. 2011), *Guadua angustifolia* by means of RAPD and ISSR markers (Nadha et al. 2011), while very recently Singh et al. (2013) used various DNA-based markers for *D. asper*. Till date, though there are few reports on tissue culture of *D. strictus*, but no attempt has been made to ascertain their clonal fidelity.

Keeping all these in mind, the present investigation was aimed to develop an efficient and reproducible for the production of genetically identical and stable plantlets coupled with the use of RAPD and ISSR markers to ascertain whether true to type plants are generated or not before they are released for large-scale plantation.

Materials and methods

Plant material collection

Single nodal segments without any sprouted buds of *D. strictus* were collected from the forests of Sukna, Darjeeling district of West Bengal, India and used as explant. The plant material was authenticated by bamboo taxonomist. A voucher specimen (SUK/KRR/002) is deposited at Bambusetum, Kurseong Research Range, Sukna, Darjeeling, West Bengal (Goyal et al. 2012).

Initiation of aseptic culture

The nodal segments were then brought to the laboratory and processed for aseptic culture. They were washed carefully under running tap water. The culm sheets were removed carefully and sterilization was carried out. The nodal segments were surface sterilized by immersing in 1 % extran for 10 min and the washed several times with double-distilled water (DDW) for 5 min each. After this, these were dipped in 0.1 % mercuric chloride (HgCl_2) for 5 min and rinsed several times with sterile DDW to remove the traces of HgCl_2 under the laminar air flow cabinet. Next, they were treated with 70 % ethyl alcohol for 1 min and finally washed several times with sterile DDW. After surface sterilization, the nodal segments were trimmed in 2–3 cm height and blotted dry on sterile blotting paper (Goyal et al. 2010b).

Culture media and conditions

Nodal segments were aseptically cultured in culture tubes containing Murashige and Skoog medium (Murashige and Skoog 1962) and Woody plant medium (Lloyd and McCown 1980) supplemented with 3 % sucrose for shoot and root development. Different concentrations of cytokinins like BAP (6-benzyl amino purine) and Kn (Kinetin) were experimented in this study. Agar at the rate of 0.8 % was used as a solidifying agent in the culture media. The pH of the medium was adjusted to 5.7 ± 0.1 with 0.1 N NaOH or 1 N HCl prior to the addition of agar, followed by autoclaving at 121 °C for 20 min at 15 psi. The growth regulators were filter sterilized and supplemented to the culture media in desired concentration. The media were then poured into sterile test tubes (to form slants) as well as jam bottles under laminar air flow cabinet. After 20 min, the media became solidified and inoculation of the explants was done carefully.

Cultures were incubated at 25 °C with a photoperiod of 16 h at 2,000–3,000 lux light intensity of cool white fluorescent light.

Subculturing was done at 2-week interval in the same media having the same composition. BAP and Kn at the rate of 1, 2, 3, 4, 5 mgL⁻¹ were experimented to induce bud break and sprouting.

In vitro rooting

For in vitro rooting studies, propagules consisting of three shoots were transferred to full-strength MS medium as well as half-strength MS medium supplemented with different concentration of IBA (indole-3-butyric acid) and NAA (Naphthalene acetic acid) either alone or in combination. Data related to root number and root length were recorded every 2 weeks up to 3rd subculturing.

Hardening and transfer of plant to soil

After rooting in vitro, the shoots with roots were taken out of the culture bottle carefully so that the root system is not damaged. The roots were washed gently under running tap water to remove the medium completely. The plantlets with roots were acclimatized in substrates having perlite, soil and farm yard manure with a ratio of 1:1:1 (by volume) for 30 days in hardening shed for both primary and secondary hardening and finally to the field conditions.

Isolation of genomic DNA and PCR amplification

The genomic DNA was isolated from the leaves of both the mother donor plant and the in vitro-propagated plantlets using Genelute Plant Genomic DNA kit (Sigma Cat# G2 N-70) following the protocol provided in the kit. The quality and quantity of the isolated genomic DNA were estimated using two methods. First, gel analysis method, i.e., where the DNA samples were run in 0.8 % agarose gel using λ DNA/*EcoRI/HindIII* double digest as molecular weight marker and second, spectrophotometrically recording the optical density at 260 and 280 nm. Initially, a total of 24 RAPD and 15 ISSR markers were used for screening. PCR reaction was carried out in a volume of 25 μ l containing 2 μ l (25 ng DNA). The reaction buffer for RAPD contained 12.5 μ l of PCR master mix (GeNeiTM Cat# 610602200031730 Pl. No. MME22), 1.25 μ l primer and Pyrogen-free water to a final volume of 25 μ l. PCR amplification was performed on a Perkin-Elmer Thermocycler 2400 with the following specifications: initial denaturation at 94 °C for 4 min, followed by 44 cycles of denaturation at 94 °C for 1 min, primer annealing at 37 °C for 1 min, primer extension at 72 °C for 2 min with final extension at 72 °C for 10 min. In case of ISSR, the reaction mixture was made similar to RAPD, however, the amplification was carried out with the following specifications: initial denaturation at 94 °C for 5 min followed by 34

cycles of denaturation at 94 °C for 45 s, primer annealing at 50–52 °C (depending upon the primer) for 1 min, Primer extension at 72 °C for 1 min with the final extension at 72 °C for 10 min. The amplified products using both RAPD and ISSR markers were resolved on 1.8 % (w/v) agarose gel containing Ethidium bromide solution (0.5 μ g/ml) run in 0.5 \times TBE (Tris–borate EDTA) buffer. The fragment size was estimated using 0.1–10 kb DNA ladder as molecular weight marker.

Results and discussion

Plant material

In this experiment, single nodal segments were used as explants for in vitro regeneration. The nodal segments were opted since they have active meristems and have the potential to produce axillary buds which in turn can develop into plantlets. This property of nodal culture forms the basis of vegetative propagation. However, using tissue culture techniques, the rate of shoot multiplication can be increased to many folds. The phytohormone (cytokinins and auxins) accelerates the event. It is due to the continuous availability of the cytokinin in the growth medium that the nodal segment gives rise to axillary bud which grows into shoot. Thus, the nodal segment proves to be suitable explants for in vitro regeneration (Aruna et al. 2009; Ramadevi et al. 2012).

Culture initiation

Contamination by fungi and bacteria was the main problem that posed during the early stage of the culture initiation. To get rid of this, various pre-disinfection treatments (extran, 70 % ethanol, cetrimide and mercuric chloride) were employed, but it could not eliminate the contaminants totally but, however, was effective in reducing the rate of bacterial and fungal infection. Extran in combination with HgCl₂ has been used in the tissue culture study of *Thamnocalamus spathiflorus*, the Asian bamboo by Bag and his co-workers (Bag et al. 2000) previously. Mercuric chloride and 70 % ethanol have been used to disinfect the explants of *B. wamin* and *D. farinosus* (Arshad et al. 2005; Hu et al. 2011), respectively. Other than these, different chemicals have been used by different workers in tissue culture to get rid of contaminations in several bamboo species belonging to different genera (Arya et al. 1999; Ndiaye et al. 2006; Mehta et al. 2011; Bejoy et al. 2012; Devi et al. 2012). The contamination of the culture was observed parallel to the separating of the sheaths and sprouting of the explants. It can be inferred from the above observation that the contaminants are housed within the

layers of the sheaths and just mere surface sterilization failed to remove them. Pre-treatment of the explants with 0.1 % HgCl₂ for 5 min could help to eliminate bacterial infection, but to eradicate the fungal infection, the duration of HgCl₂ treatment was increased to 10 min. These explants remained green and healthy growth and proliferation of the axillary shoots were observed.

In vitro bud breaking and shoot initiation

Only 20 % of the explants sprouted when cultured in basal medium. The percentage of bud breaking frequency was increased by supplementing the basal medium with plant growth regulators like BAP and Kinetin. Regeneration of the plantlets also depended upon the type of medium used. MS medium was found to be suitable in comparison with the wood plant medium (WPM) for bamboo. Since the WPM was not found to be effective, it was not used anymore and only MS was employed for further regeneration. This indicates that some of the essential component required by bamboo for its regeneration is not available in WPM. Highest sprouting rate (49 %) was found to be in MS medium with 4 mg/l BAP. Further increase in the hormone concentration resulted in reduction of bud sprouting. Of the two hormones, BAP was found to be more suitable (Table 1). The data pertaining the effect of

BAP and Kn individually on the shoot multiplication of *D. strictus* are depicted in Table 1. It was observed that different concentration of BAP had a significant effect on shoot multiplication. The maximum rate of shoot multiplication (3.68 shoots per explant) was observed on medium supplemented with 4 mg/l BAP which was significantly higher than other BAP treatments after 6 weeks. The effect of Kn on shoot multiplication was found to be moderate up to 5 mg/l with highest (2.66 shoots per explant) at 4 mg/l Kn after 6 weeks. The hormones also had considerable effect on the shoot length. The highest shoot length after 6 weeks (3.11 cm) was noted with 5 mg/l BAP followed by 3.07 cm with 5 mg/l Kn, while decrease in the shoot length was noted with other treatments. The new shoots were excised from the in vitro-developed shoots for subculture. The subculture was done every 2 weeks. The increase in shoot number was observed in the first two subsequent subcultures in all the treatments with BAP (i.e., 1–5 mg/l) and then reduced thereafter (Table 2). However, the best multiplication of 3.86 shoots per explant was recorded during the 2nd subculture in MS medium supplemented with 4 mg/l BAP (Table 2). Both the cytokinins behaved differently in the present investigation with BAP being more effective compared to Kn. BAP is most commonly used cytokinin mainly due to twofold reasons, first, it is cheap and second, it can be

Table 1 Effect of BAP and Kinetin on bud breaking frequency, shoot bud proliferation and shoot length

Hormone	mg/l	Bud break frequency (%)	No. of primary shoots per explant			Shoot length after 6 weeks (cm)
			2 weeks	4 weeks	6 weeks	
BAP	1	32	1.25 ± 0.31	1.38 ± 0.20	1.46 ± 0.07	1.67 ± 0.11
	2	36	1.93 ± 0.24	1.99 ± 0.11	2.23 ± 0.21	1.89 ± 0.18
	3	45	2.32 ± 0.49	2.46 ± 0.24	2.89 ± 0.20	2.04 ± 0.14
	4	49	3.18 ± 0.12	3.40 ± 0.17	3.68 ± 0.37	2.61 ± 0.14
	5	46	2.88 ± 0.27	3.11 ± 0.54	3.46 ± 0.32	3.11 ± 0.41
Kinetin	1	27	1.06 ± 0.13	1.22 ± 0.19	1.46 ± 0.16	1.74 ± 0.11
	2	33	1.69 ± 0.10	1.84 ± 0.13	2.14 ± 0.21	1.96 ± 0.18
	3	38	2.02 ± 0.09	2.16 ± 0.15	2.45 ± 0.17	2.13 ± 0.09
	4	36	2.31 ± 0.43	2.43 ± 0.32	2.66 ± 0.29	2.58 ± 0.30
	5	32	2.16 ± 0.27	2.31 ± 0.18	2.48 ± 0.21	3.07 ± 0.28

Table 2 Effect of BAP on shoot proliferation in the subsequent subculture

Hormone	mg/l	No. of primary shoots per explant (after 2 weeks)	Subculture (shoots per explant after 2 weeks)		
			1st	2nd	3rd
BAP	1	1.25 ± 0.06	1.32 ± 0.09	1.49 ± 0.11	1.33 ± 0.15
	2	1.93 ± 0.11	2.03 ± 0.08	2.18 ± 0.17	2.29 ± 0.08
	3	2.32 ± 0.09	2.54 ± 0.19	2.78 ± 0.22	2.72 ± 0.10
	4	3.18 ± 0.17	3.41 ± 0.22	3.86 ± 0.19	3.72 ± 0.13
	5	2.88 ± 0.10	3.19 ± 0.27	3.77 ± 0.21	3.74 ± 0.19

autoclaved (Thomas and Blakesley 1987). In the present study, BAP was found to be effectual for shoot multiplication. These findings are in accordance with the earlier work on in vitro propagation of different bamboo species like *B. arundinacea* (Ansari et al. 1996), *D. asper* (Arya and Arya 1997), *B. ventricosa* (Huang and Huang 1995), *B. nutans* and *D. membranaceous* (Yasodha et al. 1997), *B. bambos* (Arya and Sharma 1998), *B. glaucescens* (Shirin and Rana 2007), *B. balcooa* (Mudoj and Borthakur 2009), and *D. hamiltonii* (Arya et al. 2012) where BAP has been widely used and was found to be effective. The less effectiveness of Kn on in vitro propagation of *D. strictus* was also noted by Nadgir and his co-workers (Nadgir et al. 1984) and Das and Rout (1991).

In vitro rhizogenesis and acclimatization

The results of root induction among the in vitro-regenerated plantlets achieved by culturing on either MS medium or half-strength MS medium supplemented with different auxins like IBA and NAA either singly or in combination are depicted in Table 3. About 87 % of the shoots rooted in MS medium supplemented with either IBA or NAA or combination of both (1–3 mg/l). The highest number of root (1.36) was regenerated on full-strength MS medium supplemented with 3 mg/l NAA (Table 3), while maximum length of 1.64 cm of roots was recorded with combination of 1 mg/l IBA and 3 mg/l NAA (Table 3). The half-strength MS medium was found to be less effective for

in vitro rooting in comparison to full-strength MS medium. This result was in accordance with previous reports (Rout et al. 1999; Kumaria et al. 2012). The plantlets with well-developed roots after transplantation to potting mixture containing perlite, soil and farm yard manure with a ratio of 1:1:1 (by volume) exhibited 70 % survival rate and grew in the greenhouse (Fig. 1). After a month, these acclimatized plants were transferred to the field.

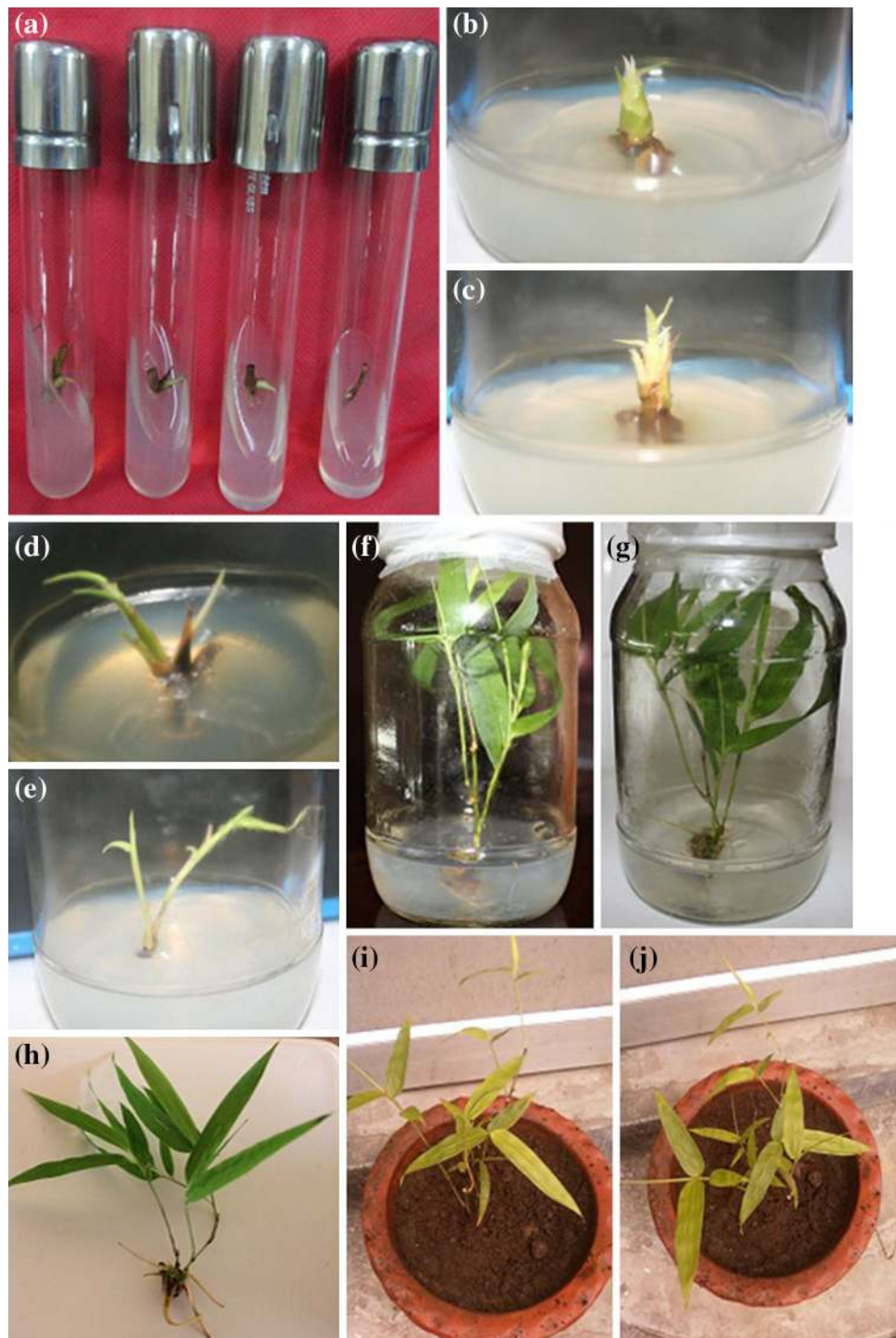
Somaclonal variation among the in vitro-raised plantlets

In bamboo micropropagation through axillary bud proliferation where no intermediary callus formation occurs has been largely attempted (Saxena 1990; Saxena and Bhojwani 1993; Arya et al. 1999; Jiménez et al. 2006). The in vitro-raised plants are expected to be genetically identical to the mother plant (used as explants), but, however, possibility of having some genetic variations cannot be ignored. Somaclonal variation is thought to be a common event in the in vitro-raised plants which include an array of genetic and epigenetic variations (Peredo et al. 2006). In this study, an attempt was made to screen the in vitro-raised *D. strictus* plantlets for somaclonal variations (if any) by employing both RAPD and ISSR markers analysis. A total of 24 RAPD and 15 ISSR primers were used to screen somaclonal variations, out of which only 10 RAPD and 9 ISSR primers were successful in amplifying the genomic DNA. The amplification using all the 10 RAPD

Table 3 Effect of IBA and NAA on various parameters of in vitro rooting in *D. strictus*

Rooting media	Hormone	Concentration (mg/l)	No. of primary roots per explant	Root length (cm)
MS	IBA	1	0.32 ± 0.01	0.88 ± 0.03
	IBA	2	0.78 ± 0.03	1.12 ± 0.02
	IBA	3	1.24 ± 0.09	1.56 ± 0.07
	NAA	1	0.29 ± 0.01	0.64 ± 0.01
	NAA	2	0.81 ± 0.02	0.98 ± 0.09
	NAA	3	1.36 ± 0.04	1.44 ± 0.07
½ MS	IBA	1	0.21 ± 0.01	0.58 ± 0.02
	IBA	2	0.69 ± 0.03	0.95 ± 0.08
	IBA	3	0.98 ± 0.04	1.09 ± 0.05
	NAA	1	0.18 ± 0.01	0.36 ± 0.01
	NAA	2	0.56 ± 0.04	0.69 ± 0.02
	NAA	3	0.74 ± 0.07	0.88 ± 0.04
MS	IBA + NAA	1 + 1	0.54 ± 0.01	1.02 ± 0.03
	IBA + NAA	1 + 2	0.87 ± 0.01	1.21 ± 0.06
	IBA + NAA	1 + 3	1.32 ± 0.03	1.64 ± 0.03
	IBA + NAA	2 + 1	0.62 ± 0.01	0.88 ± 0.01
	IBA + NAA	2 + 2	1.11 ± 0.06	1.16 ± 0.02
	IBA + NAA	2 + 3	1.06 ± 0.02	1.29 ± 0.04

Fig. 1 Stages in development of in vitro-cultured *Dendrocalamus strictus*. **a** Axillary bud breaking. **b** Healthy axillary bud increasing its volume. **c**, **d** Multiple shooting. **e** Development of leaves. **f**, **g** Root induction in rooting medium. **h** Plantlet with well-developed roots. **i**, **j** Acclimatized plant in the green house



primers resulted in 58 scorable bands whereas the ISSR primers resulted in producing 66 distinct and scorable bands (Table 4). The number of bands varied from 3 to 11 in case of RAPD primers and 5–11 in case of ISSR markers with an average of 5.8 and 7.33 bands, respectively. All the bands generated were found to be monomorphic across all

the in vitro-raised plantlets and the parent plant analyzed irrespective of whether RAPD primers or ISSR markers were used. A representative of RAPD and ISSR profile is depicted in Fig. 2. The band size ranged in between 240–1,455 and 183–1,544 bp in case of RAPD and ISSR markers, respectively. This uniformity in the banding

Table 4 PCR amplicons obtained from RAPD and ISSR markers in in vitro-raised *Dendrocalamus strictus*

Primer ID	Primer sequence (5′–3′)	Total bands amplified	No. of monomorphic bands	Band size (bp)
OPA03	AGTCAGCCAC	7	7	240–1,145
OPA07	GAAACGGGTG	6	6	245–892
OPA11	CAATCGCCGT	4	4	545–1,455
OPA20	GTTGCGATCC	6	6	344–667
OPF09	CCAAGCTTCC	5	5	435–710
OPG19	GTCAGGGCAA	11	11	249–893
OPH04	GGAAGTCGCC	3	3	451–864
OPN04	GACCGACCCA	7	7	409–851
OPN13	AGCGTCACTC	6	6	358–1,375
OPN19	GTCCGTACTG	3	3	315–1,444
Total bands		58	58	
UBC810	(GA)8T	8	8	309–845
UBC815	(CT)8G	9	9	190–1,554
UBC818	(CA)8G	11	11	137–1,011
UBC822	(TC)8A	5	5	587–1,214
UBC824	(TC)8G	7	7	305–1,237
UBC825	(AC)8T	7	7	330–1,152
UBC841	(GA)8YC	9	9	312–1,163
UBC856	(AC)8YA	5	5	183–1,108
UBC873	(GACA)4	5	5	294–857
Total bands		66	66	

pattern confirms the genetic fidelity of the in vitro-raised *D. strictus* plantlets. Detection of clonal fidelity among in vitro-raised bamboo has been attempted by Das and Pal (2005), Negi and Saxena (2010), Mehta et al. (2011), Nadha et al. (2011) and Singh et al. (2013). In *B. balcooa* and *B. tulda*, Das and Pal (2005) used merely 5 RAPD decamers to establish the genetic stability among the micropropagated plantlets. Later in 2010, Negi and Saxena made an attempt to access the genetic fidelity among the regenerants of *B. balcooa* employing 15 ISSR markers. In 2011, in two separate studies, Mehta and her co-workers and Nadha and her co-researchers used AFLP markers and DNA-based markers (RAPD and ISSR) for *B. nutans* and *G. angustifolia*, respectively. Recently, Singh and his colleagues (Singh et al. 2013) used different DNA-based markers (RAPD, ISSR, AFLP, SSR) to study the genetic fidelity of *D. asper*. Though both RAPD primers and ISSR markers help in detecting polymorphism, ISSR markers dominate the RAPD primers. ISSR markers showed high polymorphism and have good reproducibility because of the presence of large number of SSR region as compared to RAPD (Ray et al. 2006). Moreover, ISSR markers are much larger in size than RAPD which are decamers and hence have higher annealing temperature. Higher annealing temperature is considered to result in greater consistency

whereas lower annealing temperature might produce artifact amplicons because of non-specific amplification (Bornet and Branchard 2001). Both the fingerprinting techniques have their own advantages and disadvantages. Thus, keeping this in mind, both RAPD primers and ISSR markers were used to screen the clonal stability of *D. strictus*. This analysis showed that there was virtually no variability among the micropropagated plantlets of *D. strictus* and thus can be concluded that the in vitro-raised plants avoided the genomic aberrations and did not lead to any somaclonal variation.

Conclusion

In conclusion, this study led to the establishment of standard protocol for in vitro regeneration of *D. strictus* in particular and bamboo in general. The production of monomorphic bands by the mother plants and the in vitro-raised plantlets against both the RAPD and ISSR primers showed that there was virtually no variability among the micropropagated plantlets and the mother plant of *D. strictus* and thus can be concluded that the in vitro-raised plants avoided the genomic aberrations and did not lead to any somaclonal variation.

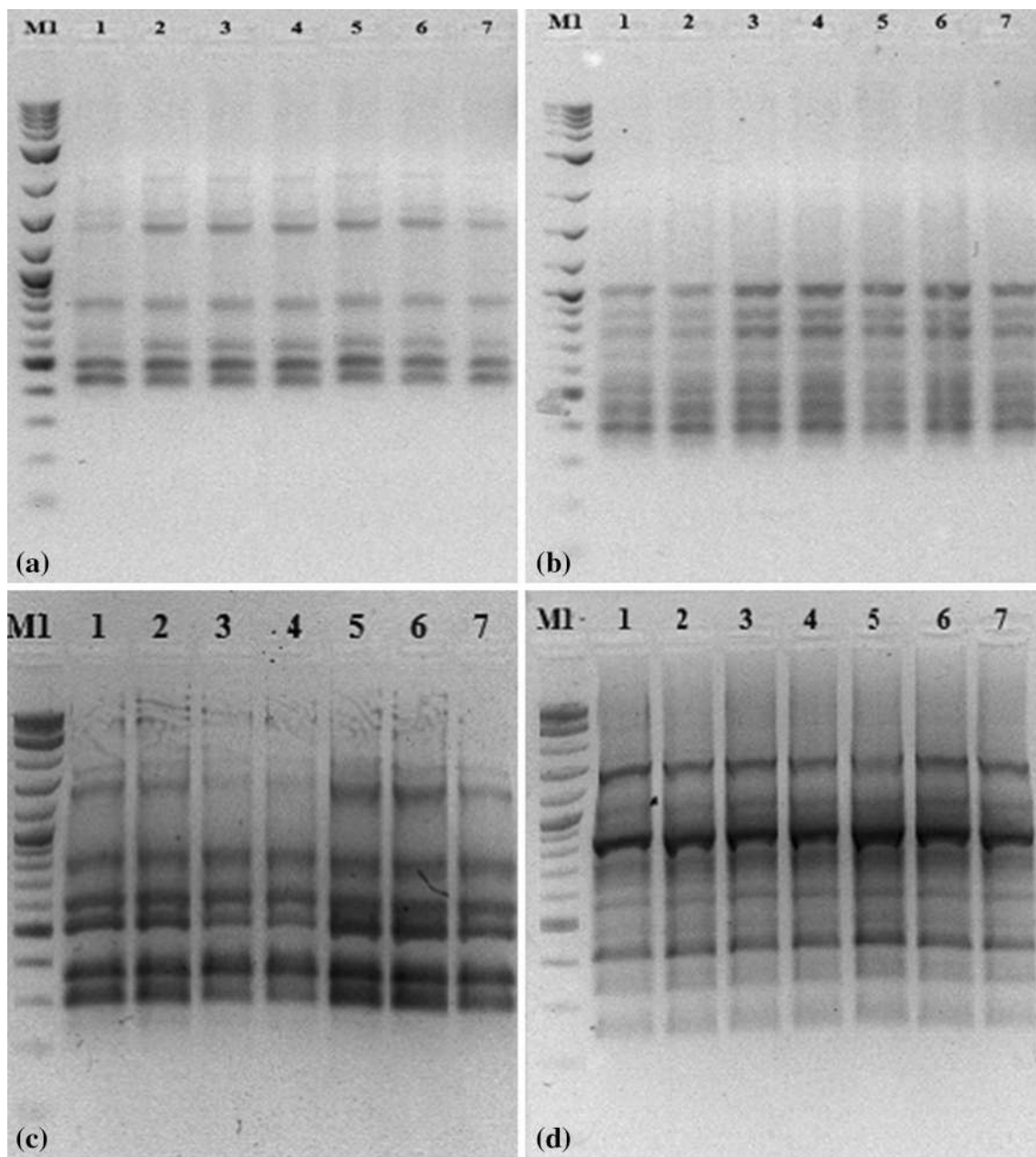


Fig. 2 DNA fingerprinting patterns of in vitro-raised *D. strictus*. ISSR primers, **a** UBC824; **b** UBC810 and RAPD primers; **c** OPN04; **d** OPG19, among in vitro-regenerated plantlets compared with the

donor plant: Donor plant (*lane 1*), micropropagated plants (*lanes 2–7*) and molecular weight markers 0.1–10 kb DNA ladder (M1)

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Conflict of interest The authors declare no conflict of interest.

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