IN-VITRO PROPAGATION, BIOCHEMICAL STUDIES AND ASSESSMENT OF CLONAL FIDELITY THROUGH MOLECULAR MARKERS IN *BAMBUSA BALCOOA*

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BRAR J, SHAFI A, SOOD P, ANAND M & SOOD A. 2014. In-vitro propagation, biochemical studies and assessment of clonal fidelity through molecular markers in *Bambusa balcooa*. Micropropagation protocol of *Bambusa balcooa* has been established using nodal explants with 92.5% success after acclimatisation. The extraction of chlorophyll from the epidermis of culms using acetone, dimethylformamide and dimethyl sulfoxide as solvents and the use of auxin in shoot multiplication are also reported for the first time in this species. Due to stringent environmental conditions in the greenhouse, electrolyte leakage percentage (67.4 ± 2.4%) was found to be higher in ex-vitro raised plants whereas relative water content percentage (72.7 ± 2.3%) was higher in in-vitro grown plants. Analysis of total soluble sugars (16.5 ± 0.78 mg g^{-1}) and starch content (6.32 ± 0.55 mg g^{-1}) displayed in-vitro plants to be richer in carbohydrates reserves. However, lignin content (28.74 ± 1.59 mg L^{-1}), stomatal density (56.0 ± 2.64 in 140 μm^2) and leaf area index (4.66 ± 0.81 cm^2) were greater under ex-vitro conditions. Molecular characterisation using 25 random amplified polymorphic DNA and 15 inter-simple sequence repeats markers exhibited 89 amplified products depicting no polymorphism between parent clump and in-vitro raised plants.

Keywords: Acclimatisation, axillary proliferation, chlorophyll, ISSR, micropropagation, mother plant, nodal segment, RAPD

BRAR J, SHAFI A, SOOD P, ANAND M & SOOD A. 2014. Pembiakan *in vitro*, kajian biokimia serta pentaksiran fideliti klon dengan penciri molekul dalam *Bambusa balcooa*. Protokol pembiakan mikro *Bambusa balcooa* telah dibangunkan menggunakan eksplan nod dengan kejayaan sebanyak 92.5% selepas pengikliman. Pengekstrakan klorofil daripada epidermis kulma menggunakan aseton, dimetilformamida dan demetil sulfoksida sebagai pelarut serta penggunaan auksin dalam penggandaan pucuk dilaporkan untuk pertama kali bagi spesies ini. Oleh sebab keadaan persekitaran dalam rumah kaca adalah terkawal ketat, peratusan kebocoran elektrolit (67.4 ± 2.4%) adalah lebih tinggi dalam tanaman yang dibiakkan secara *ex vitro* manakala peratusan kandungan air relatif (72.7 ± 2.3%) lebih tinggi dalam tanaman *in vitro*. Analisis jumlah gula terlarut (16.5 ± 0.78 mg g^{-1}) dan kandungan kanji (6.32 ± 0.55 mg g^{-1}) menunjukkan bahawa tanaman *in vitro* lebih kaya dalam simpanan karbohidrat. Bagaimanapun, kandungan lignin (28.74 ± 1.59 mg L^{-1}), kepadatan stoma (56.0 ± 2.64 pada keluasan in 140 μm^2) dan indeks keluasan daun (4.66 ± 0.81 cm^2) lebih tinggi di bawah keadaan *ex vitro*. Pencirian molekul menggunakan 25 penciri *random amplified polymorphic DNA* (RAPD) serta 15 penciri *inter-simple sequence repeats* (ISSR) menghasilkan 89 produk diperbesar yang tiada polimorfisme antara rumpun induk dengan tanaman *in vitro*.

INTRODUCTION

Recently, bamboos have become a source of prime concern according to the amendments in the Forests Right Act 2006. In a study of 25 edible bamboo species, protein content was highest in *Bambusa balcooa* (Bhat et al. 2005). The shoots are quite popular with Vietnamese as a vegetable food and leaves as fodder for animals. It is the best and strongest species for building purposes and is much used for scaffolding (Gillis et al. 2007). *Bambusa balcooa* is a clumping bamboo of Indian origin which can grow up to a height of 25 m and thickness of 15 cm at an altitude of 700 to 1500 m in any type of soil but prefers moist alluvial/clay soils with good drainage. Gregarious flowering is reported in this species and the clump generally dies immediately after flowering without setting any seeds. Hence, propagation by seeds is a limitation and devising a method for in-vitro propagation holds great promise.

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Although some protocols for micropropagation of this species from nodal explants are already available (Das & Pal 2005, Mudoi & Borthakur 2009, Negi & Saxena 2010), many aspects of research are still needed including potential of axillary buds for micropropagation, improvements in rooting and acclimatisation processes, biochemical and physiological parameters and modifying in-vitro and ex-vitro conditions according to the needs of the species. These will prove beneficial for large-scale propagation of this species. Use of shoot tips in most plant species is generally considered as genetically stable systems but confirmation of producing true to type plants by molecular markers will ensure quality production of elite genotypes. The present study described the reproducible and effective protocol for in-vitro propagation of *B. balcooa*.

MATERIALS AND METHODS

Establishment of micropropagation protocol

Single node segments (3–4 cm long) from healthy 4-year-old culms of *B. balcooa* at the experimental farm of the Institute of Himalayan Bioresource Technology, Palampur, India (1300 m above sea level, 32° 7' N, 76° 31' E) were used as explants after surface sterilisation. Segments were inoculated on Murashige and Skoog (MS) medium (Murashige & Skoog 1962) containing 2% (w/v) sucrose and 0.8% (w/v) agar and having a pH of 5.75 before autoclaving. The basal MS media was supplemented with cytokinin (BAP) (0.0 to 13.2 µM), auxin (NAA) (0.0 to 2.2 µM) and sucrose (0.5 to 3% w/v) for shoot multiplication. Shoots were subcultured on fresh medium at a regular interval of 20 days. These shoots were then excised in clumps of 4–5 from multiple shoot bunches and transferred to MS media in full, half and quarter strengths supplemented with NAA (0.0 to 24.16 µM) and 0.2% (w/v) gelrite as gelling agent for induction of roots. After 30 days, rooted plantlets were removed from the culture tubes and washed in lukewarm water. Then the plants were placed inside a 300 cm × 210 cm polytunnel under shade for acclimatisation initially in the river bed sand. After 14 days, plantlets were transferred to 17.5 cm × 12.5 cm polybags containing potting mixture of soil, sand and farmyard manure in equal proportion.

Physiological changes in response to ex-vitro acclimatisation

For determination of stomatal density, the method described by Bag et al. (2000) was used. Scanning electron microscopy (SEM) was performed following the method by Saha et al. (2011). Relative water content was determined according to Perl-Treves and Galum (1991) using nine leaf discs of 0.5 cm diameter. Percentage of leakage was measured using conductivity meter (Wright & Simon 1973). About 2 cm basal portions (50 mg) of young culms were taken for the determination of lignin using acetyl bromide method (Iiyama & Wallis 1988). Leaf area index was measured by traditional graph paper method.

Biochemical changes in response to ex-vitro acclimatisation

Epidermis layer was peeled from the culms and ground to powder. The power (40 mg fresh weight) was added to a vial containing 25 ml of acetone (MacKinney 1941), dimethylformamide (DMF) (Moran & Porath 1980) or dimethyl sulfoxide (DMSO) (Hiscox & Israelstam 1979) and chlorophyll was extracted using an ultrasonicator for 3 min. The chlorophyll solutions were analysed using ultraviolet-visible spectrophotometer. Total soluble sugars were measured using anthrone reagent (McCready et al. 1950). Starch was measured as liberated glucose using anthrone reagent after hydrolysing the extracted tissues with perchloric acid (Adams et al. 1980).

Molecular characterisation

In a bid to ascertain genetic fidelity of tissue culture-raised plants, random samples from leaves were taken from 6-month-old plants growing in the greenhouse for isolation of DNA. DNA was extracted from fresh expanded leaves (100 mg) using cetyltrimethyl ammonium bromide (CTAB) (Doyle & Doyle 1990). Aliquots of 5 µL sample plus gel loading buffer (6×) were loaded on 1.8% agarose gel for electrophoresis in 1× TBE buffer for random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) analysis. Gel photographs were analysed through gel documentation system.

Statistical analysis

All investigations were based on complete randomised block design with minimum of five replicates. Data are reported as mean ± significance difference (SD). Significance of treatment was determined by one-way ANOVA, and the means were compared with Duncan's test at a significance of $p \leq 0.05$ using STATISTICA, release 7, Statsoft Wipro.

RESULTS

Establishment of micropropagation protocol

In-vitro bud break was achieved on basal MS media with 2% sucrose in about 15 days (Figure 1a). Best results for shoot multiplication were obtained on agarified MS medium supplemented with 4.4 µM of BAP and 0.53 µM of NAA (Figures 1b and c) where 19.8 ± 1.4 shoots per explant grew in about 30 days. Shoot length was 3.44 ± 0.23 cm and number of leaves was 19.5 ± 1.26 with no signs of albinism (Table 1). Higher doses of NAA (2.2 µM) induced thin leaf like shoots, majority of which did not develop further. Shoot proliferation in media with 1% (w/v) sucrose was higher than 3% (Figure 2a). No rooting was observed on the control basal MS medium. NAA was more effective for rooting of microshoots than IBA or a combination of NAA and BAP. Full strength MS media (Figure 2b) fortified with 16.11 µM of NAA and 2% (w/v) of sucrose gave the best response for induction of roots with mean number of roots 11.5 ± 1.58, root length 4.09 ± 0.4 cm and rooting percentage 76.6% (Table 2). NAA when used in low concentrations (0 to 8.05 µM) or with BAP produced low rooting. Generally, five to six roots developed from the basal cut end of the propagule within 25–30 days. Root growth was more pronounced in shoot clumps than in single shoot. The transfer of in-vitro rooted shoots to basal MS after 20–25 days produced good quality roots (i.e. roots were not very thin and brittle and could be pulled out without breakage). After a month, plantlets were acclimatised in a greenhouse and the rate of survival after acclimatisation was 92.5%. A total of 50 plants had been successfully transferred to the pots so far. Hence, a complete protocol for micropropagation was established (Figures 1a–g). There was significant difference in the number of stomata and density of in-

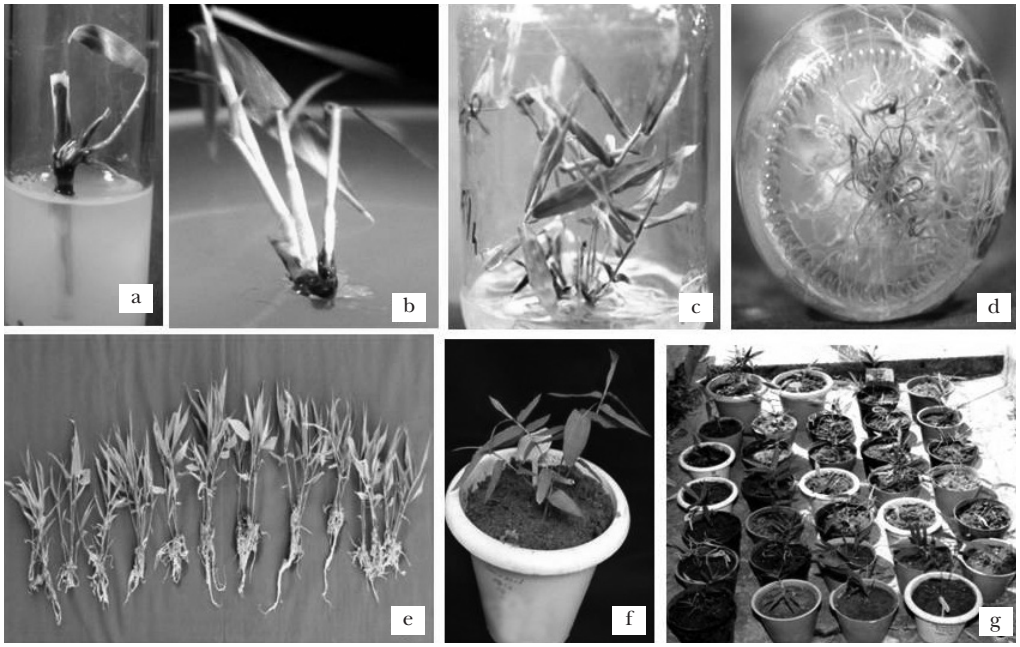

Figure 1 Micropropagation protocol of *Bambusa balcooa*: (a) sprouted bud on the 15th day, (b) sprouted bud with 3–4 shoots subcultured onto shoot proliferation media, (c) a cluster of axillary shoots showing multiple shoot proliferation, (d) root induction in rooting media, (e) root development after transfer to growth regulator-free media, (f) hardened plantlets in soil:sand:farm yard manure of 1:1:1 and (g) greenhouse acclimatised plants

Table 1 Effects of different BAP and NAA concentrations on the rate of proliferation of shoots in *Bambusa balcooa*

BAP/NAA (µM)	No. of shoots	Shoot height (cm)	Total no. of leaves
0/0	8.0 ± 1.1 g	1.24 ± 0.17 g	9.0 ± 1.41 h
0/0.46	6.2 ± 1.3 h	1.23 ± 0.18 g	6.9 ± 1.59 i
0/1.16	4.2 ± 0.9 i	1.12 ± 0.09 gh	5.5 ± 1.17 j
0/2.2	3.0 ± 0.6 j	0.94 ± 0.14 h	3.9 ± 1.59 k
4.4/0	8.8 ± 1.3 g	1.54 ± 0.16 f	11.5 ±1.50 fg
4.4/0.46	14.2 ± 1.3 c	1.92 ± 0.10 de	13.6 ± 1.71 de
4.4/1.16	10.9 ± 1.1 ef	1.75 ± 0.16 ef	13.4 ± 1.77 de
4.4/2.2	10.0 ± 1.1 f	1.60 ± 0.19 f	10.2 ± 1.39 gh
8.8/0	16.8 ± 1.1 b	2.84 ± 0.42 b	16.5 ± 1.50 b
8.8/0.46	19.8 ± 1.4 a	3.44 ± 0.23 a	19.5 ± 1.26 a
8.8/1.16	15.1 ± 1.9 c	2.40 ± 0.22 c	14.6 ± 1.64 cd
8.8/2.2	14.8 ± 1.6 c	2.03 ± 0.17 d	14.4 ± 0.84 d
13.2/0	14.9 ± 2.0 c	2.11 ± 0.27 d	15.9 ± 1.66 bc
13.2/0.46	12.7 ± 1.2 d	1.66 ± 0.21 g	14.2 ± 2.74 d
13.2/1.16	11.7 ± 1.2 de	1.62 ± 0.18 g	12.6 ± 1.26 ef
13.2/2.2	8.7 ± 1.4 g	1.55 ± 0.27 g	11.9 ± 1.52 f

Values are means of five replicates where different letters within a column indicate significant difference by Duncan's multiple range test, $p \leq 0.05$

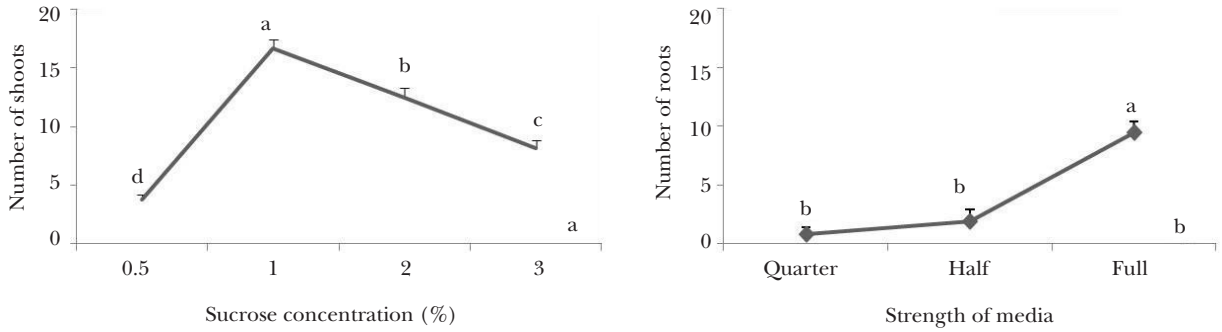

Figure 2 (a) Mean number of axillary shoots in a single node segment from field-grown culms of *Bambusa balcooa* at varying sucrose concentrations and (b) mean number of roots per nodal segment at different strengths of MS media supplemented with 16.11 µM NAA

vitro and ex-vitro grown plants (Figures 3a–d). SEM analysis indicated that stomatal density in 140 µm^2 was highest on abaxial side of ex-vitro hardened plants. More stomata were open on the abaxial side compared with the adaxial side. Circular stomata changed shape to elliptical when facing water stress under ex-vitro conditions. Silica cells were present throughout both surfaces but more clearly visible on the abaxial side. In addition, abaxial surface had conspicuous papillae present in costal and intercostal regions of the leaves (Figures 3e–h).

Physiological parameters

Percentage of relative water content was higher (72.7%) in in-vitro grown plantlets than ex-vitro (67.7%) (Table 3). Growth and development under controlled environmental conditions contributed to this higher value. There was an inverse relationship of water saturation deficit with relative water content. Low relative water content in in-vivo conditions indicated a lag phase in the growth of plants when relative humidity in the greenhouse shifted from 70

Table 2 Effect of NAA on rooting response in *Bambusa balcooa*

Growth regulator	Concentration (µM)	No of roots	Root length (cm)	Rooting (%)	Root growth*
NAA	0	0.0 ± 0.0 f	0.0 ± 0.0 e	0	-
NAA	2.68	0.0 ± 0.0 f	0.0 ± 0.0 e	0	-
NAA	5.37	0.0 ± 0.0 f	0.0 ± 0.0 e	0	-
NAA	8.05	0.0 ± 0.0 f	0.0 ± 0.0 e	0	-
NAA	10.74	0.80 ± 0.6 f	1.0 ± 0.2 d	5.33	+
NAA	13.42	1.6 ± 0.51 e	2.57 ± 0.3 c	10.60	++
NAA	16.11	11.5 ± 1.58 a	4.09 ± 0.4 a	76.60	++++
NAA	18.79	5.50 ± 1.26 b	2.71 ± 0.3 c	36.60	++++
NAA	21.48	4.50 ± 0.84 c	2.03 ± 0.3 b	30.00	++++
NAA	24.16	3.60 ± 1.34 d	1.18 ± 0.3 d	24.00	+++
NAA/BAP	2.68/0.44	0.0 ± 0.0 c	0.0 ± 0.0 c	0	-
NAA/BAP	5.37/0.44	0.8 ± 0.6 b	1.25 ± 0.48 b	5.30	+
NAA/BAP	10.74/0.44	1.9 ± 0.8 a	1.60 ± 0.39 a	12.60	++
IBA	4.90	0.0 ± 0.0 f	0.0 ± 0.0 f	32.60	-

Values are means of five replicates where different letters within a column indicate significant difference by Duncan's multiple range test, $p \leq 0.05$; *indicates number of roots found (– absence of roots, + single root, ++ two roots, +++ three roots, ++++ four or more roots)

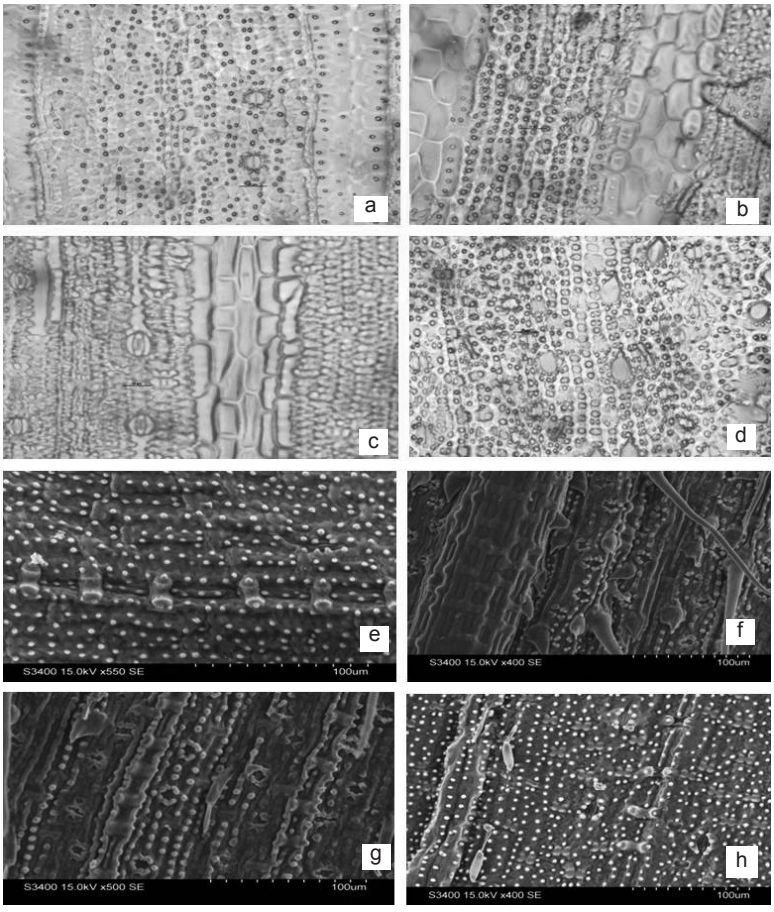

Figure 3 (a–d) Stomata in 68 µm^2 (a) adaxial surface of in-vitro grown leaf showing 18.0 ± 2.64 stomatal density, (b) adaxial surface of ex-vitro grown leaf showing stomata with density of 24.0 ± 2.0 and finger-like microhairs, (c) abaxial surface of in-vitro grown leaf showing stomatal density 30.66 ± 4.04, (d) abaxial surface of ex-vitro grown leaf having highest stomatal density of 41.33 ± 3.21; (e–h) scanning electron microscopy images showing stomata in 140 µm^2 (e) adaxial surface of in-vitro leaf showing stomatal density of 32.0 ± 3.0, (f) adaxial surface of ex-vitro grown leaf showing 42.3 ± 2.5 stomatal density, (g) abaxial surface of in-vitro grown leaf showing stomatal density of 48.6 ± 3.5 and (h) adaxial surface of ex-vitro grown leaf showing stomatal density of 56.0 ± 2.64

Table 3 Biochemical and physiological parameters in *Bambusa balcooa*

Physiological parameter	In-vitro	Ex-vitro
Relative water content (%)	72.7 ± 2.3 a	67.7 ± 3.3 b
Water saturation deficit (%)	27.3 ± 2.3 a	32.3 ± 3.3 b
Electrolyte leakage (%)	57.7 ± 2.7 b	67.4 ± 2.4 a
Leaf area index (cm^2)	1.06 ± 0.44 b	4.66 ± 0.81 a
Lignin content (mg L^{-1})	17.90 ± 2.16 b	28.74 ±1.59 a
Total soluble sugars (mg g^{-1} fw)	16.5 ± 0.78 a	13.38 ± 2.03 b
Starch (mg g^{-1} fw)	6.32 ± 0.55 a	4.76 ± 0.51 b

Values are means of five replicates where different letters within a column indicate significant difference by Duncan's multiple range test, $p \leq 0.05$; fw = fresh weight

to about 60%. Exposure of plants to stressed environment of ex-vitro in a polyhouse resulted in significant increase in electrolyte leakage percentage of 67.4% compared with in-vitro grown plants (57.7%) (Table 3). Leaf area index was significantly higher (4.66 cm^2) in ex-vitro hardened plantlets showing small physiological differences in growth and metabolism.

Biochemical parameters

Maximum values for chlorophyll a and b were 7.13 and 2.55 mg g^{-1} fresh weight respectively under ex-vitro conditions when acetone was used as solvent (Table 4). Increased levels of CO_2 in greenhouse promoted the synthesis of chlorophyll and carotenoids. Efficiency of extraction was in the order acetone > DMSO > DMF due to differences in stability and viscosity of these solvents. Lignin content (28.74 ± 1.59 mg L^{-1}) was higher in the acclimatised plants due to more pronounced growth and metabolism while total soluble sugars (13.38 ± 2.03 mg g^{-1} fresh weight) and starch (4.76 ± 0.51 mg g^{-1} fresh weight) contents were lower (Table 2).

Molecular characterisation

In ascertaining clonal fidelity, of the 25 scanned RAPD markers, 21 primers produced 61 amplicons (Table 5). In the case of ISSR analysis using 15 markers, 10 ISSR primers gave 28 scorable bands (Table 6). From the total of 31 scanned markers, 89 amplified products were obtained. Size of fragments varied between 100 and 1500 kb (Figure 4). Optimum melting temperature of primers used for RAPD markers fell near 37 °C and for ISSR markers, between 41.5 and 50.5 °C. For RAPD analysis, OPO series gave the best amplification followed by OPT and OPA series. For ISSR markers UBC 810, 811 and 888 gave maximum amplified products in the range of 250 to 1500 bp. In our study, no variation was reported among in-vitro raised plants and the mother plant banding profiles. Hence, axillary bud proliferation was the method of choice for micropropagation.

DISCUSSION

The present study reports a significant improvement in growth and multiplication of *B. balcooa* in the shoot multiplication medium having a combination of BAP and NAA compared with control and when these were used individually. NAA has been used for multiple shoot formation in *Dendrocalamus hamiltonii* and some other bamboo species (Agnihotri et al. 2009). Our study showed that low concentrations of NAA were required for maintaining proper cytokinin:auxin ratio for proliferation of cultures of this species. Increased cytokinin:auxin ratio enhanced the ability for shoot organogenesis (Smigocki & Owens 1988). Sugars have been shown to regulate the expression of components of phytohormone response pathways (Gibson 2004). The requirement for low sucrose in *B. balcooa* compensates for high levels of endogenous cytokinins. Rooting in general has been a bottleneck in bamboos (Dekkers & Rao 1989). In some species such as *D. strictus,* BAP alone induced rooting (Shirgurka et al. 1996). The requirement for exogenous supply of high levels of NAA (16.11 µM) indicated that endogenous levels of auxins in the rooting zone were suboptimal for rooting response. Gelrite

Table 4 Chlorophyll content in *Bambusa balcooa* by ultrasonics

Solvent	In-vitro (mg g^{-1} fw)	Ex-vitro (mg g^{-1} fw)
Acetone	Chlorophyll a: 4.97 ± 0.5 a Chlorophyll b: 1.71 ± 0.62 a	7.13 ± 1.96 a 2.55 ± 0.78 a
DMSO	Chlorophyll a: 3.61 ± 0.64 b Chlorophyll b: 1.31 ± 0.73 a	4.48 ± 0.75 b 2.52 ± 0.67 a
DMF	Chlorophyll a: 2.77 ± 0.59 c Chlorophyll b: 1.18 ± 0.64 a	3.93 ± 1.17 b 2.28 ± 1.43 a

DMF = dimethylformamide, DMSO = dimethyl sulfoxide; values are means of five replicates where different letters within a column indicate significant difference by Duncan's multiple range test, $p \leq 0.05$; fw = fresh weight

Table 5 RAPD primers used to verify *Bambusa balcooa* clones

Primer	5'–3' motif	No. of scorable bands	No. of monomorphic bands	No. of polymorphic bands	Range of amplication (bp)
CAG GCC CTT C	4	4	4	0	550, 600, 700, 800
TGC CGA GCT G	2	2	2	0	300, 500
AAT CGG GCT G	3	3	3	0	450, 550, 700
TCG GCG ATA G	4	4	4	0	250, 350, 500, 600
GAC CGC TTG T	4	4	4	0	400, 900, 1000, 1200
CAA ACG TCG G	3	3	3	0	700, 800, 1100
ACG TAG CGT C	2	2	2	0	300, 500
CCC AGT CAC T	4	4	4	0	200, 300, 700, 900
CAG CAC TGA C	2	2	2	0	600, 1000
CCT CCA GTG T	6	6	6	0	100, 150, 250, 350, 800, 900
AGC ATG GTC C	1	1	1	0	1200
TGG CGT CCT T	5	5	5	0	200, 250, 500, 600, 1000
TCG GCG GTT C	2	2	2	0	500, 1100
CTC GCT ATC C	1	1	1	0	500
CAA GGG CAG A	3	3	3	0	500, 700, 800
CAC CCC TGA G	1	1	1	0	500
CCT TCG GAA G	1	1	1	0	300
GGG TGT GTA G	4	4	4	0	400, 450, 500
AGG ACT GCC A	2	2	2	0	100, 250
GGT GAA CGC T	4	4	4	0	500, 600, 900, 1100
GAT GCC AGA C	3	3	3	0	400, 700, 1200
Total	21	61	61	0	100–1200

was the preferred base for rooting in the current study due to its clarity, less requirement (0.2%), fineness of texture and it being a highly purified polysaccharide in contrast to agarified medium. Our study showed positive influence of in-vitro conditions on relative water content and electrolyte leakage depicting the role of controlled conditions in maintaining the stability of biomembranes (e.g total soluble sugars and starch). Our observations were similar to findings by Hirose et al. (1997) where increased leaf area index of hardened plants was attributed to increased CO_2 levels and rate of photosynthesis. The extraction efficiency of solvents used for chlorophyll extraction can be attributed to their different stability (Wu et al. 2002). Ex-vitro hardened plants had more pronounced growth due to photoautotrophic mode of photosynthesis and hence, more biomass in terms of lignin content (McKendry 2002). Appropriate assimilation of biomolecules bring about formation of new structures in

Table 6 ISSR markers utilised to verify *Bambusa balcooa* clones

Primer	5'–3' motif	T_m (°C)	T_a (°C)	Scorable band	Monomorphic band	Polymorphic band	Range of amplification (bp)
UBC 810	$(GA)_9T$	45.4	42.5	4	4	0	400, 700, 900, 1500
UBC 811	$(GA)_8C$	46.8	44.0	4	4	0	250, 500, 600, 1400
UBC 812	$(GA)_8A$	45.7	41.5	3	3	0	400, 500, 800
UBC 815	$(CT)_8$ G	46.8	44.0	2	2	0	900, 1100
UBC 818	$(CAC\ ACA)_2$ CAC AG	51.0	48.5	3	3	0	500, 700, 1100
UBC 834	$(AG)_8YT$	49.2	46.0	3	3	0	150, 300, 350
UBC 844	$(CT)_8RC$	48.6	45.0	1	1	0	400
UBC 850	$(GT)_8YC$	52.7	50.0	3	3	0	200, 400, 900
UBC 857	$(AC)_8YG$	54.3	50.5	1	1	0	100
UBC 888	$BDBC(AC)_5A$	47.3	43.5	4	4	0	300, 400, 600, 1400
Total	10	–	–	28	28	0	100–1500

B = (C,G,T, i.e. not A), D = (A, G, T, i.e. not C), R = (A, G), Y = (C, T); UBC series sequences of University of British Columbia, Canada; T_m = melting temperature of primers used, T_a = annealing temperature

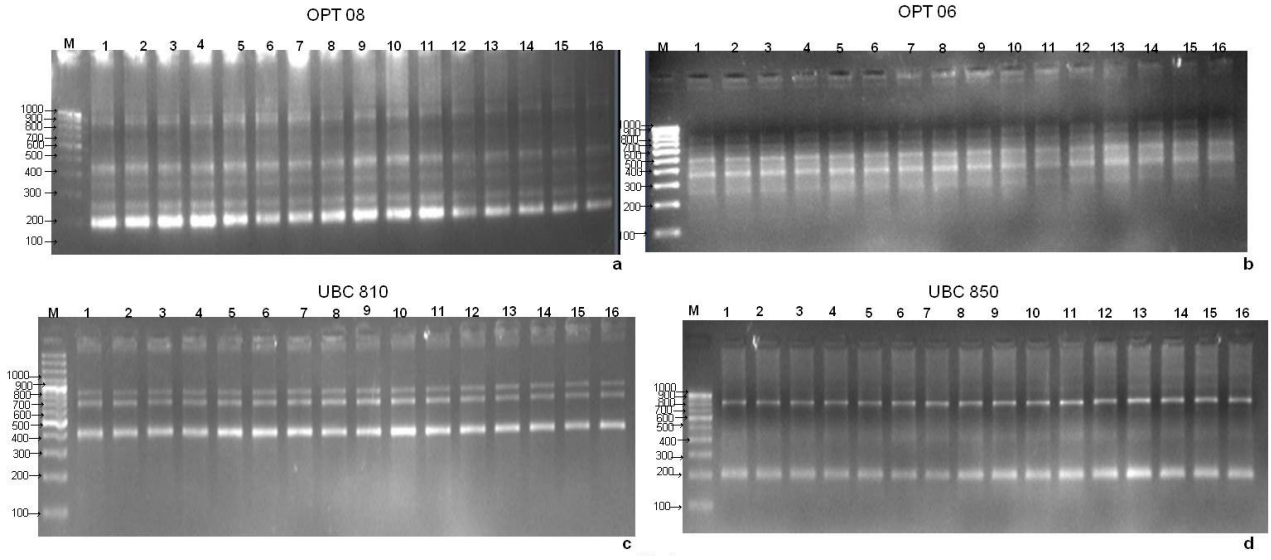

Figure 4 Amplifications produced using RAPD and ISSR markers; primers (a) OPT 08, (b) OPT 06, (c) UBC 810 and (d) UBC 850; lanes M = ladder, 1 = mother plant, 2–16 = tissue culture-raised plants

plants (Niklas & Enquist 2002) and thus accounting for more vigorous growth. This may be the possible reason for decreased levels of biomolecules content in ex-vitro grown plants in the present investigations. The selection of elite genotypes is the basis of clonal forestry and molecular markers have been proposed as tools for assessment of variation. Off types have been detected in clonal propagation in many species including *Rosa damascena* (Kaur et al. 2007) and *Camellia* spp. (Devarumath et al. 2002). Hence, it is important to regularly check the fidelity of in-vitro raised clones using different molecular markers. RAPD and ISSR results of the present investigations demonstrated that no major genetic variation occurred during in-vitro propagation through axillary bud proliferation in *B. balcooa*. This, once again, proved it to be the safest method to produce true to type plants (Das & Pal 2005, Agnihotri et al. 2009, Bopana & Saxena 2009, Negi & Saxena 2010).

CONCLUSIONS

Our study showed axillary bud proliferation as an effective means to propagate true to type progenies. Sucrose was found to significantly alter the efficiency of in-vitro propagation depending upon the type of species studied. However, role of biochemical, physiological and molecular factors in micropropagation of bamboos needs further investigation so as to modify culture conditions for better growth and survival. Clonal fidelity of tissue culture-raised and mother plants was established and hence, micropropagation protocol developed here can be used for large-scale multiplication.

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