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In Vitro Micropropagation of 54 Species from 15 Genera of Bamboo

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Abstract. **Fifty-four out of 67 species of bamboo tested were successfully propagated in vitro. For nearly every species, multiple shoots were produced from axillary buds on stem node segments cultured on Murashige and Skoog medium containing BA. In a very few species plants could be regenerated adventitiously from callus. This method of propagation was not very efficient or reliable. Rooting occurred in media containing NAA at 2.7 to 5.4 µM. Several species could be stored in vitro on half-strength medium at room temperature > 15 months without transfer. Chemical names used: N6-benzyl-amino purine (BA); napthyleneacetic acid (NAA).**

Bamboo is an economically important multipurpose crop. It is used as a source of food, fiber, building materials, and biofuel. Most bamboo is harvested from naturally occurring stands with minimal conservation or reforestation. Many species of bamboo are endangered because of harvesting and the lack of knowledge on propagation methods and flowering control (most species die after flowering). Current methods of bamboo propagation rely on culm cutting, rhizome division, or seed. These methods are expensive and inefficient. Micropropagation offers the potential for rapidly increasing select bamboo clones for reforestation and conservation. At a recent conference in Singapore there were several oral reports describing the successful use of tissue culture to microprop agate bamboo (Rao et al., 1990). In only two instances have sufficient data been obtained to allow the publication of the results (Huang et al., 1988; Hassan and Debergh, 1987). In our investigation, we studied the response of an additional 52 species and 12 genera (Table 1).

Explants consisted of either seed, inflorescences, stem-node sections, meristem domes, or leaves from underground shoots. Explants were sprayed with 70% ethanol, surface-sterilized for 30 min in 1% sodium hypochlorite, and washed three times in sterile water. All cultures were maintained at 25C with 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ cool-white fluorescent illumination for 16 $h \cdot day^{-1}$.

Node sections containing an axillary bud were placed on media containing Murashige and Skoog (1962) (MS) salts and vitamins supplemented with 88 µM sucrose, 6 g agar/liter, NAA (2.7, 5.4, or 10.8 µM) and BA (2.2, 4.4, 8.8, 22.0, or 44.0 µM). Leaf sections (1 cm^2) from young underground shoots were placed on MS medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) (4.5, 13.5, 27.0, 40.5, or 81.0 µM). Mature and immature seed were placed on MS medium supplemented with BA (0.44, 1.1, 2.2, 4.4, or 8.8 µM) and NAA (2.7 or 5.4 µM). In addition, 2,4-D (0.45, 2.3, 4.5, 9.0, 13.5, or 27 µM) was used with immature seed explants. Immature inflorescences containing flower buds smaller than 0.1 cm were cultured in darkness and light on MS medium containing 2,4-D (11.3, 22.5, or 45.0 µM) and NAA (5.4 µM). Meristem domes (0.1 cm) were excised from young shoots and placed on MS medium supplemented with 2,4-D (0.45, 2.3, 4.5, or 9.0 µM), NAA (0.54, 2.7, or 5.4 µM), BA (2.2 or 4.4 µM), and coconut water (10% or 20% v/v). Rooting experiments were conducted with full-strength, half-strength and quarter-strength media supplemented with NAA (13.5, 27.0, or 54.0 µM), BA (0.44, 4.4, or 8.8 µM), or 1-H-indolebutanoic acid (IBA) (2.5, 10.0, 12.5, or 25.0 µM).

Most species produced multiple shoots from

Fig. 1. Proliferating culture of *Dendrocalmus asper* clone 16. Culture was derived from an explant with shoots after 20 days on medium containing 13.2 µM BA.

Table 1. Species of bamboo used for micropropapation.

1.	*Arundinarea auriculata*	+[z]
	A. ciliata	–
	A. pusilla	+
	A. superecta	+
2.	*Atatea aztecorum*	–
3.	*Bambusa arundinacea*	+
	B. brandisii	+
	B. burmannica	+
	B. beechyama var. *pubescens*	–
	B. flexuosa	+
	B. gracilis	–
	B. glauescens	+
	B. humilis	–
	B. longispiculata	+
	B. multiplex[y]	+
	B. multiplex variegata	+
	B. nigra	+
	B. oldhamii[y]	+
	B. polymorpha	+
	B. venticosa	+
	B. venticosa variegata	+
	B. vulgaris[y]	+
	B. cv. Dam Khan[y]	+
	B. cv. Bong Ban[y]	+
	B. cv. Bong Naew[y]	+
4.	*Cephalostachym pergracile*[y]	+
	C. viratum	+
5.	*Dendrocalmus asper*[y]	+
	D. giganteus[y]	+
	D. latiflorus[y]	+
	D. membranaceus[y]	+
	D. sericeus	–
	D. strictus	+
	D. nutans[y]	+
	D. hamiltonii	–
	D. cv. Bong Kaiy	+
6.	*Dinochloa scandens*	+
7.	*Gigantochloa auricurata*	+
	G. apus	+
	G. albociliata	+
	G. compressa	+
	G. densa	–
	G. hasskarliama	+
	G. hosseusii	+
8.	*Hibanobambusa trianguillans*	+
9.	*Melocalamus compactiflorus*	–
10.	*Phyllostachys humilis*	–
	P. gramineus	+
	P. bambusoides	+
	P. nana	–
	P. nigra	–
	P. nigra f. *megurochiku*	+
	P. aurea	+
	P. sulphurea	+
	P. pubescens	+
11.	*Pleioblastus fortunee*	+
12.	*Pseudosasa japonica*	+
13.	*Oxytenenthera albociliata*	+
14.	*Sasa fortunei*	+
15.	*Sasaella suwekoana*	+
16.	*Semiarundinaria fastuosa*[y]	+
17.	*Schizostachym aciculare*	+
	S. brachycladium	+
	S. zollingeri	–
18.	*Thyrsostachys oliveri*[y]	+
	T. siamensis[y]	+

[z]+, Successfully micropropagated; –, unsuccessfully micropropagated.
[y]Species used to develop the micropropagation protocol.

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Table 2. The effects of BA and NAA on shoot proliferation from stem-node segments of a representative species (*Dendrocalamus asper*). Values are reported as the mean number of shoots produced per nodal explant after 30 days in culture.

NAA (μM)	BA concn (μM) 0	22	44	66
	Shoots produced (no.)			
0	0.8 bc[z]	2.1 a	2.6 a	3.0 a
2.7	0.6 bc	1.7 ab	2.3 a	2.6 a
5.4	0 c	1.7 ab	1.9 ab	2.6 a

[z]Means were obtained from 10 replicates and separated by Duncan's new multiple range test, $P = 0.05$.

Table 3. Effect of genotype on multiplication rate of proliferating cultures after the fifth subculture of three different clones of *Dendrocalmus asper*. Data were collected after a three-shoot explant was cultured for 20 days.

Clone	BA concn (μM)	Shoots (no.)	Shoot length (cm)	Leaves (no.)
3	13.2	10.5[z]	2.05	30.25
	22.0	15.5	1.84	30.75
16	13.2	39.0	2.05	51.25
	22.0	43.2	1.84	45.51
20	13.2	12.8	2.63	30.75
	22.0	21.0	2.55	60.75

[z]Means were obtained from 10 replicates.

the axillary bud on a stem node from young shoots when placed on medium containing 22 μM BA. Multiple shoots developed within 30 days. Other cytokinins [N-(3-methyl-2-butenyl)-1- *H*- purine-6-amine (2iP) and 6-furfurylaminopurine (kinetin)] and coconut water were inferior to BA in inducing proliferation. Addition of auxin (NAA) had little effect on the multiplication rate of most species (Table 2). *Arundinacea pusilla, Bambusa polymorpha, B. venticosa,* and *Sasaella suwekoana* required 5.4 μM NAA for proliferation. Several species, such as *B. multiplex, Gigantochloa ligulata, G. hosseusii, Dendrocalmus nutans,* and *Cephalostachym pergraciles,* had a high proliferation rate (>10 shoots per node). The degree of proliferation depended on the genotype. The amount of multiplication for three clones of *D. asper* varied 4-fold (Table 3). The degree of proliferation also depended on the propagule size. Propagules that contained at least three shoots proliferated at a maximum rate. Single shoots did proliferate, but at a much slower rate. The rate of proliferation increased after several subcultures. For example, in *D. asper* the first subculture resulted in about three shoots per propagule; while after the third subculture about nine shoots per propagule were observed (Fig. 1).

The shoots rooted on media containing NAA between 2.7 and 5.4 μM depending on the species and the length of time the shoots were in culture. Multiple shoot development occurred within 30 days, and roots appeared after 2 to 3 weeks. Several species could not be micropropagated because of the quality of the shoots; the shoots that formed were abnormal and few. Preliminary experiments have suggested that woody plant medium (Lloyd and McCown, 1981) might be able to induce multiplication of those species that could not be proliferated on MS medium.

All species formed callus from leaf sections placed on medium containing 13.5 to 27.0 μM 2,4-D. The presence of charcoal, BA, or casein hydrolsate decreased callus formation. Shoots could be regenerated only from leaf callus of *B. flexicosa.* Callus could also be produced from immature inflorescences of *B. oldmanii, D. latiflorus,* and *D. asper* cultured on media containing 13.5 to 27.0 μM 2,4-D. Shoots could be regenerated from these callus tissues on media containing 2.2 to 8.8 μM BA and 1.4 to 5.4 μM NAA at a ratio of 2:1. In addition, shoots could be produced directly if the explants were placed on medium containing 22 μM BA instead of 2,4-D.

Callus was produced from meristem domes cultured on medium containing 2,4-D and coconut water. The optimal concentrations varied with species. Quality and proliferation of such callus were inferior to callus derived from either leaves or immature inflorescences. We were not successful in producing regenerable callus using BA and NAA. Huang et al. (1988), however, found that regenerable callus could be produced from shoot tips on medium containing 5.4 μM NAA and 4.4 μM BA for *B. multiplex* and 5.4 μM NAA and 44 μM BA for *P. aurea.* This callus differentiated into shoots when cultured on 5.4 μM NAA and 13.2 μM BA for *B. multiplex* and 44 μM BA for *P. aurea.* They found, as we did, that cultures placed on media containing 2,4-D were not able to regenerate shoots. Hassan and Debergh (1987), however, found that *P. viridis* when cultured on 9 μM 2,4-D produced callus that was able to regenerate shoots when transfered to hormone-free medium.

Bamboo grew very rapidly in vitro and required frequent subculture. However, cultures could be maintained for > 1 year at 25C without subculture if placed on half-strength MS media. During the year, the older shoots turned brown and eventually died. Before the older shoots died, new small green shoots developed that did not elongate until subcultured on fresh medium. These shoots were regenerated into healthy, normal-appearing plants.

Flowering occurred sporadically during in vitro cultures of *B. nana, B. arundinacea, B.* sp. Dam Khan, *D. membranaceus, C. pergracil, B. glaucescens, B. brandisii,* and *B. multiplex* All shoots died after flowering, except those of *B. multiplex.* Culture conditions (light, medium, temperature, ect.) did not have any effect on the flowering response, which was random and at low frequency. Nadguada et al. (1990) have also reported in vitro flowering of *B. arundinacea, D. stricta* and *D. brandisii.* They were able to induce flowering by three subcultures on media containing 5% (v/v) coconut water and 2.2 μM BA.

We recommend micropropagating bamboo from axillary buds of young, nonflowering stems. These buds should be cultured as nodal segments on MS medium containing 22 μM BA. Resulting shoots should be rooted on MS medium containing 5.4 μM NAA. Inclusion of auxin (5.4 μM NAA) in proliferation medium may help multiply recalitrant species.

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