European Journal of Biotechnology and Bioscience
ISSN: 2321-9122, Impact Factor: RJIF 5.44
Received: 07-01-2021, Accepted: 22-01-2021, Published: 01-04-2021
www.biosciencejournals.com
Volume 9, Issue 2, 2021, Page No. 10-17

In vitro cloning of *Guadua angustifolia* kunth–an exotic, fast growing and industrially important bamboo species

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Abstract
Micropropagation studies were conducted on the effect of media, growth hormones for shoot initiation, shoot multiplication, *in vitro* and *ex vitro* rooting. Nodal explants from mature culms of *Guadua angustifolia* were used for shoot induction. Maximum of 4-5 shoots exhibited on MS liquid medium fortified with additives + NAA 0.25 mg/l + BAP 5.0mg/l. *In vitro* differentiated shoots were further multiplied on MS liquid medium supplemented with additives + NAA 0.25 mg/l + BAP 2.5 – 5.0 mg/l. The Shoot multiplication rate was 4-5 folds within a 4 week period. Liquid medium proved better than agar gel medium for shoot initiation and multiplication. *In vitro* raised propagules (2-3 shoots/clump) exhibited 100% *in vitro* rooting on MS/4 basal salts medium fortified with NAA 1.0 mg/l within a 4 week period. For *ex vitro* rooting, *in vitro* shoots were pulse treated with NAA (1000 ppm) for 5 mins and transplanted in the sand as rooting media. Pulse treated shoot clumps exhibited high frequency (95.55%) root induction. The survival rate of *in vitro* plants in the field was 100 percent at six months from planting.

Keywords: *Guadua angustifolia*, exotic, bamboo, cloning, mature clump, in *vitro* and *ex vitro* rooting

Introduction
The genus *Guadua* comprises 29 species, distributed in South America and the Philippines [1]. In India, it has been introduced in Forest Research Institute, Dehra Dun [2]. In Karnataka, the Bamboo Society of India, Bangalore, Karnataka India introduced it in India. Karnataka State Forest Department has planted few clumps in Research Field Station at Nallal near Bangalore, Karnataka India.

Guadua angustifolia Kunth is a large sized spectacular sympodial bamboo. The culm attains a height of 30m and a diameter of 20cm. The culm is dark green with white bands at the nodes, has short internodes and thorny branches. It is one of the fastest growing and most valuable species used for various purposes: scaffolding, building material, a source of pulp for the paper industry, making furniture and the young shoots are edible [3].It has many uses and supports local economy wherever it is grown. Guadua have a great potential to fix atmospheric carbon dioxide. Due to its versatility, lightness, flexibility, endurance, hardness, strength, climatic adaptability, seismic-resistance, rapid growth, and easy handling, it is widely employed in pharmaceutical, paper, charcoal, and construction industries [4]. It has excellent strength properties and is considered as one of the most useful, unexploited species for social forestry and agro forestry in India. *G. angustifolia* is propagated by seed and cuttings, but seed availability is uncertain [3]. For large-scale propagation (>50,000 plants/ year) of bamboo species, classical techniques of vegetative propagation are largely insufficient and inefficient and plant tissue culture is the only reliable attractive [5].

Very few studies have been reported on the micropropagation of *G. angustifolia* with a poor rate of shoot multiplication [6, 7, 8, 9, 10]. Micropropagation of various bamboo species through axillary shoot proliferation from explants of field grown clumps has been reported [11, 12, 13, 14, 15, 16, 17]. Commercially feasible micropropagation for large-scale propagation is in practice for the species of *Arundanaria*, *Chimono bambusa*, *Fragesia* and *Yushania* [5]. Large-scale production of planting material and field trials has been reported from seedling explants in *Bambusa bambos*, *B. tulda* and *Dendrocalamus strictus* [18, 19]. Swaroop and Gambier [20] reported field trials of micropropagated planting material of selected, industrially important bamboo species of India.

Based on the importance of the species, its demand, potential uses, limitation of the traditional methods of propagation and lack of complete protocol for large scale production through *in vitro* propagation, the present studies were initiated with the aim of developing a protocol for rapid and mass production of quality planting material of *G. angustifolia* through axillary shoot proliferation from selected mature clumps.

Materials and Methods
Collection of plant material, processing and surface sterilization
Nodal shoot segments were collected from mature plant (Clump) Culm branches from the research field station of Karnataka State Forest Department, Nallal, Bangalore (Fig. 1), during February to July. Shoot segments were swabbed with 70% ethanol using absorbent cotton to remove dust and reduce microbial load. Shoot segments were kept in polythene bags and placed in an icebox with ice packs and brought into the plant tissue culture laboratory at the Institute of Wood Science and Technology, Bangalore. Leaf sheath was removed and single node shoot segments of 2.0-3.0 cm length and 2-3 mm diameter were prepared (Fig. 2). Explants were at first surface sterilized with 70% ethanol (v/v) for 20-30 seconds, followed by washing 3-4 times with sterile distilled water under aseptic conditions under a laminar airflow bench. This was followed by surface sterilization with0.1% (w/v) Mercuric chloride for 4-5

minutes. Surface sterilized explants were thoroughly (6-8 times) washed with sterile distilled water. Explants were inoculated vertically in the medium for various experiments for shoot initiation. Cultures were kept under 25±2 º C temperature at 37.5µ.mol m^{-2} s^{-1} intensity light for 12 h photoperiod.

Fig 1: Shoot clumps (Source material from Karnataka Forest Department)

Fig 2: Nodal explants for shoot initiation

Shoot initiation

Effect of growth regulators

To work out the best auxin and cytokinin and their concentrations in the medium, various auxins[IAA, IBA(0.1mg/l) and NAA(0.1 and 0.25 mg/l]and cytokinins [Kn and BAP(1.0 -10.0 mg/l)] were used in MS [31] liquid medium with additives (ascorbic acid 25mg/l + citric acid 25mg/l + L-cystein25mg/l +glutamine100mg/l) for high frequency multiple shoot induction and subsequent shoot growth.

Effect of nutrient media

In order to ascertain optimum nutritional requirement for high rate of multiple shoot induction and subsequent shoot growth, various types of nutrient media *viz*; MS, MS/2, B5 [21], WP medium [22] and Hellers [23] were used in liquid form. Various liquid media were fortified with additives + NAA 0.25 mg/l +BAP 5.0 mg/l.

Shoot multiplication

Effect of growth regulators

Cytokinins; Kn and BAP (1.0 – 5.0 mg/l) were used with NAA (0.1 or 0.25 mg/l in MS liquid medium with additives to standardize cytokinin concentration for further shoot multiplications of the *in vitro* differentiated shoots.

Effect of various media

In order to arrive at the best nutrient medium for rapid shoot multiplication and better shoot growth, various nutrient media *viz*; MS, MS/2, WP, B5 and Heller's media in the form of liquid as well as agar gelled were used. Media were supplemented with additives + NAA 0.25 mg/l + BAP 5.0 mg/l for further shoot multiplication.

***In vitro* rooting**

Effect of various auxins and their concentrations

To work out the most suitable auxin and its concentration for high frequency rooting, MS/4 basal salts, agar-gelled medium with various auxins *viz*; IAA, IBA, NAA, NOA (0.5 – 2.5 mg/l) were tested. Clumps with 2-3 shoots were used for root induction.

***Ex vitro* rooting**

To bypass one step of *in vitro* rooting, single shoot as well as shoot clumps (2-3 shoots/clump) were used as inoculum for *ex vitro* rooting. Two experiments, *viz*; i) effect of various auxins and their concentrations and ii) rooting media were conducted for *ex vitro* rooting.

Effect of various auxins

To find out the best auxin to obtain a high rate of rooting, various auxins *viz*; IAA, IBA, NAA and NOA at 1000ppm were used separately in the form of pulse treatment for 5 minutes. Pulse treated shoot clumps (2-3 shoots/clump) with auxins solutions were planted in soilrite in iron trays (l18"xW12"xH6"). Soilrite was moistened with 0.1% (w/v) Bavestin (systemic fungicide) solution and covered by a plastic sheet to maintain high humidity inside the trays. Trays were kept in the green house at 30 $\pm$ 5º C temperature and 80 ±5% relative humidity. The plastic sheet was removed after 3 weeks from the trays.

Effect of rooting media

To find out the best rooting medium for *ex vitro* rooting from *in vitro* shoots, various rooting media *viz*; sand, soil, soilrite and vermiculite were tested either alone or in combination. Shoot clumps were pulse treated with IBA (1000ppm) for 5 minutes. Pulse-treated shoot clumps were planted in iron trays consist of various rooting media. Trays were covered with polythene sheet to maintain high humidity inside the trays. This experiment was conducted in the green house at 30 $\pm$ 5ºC temperature and 80 $\pm$ 5% relative humidity.

Simultaneously, pulse treated shoot clumps (2-3 shoot/clump) with IBA (1000 ppm for 5 minutes) were also planted directly in sand bed medium consisted of a bottom gravel layer and the top layer with sand. In order to maintain high humidity, shoots were covered with polythene sheet using iron frame (poly tunnel) inside the greenhouse.

Hardening and acclimatization

In vitro rooted shoots were hardened by two approaches. In the first approach, *in vitro* rooted shoots were carefully transferred into culture bottles consist of autoclaved and moisten hardening media; sand, soilrite, vermiculite and a mixture of these and kept in the greenhouse for 3 weeks.

Later the plantlets were transplanted into polybags and root trainers (600cc capacity) and kept in the Green House for3 weeks, followed by 2 weeks under 50% shade (using Agro shade net house) before keeping in to open nursery. In the second approach, *in vitro* rooted plantlets were transplanted directly into potting medium consisted of sand, soil, and compost (4:1:5, v/v) in polybags (600cc) and root trainers (600 cc). Initially, plantlets were covered by the polythene sheet inside the poly tunnel for 2 weeks in the greenhouse, followed by keeping for 2 weeks in 50% shade (in Agro shade net house).

Experimental design, data collection and statistical analysis

For shoot initiation, one nodal shoot segment in each culture tube was inoculated and 12 replicates were used for each treatment. Observations on a percentage of shoot induction and number of shoots per explant was recorded at two weeks from initiation. For shoot multiplication and rooting experiments, 4 shoot clumps (each of 2-3 shoots) were used in each culture bottle and 5 replicates were maintained for each treatment. Number of shoots produced from each shoot clump and shoot length was recorded after three-week period. In rooting experiments, percentage of rooting, root number, and root length were recorded after 4 weeks. For *ex vitro* rooting, 24 shoot clumps were used for each replicate and three replicates were used in each treatment. All the experiments were repeated three times to confirm the results. One-way Analysis of Variance (ANOVA) using Excel version 5.0 analyzed the data and means were compared using Tukey's t test at 0.05 % level of significance (Panse and Sukhatme, 1978). All the means are presented with ± SE

$SE= \sqrt{2me^2/r}$

CD= Tab x SE

Results and Discussion

Shoot initiation

Effect of growth regulators

Out of 22 treatments with auxins, IAA and IBA(0.1mg/l) and NAA(0. 1 or 0.25mg/l) and cytokinins, Kn and BAP (1.0-10mg/l) tested in MS liquid medium with additives, medium consist of NAA0.25mg/l + BAP5.0mg/l+ additives(acetic acid 50mg/l+ citric acid 25mg/l+ Cysteine 25mg/l+glutamin100mg/l) favored high rate (86.67 %) of multiple shoots (5.3 shoots/nodal shoot segment) induction within two weeks period (Table.1, Fig.3). Further increase in the concentration of BAP did not improve shoot number and length, but necrosis of shoots was observed. A lower concentration of Kn and BAP were not effective in multiple shoot induction. Jimènez *et al.*[8] Reported low rate (37.5%) of shoot initiation form nodal shoot segment obtained from greenhouse plants in MS agar gelled medium with BAP 3.0mg/l. Whereas in the present study, MS liquid medium with additives and combined use of NAA (0.25mg/l) and high BAP (5.0mg/l) produced 86.7% multiple shoots (4-5shoots/explant). Similar to our finding, Sanjay *et al.* [16] found that combined use of NAA + BAP favored more number of shoot and shoot length as compared to Kn or BAP alone in the MS liquid medium in *Pseudoxytenanthera stocksii*. They found that BAP was superior to Kn for shoot initiation. Chaturvedi *et al.* [13] found the axillary effect of IAA + adenine sulphate in MS medium on shoot initiation from nodal shoot segment of 10-year-old clump of *D. strictus.* However, Ravikumar *et al.* [24] and Mishra *et al.* [25] found that MS liquid medium with BAP + Kn + coconut milk was best for multiple shoot initiation in *Dendrocalamus strictus*. Bag *et al.* [15] found that combined use of IBA + BAP in MS medium was better for multiple shoot initiation in *Thamnocalamus spathiflorus*.

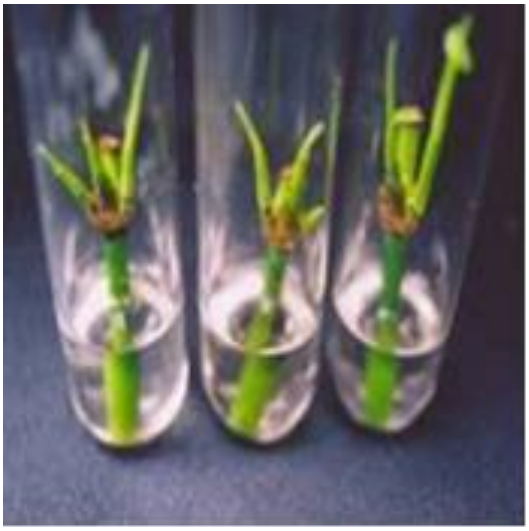

Fig 3: Shoot initiation

Table 1: Effect of growth regulators on shoot initiation from nodal shoot segment on MS liquid medium with additives

Treatments (PGR's mg/l)	Response (%)	No. of shoots/ explant	Length of shoots (cm)
Control (HF)	20.00±8.62^{e}	1.33±0.19^{i}	5.57±0.21^{a}
NAA 0.1 + BAP 1.0	33.33±8.62^{d}	1.67±0.19^{h}	3.69±0.21^{c}
IAA 0.1 + BAP 1.0	26.67±8.62^{e}	1.44±0.19^{h}	3.47±0.21^{cd}
IBA 0.1 + BAP 1.0	26.67±8.62^{e}	1.56±0.19^{h}	3.38±0.21^{cd}

NAA 0.1 + Kn1.0	26.67±8.62^{e}	1.43±0.19^{h}	3.03±0.21^{e}
NAA 0.1 + Kin 2.5	33.33±8.62^{d}	1.78±0.19^{gh}	3.17±0.21^{d}
NAA 0.1 + Kin 5.0	40.00±8.62^{d}	2.17±0.19^{f}	3.41±0.21^{cd}
NAA 0.1 + Kin 7.5	53.33±8.62^{b}	2.61±0.19^{ef}	3.53±0.21^{cd}
NAA 0.1 + Kin 10.0	40.00±8.62^{d}	2.16±0.19^{g}	3.38±0.21^{cd}
NAA 0.1 + BAP 2.5	46.67±8.62^{c}	3.00±0.19^{de}	3.73±0.21^{c}
NAA 0.1 + BAP 5.0	60.00±8.62^{b}	3.78±0.19^{c}	3.34±0.21^{d}
NAA 0.1 + BAP 7.5	66.67±8.62^{b}	4.31±0.19^{b}	2.19±0.21^{g}
NAA 0.1 + BAP10.0	53.33±8.62^{b}	3.44±0.19^{d}	1.81±0.21^{h}
NAA 0.25 + BAP 1.0	35.56±8.62^{d}	1.79±0.19^{g}	3.98±0.21^{c}
NAA 0.25 + BAP2.5	46.67±8.62^{c}	2.06±0.19^{g}	3.76±0.21^{c}
NAA 0.25 + BAP 5.0	80.00±8.62^{a}	4.8±0.191^{bc}	4.73±0.21^{ab}
NAA 0.25 + BAP 7.5	66.67±8.62^{b}	5.02±0.19^{a}	3.58±0.21^{c}
NAA 0.25 + BAP 10.0	52.33±8.62^{b}	4.33±0.19^{d}	2.61±0.21^{f}
NAA 0.25 + BAP 2.5+ add	48.67±8.62^{c}	3.69±0.19^{c}	5.06±0.21^{b}
NAA 0.25 + BAP 5.0+ add	86.67±8.62^{a}	5.30±0.19^{a}	4.67±0.21^{b}
NAA 0.25 + BAP 7.5+ add	73.33±8.62^{a}	5.26±0.19^{a}	3.93±0.21^{c}
NAA 0.25 + BAP 10.0+ add	64.44±8.62^{b}	4.78±0.19^{c}	3.32±0.21^{cd}
CD(0.05)	17.36	0.38	0.43

*Additives: Ascorbic acid 50mg/l+citric acid 25mg/l +L-cysteine25mg/l+ glutamine 100mg/l Treatment, followed by the same/alphabet does not differ significantly from each other.

Effect of various nutrient media

It was revealed from the results of the experiment on the effect of various liquid and agar gelled media (MS, MS/2, WP, B5 and HE) that liquid media proved better than the agar gelled media. Among the various media, MS liquid medium with additives + NAA 0.25 mg/l + BAP 5.0 mg/l proved the best and produced average 5.17 shoots/explant with 5.87 cm shoot length (Table 2).Whereas, MS agar gelled medium induced less number of shoots (3.46 shoots/explant) and shoot length (4.36 cm).

Sanjay *et al.* [16] reported multiple shoot initiation from nodal shoot segment of *P. stocksii* in MS liquid medium with NAA + BAP. Arya and Sharma [26] used MS liquid and WP (woody plant medium) for high frequency shoot initiation from nodal shoot segments of 3-year-old clump of *Bambusa bamboos* with cytokinin (BAP/Kn) and auxin (NAA and IAA) either alone or in combination. They found that MS liquid medium was better than WP medium for high frequency multiple shoot initiation. Lin and Chang [27] used MS medium solidified with gelrite 2g/l and sucrose 3% with TDZ for multiple shoot initiation in *B. edulis* in nodal shoot segment from field grown culms. Similarly, in *D. strictus*, Chaturvedi *et al.* [13] used MS agar gelled medium with IBA + adenine sulphate for shoot initiation from nodal shoot segment of 10 year old clump and they obtained 2-4 Shoots from the nodal shoot segment.

Table 2: Effect of various nutrient media (liquid and agar gelled) supplemented with additives*+NAA0.25mg+BAP5.0mg/l on shoot initiation from nodal shoot segments

Treatments (Media)	Agar gelled		Liquid	
	Number of shoots/explant	Shoot length (cm)	Number of shoots/explant	Shoot length (cm)
MS	3.46±0.46^{a}	4.36±0.36^{a}	5.17±0.43^{a}	5.87±0.20^{a}
MS/2	1.69±0.46^{c}	2.80±0.36^{c}	3.68±0.43^{b}	4.35±0.20^{c}
WP	1.96±0.46^{c}	2.98±0.36^{b}	3.89±0.43^{a}	4.65±0.20^{c}
B5	2.68±0.46^{b}	3.60±0.36^{ab}	4.25±0.43^{a}	5.05±0.20^{b}
HE	1.43±0.46^{c}	2.35±0.36^{c}	2.27±0.43^{b}	3.70±0.20^{d}
CD (0.05)	1.13	0.75	1.33	0.32

*Additives: Ascorbic acid 50mg/l, citric acid 25mg/l, Cysteine 25mg/l and glutamine 100mg/l. Treatment followed by the same alphabet does not differ significantly from each other.

Shoot multiplication

Effect of growth regulators

Incorporation of additives *viz*; ascorbic acid (50mg/l) + citric acid (25mg/l) + Cysteine (25mg/l) + glutamine (100mg/l) in the medium with NAA+ BAP showed auxiliary effect on shoot multiplication and growth (12- 14 shoots/clump). Combined use of NAA (0.25mg/l) + BAP (5.0mg/l) in the medium was found to be best for shoot multiplication (12.97shoots/clump) from 3-4 shoots/clump in 3 weeks period (Table 3, Fig.4 a& b). Similar to shoot initiation, a combined use of NAA + BAP and additives in the MS liquid medium proved better than NAA + Kinetin (Kn). Low rate (2.5 fold) of shoot multiplication was observed in MS agar gelled medium with BAP 3.0mg/l in 6 weeks period in *G. angustifolia* [8]. Marulanda *et al.* [29] found an average multiplication rate of 2 shoots per original explants for *G. angustifolia* cultured on solid media.

A high rate of shoot multiplication in the present studies is attributed to the use of liquid medium, inclusion of additives and combined use of NAA and BAP in the shoot multiplication medium. Saxena [29] tested various combinations of the auxins IAA, IBA, NAA and the cytokinins Kn, BAP and 2ip in MS liquid medium and found that MS liquid medium with BAP alone proved best for high rate (4-5 fold) of shoot multiplication in *B. tulda*. Chaturvedi *et al.* [13].

used IBA + adenine sulphate in MS agar gelled medium for shoot multiplication from *in vitro* differentiated shoots of *D. strictus* from field grown clump. However, on this medium, they found deterioration of shoot within 20 days and ultimate death. Incorporation of BAP, GA_3, casein hydrolysate and malt extract also attempted by them for shoot multiplication, did not help in multiplication of shoots. Shirgurkar *et al.* [30] and Ravikumar *et al.* [24] observed combined effect of Kn + BAP to be better for shoot multiplication in *D. strictus.*

Table 3: Effect of growth regulators on shoot multiplication and growth from shoot clump in MS medium with additives

Treatment (PGR's mg/l)	No. of shoots/clump	Shoot length (cm)
Control	2.70±0.79^{g}	4.30±0.27^{a}
NAA0.1+BAP0.5	8.22±0.79^{d}	3.70±0.27^{a}
NAA0.1+BAP2.0	9.30±0.79^{c}	3.40±0.27^{a}
NAA0.1+BAP2.5	9.93±0.79^{c}	3.09±0.27^{b}
NAA0.1+BAP5.0	10.23±0.79^{b}	2.70±0.27^{c}
NAA0.25+BAP1.0	9.36±0.79^{c}	3.92±0.27^{a}
NAA0.25+BAP2.5	11.5±0.79^{a}	3.65±0.27^{a}
NAA0.25+BAP5.0	12.97±0.79^{a}	3.43±0.27^{a}
NAA0.25+Kin1.0	5.10±0.79^{f}	2.80±0.27^{c}
NAA0.25+Kin2.5	6.50±0.79^{e}	2.75±0.27^{c}
NAA0.25+Kin5.0	7.89±0.79^{d}	2.20±0.27^{d}
CD(0.05)	1.60	0.54

Additives: Ascorbic acid 50mg/l, citric acid 25mg/l, Cysteine 25mg/l and glutamine 100mg/l. Treatment followed by the same alphabet does not differ significantly from each other.

Effect of various nutrient media

Liquid media proved better than the agar gelled media. MS liquid medium with additives + NAA 0.25mg/l + BAP 5.0mg/l produced maximum 10.16 shoots/ clump with shoot length of 3.8cm in 3weeks period. Shoot multiplication rate of 3 fold in 3 weeks in liquid MS medium was observed, whereas 2 fold multiplication rates was found in agar gelled medium. In general, B5, WP and HE media exhibited poor shoot multiplication in liquid as well as agar gelled media as compared to MS liquid and agar gelled media (Table 4 and Fig.4 a & b).

In accordance with our results, Saxena [29] reported that MS liquid medium was better than B_5liquid and agar gelled media with BAP on shoot multiplication in *B. tulda*. Similarly, In *Pseudoxytenanthera stocksii*, among the MS, WP and B5 liquid and agar gelled media used, MS liquid medium with additives +NAA + BAP favored high rate of shoot multiplication [17].

Table 4: Effect of nutrient media (liquid and agar gelled) supplemented with NAA 0.25mg/l+ BAP 5.0 mg/l +additives* on shoot multiplication from shoot clump

Treatments (media)	Agar gelled		Liquid	
	No. of shoots/clump	Shoot/length (cm)	No. of shoots/clump	Shoot/length (cm)
MS	7.46±0.86^{a}	3.20±0.36^{a}	10.16±0.83^{a}	3.80±0.20^{a}
MS/2	3.00±0.86^{c}	2.15±0.36^{b}	5.98±0.83^{c}	2.70±0.20^{c}
WP	4.67±0.86^{b}	2.50±0.36^{a}	6.50±0.83^{b}	2.75±0.20^{c}
B5	6.68±0.86^{a}	2.60±0.36^{a}	7.55±0.83^{b}	3.05±0.20^{b}
HE	2.73±0.86^{c}	1.80±0.36^{b}	4.27±0.83^{c}	2.35±0.20^{c}
CD (0.05)	1.79	0.75	1.73	0.42

Additives: Ascorbic acid 50mg/l, citric acid 25mg/l, Cysteine 25mg/l and glutamine 100mg/l. Treatment followed by the same alphabet does not differ significantly from each other

Fig 4: Shoot multiplication (a. Liquid medium; b. Agar gel medium)

***In vitro* Rooting**

Effect of auxins

Among the various auxins *viz*; IAA, IBA, NAA and NOA used in various concentrations (0.5 – 2.5mg/l) in MS/4 basal salts medium, medium consists of 1.0mg/l NAA favored 100% rooting with highest number (16.55) of the roots per shoot clump, followed by the NAA 2.5mg/l in 4 week period (Table 5 and Fig.5). Effect of various auxins on root induction and root numbers/clump was significantly higher compared to control. In an earlier report, spontaneous rooting has been observed in MS agar gelled medium [8].

Saxena [16] used MS, White's and modified MS (half reduced NH_4 NO_3) media with 2% sucrose and supplemented with IAA, IBA and coumarone, and solidified with gelrite (0.2%) for *in vitro* rooting from shoot clump and found that modified MS medium was the best in terms of rooting frequency and general condition of the shoots during rooting in *D. tulda*. Bag *et al.* [15] used MS/2 basal salts medium with 2% sucrose and IBA for *in vitro* rooting in *T. spathiflorus.* Arya and Sharma [26] used only MS medium with IBA or NAA for *in vitro* rooting from shoots of *B. bambos*. Similarly, in *D. asper*, Arya *et al.* [14] used only MS medium with IBA or NAA for root induction from shoot clump of seedling origin. Lin and Chang [27] used MS medium with TDZ and 2, 4-D for *in vitro* rooting from shoot clump of *B. edulis.*

Table 5: Effect of various auxins on *in vitro* rooting from shoot clump of *G.angustifolia* on MS/4 medium

Treatment (Auxin mg/l)	Response (%)	Roots		Shoots	
		Number of roots/clump	Root length (cm)	Number of Shoots/clump	Shoot length (cm)
MS/4 (no pgr's)	48.33±2.11^{j}	1.75±1.25^{g}	5.63±0.92^{a}	1.75±0.43^{c}	6.25±0.61^{a}

MS/4+IAA0.5	63.33±2.11^{h}	2.62±1.25^{g}	6.08±0.92^{a}	1.98±0.43^{c}	5.19±0.61^{a}
MS/4+IAA1.0	73.33±2.11^{f}	3.77±1.25^{e}	4.98±0.92^{a}	1.47±0.43^{d}	4.18±0.61^{bc}
MS/4+IAA2.5	78.33±2.11^{de}	3.03±1.25^{f}	5.52±0.92^{a}	1.28±0.43^{d}	5.59±0.61^{a}
MS/4+IBA0.5	68.33±2.11^{g}	2.33±1.25^{g}	6.51±0.92^{a}	1.68±0.43^{c}	4.82±0.61^{b}
MS/4+IBA1.0	78.33±2.11^{d}	3.88±1.25^{ef}	5.98±0.92^{a}	1.58±0.43^{c}	5.11±0.61^{a}
MS/4+IBA2.5	83.33±2.11^{c}	3.80±1.25^{ef}	6.31±0.92^{a}	2.15±0.43^{c}	5.27±0.61^{a}
MS/4+NAA0.5	91.67±2.11^{b}	10.90±1.25^{c}	4.47±0.92^{b}	3.30±0.43^{a}	4.96±0.61^{ab}
MS/4+NAA1.0	100.00±2.11^{a}	16.55±1.25^{a}	4.66±0.92^{ab}	4.06±0.43^{a}	4.71±0.61^{b}
MS/4+NAA2.5	100.00±2.11^{a}	12.30±1.25^{b}	3.46±0.92^{b}	3.65±0.43^{a}	5.35±0.61^{a}
MS/4+NOA0.5	56.67±2.11^{i}	3.15±1.25^{f}	4.62±0.92^{b}	2.45±0.43^{b}	4.46±0.61^{b}
MS/4+NOA1.0	58.33±2.11^{i}	3.60±1.25^{e}	2.23±0.92^{c}	1.77±0.43^{c}	3.99±0.61^{bc}
MS/4+NOA2.5	68.33±2.11^{g}	4.30±1.25^{e}	2.26±0.92^{c}	3.15±0.43^{b}	4.48±0.61^{b}
CD(0.05)	4.30	2.51	1.84	0.87	1.22

Treatment followed by the similar alphabets does not differ significantly from each other.

Fig 5: In Vitro rooted plantlets

Ex vitro rooting

Effect of auxins

Rooting percentage varied with auxins from 40.11% - 95.55%. Various auxin treatments significantly improved rooting frequency and root numbers/clump compared to control. Among the various auxins, pulse treated shoot clumps with NAA1000 ppm exhibited highest frequency (95.55%) of rooting and maximum number (4.56/clump) of roots, followed by IBA (90.56%) and IAA (73.33%). Among auxins, NOA proved least effective and induced 62.78% rooting in the shoot clump (Fig.6).

There is no report on *ex vitro* rooting in *G. angustifolia*. Saxena [29] attempted *ex vitro* rooting in *B. tulda* but did not get success. Ravikumar *et al.* [24] reported high rate of *ex vitro* rooting (70-80%) at high (85-90%) relative humidity and temperature (27-30ºC) conditions. They observed that 20-25 days period was required for initiation of roots.

Fig 6: *Ex Vitro* rooted plantlets

Effect of rooting media

Rooting media play a crucial role by providing physical support, retention of moisture, providing drainage and aeration. There was no significant difference in rooting percentage and root number in sand and Soilrite as a rooting medium. Relatively, sand exhibited highest rate of rooting (97.78%). Soil alone was not found suitable for rooting *ex vitro* rooting revealed that, sand, vermiculite and Soilrite were at par with each other. Sand is most economic and easily available. There is no earlier report on the effect of rooting media on *ex vitro* rooting in bamboo species.

Hardening and acclimatization

In vitro rooted shoots were hardened by two approaches: In the first approach, *in vitro* rooted plantlets were carefully transferred to culture bottles consists autoclaved and moisted hardening media (Sand, Soilrite, Vermiculite and mixture of these). Later, the plants were transplanted into containers (polybags and root trainers of 600cc capacity) and kept in a greenhouse for 2-3days followed by placement under shade (50%) before keeping in the open nursery. In the second approach, *in vitro* rooted shoots were transplanted directly into potting medium consists of sand soil, and compost (4:1:5, v/v) in polybags (600cc) and root trainers (600 cc). Initially, plantlets were covered by a polythene sheet for 3 weeks in a poly tunnel in the mist chamber. Survival rate was > 90 % during the hardening phase. Hardening was essential for 4 weeks in the greenhouse and 2 weeks under 50% shade (in Agro shade net house) before transferring to open nursery. Comparing the two approaches of hardening, the latter approach was better i.e. direct transplanting plants in container was better as it was less cumbersome. About 3000 micropropagated plants produced based on the protocol developed and used for field trials, (Fig.7) were provided to the Forest Departments of different States and private organizations. Protocol thus developed will be useful for rapid and mass production of Clonal planting material of *G. angustifolia* for establishment of industrial plantations and agro-forestry trials.

Fig 7: Hardened plants

Field trials

Initial field trial conducted at Gottipura exhibited 100 percent survival despite the trial base laid during off monsoon period. Plantlets maintained their growth phase; initially the average shoot numbers were 6.32 with shoot length 26.33cm at the time of planting. Five months after planting, shoot numbers were 15.84 with shoot length of 63.45cm.At 10 months from planting the shoot number are 18.93 with shoot growth 114.20(length) cm (Table 6 and Fig.8 & 9).

Table 6: Growth performance of micropropagated plants of *G. angustifolia* in the field after 5 months of planting

Treatments (Age of plants in months)	No. of shoots/clump	Length of shoots (cm)
Zero (at the time of planting)	3.62 ± 0.83^{c}	26.33^{c}
Five	15.84 ± 0.83^{b}	63.45^{b}
Ten	18.93 ± 0.83^{a}	114.20^{a}
CD (0.05)	2.04	15.55

Treatment followed by the similar alphabets does not differ significantly from each other.

Fig 8: Five months old plant

Fig 9: Ten Months Old Plant.

Conclusion

Due to increasing demand of bamboo and its products exponentially and to circumvent cumbersome convention propagation methods. The present study will be useful for large-scale production of high-quality plants of *G. angustifolia* for plantations and establishment of Germplasm in short duration of time.

Acknowledgement

Authors are thankful to the Director and Group coordinator research, Institute of Wood Science and Technology, Bengaluru, for encouragement to carry out the present study. Karnataka State Forest Department, Bengaluru, is acknowledged for providing the plant material of *Guadua angustifolia.*

References

1. Grosser D, Liese. Present status and problems of bamboo classification. Amold Arboretum,1973:54(2): 93-308.
2. Bennet SSR, Gaur RC. "*Thirty seven bamboos growing in India".* Forest research, Institute, Dehra Dun, India 1990, 75.
3. McClure FA. "*The Bamboos a fresh perspective"*, Harvard University Press, Cambridge, Massachusetts, USA 1966, 347.
4. Harleen Kaur Nadha, Richa Salwan, Ramesh Chand Kasana, Manju An and, and Anil Sood. Identification and elimination of bacterial contamination during *in vitro* propagation of *Guadua angustifolia* Kunth. Pharmacogn Mag,2012:8(30):93-97.
5. Geilis J, Oprins J. The strategic role of biotechnology in mass scale production of woody bamboos. In: *Sustainable agriculture for food, energy and industry*, El Bassam, N., Bhel, R. K., and Prochnow, B. (eds.), James and James, London 1998, 165-171.
6. Manzur D. Bosques de guadua (Bambusa) en el laboratorio. Rev. Agric. Trop. (Bogotá) 1989, 53-55.
7. Marulanda MM, Gutierrez LG, Márquez M. Micropropagación de *Guadua angustifolia* Kunth. Actual. Biol,2005:27:5-15.
8. Jimenez VM, Castillo J, Tavares E, Guevara E, Montiel M. *In vitro* propagation of the Neotropical giant bamboo, *Guadua angustifolia* Hunth, through axillary shoot proliferation. Plant Cell Tiss. Org. Cult. 2006:86:389-395.
9. Daquinta M, Gregori A, Cid M, Lezcano Y, Sagarra F. Formación de callos e inducción de brotes a partir de tejido intercalar de ramas de plantas adultas de *Guadua angustifolia* Kunth. Biotecnol. Veg.2007:7:119-122.
10. Luis Gonzaga Gutierrez, Rodolfo Lopez-Franco, Tito Morales-Pinzon. Micropropagation of *Guadua angustifolia* Kunth (Poaceae) using a temporary immersion system RITA®. African journal of Biotechnology,2016:15(28):1503-1510.
11. Hassan AAEL, Debergh P. Embryogenesis and plantlet development in the bamboo *Phyllostachys viridis* (Young). McClure. Plant Cell Tissue and Organ Culture.1987:10:73-77.
12. Prutpongse P, Gavinlertvatana P. *In vitro*

micropropagation of 54 species from 15 Genera of bamboo. Hort. Sci,1992:27(5):453-454.
13. Chaturvedi HC, Sharma M, Sharma AK. *In vitro* regeneration of *Dendrocalamus strictus* Nees through nodal segment taken from field grown culm. Plant Sci,1993:91:97-101.
14. rya S, Sharma S, Kaur R, Arya ID. Micropropagation of *Dendrocalamus asper* by shoot proliferation using seeds. Plant Cell Rep,1999:18:879-882.
15. Bag N, Chandra S, Palani LMS, Nandi SK. Micropropagation of Devringal (*Thamnocalamus spathiflorus* (Trin) Munro) – a temperate bamboo and comparison between *in vitro* propagated plants and seedlings. Plant Sci,2000:156:125-135.
16. Sanjaya, Rathore TS, Rai VR. Micropropagation of *Pseudoxytenanthera stocksii* Munro. *In vitro* Cell. Dev. Biol-Plant.2005:41:333-337.
17. Somashekar PV, Rathore TS, Shashidhar KS. Rapid and simplified method of micropropagation of *Pseudoxytenanthera stocksii. Forest biotechnology in India. Ed*. Ansari *et al.*, Satish serial publishing house. Delhi 2008, 165-182.
18. Rao IU, Rao IVR, Narang V, Jerath R, Pillai KG. Mass propagation of Bamboo from somatic embryos and their successful transfer to forest. In: *Bamboo Current Research, Proc.of Intl. Bamboo Workshop*, [Nov. 14-18] 1988, KFRI, Peechi 1990, 167-172.
19. Saxena S, Dhawan V. Regeneration of large-scale propagation of bamboo (*Dendrocalamus strictus* Nees) through somatic embryogenesis. Plant Cell Report,1999:18:438-444.
20. Swarup R, Gambhir A. Mass production, certification and field evaluation of bamboo planting stock produced by tissue culture. In: proceeding of the international conference on improvement of bamboo productivity and marketing for sustainable livelihood, April 15 to 17th, 2008, New Delhi, Published by Cane and Bamboo Technology Centre, Guwahati 2008, 22-27.
21. Gamborg OL, Miller RA, Ojima K. Nutrient requirements of suspension cultures of soyabean root cells. Exp. Cell. Res,1968:50:151-158.
22. Lloyd G, McCown BH. Commercially-Feasible Micropropagation of Mountain Laurel, *Kalmia latifolia*, by Use of Shoot-Tip Culture. Combined Proceedings-International Plant Propagator's Society,1980:30:421-427.
23. Heller R. Recherchessur la nutrition minerale de tissues veg etaucultivees *in vitro*. Ann. Sci. Nat. Bot. Et Bio. Veg. 11th Series,1953:14:1-223.
24. Ravikumar R, Ananthakrishnan G, Kathiravan K, Ganapathi A. *In vitro* propagation of *Dendrocalamus strictus* Nees. Plant Cell Tissue and Organ Cult,1998:52:189-192.
25. Mishra Y, Ram PK, Shirin F, Ansari SA. Augmenting *in vitro* shoot multiplication by vipul (triacontanol) and adventitious rhizogenesis by rice bran extract in *Dendrocalamus strictus*. Ind. J. Exp. Biol,2001:39(2):148-154.
26. Arya S, Sharma S. Micropropagation technology of *Bambusa bambos* through shoot proliferation. Ind. For,1998:124:725-731.
27. Lin CS, Chang WC. Micropropagation of *Bambusa edulis* through nodal explants of field grown culms and flowering of regenerated plantlets. Plant Cell Rep,1998:17:617-620.
28. Marulanda ML,Carvajalino M, Vargas C, Londono X. La biotechnologia apliadal al studio y aprocechaniento de la Guadua. In: Seminario-Taller Avances en la Investigacion sobre Guadua,Pereira, Colombia, 2002, 5.
29. Saxena S. *In vitro* propagation of the bamboo (*B. tulda* Roxb.) through shoot proliferation. Plant cell Rep,1990:13:683-686.
30. Shirgurkar MV, Thengane SR, Poonamwala IS, Jana MM, Nadagauda RS, Mascarenhas AF. A simple *in vitro* method of propagation and rhizome formation in *Dendrocalamus strictus* Nees. Current Science,1996:70:940-943.
31. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant,1962:15:473-497.