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IN VITRO REGENERATION OF *BAMBUSA VULGARIS* VAR. *STRIATA* USING NODAL EXPLANTS

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Abstract

The current study aimed to develop an effective micropropagation protocol for *Bambusa vulgaris* var. *striata* (Lodd. ex Lindl.) Gamble through direct organogenesis using nodal explant. The maximum axillary bud break (84%) with the greatest number of shoots per explant (5.20 ± 0.37) was achieved using liquid MS medium containing 2.5 mg/l BAP and 1.5 mg/l TDZ, while the maximum length of shoots (7.12 ± 0.27 cm) was induced on 2.0 mg/l BAP. Shoots were multiplied in stirred liquid MS medium with 3.0 mg/l BAP, resulting in the highest number of shoots per culture (13.20 ± 0.58) with the shoot length of 7.32 ± 0.48 cm. BAP in combination with TDZ resulted in a feeble response to shoot proliferation; although in some cases, the presence of TDZ with BAP was inhibitory. The shoots progressively increased in number and length over the first four subculture cycles in the aforementioned medium, but declined in the 5th sub-culture cycle. Following the 4th sub-culture cycle, each culture produced 16.60 ± 0.60 shoots with an average length of 8.96 ± 0.61 cm. Adding 10% coconut water to the medium resulted in excellent shoot growth and development, with an average of 18.40 ± 1.44 shoots per culture, measuring 8.56 ± 0.79 cm. Half-strength MS medium with 3.0 mg/l IBA resulted in the highest rooting percentage (85%) and the most roots per culture (8.00 ± 0.71). Successful acclimatization of well-rooted clumps of 3-4 shoots was achieved in a mixture of soil, sand, and compost (2:1:1) with 85% survival rate.

Introduction

Bambusa vulgaris var. *striata* (Lodd. ex Lindl.) Gamble (Poaceae) is the most frequently grown species in the genus *Bambusa*, which contains roughly 157 species overall and a number of infra-specific taxa known as varieties, including aureovariegata, striata, waminii, vittata, latifolia, and others (The Plant List 2010, Sharma *et al*. 2014). *Bambusa vulgaris* var. *striata* (Lodd. ex Lindl.) Gamble is a medium sized tropical and subtropical clumping bamboo native to China and the Indochina regions. It is the most visually appealing of the aforementioned varieties owing to its bright yellow internodes that are randomly marked with wide and narrow green vertical stripes (Jhon and Nadgauda 2007). It is widely cultivated as an ornamental in towns and is often used as attractive hedges along property borders (Ohrnberger 1999, Roxas *et al*. 2000). It is also widely grown as a source of pulp and paper-making materials, and its culms are used to make poles, fences, and props, exquisite handicrafts, small irrigation pipes, water or other liquid containers, and baskets (Roxas 2012, Mulatu *et al*. 2016, Junior *et al*. 2019).

Regeneration of *B. vulgaris* var. *striata* through seeds is impractical since it flowers irregularly and does not set seeds because the lemma and palea fail to open properly and the pollen is not viable (John and Nadgauda 1993). This variety can only be propagated by vegetative means, such as cutting, layering, offset planting, and stump sprouting, with rhizome-cutting being the most prevalent (Satapathy *et al*. 2020). However, vegetative propagation methods have some limitations for large-scale propagation such as, propagule extraction being laborious and cumbersome to transport, having inadequate numbers and a reduced survival rate, and not always

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being available due to seasonal specificity (Singh *et al*. 2012, Mudoi *et al*. 2013). So, the ineffectiveness of the sexual and vegetative propagation method is one of the challenges in the commercial production of this ornamental bamboo variety. In this regard, propagation using tissue culture appears to be the most effective means for its large-scale propagation. Thus, the current study was mainly focused on developing an efficient *in vitro* regeneration protocol for large-scale production of plantlets of the economically significant ornamental bamboo *B. vulgaris* var. *striata* using nodal explants.

Materials and Methods

The nodal segments with pre-existing axillary buds from *Bambusa vulgaris* var. *striata* were employed as explants to initiate the *in vitro* culture. This specific variety of bamboo (Accession no. DACB 87300) was successfully identified by the authorities of the Bangladesh National Herbarium located in Mirpur, Dhaka. Explants were surface sterilized following the approach of Sharothi *et al*. (2022), with minor modifications. Then they were inoculated separately in liquid MS medium (Murashige and Skoog 1962) containing varying strengths of BAP alone or in conjunction with TDZ for direct shoot induction. Directly induced shoots were subdivided into smaller clumps, each with 2-3 shoots, and then cultured on freshly prepared MS medium containing various strengths of BAP singly or in combinations with Kn, TDZ, and NAA for shoot multiplication. This experiment also investigates the effect of successive sub-culture cycles and varying coconut water concentrations (5-15%) on shoot multiplication. The cultures were subjected to a 16 hrd light and 8 hrs dark cycle daily at a constant temperature of 24 ± 2°C, with a light intensity of 2000-3000 lux.

To promote roots, shoots of about 6-7 cm were divided into shoot clumps (3-4 shoots) and cultured on half-strength MS medium fortified with different concentrations of IBA and NAA, either singly or in combination. For root induction, the newly transferred cultures were kept in dim light for 3-4 days and then they were kept in the light. The plantlets with strong and stout root system were acclimatized using a method followed by Raju *et al*. (2023). Afterward, fully established plants in the soil condition were transferred to larger pots for further growth and development. The means and standard errors of all dependent variables were analysed by one-way analysis of variance (ANOVA) using SPSS version 16.0 software followed by a post hoc comparison of means using DMRT with a significance level of 5%.

Results and Discussion

During direct shoot induction, the combined effect of BAP and TDZ outperformed the single effect of BAP in terms of responding explants and shoot bud induction per culture. Although the nodal explants sprouted in all media combinations, the liquid MS medium containing 2.5 mg/l BAP with 1.5 mg/l TDZ exhibited the maximum percentage of direct shoot induction (84%) and the highest number of shoots per explant (5.20 ± 0.37), after 20-25 days of culture (Table 1, Fig. 1a). Similar studies by Raju *et al*. (2023), Sharothi *et al*. (2022), and Raju and Roy (2016) found that liquid MS medium in combination with 1.5-2.0 mg/l BAP and 1.0 mg/l TDZ was sufficient for direct shoot induction in the cases of *Dendrocalamus giganteus*, *B. tuldoides* and *B. bambos*, respectively. However, the directly induced shoots reached their maximum length of 7.12 ± 0.27 cm, when 2.0 mg/l BAP was given singly to the medium (Table 1; Fig. 1b). These results agree with the findings of Mishra *et al*. (2022) in the case of *B. nutans*, and Chavan *et al*. (2021) in the case of *B. balcooa*.

Table 1. Effects of different BAP concentrations, either alone or with TDZ, on direct shoot induction from nodal explants of *Bambusa vulgaris* var. *striata*.

Growth regulators (mg/l)	Responding explants (%)	No. of shoots/explant (Mean ± S.E.)	Shoot length (cm) (Mean ± S.E.)
BAP			
1.0	36	$2.20^{b} \pm 0.20$	$2.92^{d} \pm 0.34$
1.5	56	$3.00^{ab} \pm 0.45$	$4.94^{bc} \pm 0.63$
2.0	**76**	$\mathbf{4.00^{a} \pm 0.63}$	$\mathbf{7.12^{a} \pm 0.27}$
2.5	60	$3.20^{ab} \pm 0.37$	$5.22^{b} \pm 0.56$
3.0	48	$2.60^{b} \pm 0.40$	$3.74^{cd} \pm 0.49$
3.5	40	$2.60^{b} \pm 0.24$	$3.42^{d} \pm 0.44$
BAP+TDZ			
1.0+1.0	24	$1.60^{e} \pm 0.40$	$1.80^{f} \pm 0.14$
1.0+1.5	28	$2.00^{de} \pm 0.32$	$1.96^{f} \pm 0.16$
1.5+1.0	32	$2.40^{cde} \pm 0.24$	$2.30^{ef} \pm 0.15$
1.5+1.5	40	$2.20^{de} \pm 0.49$	$2.94^{de} \pm 0.33$
2.0+1.0	60	$3.00^{bcde} \pm 0.63$	$4.32^{bc} \pm 0.22$
2.0+1.5	68	$3.80^{b} \pm 0.37$	$4.54^{b} \pm 0.29$
2.5+1.0	72	$3.60^{bc} \pm 0.40$	$5.14^{ab} \pm 0.35$
2.5+1.5	**84**	$\mathbf{5.20^{a} \pm 0.37}$	$\mathbf{5.68^{a} \pm 0.45}$
3.0+1.0	52	$2.80^{bcde} \pm 0.37$	$3.56^{cd} \pm 0.38$
3.0+1.5	60	$3.20^{bcd} \pm 0.37$	$4.38^{bc} \pm 0.35$
3.5+1.0	32	$1.80^{de} \pm 0.58$	$2.28^{ef} \pm 0.31$
3.5+1.5	44	$2.40^{cde} \pm 0.40$	$3.14^{de} \pm 0.18$

Values with different letters within a column represent significant difference at 5% level by DMRT.

The inclusion of various concentrations and combinations of growth hormones in the medium had an effect on the proliferation rate of the directly induced axillary shoot. However, BAP was proved to be the most effective cytokinin for *B. vulgaris* var. *striata* shoot proliferation out of all the cytokinin types studied. A maximum of 88% of the cultures in the stirred liquid MS medium containing only 3.0 mg/l BAP showed shoot proliferation, resulting in 13.20 ± 0.58 shoots per culture with an average length of 7.32 ± 0.48 cm (Table 2, Figs 1c and 1d). However, an increase or decrease of BAP concentration over 3.0 mg/l reduces the multiplication rate (Table 2). This is consistent with the findings of Ramanayake *et al*. (2006), who studied similar varieties of bamboo and observed continuous shoot proliferation in MS medium fortified with 4.0 mg/l BAP only. Similar to this, Saini *et al.* (2016) demonstrated that *Drepanostachyum falcatum* was able to produce 7-9 folds shoot multiplication on MS medium containing 3.5 mg/l BAP.

The results of this study also indicated that using BAP alone was more effective than combining it with other hormones like Kn, TDZ, and NAA. Moreover, BAP in conjunction with TDZ resulted in a very weak response to shoot proliferation; in some cases, the presence of TDZ with BAP was inhibitory (Table 2). Whereas, Raju and Roy (2016), Sharothi *et al*. (2022), and Raju *et al.* (2023) reported 84-90% shoot proliferation in *B. bambos*, *B. tuldoides*, and

Dendrocalamus giganteus on MS medium containing 2.0-3.0 mg/l BAP with 1.0-1.5 mg/l TDZ, respectively. The current study also found that the combination of BAP with Kn and NAA in shoot multiplication resulted in a moderate multiple shoot formation (Table 2). Whereas, Waikhom and Louis (2014) reported that, the combined effect of BAP and Kn increased the shoot multiplication rate in *B. tulda* and *Melocanna baccifera.*

Table 2. Effects of BAP, either alone or with Kn, TDZ, and NAA on shoot proliferation of *Bambusa vulgaris* var. *striata.*

Growth regulators (mg/l)	Percentage of shoot proliferation	No. of regenerated shoots (Mean ± S.E.)	Shoot length (cm) (Mean ± S.E.)
Control	00	00	00
BAP			
1.0	24	$2.60^{d} \pm 0.24$	$2.70^{c} \pm 0.37$
1.5	36	$3.00^{d} \pm 0.32$	$2.94^{c} \pm 0.44$
2.0	60	$4.80^{c} \pm 0.37$	$3.78^{bc} \pm 0.57$
2.5	72	$7.00^{b} \pm 2.45$	$4.54^{b} \pm 0.64$
3.0	**88**	**$13.20^{a} \pm 0.58$**	**$7.32^{a} \pm 0.48$**
3.5	64	$6.40^{b} \pm 0.68$	$4.02^{bc} \pm 0.40$
4.0	56	$4.00^{cd} \pm 0.63$	$3.62^{bc} \pm 0.34$
BAP+Kn			
1.0+0.5	32	$3.00^{b} \pm 0.45$	$3.16^{b} \pm 0.52$
1.0+1.0	44	$3.40^{b} \pm 0.51$	$3.66^{ab} \pm 0.31$
2.0+0.5	60	$4.40^{b} \pm 0.75$	$4.56^{ab} \pm 0.49$
2.0+1.0	64	$4.00^{b} \pm 0.63$	$4.74^{ab} \pm 0.60$
3.0+0.5	**72**	**$7.20^{a} \pm 1.07$**	**$5.04^{a} \pm 0.68$**
3.0+1.0	56	$4.60^{b} \pm 0.68$	$4.36^{ab} \pm 0.41$
4.0+0.5	48	$3.40^{b} \pm 0.75$	$3.28^{b} \pm 0.50$
4.0+1.0	32	$3.00^{b} \pm 0.32$	$3.16^{b} \pm 0.36$
BAP+TDZ			
1.0+0.5	16	$2.40^{ab} \pm 0.24$	$2.74^{a} \pm 0.27$
1.0+1.0	24	$2.20^{b} \pm 0.37$	$2.66^{a} \pm 0.28$
1.0+1.5	00	00	00
2.0+0.5	32	$2.80^{ab} \pm 0.58$	$2.78^{a} \pm 0.25$
2.0+1.0	40	$3.80^{a} \pm 0.49$	$3.32^{a} \pm 0.22$
2.0+1.5	00	00	00
3.0+0.5	36	$3.20^{ab} \pm 0.58$	$3.12^{a} \pm 0.24$
3.0+1.0	00	00	00
3.0+1.5	00	00	00
BAP+NAA			
1.0+0.2	24	$2.40^{d} \pm 0.24$	$2.94^{c} \pm 0.31$
1.0+0.5	40	$2.80^{cd} \pm 0.37$	$3.76^{bc} \pm 0.48$
2.0+0.2	44	$3.80^{cd} \pm 0.37$	$3.72^{bc} \pm 0.48$
2.0+0.5	52	$4.60^{bcd} \pm 0.98$	$4.20^{bc} \pm 0.62$
3.0+0.2	60	$6.60^{ab} \pm 0.51$	$5.16^{ab} \pm 0.62$
3.0+0.5	**76**	**$7.80^{a} \pm 0.97$**	**$5.86^{a} \pm 0.48$**
4.0+0.2	52	$4.40^{bcd} \pm 0.68$	$3.92^{bc} \pm 0.16$
4.0+0.5	56	$4.80^{bc} \pm 1.16$	$4.08^{bc} \pm 0.35$

Values with different letters within a column represent significant difference at 5% level by DMRT.

Fig. 1. *In vitro* regeneration of *Bambusa vulgaris* var. *striata* through direct organogenesis. a-b. Direct shoot induction on liquid MS medium with 2.5 mg/l BAP + 1.5 mg/l TDZ (a); 2.0 mg/l BAP (b), c-e. Multiple shoot formation in liquid MS medium with 3.0 mg/l BAP after 15 days of 1st subculture (c); 30 days of 1st subculture (d); 30 days of 4th subculture (e), f. Incorporation of 10% CW with the aforementioned medium for rapid shoot proliferation, g. *In vitro* rooting on half-strength of solid and liquid MS medium with 3.0 mg/l IBA, h. Complete plantlets, i. 2-weeks old hardened plants, j. Fully acclimatized plants in the clay pot after 6-months.

To investigate the influence of periodic sub-culture on shoot multiplication, fresh shoots from the multiplication stage were dissected into segments comprising 2-3 shoots and sub-cultured into the responsive optimum medium for multiplication. The number and length of shoots increased progressively throughout the first four sub-cultures, and then declined in the 5th sub-culture. After the 4th subculture cycle, the maximum number and length of regenerated shoots per culture were 16.60 ± 0.60 and 8.96 ± 0.61 cm, respectively (Table 3, Fig. 1e). Similarly, Hossain *et al.* (2018) and Sharothi *et al*. (2022) showed that the number and length of shoots gradually increased over the first four subcultural cycles of shoot multiplication in *B. tuldoides* and *D. giganteus*. However, Mudoi *et al*. (2014) maintained the shoot multiplication rate in *B. nutans* up to the 6th sub-culture cycle.

The addition of coconut water considerably increased the rate of multiple shoot formation. With the highest number of regenerated shoots per culture was observed 18.40 ± 1.44 when 10% CW was added to the stirred liquid MS medium with 3.0 mg/l BAP. The average shoot length in the same combination was found to be 8.56 ± 0.79 cm, although the length of the shoot was not significantly affected by the different concentrations of coconut water (Table 4, Fig. 1f). However, as the concentration of coconut water increases over 10%, both the number of regenerated shoots per culture and the length of the shoots decreased. Similar to this, Sharothi *et al*. (2022) in *B. tuldoides* and Raju and Roy (2016) in *B. bambos* found that using 10% coconut water in the medium was the most efficient in increasing shoot proliferation rate. Although Raju *et al*. (2023) in *D. giganteus* and Das and Pal (2005) in *B. balcooa* reported that 8% coconut water increased shoot proliferation.

Table 3. Effects of repeated sub-culture cycles on shoot proliferation of *Bambusa vulgaris* var. *striata* in liquid MS medium with 3.0 mg/l BAP.

Sub-culture cycle	No. of regenerated shoots (Mean ± S.E.)	Percentage of change in number of shoots per subculture	Shoot length(cm) (Mean ± S.E.)
1st	13.20^{b} ± 0.58	0	7.32^{b} ± 0.48
2nd	14.00^{b} ± 0.84	+ 6.06	7.74^{ab} ± 0.47
3rd	15.40^{ab} ± 0.68	+ 10.00	8.42^{ab} ± 0.34
4th	**16.60^{a} ± 0.60**	**+ 7.79**	**8.96^{a} ± 0.61**
5th	14.20^{b} ± 0.92	- 14.46	7.88^{ab} ± 0.49

Values with different letters within a column represent significant difference at 5% level, by DMRT.

Table 4. Effects of coconut water with constant 3.0 mg/l BAP and 3% sucrose on shoot proliferation of *Bambusa vulgaris* var. *striata* in liquid MS medium.

Coconut water (%)	No. of regenerated shoots (Mean ± S.E.)	Shoot length (cm) (Mean ± S.E.)
0	13.20^{b} ± 0.58	7.32^{a} ± 0.48
5	15.80^{b} ± 0.49	7.78^{a} ± 0.69
10	**18.40^{a} ± 1.44**	**8.56^{a} ± 0.79**
15	13.80^{b} ± 0.58	7.54^{a} ± 0.47

Values with different letters within a column represent significant difference at 5% level by DMRT

Regardless of the liquid or solid medium, considerable rooting was achieved within 2-3 weeks when 3.0 mg/l IBA was used, resulting in 85% rooting efficiency and 8.00 ± 0.71 roots per culture with 10.06 ± 0.43 cm in length (Table 5, Fig. 1g). When the concentration of IBA exceeded 3.0 mg/l, the rate of root induction dropped (Table 5). Confirming results have been reported by Sharothi *et al*. (2022) on *B. tuldoides*, Venkatachalam *et al*. (2015) on *B. arundinacea*, and Waikhom and Louis (2014) on *B. tulda*. They observed that increasing the concentrations of IBA up to 3.0 mg/l promoted rooting. Compared to the IBA-containing medium, there was less rooting on the NAA-containing medium, even the interactive effect of IBA with NAA was less satisfactory (Table 5). In contrary, Maiya *et al*. (2021) in *B. nutans*, Nurhayani *et al*. (2018) in *B. balcooa*, and Raju and Roy (2016) in *B. bambos* observed maximum number of rooting by using the combination of IBA and NAA.

The rooted plantlets of *B. vulgaris* var. *striata* (Fig. 1h) were efficiently acclimatized with 85% survival rate in a soil combination containing garden soil, sand, and compost at 2:1:1 (Fig. 1i). Using the same potting mixture, 90-100% survival rates were reported by Raju *et al*. (2023) in *D. giganteus*, Sharothi *et al*. (2022) in *B. tuldoides*, and Raju and Roy (2016) in *B. bambos*. On the other hand, using the same potting mixture, Shood *et al*. (2014) observed a 77% survival rate for *Phyllostachys pubescens*. After a month, once new leaves appeared, the plants were moved to a bigger clay pot with a 1:1 ratio of compost and garden soil. This allowed for sufficient growth before transferring the plants to their field conditions (Fig. 1j).

Table 5. Effects of different concentrations of IBA and NAA, either alone or in combination, on the rooting of *in vitro* raised shoots of *B. vulgaris* var. *striata*.

Concentrations of IBA and NAA (mg/l)		Rooting efficiency (%)	No. of roots per culture (Mean ± S.E.)	Root length (cm) (Mean ± S.E.)
IBA	NAA			
1.0	-	35.00	$3.20^{cde} \pm 0.37$	$3.08^{e} \pm 0.27$
2.0	-	60.00	$4.80^{bc} \pm 0.97$	$5.32^{c} \pm 0.28$
3.0	-	**85.00**	$\mathbf{8.00^{a} \pm 0.71}$	$\mathbf{10.06^{a} \pm 0.43}$
4.0	-	70.00	$5.80^{b} \pm 0.73$	$6.84^{b} \pm 0.51$
-	1.0	20.00	$2.40^{e} \pm 0.24$	$2.90^{e} \pm 0.34$
-	2.0	45.00	$3.60^{cde} \pm 0.24$	$3.76^{de} \pm 0.25$
-	3.0	55.00	$4.00^{cde} \pm 0.63$	$4.50^{cd} \pm 0.36$
-	4.0	40.00	$2.80^{de} \pm 0.37$	$3.02^{e} \pm 0.22$
2.0	0.5	35.00	$3.60^{cde} \pm 0.68$	$3.34^{e} \pm 0.32$
3.0	0.5	50.00	$4.40^{bcd} \pm 0.51$	$3.66^{de} \pm 0.30$
5.0	2.0	30.00	$2.80^{de} \pm 0.37$	$2.84^{e} \pm 0.26$
5.0	3.0	40.00	$2.60^{de} \pm 0.24$	$3.20^{e} \pm 0.35$

Values with different letters within a column represent significant difference at 5% level by DMRT.

The current work describes an efficient *in vitro* regeneration strategy for *Bambusa vulgaris* var. *striata* from the nodal segments of mature field-grown plants with high multiplication efficiency. This technique will help in the large-scale plant regeneration of this ornamental bamboo variety faster than any other traditional way of propagation. Furthermore, this technique will assist stakeholders in the ornamental bamboo trade in conserving gene pools and increasing productivity.

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