J. Bamboo and Rattan, Vol. 16, Nos. 4, pp. 147-160 (2017)

Meta-topolin overcomes seasonal dormancy and enhances *in vitro* axillary shoot proliferation in nodal explants of *Pseudoxytenanthera ritcheyi* - a commercially valuable bamboo

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Abstract: The effect of selected parameters, which influence the establishment of *in vitro* shoot cultures through axillary bud proliferation from nodal explants of field grown clumps of *Pseudoxytenanthera ritcheyi* was studied. Single node explants collected across various seasons and cultured on a MS basal medium supplemented with various Plant Growth Regulators (PGRs) in CRD experiment. Explant size (diameter and length), position of the node on secondary branches as well as season of collection were important determinants of the time taken for frequency of axillary bud break as well as number and the length of shoots produced. Nodal explants of diameter 2-3 mm and 2.5-3 cm length were found to give the best results in all the parameters studied. Maximum frequency of bud break was obtained on 1 uM thidiazuron (TDZ) but shoot length was significantly reduced. The least effective PGR was Kinetin. BAP and meta-topolin gave similar results in the parameters studied, but the latter was superior in terms of its ability to overcome season-induced dormancy of buds. Incorporation of meta-topolin (10 μM) in culture media resulted in a consistently high axillary bud break throughout the year (86% to 98%).

Key words: Pseudoxytenanthera ritcheyi, meta-topolin, bud break, multiple shoot formation, micropropagation

INTRODUCTION

Pseudoxytenanthera ritcheyi known as Erankol (Kerala), Chewa or Choomaree (Karnataka) and Manga or Udhe (Maharastra) is a medium-sized, loosely spaced, clump forming bamboo distributed naturally in the Western Ghats region extending from Maharashtra to northern Kerala, India. It prefers tropical climate and can grow on rocky, shallow soils on ridges and slopes of hills. The culm of this bamboo is solid and strong and is considered an important commercial species for fencing and as stakes for cultivation of vegetables. It is also preferred for making walking sticks, umbrella handles and bicycle frames. The species is however not in cultivation and due to unregulated harvesting, wild populations have been over-exploited. This has led to a ban on its harvest in Kerala state and hence conservation of this species has to be accorded high priority (Kumar *et al*., 2009).

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Propagation in bamboo through seeds is considered an inexpensive and rapid method, but the nature of flowering (monocarpic) and short viability period (3-6 months) hinder planting stock production in most commercially important bamboo species. *P. ritcheyi* also has been reported to have monocarpic flowering (Beena *et al.,* 2007) and hence the need to develop alternate means of generating planting stock for raising commercial plantations of the species. The response of bamboo to vegetative propagation techniques vary widely across species and year-round vegetative propagation in bamboo is complicated due to seasonal specificity of material (Saxena and Bhojwani, 1993). Propagation through stem cuttings was attempted in *P. ritcheyi.* Only culm cuttings from the basal portion kept as control gave the maximum rooting (31 %) during the summer season (Kuruvilla, 2017). Hence, mass-multiplication of *P. ritcheyi* requires special attention and may justify the need for exploring in *vitro* culture options.

There are several successful reports on clonal multiple-shoot proliferation through nodal explants of juvenile seedlings in bamboo (Nadgir *et al*., 1984; Saxena 1990; Chambers *et al*., 1991; Prutpongse and Gavinlertvatana 1992). Cloning of adult bamboo through micropropagation require optimization of various endogenous and exogenous factors and the relative lack of success has been due to limitations such low multiplication rates, poor rooting, and a tendency of shoots to become necrotic (Gutierrez *et al.,* 2016; Singh *et al.,* 2012; Sharma and Sarma, 2013).

PGR play an important role in the processes controlling dedifferentiation and redifferentiation. The two major properties of cytokinins towards this include stimulation of cell division and release of axillary buds from dormancy (George *et al.,* 2008). There is, however, difficulty in predicting the effects of plant growth regulators and great differences in *in vitro* response is seen between species, cultivars and even plants of the same cultivar grown under different conditions. While synthetic analogues, kinetin (Kn) and Benzyl Amino Purine (BAP), are used more frequently in plant tissue culture including that of bamboo, the morpho-regulatory potential of Thidiazuron (TDZ) has led to its increased application in a wide array of plant species (Guo *et al.,* 2011). Lin and Chang (1998) have demonstrated that TDZ can be an effective promoter of shoot proliferation in micropropagation and adventitious shoot regeneration of *Bambusa edulis* through nodal explants of field-grown culms. Ornellas *et al.* (2017) has provided new insights on the positive effect of *meta*-topolin (mT) (an aromatic cytokinin) on branch height in micropropagation of *Dendrocalamus asper*. The effect of different cytokinins viz. mT and TDZ in addition to the commonly used 6-benzyladenine (BA), kinetin (Kn) on induction of axillary bud proliferation and multiple shoot in nodal explants collected from adult, field grown clumps of *P. ritcheyi* was examined in this study in an effort to develop a micropropagation procedure.

MATERIALS AND METHODS

Explant source

Dormant axillary buds were collected from actively growing secondary branches (<1 yr. old) of clumps of *P. ritcheyi* located at the KFRI Bambusetum, Thrissur, India. Leaf sheaths covering the nodes were removed with a sharp surgical blade and the stem washed thoroughly under running tap water for one hour. After that they were trimmed to different sizes and washed again in tap water with a few drops of Tween 20 (Himedia, Mumbai, India) for 20 min followed by 60 min treatment with 1 % (w/v) Bavistin (BASF), 250 ppm each of the antibiotic cefotaxime and tetracyclin. The explants were then washed three times with sterile water and under sterile conditions treated with 0.1 % (w/v) $HgCl_2$ with continuous shaking for 8 min followed with three rinses in sterile water after which the nodal explants were inoculated into appropriate media.

Culture conditions

A semi solid basal medium (MS) consisting of salts, vitamins of the Murashige and Skoog (1962) and 3 % sucrose, was used after solidifying with 0.7 % (w/v) agar. Different plant growth regulators like BAP, Kn, TDZ and mT were added at various concentrations to MS medium before the pH of the medium was adjusted to 5.7. 15 ml of media in test tubes (150 X 25 mm) and culture bottles (450 ml). Media were autoclaved at 1.06 kg cm^{-2} and 121 °C for 15 min. Cultures at all growth stages were incubated under 25±2 °C, 60 % RH and a 16-h photoperiod illumination provided by LED tubelights with an average photosynthetic photon flux density of 40 $\mu molm^{-2} s^{-1}$.

Axillary bud initiation and multiple shoot proliferation

Semi-solid MS medium were used to initiate multiple shoots from the nodal explants. Sprouted buds were sub-cultured in fresh media in bottles after 7 d to permit multiple shoot formation. To determine the most suitable hormonal combination for explant establishment and bud proliferation, explants were cultured on MS medium supplemented with BAP, Kn, mT (5-20 μM) or TDZ (0.5-4.5 μM), whereas medium without any PGR served as a control. The performances of media were evaluated in terms of time taken for bud break, frequency of bud break, number and length of shoots after 21 d of culture.

To investigate the seasonal effects on *in vitro* axillary bud proliferation, the collection time in the calendar year was divided in four seasons viz. March to May, June to August, September to November and December to February. Nodal segments were collected in the first week of every season. Influence of different cytokinins was evaluated on bud break seasonality. The effects of diameter (1-2, 2-3, 3-4 and 4-5 mm), length of explant (1.5, 2, 2.5 and 3 cm.) and position of explants [Lower (1^{st} to 3^{rd}), Middle (4^{th} to 7^{th}) and Upper (8^{th} to 10^{th}) on the branch] on axillary bud break were

studied. MS medium supplemented with different cytokinins (BAP: 10μM, Kn: 15μM, TDZ: 1μM, mT: 10 μM) were used for all the experiments.

Experimental design and statistical analysis

The experiments were carried out following a completely randomized factorial design. Each explant was considered as an experimental unit. All the experiments were repeated thrice using 20 explants in each replication. The length of the sprouted shoots was determined by placing the explants on sterile graph paper and measuring the length from the common base of the shoots to the individual tips. The data collected after 21 days were subjected to the Analysis of variance, and significant differences among the treatments were tested using Duncan's multiple range test (Duncan, 1955) at 5 % level using SPSS (Version 11, SPSS Inc. Chicago, USA) software package.

RESULTS AND DISCUSSION

Surface sterilization with $HgCl_2$ resulted in aseptic and viable cultures in 98% of the nodal explants. A high level of bud break (97.85%) was obtained in explants cultured on MS basal medium without any growth regulators (Table 1). On media supplemented with cytokinin, bud break was inhibited especially on kinetin and TDZ treatment whereas on BAP and mT, there was only a marginal reduction in number of sprouted buds, except for mT at 10 μM where it improved the response compared to control. However, in spite of a high level of bud-break in hormone free media, shoot number and length of shoots were lower when compared to media with PGR. It was also observed that there was an inhibition in bud break with increase in concentration within each of the cytokinins. Shoot necrosis was induced in new sprouts of *P. ritcheyi* in all the cytokinins except for mT, which gave higher sprouting response (92-98.42%) at different concentrations.

Despite the well-documented function of cytokinins in delaying senescence, there are several lines of evidence linking cytokinins to the induction of cell death. Although BAP is currently the most common and affordable cytokinin used in micropropagation, its utilization has several drawbacks such as shoot-tip necrosis, inhibition of rooting and problematic acclimatization of plants in the greenhouse (Werbrouck *et al.,* 1996). Kunikowska *et al.* (2013) reviewed the use of cytokinins in plant tissue culture and found that the two natural cytokinins Kn and BAP at high concentrations (>44μM) are able to induce programmed cell death (PCD) in plants and also reported that BAP is able to induce PCD in plant cultured cells whereas Kn induces this process. only in living plant tissues. Among various cytokinins used in the present study, Kn gave the lower rate of explant establishment as well as lower number of shoots. TDZ, a phenyl urea derivative, is the other PGR, which induces maximum shoot number during sprouting. Treatment with this cytokinin however resulted in shortening of the shoots derived from the sprouted buds to as low as 0.18 cm. Lin and

Chang (1998) have demonstrated that TDZ at higher concentration inhibited shoot elongation and also induced considerable vitrification in *B. edulis*. While in our studies mT improved not only the bud break, and number of shoots, but also higher shoot length, Ornellas *et al.* (2017) had showed that increasing concentrations of mT caused a reduction in culm size and an increment in branch size, with an overall increase in the proliferation rate of cluster of *D. asper*. In most of the studies on bamboo micropropagation viz.(Sood *et al.,* 2002; Agnihotri and Nandi, 2009;Jimenez *et al.*, 2006), BAP is used because it is the most effective and affordable cytokinin, but in this study, application of mT in inducing axillary bud break from nodal explants was found to be more effective.

Table 1. Effect of different cytokinins supplemented in MS solid medium on axillary bud proliferation of *Pseudoxytenanthera ritcheyi**

Cytokinins	Concentration	Days required for bud break	% bud break	No. of shoots	Length of shoots (cm)
0	0.0	7.02c	97.85ab	1.89cde	6.01a
BAP	5.0	9.12d	91.02e	2.33cd	6.0a
	10	5.17bc	97.00ab	4.50b	4.0bc
	15	3.67a	94.33c	4.83ab	3.83c
	20	10.0de	86.77fg	2.0cd	1.37de
Kn	5.0	13.67f	93.23cd	1.67cde	5.33ab
	10	11.17e	81.67h	2.0cd	6.0a
	15	11.33ef	81.10h	3.33c	4.33bc
	20	18.67fg	73.57i	1.83cde	3.67c
TDZ	0.5	7.18c	89.92f	4.72ab	1.74d
	1.0	6.74bc	87.02f	4.81ab	1.06de
	2.0	5.12bc	84.72fgh	4.92ab	0.89f
	4.0	5.08b	82.03gh	5.06a	0.18g
mT	5.0	5.16bc	94.60c	3.95bc	4.10bc
	10	5.02b	98.42a	4.39b	4.32bc
	15	4.97ab	96.16b	4.41b	4.71b
	20	4.82ab	92.04d	4.48b	4.76b

Data recorded after 4 weeks of inoculation (±SE). Different letters within a column indicate significant differences at p = 0.05 by Duncan's multiple range test (DMRT).

Explants across the different diameter classes showed bud break within 4.2 to 21.02 days and ranged between 27.42 to as high as 98.42% (Table 2.). Across all diameter

classes, mT gave the best results in time taken for bud break, frequency of bud break, shoot number and shoot length. Generally, explants with larger diameter (> 3 mm) gave higher shoot number but with delayed response and reduced frequency of bud break. However, the length of the shoots was not influenced by this parameter. Higher shoot length was obtained on explants of size > 2 mm.

Table 2. Effect of diameter of the explant on axillary bud proliferation from nodal explants of *Pseudoxytenanthera ritcheyi*

Diameter of the explant (mm)	Cytokinins	Days required for bud break	% bud break	No. of shoots	Length of shoots (cm)
1-2	0	6.94cd	97.02ab	0.93ghi	4.14b
	BAP	4.26a	97.40ab	0.94gh	4.20b
	Kn	8.19e	94.86abc	0.95g	4.46b
	TDZ	6.01c	97.31ab	0.94gh	2.52d
	mT	4.56ab	97.76ab	0.98g	4.06b
2-3	0	7.02d	97.85ab	1.89efg	6.01a
	BAP	5.17b	97.0ab	4.50c	4.00bc
	Kn	11.33f	81.10cd	3.33e	4.33b
	TDZ	6.74cd	87.02c	4.81bc	1.06ef
	mT	5.02b	98.42a	4.39cd	4.32b
3-4	0	7.33d	93.04bc	1.99efg	6.24a
	BAP	5.83bc	95.26abc	4.87b	4.42b
	Kn	11.52f	79.28e	4.29cde	4.51ab
	TDZ	6.94cd	84.15cd	4.95ab	1.32e
	mT	5.27b	96.03b	4.46cd	4.48b
4-5	0	21.02i	27.42g	2.26ef	6.26a
	BAP	18.17g	67.72f	4.92ab	4.46b
	Kn	18.33g	61.05f	4.39cd	4.53b
	TDZ	19.74h	67.16f	4.98a	1.41e
	mT	8.02e	72.31ef	4.93ab	4.62ab

***** Data recorded after 4 weeks of inoculation (±SE).Different letters within a column indicate significant differences at p = 0.05 by DMRT.

There are several reports that reveal the significance of diameter of the explant on the bud break and shoot growth in different bamboo species. Nodal segments of 1.5-2.5 mm diameter from 2-year old plants resulted in highest shoot initiation in *Thamnocalamus spathiflorus* (Bag *et al.,* 2000). Similarly in *Pseudoxytenanthera stocksii* (Sanjaya *et al.,* 2005; Somashekar *et al.,* 2008) and *Guadua angustifolia*

(Jimenez *et al*., 2006), nodal explants of 2-3 mm diameter resulted in highest shoot initiation. Kabade,(2009) used different diameter classes of explants (1-2, 2-3, 3-4 and 4-5 mm), and reported that nodal shoot segments of 2-3 mm diameter favoured maximum shoot initiation within 3 weeks in *B. bambos* and *D. strictus*.

The size of the nodal explants was observed to influence all the studied parameters in culture establishment (Table 3). The time taken for bud break had an inverse relation to the length of the explant. With the PGR treatments, it was seen that PGR decreased the time taken for bud break except for Kn, which delayed it beyond that of the control. In the case of frequency of bud break, the size of the explant did not have a notable effect except for the 1.5 cm class where it was significantly lower. The number of shoots formed as well as length was influenced by the size of explant and use of PGRs.

Table 3. Effect of length of the explant on axillary bud proliferation from nodal explants of *Pseudoxytenanthera ritcheyi* *

Length of the explant (cm)	Cytokinins	Days required for bud break	% bud break	No. of shoots	Length of shoots (cm)
1.5	0	8.43e	92.34d	1.72de	3.83c
	BAP	6.52cd	94.01cd	4.34ab	3.28de
	Kn	14.10g	80.00fg	3.08bc	4.08cd
	TDZ	7.98de	84.08ef	4.63ab	0.92g
	mT	5.96bc	96.15bc	3.53bc	3.76d
2	0	7.02d	97.85abc	1.89de	4.01d
	BAP	5.17b	97.00abc	4.50ab	4.0d
	Kn	11.33f	81.10f	3.33bc	4.33c
	TDZ	6.74cd	87.02e	4.81a	1.06ef
	mT	5.02ab	98.42a	3.99b	4.32c
2.5	0	6.96cd	97.88abc	1.97d	4.13a
	BAP	5.12b	97.08abc	4.58ab	4.12cd
	Kn	11.12f	81.16ef	3.42bc	4.38c
	TDZ	6.62c	87.50e	4.87a	1.14f
	mT	5.00ab	98.44a	3.99b	4.41c
3	0	6.92cd	98.01ab	1.99d	4.21cde
	BAP	5.08ab	97.98abc	4.58ab	4.24cde
	Kn	11.06f	82.30ef	3.47bc	4.42cd
	TDZ	6.57cd	87.96e	4.89a	1.18ef
	mT	4.98a	98.62a	3.99b	4.52c

***** Data recorded after 4 weeks of inoculation (±SE).Different letters within a column indicate significant differences at p = 0.05 by DMRT.

Longer explants gave more number as well as longer shoots. As in the previous experiments, TDZ reduced the shoot length. Across all the size classes, mT was found to reduce time taken for bud break, improve the frequency of bud break, shoot number and length of new shoots.

The effect of the explant size on axillary bud proliferation has been studied in *Thamnocalamus spathiflorus* (Bag *et al.,* 2000) and in several other plant species, in particular *Cynara cardunculus* (El Boullani *et al*., 2017) *Gerbera jamesonii* (Nhut *et al*., 2007) and cedar (*Cedrus* spp.) (Renau-Morata *et al*., 2005). Smith, (2000) suggested that the explant size has an effect on the response of the tissue and this was due to the more nutrient reserves and endogenous plant growth regulators to sustain the culture. George *et al.* (2008) reported that large explants generally survive more frequently and grow more rapidly at the outset than very small ones.

Nodes from the middle of the branch gave the best results in all the parameters (Table 4). The upper nodes were the next best for establishing the cultures since the lower most buds took longer to achieve bud break and had a lower frequency of bud break, number of shoots as well as the shoot length. Physiological age of buds i.e. the position

Table 4. Effect of position of the explant on axillary bud proliferation from nodal explants of *Pseudoxytenanthera ritcheyi* *****

Position	Concentration	Days required for bud break	% bud break	No. of shoots	Length of shoots (cm)
Lower	0	13.14g	51.24efg	1.12f	5.23ab
	BAP	12.52f	63.40de	3.64bc	3.67bc
	Kn	12.52f	54.11e	3.26c	4.01c
	TDZ	11.74fg	52.13ef	3.05cd	0.98f
	mT	8.31e	66.16d	3.21c	4.21bc
Middle	0	7.02d	97.85ab	1.89e	6.01a
	BAP	5.17ab	97.00ab	4.50ab	4.0c
	Kn	11.33f	81.10bc	3.33c	4.33b
	TDZ	6.74c	87.02b	4.81a	1.06e
	mT	5.02a	98.42a	3.99b	4.32b
Upper	0	8.47e	72.01cd	1.24f	6.13a
	BAP	6.51c	74.06cd	3.47bc	4.21bc
	Kn	11.82fg	75.16c	3.05cd	4.35b
	TDZ	7.53de	73.51cd	4.52ab	1.14e
	mT	5.67b	77.93c	3.46bc	4.39b

***** Data recorded after 4 weeks of inoculation (±SE).Different letters within a column indicate significant differences at p = 0.05 by DMRT.

of buds in mother plant, is one of the important factors that determine the morphogenetic response of the explant during culture establishment. Several reports suggest that the regeneration potential of bud explants depends on the differential meristematic activity within different plant parts with respect to position (Zulfiqar *et al*., 2009; Palanisamy and Kumar, 1997; Han *et al.,* 1997; Barcelo-Munoz *et al.,* 1999). Devi and Sharma (2009) exhibited the effect of position of the node on lateral branches in axillary bud proliferation of *Arundinaria callosa* Munro and they reported that mid-culm nodes are the most suitable for proliferation with high bud-break percentage and multiplication.

Several of the parameters studied in the establishment phase such as time taken and frequency of bud break and number of shoots formed per explant were found to be influenced by the season of explant collection (Table 5). Bud-break frequency of the

Table 5: Effect of season on axillary bud proliferation from nodal explants of *Pseudoxytenanthera ritcheyi* *

Season	Cytokinins	Days required for bud break	% bud break	No. of shoots	Length of shoots (cm)
Mar-May	0	7.02cd	97.85ab	1.89e	6.01ab
	BAP	4.81a	97.01ab	4.50ab	4.0cd
	Kn	11.33fg	81.10e	3.33bc	4.33c
	TDZ	6.74c	87.02d	4.81a	1.06g
	mT	5.02ab	98.42a	3.99ab	4.32c
Jun-Aug	0	19.16k	0.08l	1.12ef	2.00f
	BAP	15.74hi	7.00k	3.68b	2.50ef
	Kn	18.61j	8.09j	3.01cd	2.92e
	TDZ	16.85i	7.13jk	3.24bc	0.88h
	mT	5.87b	86.05de	3.43bc	4.08cd
Sep-Nov	0	13.06gh	31.06i	1.86e	2.24ef
	BAP	9.72f	46.08g	3.82b	2.76e
	Kn	14.23h	41.17gh	3.63bc	2.98e
	TDZ	12.85g	46.21g	3.61bc	1.24fg
	mT	5.38ab	89.14cd	3.46c	4.02cd
Dec-Feb	0	8.11e	90.21c	1.73e	6.32a
	BAP	7.36cd	91.16bc	4.04ab	5.22bc
	Kn	12.28g	78.04ef	2.97d	5.51b
	TDZ	6.89c	81.30e	4.52ab	1.31fg
	mT	5.19ab	92.23b	3.76b	4.47c

*Data recorded after 4 weeks of inoculation (±SE).Different letters within a column indicate significant differences at p = 0.05 by DMRT.

axillary buds was not uniform during the period under study. The best season for quick bud break was March to May and with the use of PGRs, the time taken is reduced except in the case of Kn. The frequency of bud break was least during June to August period, which was the monsoon season during which new shoots emerge. Use of PGRs did not influence the bud break significantly except in the case of media with mT where the notable effect was the consistently high frequency of bud break across all the seasons. A consistency in the number of shoots produced irrespective of the season was maintained whereas in the control and in media with other cytokinins there was a clear seasonal effect in these parameters. Although bud break frequency improves during the September to November season, there is no concomitant increase in the number or length of shoot formed until the next season. As in the previous experiments, the shoot length was high in control and inhibition by TDZ was observed.

Several studies on the suitable time of year to collect explants for initiating cultures from nodal explants of bamboo have been carried out. Similar results were reported in *P. stocksii* (Sanjaya *et al.,* 2005), that nodal shoot segments collected during April-September produced a significantly more number of shoots with better shoot growth. In *Dendrocalamus longispathus* (Saxena and Bhojwani, 1993) and *D. giganteus* (Ramanayake and Yakandawala, 1997) peak bud break were observed before the onset of monsoon rains each year as was the case in our study. February-March and September-October showed maximum bud break response in *Drepanostachyum falcatum* and in *Bambusa balcooa*, respectively (Arya *et al*., 2008). February, March and December months were found to be the best for establishment of aseptic cultures in *B. nutans* (Mehta *et al.,* 2011). Period between July-August was found to be suitable for shoot initiation in *D. strictus* (Chaturvedi *et al.,* 1993) whereas the highest bud break frequency in *Bambusa balcooa* was recorded in October (Das and Pal, 2005). Negative effect of rainy season on bud break was also reported in *A. callosa* (Devi and Sharma, 2009) which resulted in low percentage of bud-break.

Results presented in this study clearly demonstrate a distinctive response of axillary buds of *P. ritcheyi* to the growth regulator mT. This is the only cytokinin, which gave consistency in time of bud break irrespective of season, frequency of bud break, number of sprouts as well as shoot length. Being the most active cytokinin in the class of natural aromatic cytokinins, mT might be a potential alternative to BAP with clear advantages. The quick degradation rate and higher metabolic activity of mT during different stages of plant tissue culture could eliminate the toxicity caused by the application of other cytokinins resulting in shoot necrosis (Werbrouch *et al.,* 1996) and preventing shortening of shoots and inhibition of rooting observed in instances where other cytokinins are used. Therefore, mT appears to be an ideal cytokinin for the establishment of shoot cultures through axillary bud proliferation in *P. ritcheyi.*

CONCLUSION

Axillary bud proliferation in nodal explants of *P. ritcheyi* was influenced by the size, position on plant and season of collection of the explants. Explants of medium size (2-3mm diameter and 2.5cm long) from the middle of the branch were most suitable for establishing shoot cultures. The season preceding the monsoon, i.e. March to May, was found to be the best time to initiate cultures. Moreover, use of mT could overcome the seasonal dormancy shown in the rest of the year. Meta-topolin had an advantage over other cytokinins in that it could induce a constant rate of bud break as well as shoot number thereby improving the success in establishment of multiplying shoot cultures for efficient micropropagation.

ACKNOWLEDGEMENTS

The study was in part supported by the generous funding and the grant of a research project to the authors, by the Kerala Forest Department, Thiruvananthapuram. The authors are also grateful for the technical support given in collection of plant material by Ms. Nimisha Joy.

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