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CLONAL MACROPROPAGATION OF BAMBUSA NAGALANDIANA NAITHANI THROUGH CULM SEGMENTS AND BRANCH CUTTING: AN ENDEMIC BAMBOO OF NAGALAND, INDIA

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Abstract

Bambusa nagalandiana Naithani is an indigenous bamboo found in Nagaland and Mizoram, India. The species is a sympodial bamboo and identified by its yellow culm with green stripes. Because of its beautiful architecture the species is used for ornamental purpose, construction work and furniture making. The young shoots are also used as vegetable. Due to narrow distributional range and high demand for various purposes the species is under threat in the natural habitat. Present communication reports a successful attempt to develop protocol for macropropagation from culm segments and branch cuttings of this economically important threatened bamboo species.

Keywords: *Bambusa nagalandiana; Culm splitting; Endemic bamboo; Macropropagation; Nagaland; Ornamental bamboo*

Introduction

Bamboos are a group of tall, arborescent and perennial evergreen grasses that grow very fast. Most of the species of genera *Bambusa* are well known for their multipurpose use and are commercially important for varied applications viz. construction materials, furniture, fences, handicraft, paper pulp, edible shoots etc. Their versatile use is due to their excellent splitting ability, tensile and compressive strength and amenability of being harvested within 4-5 years after planting [1-4]. It is one of the fastest growing, most resourceful woody plants with high productivity. Some of the industrially important species like *Bambusa balcooa*, *B. bambos*, *B. pallida*, *Dendrocalamus brandisii, D. Giganteus* etc. and are mainly used in construction as scaffoldings, handicrafts and as alternative source of timber. *Dendrocalamus brandisii* being a fast growing species is used in afforestation, social forestry and agro forestry [5-6]. Bamboos are not only of economic importance but also of ecological importance as it helps in preventing soil erosion by its strongly developed rhizome and roots.

Bamboo being monocarpic, flowering and seed setting are accomplished at very long intervals. The length of flowering cycle varies from three years (*Schizostachyum elegantiaaium*) to 60 years (*Bambusa polymorpha*), but for most species, it lies between 30-60 years [7,8]. Since after flowering most of the bamboos dies, the possibility of raising bamboo plantation from seed is limited and difficult. Since seeds are not available most of the time, bamboos can be propagated through conventional vegetative method like offset/rhizome planting, branch and

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culm cuttings etc. Vegetative propagation seems to be the preeminent choice taking into account the encouraging results reported by many others [9-11]. Vegetative propagation through offset cutting, rhizome splitting, culm and branch cuttings are successfully tested in several bamboo species [12].

Bambusa nagalandiana Naithani is a sympodial bamboo, endemic to North-East region (Nagaland and Mizoram) of India. The species is characterized by its yellow culm with green stripes (Fig. 1a) and the species is very valuable for ornamental purpose [13]. It is also used for construction work and furniture making; young shoots are used as vegetable. Other than taxonomical studies, very little work has been done on this bamboo species. Since this species is confined only to particular geographical zones of North- East India and are used for different purposes, the natural populations of the species are under severe threat. Due to this, it is necessity to conserve and adopt alternative methods for multiplication and propagate this species.

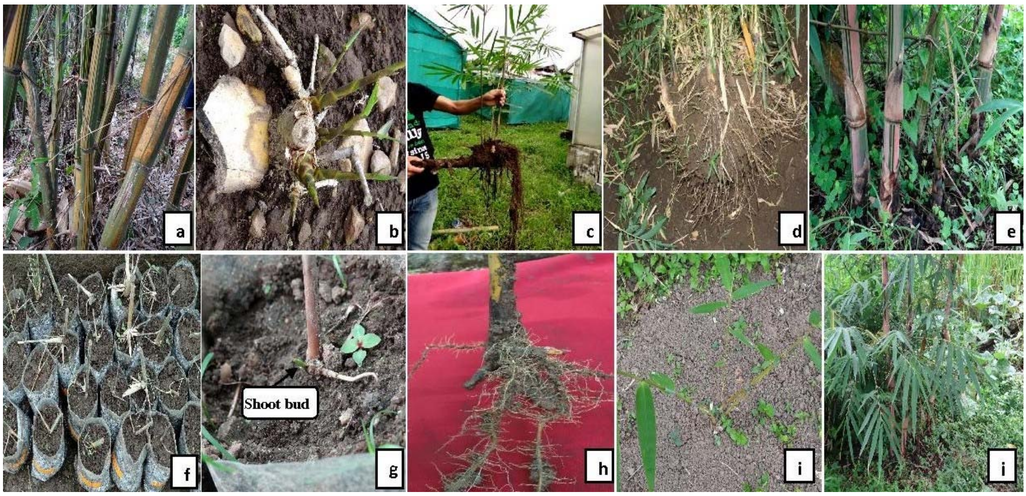

Fig. 1. Different stages of macropropagation of *Bambusa nagalandiana* from culm segmentation and branch cutting. a. A part of *B. nagalandiana* culm in the natural habitat showing the architectural phenology, b. A culm segment in the nursery plot showing emergence of new shoots, c. Root and shoots formed from the culm segment after 6 months, d. Multiple shoots formed after one year in the experimental garden, e. Multiple plants developed in the wild from the transferred culm, f. Treated branch segments in the polybags maintained in the polyhouse, g. New shoot bud formation from the branch cutting after two months of sowing, h. Multiple roots formed from the basal part of the branch cutting, i. New shoots developed from the branch cutting in the field, j. Multiple plants developed from the sowed branch in the experimental filed.

Propagation through seed is the cheapest and easiest method for production of bamboo plants. Though there have been several reports of sporadic and gregarious flowering of other bamboo species, plantation of *Bambusa nagalandiana* via seed is restricted by lack of seed as there has been no established account on flowering of this species. The present study was under taken to develop cost effective farmer friendly protocols for producing clonal planting materials of the species for mass multiplication through culm and branch cuttings under regular nursery conditions.

Materials and Methods

Source of Planting Stock

The planting materials were obtained from Mon District, Nagaland, India at an elevation of 1190m ASL. The study was carried out in the Department of Botany, Nagaland University, Lumami, Nagaland, India. The rhizomes were collected from the natural habitat in Mon District, Nagaland in the year 2011 and planted in the Experimental garden of the Department of Botany. The plants developed from the rhizomes were used as the mother plants for the present study. Vegetative propagation was under taken once the mother plant was fully established.

Types and Preparation of Planting Materials

1. Culm cutting/splitting.

Initially culms of *Bambusa nagalandiana* were obtained from the natural habitat at Wanching Village, Mon District, Nagaland but subsequently the culms were harvested from the plants raised in the Botanical garden of the Department of Botany, Nagaland University in different seasons. The culms were examined morphologically to determine the existence of buds, which may grow and form culms. Culms in their second year of growth were used for the experimental purpose. For the present study binodal (two nodes) segments cuttings extracted by discarding the tender top segments of the culm. From the segments the branches were trimmed off before use for the present study. Due care was taken to ensure that there was no injury in the axillary buds. The binodal culm segments were then sawed ensuring that each cutting has a branch with a prominent swollen basal portion.

The cut ends of the culms were kept moist during transportation. In order to prevent desiccation, the cut ends were stuffed with soil and kept in moist gunny bags. In every segment a hole was made in the internodes with the help of a hack-saw. Care was taken not to harm or make a split in the culm. The segments were then treated with two root promoting substances (1-Naphthaleneacetic acid or ***NAA*** and boric acid) singly (0-300 ppm). This root promoting solutions were injected into the culm segments through the holes followed by wrapping with thin polythene strips. The cuttings were then placed horizontally across the nursery beds in such a way that the holes face upwards. Then it was covered with soil with a spacing of 2 x 2 feet. Observations were taken after 6 months in the field for the formation of roots.

Experimental Layout:

a. Planting material - bidonal bimodal
b. Culm segments
c. Growth promoting treatment and concentrations - NAA and boric acid singly (0 to 300 ppm with an increment of 50 ppm) singly.
d. Replications - five for each treatment.
e. Size of sampling - 20 per replicate.
f. Design - Completely Randomized Design.
g. Planting seasons - four seasons (January, April, July and October).
h. Planting conditions - nursery beds.
i. Planting medium - soil, sand and decayed wood powder (4:1:1 ratio).
j. Planting spacing - 2 x 2 feet.
k. Shading - 50%.

2. Branch cutting

Disease free branches from culm of 6 to 36 months old were harvested from the plants growing in the experimental garden. The branches were cut along the rhizomatous swelling at their base. Branch cuttings were dipped in plant hormonal solutions of IBA ranging from 0- to 1000 ppm. The cuttings were dipped for 24 hours before sowing in the potting mix. Potting mix was prepared by mixing top black soil, sand and decayed wood powder at a ratio of 4:1:1 and filled in 6 inches black plastic bags. The plastic bags were maintained in the poly shade house/poly-house at 75% shade (Fig. 1f). A set of 20 segments were taken for each treatment.

After 3 months the cuttings were uprooted and observations were noted on number of cuttings rooted and root length.

Experimental Layout:

a. Planting material - branch segments of various ages (6-24 months old).
b. Growth regulator and concentrations - IBA (0-1000 ppm).
c. Replications - five for each treatment.
d. Size of sampling - 20 per replicate.
e. Design: Completely Randomized Design.
f. Planting seasons - four seasons (January, April, July and October).
g. Branch age - 6 to 36 months after sprouting (6, 12, 18, 24, 30 and 36 months).
h. Planting conditions - poly bags maintained in the poly house.
i. Planting medium - soil, sand and decayed wood powder (4:1:1 ratio).
j. Shading - 75%.

The culm and branch segments were uprooted carefully from the nursery and plastic pots maintained in the poly house after four and two months respectively and data were collected on percent rooting, number of roots formed, root length, number of sprouts and sprout length per culm and branch segment.

The newly sprouted shoots with roots from the culms and branch cuttings were transferred to the experimental garden with a spacing 5 x5 feet for plant formation. The regenerated plants were transferred in the wild after one year of growth.

Statistical Analysis

Experimental design was completely randomized. Data was analyzed by one way ANOVA using General Linear Model procedure in SAS Statistical Package (SAS Ins.) and standard deviation from mean was worked out. Means were compared using Least Significant Difference (LSD) test at $p \leq 0.05$.

Results and Discussions

Effect of season of collection of culm and branch cuttings, branch age on rooting and sprouting

During the present investigation both the culm and branches were harvested in four different seasons for planting. Considerable variations in rooting and shoot bud sprouting were recorded between the planting stocks collected in different seasons in both the types of planting materials. In general the planting materials harvested during April to July exhibited better by producing better roots and shoots. The planting stocks collected during exhibited poorly where fewer roots per node and fewer sprouts developed. Besides these, the sprouts failed to grow normally and most of the cases degenerated after few weeks. Of the different collection seasons, ~70% culm segments planted during April responded positively where ~9 sprouts yielded per node accompanied by ~15 roots per node within 6 months of planting (Table 1, Fig. 1b). A similar response was also recorded with the branch cuttings. Branches harvested during April exhibited better response compare to other three seasons. About 60% cuttings responded positively by producing roots and shoot buds (Table 1 and Fig. 1g and h).

About 6 roots per node and 2 shoot buds sprouted from the branch cutting harvested during April, while other seasons were not supported for morphogenetic response. In the past several workers have reported the effect of the harvest seasons on rooting and sprouting of bamboo culm segments and branch cuttings [4, 6]. Razvi et. al. [6] in *Bambusa vulgaris* found rainy season was ideal for rooting and sprouting of culm segments compared to other seasons. While, according to Razvi et. al. [4] March-June period was ideal for rooting and sprouting in *Chimonobambusa anceps*. Besides bamboo, effect of season of collection of planting stocks on rooting and sprouting frequency has also been reported with other species [14-15]. Palanisamy et. al. [14] reported maximum rooting in stem cuttings of *Azadirachta indica* during February while, Khali and Sharma [15] reported maximum rooting from branch cuttings of *Taxus baccata* collected during March to October.

Table 1. Effect s of different seasons on rooting and sprouting of shoots from culm segments and branch cutting of *Bambusa nagalandiana*

Planting material	Season of collection	No. of roots per node*	No. of sprouts per node*	% response*
Culm segment	January	02.2 (±0.2)^{d}	03.1(±0.1)^{c}	30.5 (±1.3)^{d}
	April	15.3 (±0.4)^{a}	09.4 (±0.4)^{a}	70.6 (±2.1)^{a}
	July	10.2 (±0.7)^{b}	09.3 (±0.6)^{a}	50.6 (±1.7)^{b}
	October	08.4 (±0.5)^{c}	06.1 (±0.3)^{b}	45.1 (±1.9)^{c}
Branch cutting	January	01.5 (±0.1)^{d}	0.70 (±0.2)^{b}	20.5 (±1.9)^{d}
	April	06.4 (±0.7)^{a}	02.6 (±0.3)^{a}	60.3 (±1.8)^{a}
	July	05.2 (±0.6)^{a}	02.2 (±0.2)^{a}	50.3 (±1.7)^{b}
	October	03.4 (±0.3)^{c}	01.6 (±0.3)^{b}	30.5 (±1.3)^{c}

* ±SE: Standard error from mean; data represents mean of five replicates; Data with the same *letters* in the column are not significantly different at 5% level. Data collected and compiled after 6 months of plantation. About 18-24 months old branches were planted.

Compared to other plants, information on relationship between rooting and collection seasons is scanty. In deciduous plants the rooting of cuttings is directly related with release of new sprouts after winter. This effect is most attributed to the mobilization of reserves and synthesis of auxin like substances in the new shoot buds [4]. Findings of the present study are also in agreement with this argument.

Further rooting of culm segments and branch cuttings are influenced by external as well as internal factors. Amongst the different external factors, season of collection plays important role in rooting of cuttings which attributes due to seasonal fluctuation like temperature, solar irradiation, humidity etc. Planting materials collected during favourable growing season favour rooting in cuttings [16].

Table 2. Effect of quality and quantity of growth promoting substances on morphogenetic response of culm segments of *Bambusa nagalandiana*

Treatment type (Conc. in ppm)	No. of roots per node*	Root length* (cm)	Sprout per cutting*	Sprouts length* (cm)	% response*
Control	-	-	-	-	-
NAA					
50	03.2 (±0.2)^{e}	12.1 (±0.3)^{e}	02.4 (±0.2)^{e}	105.4 (±3.5)^{f}	20.3 (±1.1)^{e}
100	07.4 (±0.2)^{d}	24.2 (±0.4)^{d}	03.2 (±0.3)^{d}	186.7 (±2.6)^{e}	50.5 (±2.1)^{c}
150	10.3 (±0.2)^{c}	23.7 (±0.2)^{d}	03.3 (±0.2)d	210.2 (±2.7)^{c}	47.9 (±1.7)^{d}
200	15.3 (±0.4)^{a}	39.9 (±0.3)^{a}	09.4 (±0.4)^{a}	250.4 (±2.9)^{a}	70.6 (±2.1)^{a}
250	12.4 (±0.5)^{b}	31.8 (±0.6)^{b}	05.2 (±0.3)^{b}	203.4 (±3.1)^{d}	60.4 (±2.7)^{b}
300	11.2 (±0.5)^{c}	29.4 (±0.7)^{c}	04.1 (±0.3)^{c}	228.3 (±2.1)^{b}	50.7 (±2.1)^{c}
Boric acid					
50	1.2 (±0.2)^{c}	3.5 (±0.2)^{d}	-	-	10.2 (±1.2)^{e}
100	3.2 (±0.2)^{b}	8.2 (±0.3)^{b}	02.2 (±0.3)^{b}	30.2 (±0.9)^{b}	20.5 (±1.4)^{d}
150	3.5 (±0.3)^{b}	7.9 (±0.2)^{b}	02.2 (±0.2)^{b}	32.5 (±0.7)^{a}	22.9 (±2.7)^{c}
200	4.3 (±0.3)^{a}	9.5 (±0.5)^{a}	03.1 (±0.1)^{a}	22.9 (±0.7)^{c}	40.6 (±1.7)^{a}
250	2.8 (±0.2)^{c}	4.3 (±0.2)^{c}	-	-	27.5 (±1.8)^{b}
300	3.2 (±0.4)^{b}	5.2 (±0.4)^{c}	-	-	20.6 (±2.1)^{d}

* ±SE: Standard error from mean; data represents mean of five replicates; Data with the same *letters* in the column for the same factor are not significantly different at 5% level. Data collected and compiled after 6 months of plantation.

Besides different external factors, internal physiological factors also equally important for rooting of planting materials. In the present study with rooting of branch cutting of *B.*

nagalandiana depicted the importance of age of the branches on rooting. Branches of different ages (6 months to 36 months) were harvested and planted in the potting mix (Table 2).

Of the different branch ages, branches up to 12 months old exhibited very poor morphogenetic response; though rooting of the cutting were recorded, failed to sprout shoot buds. Under the experimental condition optimum rooting as well as sprouting was achieved from the branch cuttings of 18-24 months old branches (~60%). It was observed that though rooting response of the branch cutting was quite good, sprouting of shoot buds was very poor (Fig. 1g and h). The first report of propagation of bamboo was reported by White [17] in *Gigantochloa verticillata* and *Sinocalamus oldhami*. It was found that it is very difficult to produce sprouts and culms from the branch cuttings as they failed to produce rhizome from the planted branch cuttings.

Effect of growth promoting substances on morphogenetic response of culm segments and branch cuttings

Besides season of collection, branch age on rooting and sprouting, the quality and quantity of growth promoting substances exhibited pronounced effects in both culm segments as well as in branch cuttings. In both types of planting materials studied in the present investigation, there were no response and degenerated after few weeks. In culm segments two different types of growth promoting substances were injected in different concentrations. In general NAA was found to be superior over boric acid. Of the different concentration of NAA injected, optimum morphogenetic response was registered with 200 ppm NAA. Under this condition 15.3 roots per node 9.4 shoot buds developed within 6 months of plantation (Table 3, Fig. 1c). Besides number of roots and sprouts, this concentration also supported root and shoot growth. At this concentration ~ 39.9 cm and 250.4 cm long roots and shoots respectively developed. In contrary under the similar condition with boric acid morphogenetic response was very poor where only 4.3 numbers of roots and 3.1 numbers of sprouts yielded in the same time scale. Lower as well as higher concentrations of NAA were less effective as these concentrations did not support healthy and optimal morphogenesis, while at higher concentration of boric acid did not support sprouting from the node and yielded only roots.

Table 3. Effect of ages of *B. nagalandiana* branch on rooting and sprouting of shoot buds under poly house condition

Branch age (months)	% response*	Type of response
6	15.2 (±0.6)	Sprouting of axillary buds but failed to produce roots.
12	19.7 (±0.5)	Very few roots formed but degenerated subsequently.
18	60.3 (±1.8)	Healthy roots and shoot buds formed.
24	59.8 (±1.7)	As above.
30	36.7 (±1.4)	Fewer roots formed.
36	33.5 (±2.5)	As above.

* ±SE: Standard error from mean; data represents mean of five replicate; Data collected and compiled after 3 months of plantation.

In the present study for inducing roots and sprouts from the branch cuttings, cuttings were treated with IBA (0-1000 ppm). At lower concentrations up to 400 ppm there was no sprouting. Under the given experimental conditions, as many as 6.4 roots and 2.6 sprouts per nodes formed after three months of plantation in the poly house where ~60% cuttings responded positively (Table 4).

The role of different plant hormones and other growth promoting substances are being reported with other bamboo species. In general auxins are preferred growth substances over others for both rooting and sprouting [6].

Table 4. Effect of IBA on morphogenetic response of branch cutting of *B. nagalandiana*

IBA Conc. (ppm)	No. of root formed per cutting*	Length of root* (cm)	No. of sprout per cutting*	% response*
Control	-	-	-	-
100	1.2 (±0.2)^{e}	14.5 (±0.5)^{f}	-	20.4 (±1.5)^{e}
200	1.0 (±0.2)^{e}	15.2 (±0.5)^{f}	-	15.3 (±1.6)^{f}
300	2.2 (±0.4)^{d}	15.7 (±0.6)^{f}	-	14.9 (±1.2)^{f}
400	2.4 (±0.3)^{d}	23.8 (±0.8)^{e}	-	22.7 (±1.7)^{e}
500	3.4 (±0.5)^{c}	24.7 (±1.2)^{e}	1.2 (±0.2)^{b}	30.3 (±1.9)^{d}
600	4.2 (±0.6)^{c}	33.6 (±0.4)^{d}	1.4 (±0.2)^{b}	40.9 (±1.6)^{c}
700	5.3 (±0.7)^{b}	45.6 (±2.1)^{c}	1.2 (±0.3)^{b}	50.3 (±2.1)^{b}
800	5.4 (±0.6)^{b}	46.0 (±0.7)^{c}	1.2 (±0.2)^{b}	49.8 (±2.1)^{b}
900	5.6 (±0.4)^{b}	56.5 (±1.2)^{b}	2.2 (±0.3)^{a}	51.3 (±2.4)^{b}
1000	6.4 (±0.7)^{a}	60.4 (±1.3)^{a}	2.6 (±0.3)^{a}	60.3 (±1.8)^{a}

* ±SE: Standard error from mean; data represents mean of five replicates; Data with the same *letters* in the column are not significantly different at 5% level. Data collected and compiled after 3 months of plantation. About 18-24 months old branches were planted.

The rooted culms and branch cuttings were uprooted from the bed and poly bags respectively (Fig. 1c and h) and transferred to the open space in the garden for further growth. Within 3-4 months new clums and shoots developed (Fig. 1d and i) and within one year functional bamboo clumps formed (Fig. 1e and j).

Findings of the present study open a new route for cost effective macropropagation of this endemic, economically important threatened bamboo species of North Eastern region of India.

Conclusions

The paper describes an efficient and reproducible protocol for mass multiplication of a *Bambusa nagalandiana* – an endemic threatened bamboo species through macropropagation technique. The protocols developed are cost effective and can be followed by the unskilled people for propagation of the species. Application of this protocol will minimize the stress on its natural populations in wild by meeting the demand of local furniture making industry. The regenerated plants could be relocated to the sites of natural population thereby would help in conservation the species in the natural habitats.

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