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In-vitro Nodal Bud Culture of Giant Bamboo *(Dendrocalamus giganteus)*

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ABSTRACT. Nodal segments collected from the field were cultured on Murashige and Skoog's (MS) medium supplemented with 5 mg/l BAP (6-benzylamino purine). The buds collected from the central portion of the lateral branches had the highest bud-break compared to buds collected from other parts of the branches. When the prophylls of the buds were removed, bud-break was enhanced. The buds were treated with 0-10 mg/l BAP to activate bud-break, and highest bud-break was observed with 7 mg/l BAP. Therefore this study shows that, bud-break in nodal segments collected from central portions of the lateral branches were enhanced when the prophylls of the buds were first removed before culturing with 7 mg/l BAP.

INTRODUCTION

Bamboos, which are giant, tree-like grasses have a long history. They are exceptionally versatile and widely used especially in Asia where the plant is known as the 'poor man's timber'. Although the bamboo plays an important role as a multipurpose forest tree in Asian countries, it is underutilized in Sri Lanka. This is mainly due to the paucity of suitable local species. Among the introduced species, the giant bamboo is recognized for large scale planting in Sri Lanka (Vivekanandan, 1990). The massive interlocking rhizomatous structure binds soils, whereby it promotes soil stability. Giant bamboo, has a recorded cane length of 25-30 meters and a diameter of 20-25 cms, and yields more fibres than most bamboos, furnishing good quality raw material for paper pulp (Zheng-Xing *et. al.*, 1988). This fast growing culm sold at Rs. 100-130 each in Sri Lanka, is much valued and used by rural communities for farming, construction, fuel and numerous cottage

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industries. The edible young shoots of the giant bamboo could be prepared into many delicious dishes.

Unlike many other bamboos, it cannot be easily propagated by conventional methods. The rhizomes which are often scarce and difficult to dig up without any damage, could be used to produce only a limited number of plants without disturbing the mother clump. Propagating the giant bamboo by culm cutting has proved to be difficult (Vivekanadan, 1990). *Dendrocalamus giganteus*, unlike a number of other Asian bamboos, is mast seeding and semelparous with a 76 year flowering cycle. Tissue culture which has been attempted for cloning a few other "difficult – to – propagate" bamboo species, has so far not been reported to succeed for propagating giant bamboo. The present study was undertaken with the objective of developing a technique for clonal multiplication of giant bamboo by nodal bud culture.

MATERIALS AND METHODS

Lateral branches arising from 15 to 20 meter tall culms were brought to the laboratory, and nodal segments about 30 mm long consisting of a nodal bud and two internodal pieces from either side were removed. The nodal buds were collected from duly recorded regions of the lateral branches as given in Figure 1.

Surface sterilization was done by rinsing with 70% alcohol, next with 0.3% $HgCl_2$ for 10 min and then washing with sterile distilled water. The medium used in this study was Murashige and Skoog (1962) basic salts supplemented with 5 mg/l BAP, 2% sugar, 5% coconut milk and 0.6% agar (Fluka) for solid medium. The pH of the medium was adjusted to 5.7 $\pm$ 0.1 prior to autoclaving. All three types of nodal segments were cultured in test tubes (150 x 18 mm) with 15 ml of media, and incubated at 22 $\pm$ 1 C under a photoperiod of 16 hrs and 1500 lux. Thirty replicates were used for each treatment. Observations were recorded on bud – break after 10 – 14 days.

To activate bud – break, buds were excised from the central region of the lateral branches during the dry season, and in 25 nodal segments the prophylls protecting the buds were removed before sterilization and cultured in the same medium as before. Percentage of bud – break was recorded after 10 – 14 days.

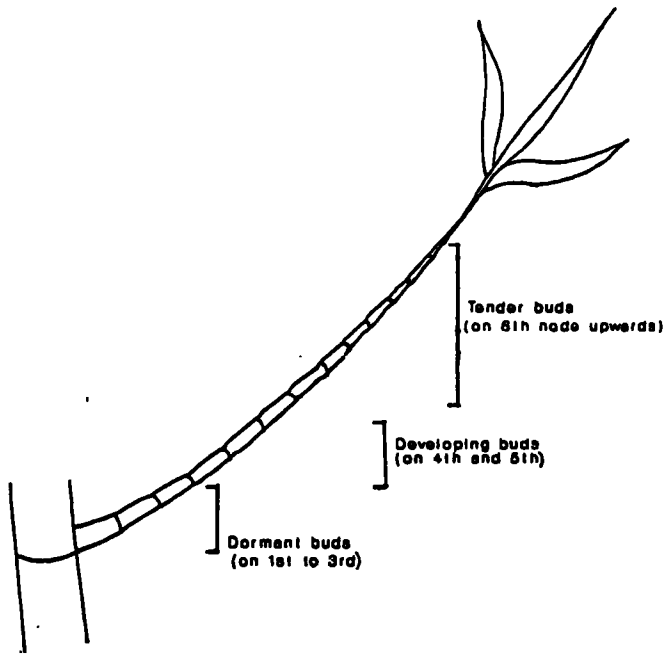

Fig 1. Position of buds on lateral branches

Next the nodal buds from the central region of the lateral branches were collected during the dry season, its prophylls removed and cultured in MS basal medium with 2% sugar, 5% coconut milk, 0.6% agar and BAP at different concentrations (0, 0.5, 1, 5, 7 and 10 mg/l). The sprouted buds were transferred to fresh medium with the same composition and kept under observation.

Chi – squared test was used in experiments to determine whether the factors tested had any effect on bud – break or not.

RESULTS AND DISCUSSION

In the field nodal buds sterilized by the given method, 68% contamination was recorded. Many other sterilents have been used, (0.1 – 0.2% streptomycin, 10% $Ca(OCl)_2$ and 0.1 – 0.3% $HgCl_2$) singly or in combination (unpublished data) but this did not provide adequate sterile materials. Bamboos usually flourish near water and their damp leaf pockets are ideal for the profuse growth of microflora. On lateral branches arising from the culm, 3 types of nodal buds can be identified according to their position on branches, age, size, maturity and the internodal length. The results in Table 1 shows that only the buds from the middle portion (*4th and 5th buds*) of the lateral branches respond to *in – vitro* conditions. On each of these nodes there were about 5 – 7 small buds developing underneath the prophylls. In *in – vitro* culture, with development of buds the prophylls are forced apart following which the buds break and shoots emerge. Out of the 7 buds, only a few buds emerge through the prophylls. Nodal buds excised from the lower region (*1st to 3rd buds*) of the lateral branches never developed in culture. They had only about 3 – 5 buds visible underneath the prophylls and remained dormant throughout. *In planta* also, the rate of bud – break in these dormant buds is low. The buds of the upper regions of the lateral branches also did not respond to culture. In this zone there could be 10 – 20 nodes for a length of 30 – 40 cm upwards from the 5th node. Towards the very tip of the branches the nodes are very tender and compact and their buds do not develop to give vegetative branches in nature too. For all subsequent experiments, buds dissected from the central region of the lateral branches were used.

Bud – break was recorded from nodal buds cultures, after removal of the leathery prophylls (Table 2). The Chi – squared test was

performed on the data and it showed that the removal of prophylls seems to help a considerable number of buds to grow out which would otherwise not develop in culture. The strong leathery prophyll could be a purely mechanical impediment to bud growth. It could be stated, that the prophylls do not act as a strong barrier for bud-break in the materials collected during the rainy season (unpublished data), a fact which could be explained by either the prophyll losing its characteristic texture with the onset of moisture or the inhibitors themselves following a seasonal rhythm of production. Therefore the removal of leathery prophylls helps the buds to grow out during dry seasons. For all future experiments, the prophylls of the buds were removed before surface sterilization.

Table 1. Physiological maturity of the bud on bud-break.

Position of buds on lateral branches.	% Bud-break[a]
1-3 buds from base	0
3-5 buds from base	50
5th bud upwards	0

a - 30 replicates/treatment
X^2 - 36 with 2 d.f, $P > 0.05$

Table 2. Effect of prophyll removal on bud-break.

Treatment	% Bud-break[a]
Nodal buds with prophylls removed	36
Nodal buds with prophylls intact	4

a - 25 replicates/treatment
X^2 - 6.1, 1 d.f, $P < 0.05$

Next the field buds of giant bamboo were cultured in BAP to induce bud-break. The results in Table 3 indicate that there is no significant difference in response to 5, 7 and 10 mg/l BAP treatments in bud-break.

Table 3. Effect of BAP on bud-break in field nodal buds.

BAP mg/l	% Bud-break[a]
0.0	3.3
0.5	3.3
1.0	0.0
5.0	26.0
7.0	43.0
10.0	23.0

[a] - 30 cultures/treatment
X^2 - 32.1 , with 5 d.f, $P < 0.05$

Sprouted nodal segments were transferred to fresh media of the same composition, after 14 days. About 1-3 shoots emerged from each node elongating from 1-3 cm. The cultures deteriorated without any further development when observed after 4 wks.

Similar work on field nodal buds have been reported on bamboos. Mascarenhas *et. al.*, (1988) reported that *Dendrocalamus strictus* nodal buds could be sprouted with Kinetin (0.5 mg/l) and BAP (1.0 mg/l) and more work is yet being carried out on subculturing, multiplication and rooting. In *Bambusa arundinacea*, *Bambusa vulgaris* and *Dendrocalamus strictus* 2-3 shoots could be developed in 0.2 mg/l Kinetin and 0.5 mg/l BAP and only 20% of the shoots developed roots with 1.0 IBA with *Dendrocalamus strictus*, while rooting was difficult in the other two species (Nadgir *et. al.*, 1984). In *Bambusa ventricosa* too, only bud-break was observed with 5 mg/l BAP (Dekker and Rao, 1987).

The work carried out in bamboo up to 1988, reveals that obtaining plantlets from field nodal buds is difficult (Mascarenhas *et. al.*, 1988). Besides, the cloning of mature trees is preferred over plants raised from

seeds, because the presence of desired qualities may be ascertained beforehand. Even though some tree species can be micropropagated from tissues collected from mature trees (Ahuja, 1987) many others can presently be propagated only from juvenile tissues.

CONCLUSIONS

When nodal buds were collected from the field, the position of the buds have turned out to be important in bud-break. The removal of prophylls and the addition of 5-10 mg/l BAP to the medium enhanced bud-break.

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