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*Short Communication*

# MICROPROPAGATION OF *DENDROCALAMUS HAMILTONII* THROUGH NODAL EXPLANTS

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**ABSTRACT**
Multiple shoots were induced from seedling and axillary buds of mature plants of *Dendrocalamus hamiltonii* on White's medium supplemented with BA and kinetin. About 20-25shoots were obtained within 15-25 days from a nodal explant of seedling and 3–8 shoots were obtained from a nodal explant of mature plants in the primary culture. The seedling- derived cultures were separated into groups of 5–7 and transferred to fresh subculture medium. Rooting of the shoots was achieved under in vitro and ex vitro conditions. 90-95% of rooting was achieved by the ex- vitro method using IBA, NAA, & IBA+NAA.

**KEY WORDS**: Micropropagation, Multiple shoots induction, nodal explants, plantlets regeneration and Root induction.

## INTRODUCTION

*Dendrocalamus hamiltonii* Munro is a remarkable clumped bamboo, that yields double harvest – nutritive green fodder for cattle in winter, and economically attractive culms of domestic and industrial importance (Sharrna, 1989). A native of tropical Eastern Himalaya and Nepal, it has long been cultivated in Himachal Pradesh (H.P.) state of India on a small scale. It is so useful that it has entered the culture of Himachal people, who call it 'Maggar'' & is a multipurpose bamboo with many well-known uses, and a source of nutritive green fodder for the cattle, especially during winter – a lean period for the greens in the hills. Like other bamboos, it is also propagated through seed, stem or rhizome cuttings. A method of mass propagation has also been developed through the use of single node culm cuttings in maggar bamboo. However, the traditional methods of propagation limit the number of propagules that can be produced, and is both labour intensive and time-consuming. Further, there is also risk involved in bamboo propagation through vegetative means using material from plants of unknown age because of the peculiar behaviour of mass flowering and death of the flowered clumps. Thus the use of tissue culture(TC) techniques for rapid multiplication of bamboos has been recommended; it offers many advantages, particularly when the explants are taken from physiologically young and field-tested elite clones. Survival rate is recorded 100 % after plantation in the field.

It is reported by Lin and Chang that the maturation of the bamboo species affects the potentiality of axillary buds and the success of clonal multiplication from adult culm is restricted by many factors. It is proposed by Byatriakova *et al*., 2003 that bamboo forests are important potential modulator of global environment.Commercial production of Bamboo is the first attempt in Eastern Himalaya Part of India through tissue culture process. The 100% survival rate is also first record in this type of study.

## MATERIALS & METHODS

Selection of healthy nodal segments of *Dendrocalamus hamiltonii* (2.0-2.5 cm in length) were collected from a natural bamboo clumps of nearby places of Tajpur (Bihar). The nodes containing the axillary buds were used to initiate and establish *in vitro* culture. Stands of donor bamboos were about 7-15years old. Leaf sheath of nodal segments were removed with sharp blades, sized and were surface sterilized by 70 percent ethanol, then disinfected with 0.1 per cent $HgCl_2$ solution for 5minutes. Many workers have found mercuric chloride satisfactory (Nadgir *et al*., 1984; Saxena, 1990; Das and Rout, 1991). Others have used sodiumhypochlorite and chlorine water for surface sterilization (Rao *et al*., 1985; Yeh and Chang, 1986 a, b). There is one report of use of sodium hypochlorite and mercuric chloride successively (Yashoda *et al*., 1997). The disinfected explants were washed thoroughly under running tap water containing 1-2 drops of Tween 20 solution and then with sterile distilled water, it was then given a 30 second dip in 70% ethanol and again rinsed in sterile distilled water. Pre-treatment of the explants were carried out with aqueous solution of 0.5 percent of Bavistin, a fungicide for 15minutes. White's media supplemented with 100mg/l myo-inositol, sucrose (3%) and 0.75 per cent agar were used for culturing the explants. Different concentrations of growth regulators like BA, IAA, NAA, IBA & Kinetin were used in medium. The pH of the medium was adjusted to 5.7. The media was sterilized in an autoclave at $121^0$ C, 15 lb pressure for 15 minutes. Sterilized nodal sections were cultured in the semi solid White's medium in culture bottle in inoculation chamber under controlled conditions

.Cultured buds sprouted within 3-4weeks.Sprouted buds were subcultured and transferred in another bottles containing same media. After some time sprouted buds elongated and developed into a number of multiple shoots. The elongated shoots were used as explants either as a single or a cluster of two- three shoots for production of clumps of multiple shoots and root induction.

When roots were well developed, plantlets were removed from culture medium and after washing the roots gently under running tap water, were transferred to hykopot containing cocopeat and Vermicompost into 3:1 ratio. They were irrigated with water and kept in closed conditions maintaining the humidity at green house for 20-30 days. The plantlets were acclimatized at green house before transferring to shade net house and then to natural condition. Within 3 months the plantlets were transferred to polybags containing soil: sand: cowdung mixture (1:1:2) and shifted to Agronet site. There are proliferation of shoots into miniclumps . Miniclumps were separated in separate polybags and were irrigated. Now they are ready for distribution.

| Growth regulators(mg/l) | No.of shoot /clumps | Shoot-length(cm) |
|---|---|---|
| Kn0.25 | 1.8±1.5 | 1.0±0.05 |
| Kn0.50 | 1.5±0.49 | 1.1±0.33 |
| Kn1.00 | 3.06±1.59 | 2.09±1.1 |
| Kn1.50 | 3.25±1.6 | 3.05±1.21 |
| Kn2.00 | 3.85±2.09 | 3.25±1.3 |
| BA0.25 | 4.09±2.4 | 3.26±0.6 |
| BA0.50 | 4.66±2.90 | 4.01±1.3 |
| BA1.00 | 8.00±1.7 | 4.5±0.3 |
| BA1.50 | 12.5±2.84 | 4.7±0.2 |
| BA2.00 | 17.57±3.37 | 4.00±0.5 |

## RESULTS

White's medium containing solidifying agent Agar with or without growth regulators like BA, IAA, IBA, NAA, Kinetin and IAA+IBA+NAA in different concentrations were prepared. The nodal explants were collected in month of September & were cultured in prepared media .After 2-3weeks cultured explants were noticed to be sprouted, but those, which were remaining green for long period did not sprout and dried up. Sprouting percentage depends on month of collection, age of bamboo-clumps at collection site, culture conditions. Seasonal effect on axillary bud-breaking was observed by Saxena and Dhawan in 1993 on *D. longispathus*. Sprouted parts are separated by sharp scalpels under controlled conditions in inocculation cabinet, *i.e.* singly or clumps of sprouted parts were used as an explant. Separated parts of clumps were cultured in different bottles having same concentration of growth regulators. In present study sprouting percentage, shoot lengths etc. vary with changes in the concentration of growth regulators. All explants didn't produce similar number of shoots, similar shoot-lengths within the same period of time in the same sps. All these things vary with changes in age of selected plants, pretreatment-procedures, culture conditions. If once the bud-break occurred, successive process was to be done by subculturing in same medium. There was variation in reading with variation in the use of treatment-concentration. Among the used growth-hormone BA (0.50mg/l,1.0mg/l) was a suitable growth-hormone for shoot-induction. Shoot-multiplication rate 4.66±2.90 and 8.00±1.7 with shoot-length 4.01±1.3 and 4.5±0.3 respectively is an ideal reading. Maximum multiplication rate is 17.57±3.37 in corporation with BA 2.00mg/l and maximum shoot-length 4.7±0.2 with the use of BA1.50mg/l. It is also observed by Saxena and Dhawan that shoots induced from nodes of *B. valgaries* multiplied at a slower rate than those from *D. Longispathus*

## DISCUSSION

Micropropagation technique is used to produce elite clones of bamboo. Most of the success achieved, either by shoot formation or by embryo formation only by juvenile (zygotic embryo and seedling explants) explants are more easily established in culture and growth and rapid proliferation than other materials .New sprouts produced through *invitro* culture were also used as an explant, for clonal propagation explants from adults should be the material of choice (Thorpe *et al.*, 1991; George, 1993). Sterilization with 0.1% $Hgcl_2$ for 5-minutes was to be used. Some workers used Sodium chlorite or chlorine water for surface sterilization (Rao *et al.*, 1985; Yeh and Chang, 1986a, b). White's media supplemented with 100mg/l myo-inositol, sucrose (3%) and 0.75 per cent agar were used for culturing the explants and 0.25mg/lKn for initiation. Maximum multiplication is observed with the use of 2.00mg/l BA.

It is also observed by Saxena and Dhawan, 1993, that shoots induced from nodes of *B. valgaries* multiplied at a slower rate than those from *D. Longispathus.* Subculture must be done after some fixed period, delaying of subculturing period resulted in gradual browning of the medium. Mudai and Borthakur reported the same result in *Bambusa balcoa*. Browning of the medium was experienced by many other Francisco J. Hidalgo and Rosario Zamora (2000). But by quick transfer of explants on fresh medium the problem was sorted-out. For root induction, there was application of IAA, IBA, NAA, giving successive results &after some time acclimatization could be done. Plantlets were transferred to trial site & was studied that survival rate is 69.8%. By different workers survival rate reporting is 70-80% (Nadgir *et al.*,

1984). Efficient rooting is the main target through micropropagation .Only 30% rooting is reported in *D. strictus* (Chaturvedi *et al*., 2011). Although it is poor man's timber, it is very important in making papers, card – boards *etc*. Thus it is very important raw material for paper-mills. Micropropagation of *D. hamiltonii* produces a large no. of clones, which is the future property of farmers to reduce the cost, further culture is needed.

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