CLONAL PROPAGATION OF SOME BAMBOO SPECIES THROUGH ADVENTITIOUS RHIZOGENESIS IN CULM CUTTINGS

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Introduction

Every node of segmented axis of a bamboo plant bears a bud or a branch. Several studies on vegetative propagation have aimed at transforming as many as possible of these innumerable buds into planting material through induction of adventitious rhizogenesis (Banik, 1980). It is a viable cloning option having the advantage of obtaining enormous number of cuttings from single clump with lower cost of transport, handling and labour (Dransfield and Widjaja, 1995). For these reasons, rooting of culm/branch cuttings has been adopted for propagation of bamboo species since long (Pathak, 1899; Dabral, 1950; McClure, 1966). However, the procedure involves several limitations, most pronounced of which are highly season-specific rooting behaviour of most bamboos and inherent rooting recalcitrance in many important species. A variety of growth regulators have been employed by the researchers with variable amount of success and differential response in bamboo species (Uchimara, 1977; Suzuki and Ordinaro, 1977; Surendran *et al.,* 1989; Seethalakshmi *et al.,* 1989; Agnihotri and Ansari, 2000; Singh *et al.,* 2002). Present study pertains to testing of graded doses of identified effective growth regulator in the best suitable planting season to optimize adventitious rhizogenesis in culm cutting with a view to evolve cloning procedure for *Bambusa multiplex* (Laur.) Raeush. Ex Schult. & Schult. F., *Bambusa tulda* Roxb., *Bambusa vulgaris* Schrader ex Wendl.

Material and Methods

Single node culm cuttings of three species of bamboo viz. *Bambusa multiplex*, *Bambusa tulda* and *Bambusa vulgaris* were prepared from the mature culms collected from a five-year-old plantation in April 2005. The nodal segments measuring about 8-12 cm in length were surface disinfected for 5 min with 0.2% (w/v) aqueous solution of mercuric chloride and subsequently washed with sterilized water. The sterilized single node segments were subjected to treatment with 3 different concentrations of NAA (naphthalene acetic acid) i. e. 1, 2 or 4 mM (mili mole) by immersing them for 24h. A suitable control was maintained wherein these were immersed in water for 24 h. The planting season and growth regulator i.e. NAA was decided on the basis of our earlier studies in these species (Nain and Singh, 2005). Twenty single node culm cuttings/treatment/replicate were used and three replicates were maintained for each species. The treated cuttings were horizontally placed and covered completely with sand (10cm deep) in ground beds of

low-cost mist chamber maintained at 70 5% relative humidity and 30 2°C temperature.

After two months, the cuttings were scored for adventitious rooting percentage, root number and length. Wherever the data were recorded in percentage, the values were converted in to arc √sine transformations prior to statistical analysis. The data obtained were subjected to statistical analysis, employing analysis of variance (ANOVA), 'F'-test for significance at P = 0.05 and computing LSD values to separate means in different statistical groups using statistical software SX (Version 2.0; NH analytical software, 1987).

Results

A comparison of induction and growth of adventitious roots in the three species of bamboo under study revealed significant differences. *B. multiplex* exhibited best rhizogenesis followed by *B. vulgaris.* Low percentage of rooting was recorded in *B. tulda* as compared to other species. Significant differences in root number and length were recorded in *B. vulgaris* (Table 1). The influence of different concentrations of NAA tried was also found significant for adventitious rhizogenesis. Best rooting occurred in 2mM NAA followed by 4 mM NAA, which were enhanced by 166 % and 153 %, respectively over the water treated control. All NAA doses resulted in better root length than control but the treatment of 4 mM NAA proved to be the best so far as the root number is concerned (Table 2).

Significant interaction of bamboo species to different concentrations of NAA tried was noticed for adventitious rhizogenesis. In *B. tulda* and *B. vulgaris,* best root induction was observed when culm cuttings were administered with 4 mM NAA. The higher concentration of NAA also produced more roots than the other treatments in *B. tulda.* However, 2mM and 1mM concentrations of NAA proved superior for rooting in *B. multiplex* (Table 3).

Table 1

Characteristics of adventitious rhizogenesis in different bamboo species.

Species	Characteristics		
	Rooting	Root number	Length (cm)
B. tulda	11.4	6.88	15.83
B. vulgaris var. green	45.5	8.25	21.50
B. multiplex	51.3	3.83	15.83
$LSD_{0.05}$	5.39	2.58	6.56

Data is mean of three replicates.

Table 2

The effect of graded doses of naphthalene acetic acid on adventitious rhizogenesis.

Treatment	Characteristics		
	Rooting	Root number	Length (cm)
Control	16.7	4.44	6.89
NAA (1 mM)	36.9	4.61	16.33
NAA (2 mM)	44.4	6.33	17.56
NAA (4mM)	42.3	9.89	16.44
$LSD_{0.05}$	6.23	2.98	7.57

Data is mean of three replicates.

Table 3

The interaction of graded doses of naphthalene acetic acid and different bamboo species for adventitious rhizogenesis.

Species	Treatments	Characteristics		
		Rooting (%)	Root number	Length (cm)
B. tulda	Control	0	0	0
	NAA (1 mM)	11.0	2.8	4.0
	NAA (2 mM)	20.0	5.3	7.7
	NAA (4mM)	29.7	19.3	10.7
B. vulgaris var. green	Control	29.7	10.3	10.3
	NAA (1 mM)	45.1	5.7	25.7
	NAA (2 mM)	50.0	10.3	28.3
	NAA (4mM)	57.8	6.7	21.7
B. multiplex	Control	39.9	3.0	10.3
	NAA (1mM)	60.1	5.3	19.3
	NAA (2mM)	65.1	3.3	19.7
	NAA (4mM)	39.9	3.7	17.0
$LSD_{0.05}$		7.02	5.17	NS

Data is mean of three replicates

Discussion

Variable response to adventitious root formation among various bamboo species generally results due to the differences in morphological features and endogenous levels of stored photosynthates and auxillary substances. Exogenous application of various growth regulators, mostly auxins, has been reported to positively influence induction and growth of adventitious roots in culm cuttings of bamboos (Uchimara, 1977; Suzuki and Ordinaro, 1977; Agnihotri and Ansari, 2000; Singh *et al.,* 2002). Exogenous application of auxins becomes effective if their endogenous level is low for example due to inactive growth phase or less accumulation in distal plant parts (Singh *et al.,* 2004). A comparison of the incidence of rooting, devoid of any exogenous NAA treatment i.e. in control condition, indicated the difference in the inherent capacity of adventitious rhizogenesis of the species in the order: *B. tulda* (0%) < *B. vulgaris* (30 %) < *B. multiplex* (40%). Interestingly, reverse pattern exists in wall thickness, culm diameter and internodal length i.e. *B. tulda* > *B. vulgaris* > *B. multiplex* (Dransfield and Widjaja, 1995). Seemingly, striking differences in the morpho-anatomical characteristics among these bamboo species influenced adventitious

rhizogenesis. These differences may also be responsible for differences in endogenous levels of auxin and auxillary substances to manifest differential rhizogenesis.

Induction of adventitious rooting only after exogenous application of auxin (NAA) and successive enhancement of rooting in higher doses in *B. tulda* indicates towards low endogenous auxin level and the need for exogenous auxins for root induction in the species. *B. tulda* has been known to be a very reluctant-to-root species in which adventitious rhizogenesis remains elusive even after exogenous treatments of various growth regulators (McClure and Kennard, 1955; Kumar *et al.,* 1990, 1994). Increasing NAA doses similarly optimized adventitious rhizogenesis in *B. vulgaris*. However, in case of *B. multiplex* NAA treatment of 4 mM became supra-optimal or inhibitory for adventitious rhizogenesis (Table 3). The positive influence of NAA on adventitious rhizogenesis has been recorded in *B. vulgaris* (Uchimara, 1977; Azizini *et al.,* 1995) and many other bamboo species, e.g. *B. arundinacea, D. strictus* (Surendran *et al.,* 1989), *B. balcoa* (Seethalakshmi *et al.,* 1989), *D. asper* (Singh *et al.,* 2004). Nonetheless, NAA-induced adventitious rhizogenesis in culm cuttings achieved in the present investigation offers a viable option for cloning of *B. tulda, B. multiplex* and *B. vulgaris*.

Conclusions

In conclusion, clonal propagation can be accomplished employing single-node culm cuttings administered with 4 mM NAA in *B. tulda* and *B. vulgaris* and 2 mM NAA in *B. multiplex*.

SUMMARY

With a view to evolve cloning procedure for *Bambusa multiplex*, *Bambusa tulda* and *Bambusa vulgaris* single node culm cuttings were subjected to four different treatments for 24h viz., water, or 1, 2 and 4 mM NAA. The treated cuttings were horizontally placed and covered completely with sand in ground beds of low-cost mist chamber. After two months, the cuttings were scored for adventitious rooting percentage, root number and length. Significant influence of graded concentrations of NAA on adventitious rhizogenesis was observed. In *B. tulda* and *B. vulgaris* the treatment of 4 mM NAA to culm cuttings proved superior for root induction while 2mM was found best for rooting in *B. multiplex*.

सन्धिस्तम्ब कलमों में आगन्तुक राइजोजनन द्वारा कतिपय बांस जातियों में कृन्तकीय प्रवर्धन कराना

एन०पी०एस० नैन, एस०एल० मीना, के०के० कुंजम, एस०पी० त्रिपाठी व संजय सिंह

सारांश

बैम्बूसा मल्टिप्लेक्स, बैम्बूसा तुल्डा और *बैम्बूसा वल्गैरिस* की कृन्तकीय विधि विकसित करने की दृष्टि से उनकी एक ग्रन्थि सन्धिस्तम्भ कलमों पर 24 घंटे के लिए चार विभिन्न उपचार अर्थात् उन्हे पानी अथवा 1, 2 और 4 mM न्यूक्लिइक एसेटिक अम्ल में डुबाए रखना, किए गए। उपचारित कलमों को धरती में पड़ा (क्षैतिक) रखाकर अल्पमोली धुन्ध प्रकोष्ठों की जमीन पर बनी क्यारियों में रेत से पूरी तरह ढक दिया गया। दो मास उपरांत कलमों को आगन्तुक जड़ें निकलने का प्रतिशत, जड़ों की संख्या और उनकी लम्बाई जानने के लिए निकाला गया। आगन्तुक राइजोनन पर न्यूक्लिइक एसेटिक अम्ल के श्रेणीगत किए संकेन्द्रणों का सार्थक प्रभाव पड़ता देखा गया। *बै० तुल्डा* और *बै० वल्गैरिस* में 4 mM न्यूक्लिइक एसेटिक अम्ल उपचार का सन्धिस्तम्भ कलमों पर पड़ता

प्रभाव जड़ें निकलना प्रेरित करने को श्रेष्ठतर रहता पाया गया जबकि *बै० मल्टिप्लेक्स* में जड़े निकलना प्रेरित करने को 2 उड उपचार सबसे अच्छा रहा।

References

Agnihotri, K. and S.A. Ansari (2000). Adventitious rhizogenesis in relation to seasonal variation, size of culm branch cuttings and IAA treatment in Bamboos. *Indian Forester*, **126** (9): 971-984.

Azzini, A., J.I. Fahl and A.B. Salgado (1995). Rooting of rejuvenated cuttings of *Bambusa vulgaris* treated with naphthalene acetic acid. *Bragantia,* **54**: 47-50.

Banik, R.L. (1980). Propagation of Bamboo by clonal methods and by seeds (Lessard, G. and A. Chouinard, eds.). *Bamboo Research in Asia.* IDRC, Canada, pp. 139-150.

Dabral, S.N. (1950). A preliminary note on propagation of Bamboos from culm segments. *Indian Forester,* **76**: 313-314.

Dransfield, S. and E.A. Widjaja (1995). *Plant Resources of South-East Asia*, *No. 7, Bamboos*. Backuys Publishers, Leiden.

Kumar, Adarsh, Meena Dhawan and B.B. Gupta (1990). Flowering and rooting of *Bambusa tulda* culm cuttings in response to growth regulating substances. *Indian Forester*, **116** (7): 547-553.

Kumar, Adarsh and M. Pal (1994). Mass propagation of *Bambusa tulda* through macro-proliferation for raising industrial and commercial plantations. *Indian Forester*, **120** (2): 152-157.

McClure, F.A. (1966). *The Bamboos, A Fresh Perspective*. Harvard University Press, Cambridge, Massachusetts, USA.

McClure, F.A. and W.C. Kennard (1955). Propagation studies (with bamboos). *Report of Federal Experimental Station.* Puerto Rico.

Nain, N.P.S. and Sanjay Singh (2005). Standardization of Macropropagation Protocols for Important Bamboo species. *Final Technical Report*. TFRI, Jabalpur.

Pathak, S.L. (1899). The propagation of common male Bamboo by cuttings in the Pinjaur-Pateala forest nurseries. *Indian Forester,* **25**: 307-308.

Seethalakshmi, K.K., C.S. Venkatesh and T. Surendran (1989). Vegetative propagation of Bamboos using growth promoting substances-1. *Bambusa balcooa* Roxb. *Ind. J. For.,* **6**: 98-103.

Singh, Sanjay, S.A. Ansari and P. Kumar (2002). Clonal propagation of *Bambusa nutans* through culm and culm-branch cuttings. *Indian Forester,* **128** (1): 35-40.

Singh, Sanjay, P. Kumar and S.A. Ansari (2004). A simple method for large-scale propagation of *Dendrocalamus asper. Scientia Horticulturae,* **100**: 251-255.

Surendran, T., K.K. Seethalakshmi and C.K. Somen (1989). Vegetative propagation of *Bambusa arundinacea* and *Dendrocalamus strictus* by culm cuttings. *Malaysian for.,* **49**: 432-456.

Suzuki, T. and F.F. Ordinaro (1977). *Some Aspects and Problems of Bamboo Forestry and Utilization in the Phiippines*. Asia Forest Industries, College, Laguna, Philippines.

Uchimara, E. (1977). *Ecological Studies on the Cultivation of Bamboo Forest in Philippines*. Forest Research Institute, College, Laguna, Philippines.