IN VITRO MORPHOGENESIS AND PLANT REGENERATION OF BAMBOOS (*Oxytenanthera abyssinica* A. Rich. Munro)

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ABSTRACT

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With aim of large production of plant material, a protocol for micropropagation of *Oxytenanthera abyssinica* was developed at the laboratory of Plant Tissue Culture, Commission for Biotechnology and Genetic Engineering, Khartoum, Sudan, during November 2006 to April 2008. Nodal explant from 12 months seedlings age was cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of benzyl adenine (BA) in combination with Naphthalene Acetic Acid (NAA). The highest shoot multiplication (4.4/shoot/explant) was achieved on six weeks on MS supplemented with 5.0 mg L^{-1} BA and 0.2 mg L^{-1} NAA. Proliferation media in liquid status shows better performance than solid media. For elongation, *in vitro* produced shoots were cultured on MS basal media (full, half, quarter) strength, MS full strength achieved the high shoot length (7.6) cm. When the propagated shoots were transferred to a rooting medium (full, half, quarter strength) MS, supplemented with (0, 0.5, 1.0, 2.0, 4.0, 8.0 mg L^{-1}) IBA concentrations, the highest rooting percentage (70%) was achieved in full strength MS media supplemented with 8.0 mg L^{-1} IBA. Plants were successfully established in soil and adapted to greenhouse conditions and field. The protocols developed in this work provide a basis to achieve massive propagation of *Oxytenanthera abyssinica* by *in vitro* culture of nodal segments.

Key words: *In vitro* regeneration, *Oxytenanthera abyssinica*, multiple shoot

INTRODUCTION

Bamboos are monocotyledonous perennial grasses belonging to the sub family Bambusoidea of the family Poaceae (Gramineae). Bamboos cover large areas of tropical and sub tropical regions and had been subjected to depletion by bad management. As a result, reproduction cannot keep pace with exploitation and there is a need for evolution of management practices to secure regeneration, protection, and maintenance of sustained production .They cover about 14 million hectares in the world containing about 1200 species belonging to 70 genera (Shudong, 1998).

In Sudan there are two indigenous bamboos: *Arundinaria alpina* found only in the upper reaches of the Imatong Mountains and *Oxytenanthera abyssinica* found on hill slopes and along Khors in southern region, Nuba Mountains, Jebel ElDair in Kordofan and Ingassana area of Blue Nile State (Elamin, 1990).

The vegetative phase of *Oxytenantherea abyssinica* the species takes a long time to flowering and consequently the production of seeds occur sporadically after a long time estimated as 30-40 years (Kigomo, 1989; El Houri, 1997). Moreover, the seed viability span is short, lasting not more than 3 months under normal storage conditions in the Sudan.

Also, unlike other bamboos it could not be easily vegetatively propagated by ordinary nursery techniques from culm cuttings (Khan, 1966; Kigomo, 1989). Therefore, vegetative propagation at micro scale should be addressed.

Micropropagation using tissue culture techniques offers substantial advantage over other methods. The plant material can be multiplied rapidly on a large scale using explants from physiologically young, field-tested elite plants. Explants for bamboo species could be taken from juvenile materials as zygotic material or it could be from adult or mature material as node, leaf, sheath (Gielies, 1995).The explants, which have been used for callus induction in bamboo, are mature embryo, young florets, inter nodal sections (including the intercalary meristem), leaf base, shoot tip and regenerated roots (El Hassan and Debergh, 1987). *In vitro* propagation of *Dendrocalamus hametonii* using nodal explants from elite seedlings of proven field performance was investigated by Tsay *et al.*, (1990). Earlier, Nadgir *et al.,* (1984) had used single nodal cuttings for shoot multiplication of *in vitro* raised seedlings of *Dendrocalamus strictus* by rooting. Similar observations have been recorded by Banik (1987) who reported growth and proliferation of dormant culm buds on MS medium supplemented with BA and NAA with extensive rooting noticed with 2.0 mg L^{-1} NAA at an activated charcoal (0.3%). Prutpongse and Gavinlertvatana (1992) successfully propagated *in vitro* 54 bamboo species out of 67 species. Multiple shoots were produced from axillary buds on stem node segments cultured on MS medium containing 5.0 mg L^{-1} BA and 0.16 mg L^{-1} NAA, rooting occurred on media containing 3.0 mg L^{-1} NAA. They indicated that several species could be stored *in vitro* on half strength MS medium for less than 15 months.

Siddiqui (1994) obtained axillary buds from the node of *Bambusa vulgaris* and *Dendrocalamus strictus*, and these were cultured on MS medium supplemented with 5.0 mg L^{-1} BA. He found that, multiple shoots were formed. However, it was difficult to get high percentage of rooting, as this dependant on the type of the species. This study was undertaken to develop an efficient and reproducible regeneration protocol for Sudanese Oxytenantherea *abyssinica*

MATERIALS AND METHODS

Plant material and explant

Nodal explants were taken from 12 month old. *Oxtyenanthera abyssinica* seedlings were grown in the nursery. Explant size was about 5 cm in length each containing one or two axillary buds.

Service sterilization

The explants obtained were first washed thoroughly under running tap water to remove all surface dust. Then they were transferred to laminar air flow cabinet. Inside the cabinet disinfections of the nodal segments were accomplished by a brief dipping in 80% ethanol for about 30 seconds followed by immersion for 30 minutes in sodium hypochlorite solution (10% v/v) plus two drops/100 ml of Tween 20.This was followed by rinsing 3 times or more with sterilized distilled water.

Effect of plant growth regulators on multiple shoot induction

Different plant growth regulators were tested to assess the morphogenetic response. Explants were cultured on test tubes containing Murashige and Skoog 1962 (MS) media supplemented with a combination of BA and NAA. BA was used at the following concentrations 0.0, 3.0, 5.0, and 7.0 mg L^{-1} and NAA was used at four levels 0.0, 0.1, 0.2, 0.4 mg L^{-1}.

Effect of media status on in vitro morphogenesis

Nodal explants were cultured on proliferation medium (5.0 mg L^{-1} BA and 0.2 mg L^{-1} NAA) supplemented with Agar 8% or with out, to assess the influence of media status on *in vitro* morphogenesis, Agar free media were supplemented with filter paper to avoid explants sinking.

Elongation of in vitro induced shoots

Shoots were excised from the multiple shoot bunches obtained from nodal explant and transferred individually to culture bottles containing different MS basal media strength supplemented with 3% sucrose and 0.8% agar, to assess their response for elongation.

Rooting of in vitro induced shoots

Shoots were excised from multiple shoot bunches obtained from nodal explant and transferred individually and rooted on to test tubes containing MS media (full, half, quarter) strength, containing different concentrations of Indole Butyric Acid (IBA) (0.0, 0.5, 1.0, 2.0, 4.0 and 8.0 mg L^{-1}).

Acclimatization

In vitro rooted plants were removed from rooting medium and washed to remove adhering gel and transplanted to plastic pots containing autoclaved garden soil and sand at 2:1 ratio. Plants were kept under culture room conditions for 15 days. Mature plantlets, which were about 8-10 cm in height, were transplanted to the green house. They were planted in to black polythene bags (20x20cm) and placed under shade, they were covered with a plastic sheet to increase the humidity for seven days and vegetative growth was observed. They were watered daily and kept for a period of 5 months after that they were planted directly in the field.

RESULTS AND DISCUSSION

Table 1 illustrates the effects of BA and NAA concentrations on multiple shoot induction. It appears that different combinations of BA and NAA have a significant effect on the shoots number. The MS medium supplemented with 5.0 mg L^{-1} BA and 0.2 mg L^{-1} NAA produced a large number of shoot/ explant (4.4). (Figure 1).The results indicated that cultures responded differently according to the supplemented concentration of BA and NAA.

The lowest concentration of both BA and NAA showed no significant difference at $P<0.05$ compared with those of highest concentration of both BA and NAA. These results agree with the findings of Prutpongse and Gavinlertvatana

(1992) who recommended that the proliferation medium of axillary buds of most bamboo species should be in MS basic media with cytokinin and auxin.

Also, Rao (1990) recommended that micropropagation of bamboo can be carried out from axillary buds of young seedlings, and it should be cultured as nodal segments on MS medium containing 6.0 mg L^{-1} BA and 0.3 mg L^{-1} NAA. These results are in line with Kashmanika and Nirianjini (1995) who carried out experiments on bud multiplication cultured on MS medium supplemented with 5.0 mg L^{-1} BA and 0.2 mg L^{-1} NAA.

The number of shoots per explant varied with the species, on Bamboo *arundinaria* 8-10 shoots/explant were developed in MS medium containing 5.0 mg L^{-1} BA and 2% sucrose and 5% coconut milk (Nadgauda *et al.,* 1990). A number of shoots decreased with increase in the concentrations of cytokinin and auxin. Also high necrosis of tissue was observed at 7.0 and 4.0 mg L^{-1} BA and NAA concentrations respectively.

The same is in line with Saxena and Bhojwani (1998) who reported that, nodal segments of *D. hamiltanii* culture in the full strength MS medium should be containing BA with concentration ranging from 1.0 mg L^{-1} to 4.0 mg L^{-1}.

The conditions required for axillary bud sprouting are different from those in similar work on other bamboo species, but the common factor is the presence of cytokinins in the medium (Ramanuja and Yakandawala, 1997).

Table 1. Influence of BA in combination with NAA on multiple shoots induction from nodal explant of *Oxytenanthera abyssinic* after six week of culture

NAA mg/l \ BA mg/l	0.0	3.0	5.0	7.0
0.0	0.0 ^{d}	0.0 ^{d}	0.9 ^{c}	1.0 ^{c}
0.1	0.0 ^{d}	1.1^{c}	1.0^{c}	1.2 ^{c}
0.2	0.0 ^{d}	2.5 ^{b}	4.4^{a}	3.2 ^{b}
0.4	0.0 ^{d}	0.0 ^{d}	1.0 ^{c}	0.0 ^{d}

Means with the same letters are not significantly different at P<0.05 using Fisher protected L. S. D.

Figure 1. Shoot induction from nodal explants of *Oxytenanthera abyssinica* cultured on MS media supplemented with 5.0 mg/l BA and 0.2 mg/l NAA after six weeks of culture

Figure 2 shows the effect of medium state on the performance of the plantlets after 6 weeks on MS media supplemented with 5.0 mg L^{-1} BA and 0.2 mg L^{-1} NAA. It appears that the shoot length was greatest on liquid medium compared with solidified medium, also the number of leaves was greater on liquid medium than on solid medium. Preatha (1992) recommended continuous production of shoots from single buds of *Dendrocalamus strictus* in stationary MS liquid medium.

The result also agrees with Sanjay and and Saxena (1990) who investigated bud sprouting from three weeks- old aseptically grown seeds of *Bambusa tulda*, which were used to initiate cultures. Multiple shoots were obtained on liquid MS medium supplemented with $8x10^{-6}$M BA and $4x10^{-6}$M Kin continuous shoots were produced and rooted on MS media with $4x10^{-6}$M IAA. These results are in line with Saxena and Bhojwani (1998) who recommended that the shoot growth was better on liquid media than on solid media for multiplication of *Dendrocalamus longispathus*. These results might be due to the greater surface area for the shoots to absorb nutrients from the liquid media, while in solid media the area is restricted. In addition the nutrients flow gradient is more continues in the liquid medium and hence these explant tend to absorb more nutrients and growth regulator from the liquid medium.

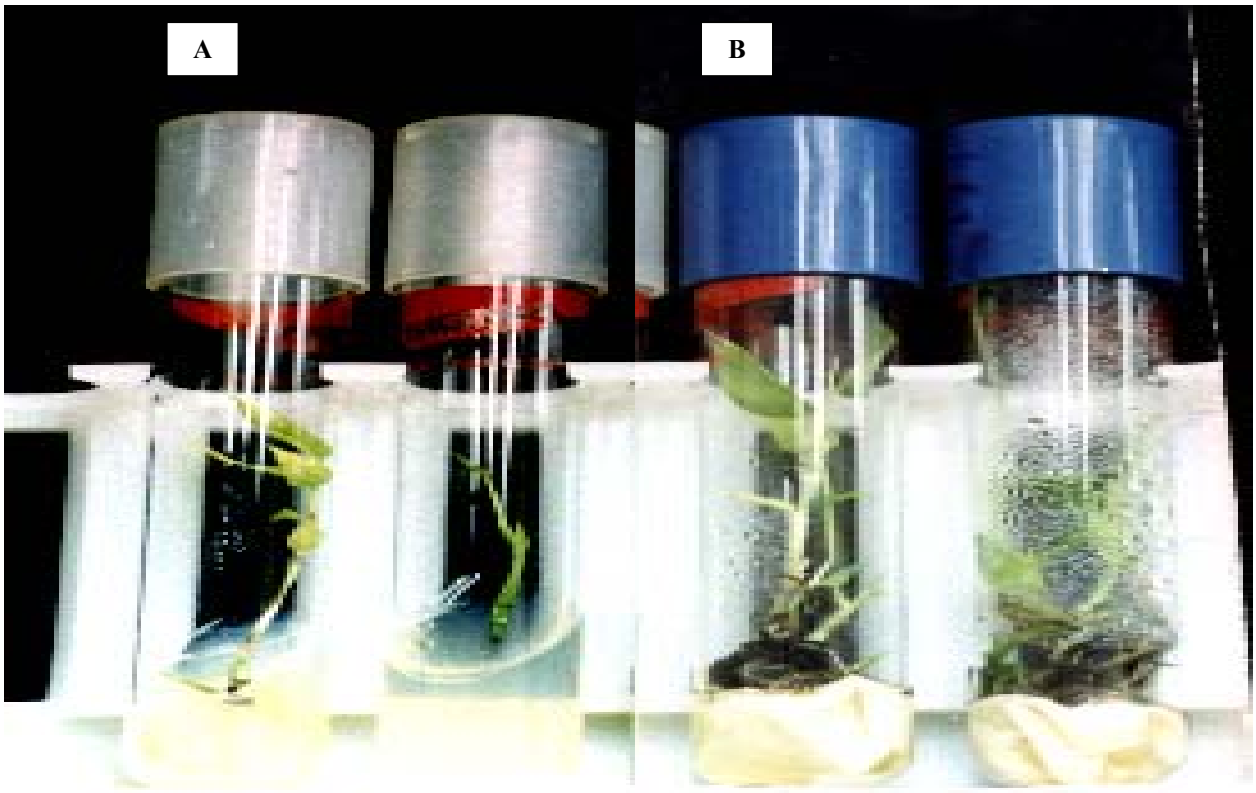

Figure 2. Effect of media status on *in vitro* morphogenesis of *Oxytenanthera abyssinica.*
A. Shoot obtained from nodal explants cultured on MS media supplemented with (5.0 mg/l BA and 0.2 mg/l NAA) solidified with Agar 8%.
B. Shoot obtained from nodal explants cultured on MS media supplemented with (5.0 mg/l BA and 0.2 mg/l NAA) agar free with filter paper

Figure 3 illustrates the effect of MS medium strength on elongation of *in vitro* derived shoots after six weeks of incubation. The shoot length on MS with full strength gave higher values, (7.5cm) compared with the shoot height 5.6 and 5.2 cm on MS with half and quarter strength respectively. The shoot height in full strength medium was significantly different at $P<0.05$ from that in the MS half and quarter strength. These differences can be explained by the presence of more nutrients in the full strength medium. The use of hormone-free medium for shoot elongation has already been reported for soybean (Kaneda *et al*., 1997) and faba bean (Khalafalla and Hattori, 2001) and cotton (Abdellatef and Khalafalla 2007).

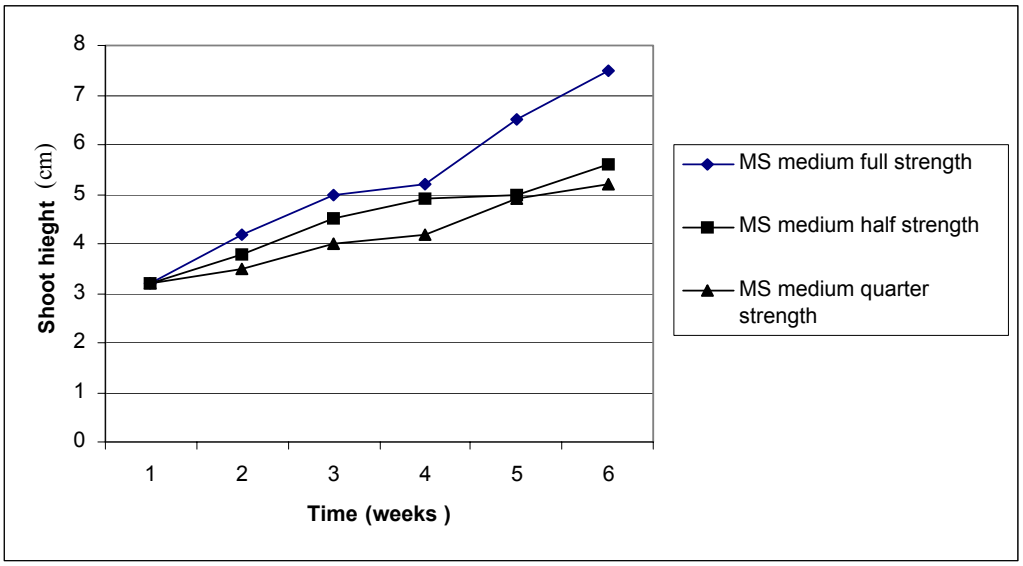

Figure 3. Effect of MS medium strength on elongation of *in vitro* derived shoots from nodal explants of *Oxytenanthera abyssinica* after six weeks of culture

The results presented in Table (2) illustrates the effect of various strengths of basic Murashige and Skoog salt media in combination with IBA at different concentrations on rooting of shoots after six weeks of culture . As IBA is a rooting hormone it increased the rooting percentage, root number and root length in all MS medium (full, half and quarter) in the highest concentrations of IBA compared to MS medium supplemented with lowest concentrations of IBA, as shown in Figure 4.

MS medium with full strength supplement with 8.0 mg L^{-1} IBA gave the highest rooting percentage (70%) compared to other treatments, using the same concentration of IBA (20% and 15%) were obtained in the half and quarter strength of MS medium.

Full strength MS medium supplemented with 1.0 mg L^{-1} IBA gave rooting percentage (16%) while no rooting was obtained in the half and quarter strength of MS medium supplemented with the same IBA concentration.

It was expected that the plantlets in MS medium with lowest strength i.e. quarter, produce more rooting percentage as stated by Prutpongse and Gavinlertvatana (1992).

This result disagrees with the results of Kashmanika and Niranjini (1995) who reported that shoots developed from axillary buds could be rooted on MS media at half strength supplemented with 5.0 mg L^{-1} IBA for *Bambusa vulgaris* and also disagrees with the findings of Saxena and Dhawan (1999) who recommended that, half strength MS medium supplemented with 4.0 mg L^{-1} NAA for rooting of *Dendrocalamus strictus*.

Table. 2: Effect of various strengths of MS medium in combination with IBA on root induction of *Oxytenanthera abyssinica* plantlets after six weeks of culture

IBA conc. (mg/l)	MS basal medium (Strength)					
	Full		Half		Quarter	
	Rooting %	RL** (cm)	Rooting %	RL (cm)	Rooting %	RL (cm)
0.0	0.0 ^{f}	0.0 ^{c}	0.0 ^{f}	0.0 ^{c}	0.0 ^{f}	0.0 ^{c}
0.5	0.0 ^{f}	0.0 ^{c}	0.0 ^{f}	0.0 ^{c}	0.0 ^{f}	0.0 ^{c}
1.0	16.0 ^{d}	0.5 ^{b}	0.0 ^{f}	0.0 ^{c}	0.0 ^{f}	0.0 ^{c}
2.0	36.0^{c}	0.9 ^{b}	2.0 ^{f}	0.3 ^{b}	2.0 ^{f}	0.0 ^{c}
4.0	66.0 ^{b}	1.2 ^{a}	18.0 ^{d}	0.3 ^{b}	10.0 ^{e}	0.2 ^{b}
8.0	70.0 ^{a}	1.4 ^{a}	20.0 ^{d}	0.8 ^{b}	15.0 ^{e}	0.5 ^{b}

** RL = Root length, Means for each parameter in the same column followed by different letters are significantly different (P<0.05) using Fisher Protected L. S. D.

Figure 4. Rooting of *in vitro* derived shoots of *Oxytenanthera abyssinica* cultured on MS media supplemented with 8.0 mg L^{-1} IBA after six weeks of culture

95% of the plants survived and all were morphologically normal planting in the field is carried out when the plants are 12-month old. The rhizome system was well developed by this time and the plants established successfully (Figure 5).

Figure 5. Acclimatization of *Oxytenanthera abyssinica in vitro* produced plants.
A. Potting up and hardening of the plantlets
B. *In vitro* produced plant established in soil

CONCLUSIONS

From the experiment it was observed that the nodal explant sprouted normally on MS media supplemented with 5.0 mg L^{-1} BA and 0.2 mg L^{-1} NAA, it took about six weeks. The explants were subculture in to the same media after the 21 day. Liquid media is high performance on *in vitro* morphogenesis of bamboo plant. *In vitro* produced shoots were successfully elongated on full strength MS basal media. The shoot formation took about 15 days then transferred to rooting media MS supplemented with 8.0 mg L^{-1} BA as rooting media i.e. full strength MS medium supplemented with 8.0 mg L^{-1} IBA, which had been shown as the best medium to initiate roots.

This protocol will pave the way for the development of *in vitro* regeneration system for Bamboo plants.

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REFERENCES

Abdellatef, E. and M. M. Khalafallah. 2007. Adventitious shoot formation and plant regeneration in medium staple cotton (*Gossypium hirsitum* L.) cultivar (Barac B-67). International Journal of Agriculture and Biology, 9 (6): 913-916.

Banik, R. L. 1980. Propagation of Bamboos by Clonal Methods and by Seeds, In., G. Lenard and A. Choumard (eds) Bamboo Research in Asia. IDRC; 139 – 150.

Elamin, H. M. 1990. Trees and Shrubs of the Sudan (Pree Exeter) page (455)

El Hassan, A. A. and P. Debergh. 1987. Embryogensis and plantlet development in the bamboo *Phyllostachys viridis*, Plant cell, tissue and organ culture. 10 (1): 73 – 77

El Houri, A. E. 1997. Review of Silviculture and Utilization of Bamboos. Forests National Corporation

Gielis, J. 1995. Bamboo and Biotechnology. European Bamboo Society Journal. 8:27-39

Kaneda, Y., Y. Tabei., S. Nishimura., K. Harada., T. Akihama and K. Kitamura. 1997. Combination of thidiazuron and basal media with low salt concentrations increases the frequency of shoot organogenesis in soybean (*Glycine* max (L.) Merr). Plant Cell Reports. 17: 8–12

Kashamanika, H. and G. Niranjini. 1995. Propagation of *Bambusa vulgaris* (Yellow bamboo) through nodal bud culture. Journal of Horticulture Science. 70 (3): 469 – 475

Khalafalla, M. M. and K. Hattori. 2001. A combination of thidiazuron and benzyladenine promotes multiple shoot production from cotyledonary node explants of faba bean (*Vicia faba* L.). Plant Growth Regulation. 27: 145–148

Khan, M.A. WC. 1996. Vegetative propagation by culm cuttings in Bamboo vulgaris and *Oxytenanthera abyssinica*, Pamphelet No 37 Repulic of The Sudan, Forest Department 14.P

Kigomo, B. N. 1989. An interim Note on the establishment of the African Mountain bamboo in Kenya. Kenya Forestry Research Institute (KFRI) Muguga. Technical Note No. 2, October 1989 Kenya Journal Science. 8(1 – 7): 5 – 13

Muraashige, T and F. Skoog. 1962. A revised medium for growth and bioassay with Tobacco Tissue Culture. Plant physiology. 15:473-479

Nadgauda, R. S. Parasharami, V. A. Mascarenhas, A. F. (1990). Precocious flowering and seeding behaviour in tissue-cultured bamboos. *Nature* 344: 335 - 336.

Nadgir, A. L., C.H. Phadke., P. K. Gupta., V. A. Parsharami., S. Nair and A. Mascarnenhas. 1984. Rapid Multiplication of bamboo by tissue culture. Silvae Genet. 33: 219-223

Preatha, N. 1992. continous shoot proliferation of *Dendrocalamus strictus* in stationary liquid culture. Journal of Tropical forestry. 8(2): 105-108

Prutpongse, P. and P. Gavinlertatana.1992. *In vitro* micropropagation of 54 species from 15 genera of Bamboo. Horticultural Science. 27(5): 453-454

Ramanuja, S.M. and K. Yakandawala. 1997. Incidence of Flowering Death and Phenology of Development in the Giant bamboo (*Dendrocalamus giganteus*). Annals of Botany 82: 7792-785

Rao, I. A. 1990. Propagation of bamboo and rattan through tissue culture. IDRC, Senior program officer forestry agriculture food and nutrition science division

Sanjay. S. and S. Saxena. 1990. *In vitro* propagation of Bamboo (*Bambusu tulda* Roxb) through shoot proliferation, Plant cell report. 431-434

Saxena, S. and S. S. Bhojwani. 1998. *In vitro* clonal multiplication of 4- year-old plants of the bamboo *Dendrocalamus longispathus*. *In vitro* Dell Dev. Biol. 29P: 135-142

Saxena, S. and V. Dhawan. 1999. Regeneration and large-scale propagation of bamboo (*Dendrocalamus strictus* Nees) through somatic embryogenesis. Plant cell report. 18(5): 438-443

Shudong, W. 1998. Cultivation and integrated utilization of Bamboo in china .China national Bamboo research center, Hangzhou. China

Siddiqui, K.M. 1994. Cultivation of BamBoo in Pakistain. Journal of Forestry 44 (2): 40-53

Tsay, H. S., C. C. Yeh and J. Y. Hscu. 1990. Embryogenesis plant regeneration from another culture of bamboo *Dendrocalamus latiflarous*. Plant Cell reports. 9(1): 349-351