RESEARCH PAPER | Biotechnology | Volume : 4 | Issue : 1 | Jan 2014 | ISSN - 2249-555X

Initiation of suspension culture and somatic embryogenesis to plant regeneration in multipurpose bamboo (Dendrocalamus tulda)

KEYWORDS: *Dendrocalamus tulda*, callus, suspension culture, somatic embryos.

C. M. Wadatkar	N.V. More	V.V. Patil
Department of Biotechnology, College of Computer Science and Information Technology Latur (MS) 413531, India.	Department of Biotechnology, College of Computer Science and Information Technology Latur (MS) 413531, India.	Department of Biotechnology, College of Computer Science and Information Technology Latur (MS) 413531, India.

ABSTRACT *The cell aggregates disperse in liquid medium under orbital shaker is known as suspension culture. The suspension culture of Dendrocalamus tulda was performed by using callus. The friable callus tease aseptically and transferred in liquid medium .The culture obtained with clumps of proliferating globular, heart stages embryo with very little non embryogenic tissue. The number and size of somatic embryo clumps was measured to quantify the growth of embryo. The suspension culture were subculture every 21 d by replacing the old medium with equal volume of fresh medium. For the establishment of callus culture and suspension culture initiation various exogenous combinations of auxins and cytokinin (2,4-D, BAP,NAA) was found to be most effective. The somatic embryos (Globular, heart stages) were cultured on MS medium containing BAP and 2, 4-D were used for shoots regeneration .The shooted plants were subculture on rooting medium. The regenerated plants were hardened. The regenerated plants were found to be phonotypicaly normal. The harden plants were ready for field trails.*

Introduction:

Dendrocalamus is the most useful multipurpose plant belonging to family Poaceae (Anonymous 1978). Dendrocalamus tulda is an ever green, deciduous bamboo with gray culms. Apart from being an important source of raw material for paper and pulp industry it is useful for many purposes (Saxena and Bhojwani, 1993). The succulent bamboo shoots is alternative source of phytosterol (Srivastava 1990).There are very few reports on reproductive biology of bamboo, possibly because of their flowering only at long interval (John et al, 1999). In vivo clonal propagation of plants is often expensive and unsuccessful. Tissue culture methods offer an alternative means of plant vegetative propagation. Murashige (1978a, 1978b) proposed four distinct stages that can be adopted for overall commercial production of clones.

In bamboo flowering occur irregularly and seeds have very short viability. Due to this large scale propagation in D. tulda because of lack of excellent breeding system (John C.K and Nadgauda 1999, Lin C. S et al 2003). In bamboo complete plantlet regeneration have been reported via micropropagation using different explants (Ravikumar R et al 1998). As a matter of course embryogenic tissue at early stages are used to initiate embryogenic suspension culture.

During the present investigation the effects of plant growth regulators, culture conditions on initiation of embryogenic suspension and plantlet regeneration in D. tulda were studied.

Materials and methods:

Fresh young leaves (leaf bid),New sprout node bud segments, root segments, shoot tip regions were excised from mother plants growing in forest garden Wadali forest station Amravati (MS). The explants were thoroughly washed under running tap water for 20 min, immerse in 1% Savlon for 2 to 3 min and thoroughly washed with distilled water. Subsequently surface sterilized with 0.1 to 1 % $HgCl_2$ for 2 to 5 min, and rinsed well with sterile distilled water for 4 to 5 times. The explants leaves (leaf bid), New sprout node bud segments, root segments, shoot tip regions were cultured on MS medium (MS 1962). MS medium was fortified with auxins and cytokinin singly as well as in combinations for callus induction. The medium were congealed with agar agar (Himedia) 0.8% and sucrose 3% was used as a sourse of carbohydrate. The PH of the medium was adjusted to 5.8 using 0.1 N NaOH and/or 0.1N HCl. The sterilized explants were cultured under laminar air flow and were incubated at 25 $\pm$ 2 ^{O}C, under 16 h photoperiod by cool white fluorescent light (1000 to 400 lux) and 8 h dark of 24 h d cycle with 55 + 5 % relative humidity. All the experiments were repeated thrice and had 10 replicates with single explants. The New sprout node bud segments, callus was maintained on MS medium, supplemented with 2.4-D, NAA and BAP 1 mg/l. The callus was subculture on fresh medium at 21 d of interval.

The callus was tease for suspension culture. For the preparation of liquid culture medium agar powder was not incorporated. The modified MS mediums fortified with 2, 4-D 1 mg/l and BAP 1 mg/l was prepared and sterilized and transfer in a laboratory Bioreactor. Precede the bioreactor (by agitation and aeration) at 25 $\pm$2 ^{O}C. Suspension were subculture every 15 d by replacing the old medium with an equal volume of fresh medium After 5 to 7 week old suspension culture, the culture was passed with the help of fine 300um sieve. The cell clumps containing globular, heart stages of embryo were observed under inverted phase contrasts microscope and light microscope.

For plantlets regeneration 3.0 to 6.0 mm somatic embryos having globular and heart shaped cell clumps were place on to fortified solid MS medium containing different combination and concentrations of plant growth regulators (BAP, Kn , 2,4-D, NAA) sucrose 30 gm/l and agar 8 gm/l.

Culture test tubes, bottles were subjected to 25 $\pm$ 2 ^{O}C, under 16 h photoperiod by cool white fluorescents light (1000 to 400 lux) and 8 h dark of 24 h d cycle with 55 + 5 % relative humidity. All the experiments were repeated thrice and had 10 replicates with single explants (Somatic embryos cell clumps).

The regenerated plantlets were transferred to MS rotting medium supplemented with auxin (IAA, IBA, NAA). Rooted plantlets thoroughly washed gently under running tap water to remove the adhering agar and transfer in 2.5 % root trainers filled with soil: Sand: FYM: Vermiculite sterilized mixture. Harden plants were placed under 30 0c $\pm$ 2 ^{O}C and 75 % RH

Volume : 4 | Issue : 1 | Jan 2014 | ISSN - 2249-555X

for 15 d in green shade house for acclimatization. Finally acclimatized plants were transfer to field.

Results and Discussion

The surface sterilization of explants was performed and optimized. It was observed that new sprout of node bud segment, leaf bid, shoot tip and roots segments stand well without any contamination when0.1% 0.5%, 0.5% and 0.8%$HgCl_2$ for 3 min respectively were used for surface sterilization. At higher concentration of $HgCl_2$ explants turn brown and no growth. In D. tulda callus formation was observed within 3 to 4 week of new sprout of node bud segments inoculation and incubations while rest of the explants leaves (leaf bid),New, root segments, shoot tip regions were don't well for callus formation. The callus induction always proceeds by swelling of the explants, as well as at cut end initiation after 10 d of incubation. The induced callus was friable, compact, yellowish white and light green in color. Various auxin viz 2.4-D, NAA and cytokinin BAP, Kn were supplemented singly as well in combination were used. 2.4-D 1 mg/l, NAA 1mg/l was found to be best for producing friable, yellowish white, light green callus, 702.90 $\pm$ 8.9 was the fresh weight after 21 days of incubation. (Photo 1. Table No. 1). Although NAA 1 mg/l induced compact callus it later turn pigmentation and showed limited growth (Photo 1b). Friable, yellowish white, light green callus was referred to as stock callus. The stock callus subculture MS medium containing 2, 4-D and NAA (1 to 2 mg/l) alone or in combination. Ogita S (2005) reported that MS medium containing 2, 4-D 4uM is the optimum concentration for callus formation by using explants cut piece shoot of Phyllostachys nigras. In present investigation we have used 2, 4-D and NAA in combination showed best callus. As well as Godbole and Sood (2002) worked on somatic embryogenesis in D.hamiltoni and reported that when new sprout node bud segments were cultured 2,4-D, BA and NAA (2mg/l each) gave compact callus. The above variations due to genetic deference in plants.

To obtained small clumps cell clumps callus should be tease mechanically when placed in liquid medium (Bioreactor), the callus were easily broken due to agitation and aeration. It was observed that MS broth containing 2, 4-D, NAA, and BAP 1mg/l each was the best for suspension culture. After 21 d of suspension culture different stages of embryo viz. globular, heart and average 33 $\pm$0.5 number of somatic embryos were observed after 21 days of suspension (Photo 2, table2). The application of suspension culture of bamboo was reported by Hung (1988).Dekker (1989) attempt to raised suspension culture but was not successful.

The somatic embryos clumps select and subculture on, MS medium containing BAP 2.5 mg/l is the optimized concentration for multiple shoots induction average 9.3 $\pm$ 0.9 number of shoots were observed after 30 days of somatic embryo clump subculture (Photo 3, Table no 3). Kn 2 mg/l and 2, 4-D 1 mg/l also showed minimum multiple shoots enrich with callus at the base. Godbole and Sood (2002) reported that MS medium containing BAP 1mg/l, 2,4-D 1mg/l, and GA 0.5 mg/l give better somatic embryogenesis and shoot bud differentiation in D. hamiltoni.

The healthy and sturdy shoots separated and transfer on rooting medium, MS containing IAA 1 mg/l is the best optimized concentration, average 5.8 $\pm$ 0.7 number of roots were observe after 15 days of shoot transfer (Photo 4). The roots develop directly at the base of shoots. Rooting response was poor on NAA and IBA. IAA containing 1 % activated charcoal power improves the in vitro rooting efficiency.

After 2 to 3 week of culture of shoots of rooting medium, the plantlets transfer to pot for hardening, Soil: Sand: FYM: vermiculite (1:1:1:1) is the best harden media. 85% plants were healthy and survive after 15 d of hardening (Photo 5). The primary harden plants were ready for field trails. This plant species can be develop through somatic embryogenesis for faster multiplication of plants for wide cultivation and their application.

Table No. 1
Effect of 2, 4-D, NAA on induction of callus (After 21 days)

Explants	Concentration of		Physical* nature of callus	**Fresh weight of callus (mg) ±SE**	**Dry weight of callus (mg) ±SE**
	2, 4-D (mg./L)	NAA (mg/L			
New sprout of node bud segment	0	0	-	-	-
	1	0.5	F, YW	510.10 ± 7.1	33.3 ± 2.3
	2	0.5	F, YW	402.33 ± 6.3	37.5± 1.9
	3	0.5	-	-	-
	4	0.5	-	-	-
	1	1	F, YW, LG	702.90 ± 8.9	86.6 ± 2.5
	2	1	F, YW	300.00 ± 5.4	33.6 ± 1.8
	3	1	-	-	-
	4	1	-	-	-

* Visual observation: F- Friable, YW – Yellowish whiter LG – Light green

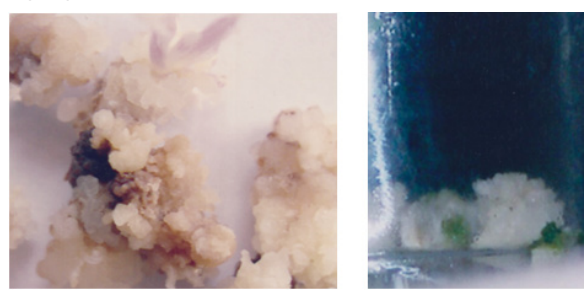

Photo 1 – Invitro Callus Induction from new sprout of node bud segment of D.tulda (MS+2, 4-D, NAA 1mg/L each)

Table No. 2
Effect of 2, 4-D, NAA and BAP on somatic embryogenesis in D. tulda (After 21 days)

Explant	Concentration (mg/L)			% of embryogenic Callus	Average No. of somatic Embriyo/ Clumps. (±SE)
	2, 4-D	NAA	BAP		
Callus	0	0	0	0	0
	0.5	0.5	0.5	30%	7.0 ± 0.2
	1.0	0.5	0.5	40%	12 ± 0.3
	0.5	0.5	1.0	40%	14 ± 0.3
	1.0	0.5	1.0	60%	17 ± 0.4
	0.5	1.0	1.0	60%	30 ± 0.5
	1.0	1.0	1.0	80%	33 ± 0.5

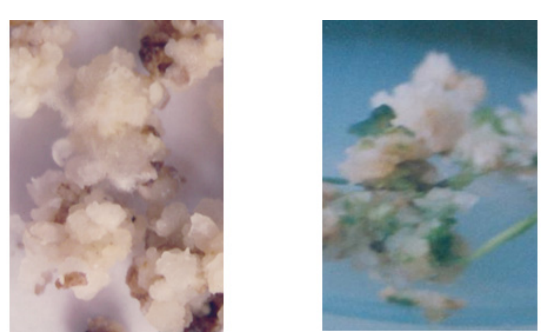

Photo 2 – Invitro Somatic embryo Induction (MS+2, 4-D, NAA, BAP 1mg/L each)
Table No. 3

Volume : 4 | Issue : 1 | Jan 2014 | ISSN - 2249-555X

Effect of BAP and 2, 4-D on shoot induction in D. tulda (after 30 days)

Explant	Concentration (mg/L)		Mean No. of shoots after 30 days (±SE)
	BAP	2, 4-D	
Somatic Embryo Clumps	0	-	-
	0.5	-	-
	1.0	-	4.5 ± 0.6
	1.5	-	5.4 ± 0.7
	2.0	-	7.0 ± 0.8
	2.5	0.5	9.3 ± 0.9
	0.5	0.5	3.0 ± 0.5
	1.0	0.5	4.0 ± 0.6
	1.5	0.5	6.0 ± 0.7
	2.0	0.5	7.2 ± 0.8
	2.5	0.5	2.4 ± 0.4

Photo 3 –Invitro Multiple shoot induction (MS+, BAP 2.5mg/L)

Table No. 4
Effect of IAA and IBA on induction of roots (after 15 days)

Explant	Concentration (mg/L)		% of rooting	No. of roots / flask (±SE)
	IAA	IBA		
Shoot of D. tulda	0	-	-	-
	0.5	-	25%	2.4 ± 0.5
	1.0	-	100%	5.8 ± 0.7
	1.5	-	60%	3.0 ± 0.5
	2.0	-	Callusing	-
	0.5	0.5	30%	2.5 ± 0.5
	1.0	0.5	80 %	5.0 ± 0.7
	1.5	0.5	50 %	4.0 ± 0.6
	2.0	0.5	-	-

Photo 4 –Rooting of Invitro shoot on MS medium with IAA 1mg/L.

Photo 5 –D. tulda Harden plants lets in green house.

Acknowledgement:
The first author is thankful to University Grants Commission New Delhi for financial assistance of Major research project.

REFERENCE
Anonymous (1978): Bamboo Forest News for Asia and the Pacific (FAQ Bankok)2 (4). | Dekker A.J. (1989): In vitro propagation and germplasm conservation of certain bamboo, ginger and costus sp Ph.D thesis National University Singapore. | Huang L.C.(1988): Bamboo callus cell in liquid suspension, protoplast and plant regeneration in vitro. In chang S.T., Chan K.Y. and Woo N.Y.S. (Eds) Recent advances in Biotechnology and applied. Chiniese university of Hongkong. Hongkong pp.177-182. | John C.K. and R. S. Nadgauda (1999): In vitro induced flowering In bamboo. In vitro cell developmental biology-plants 35.Pp309-315 | Lin C.S., Lin C.C., Chang W.C. (2003): In vitro flowering of Bambusa edulis and subsequent plantlets survival. Plant cell tissue and organ culture. 72. Pp. 71-78. | Murashige T. and F. Skoog (1962) : A revised medium for rapid growth and bioassay with tabacoo tissue culture. Physiology plant 15. Pp. 473-497. | Murashige T. a (1978 a): Principal of rapid propagation. In K.W Huges et al (Eds) of higher plants through tissue. V.S. Department energy Washington D.C. Pp 14-28. | Murashige T. a (1978 b): Impact of plant tissue culture on agricultural: In T.A Thorpe (eds) Frontier of plant tissue culture University of calgary Canada Pp. 15-26. | Saxena S. and S.S. Bhojwani (1993): In vitro cell developmental biology Plant Pp.129-132. | Srivastava R.C. (1990): Bamboo new raw materials for phytosterol. Current science vol 59 no.24, Pp. 1333. | Ogita Shinjiro (2005): Callus and cell suspension culture of bamboo plant phyllosatchys nigra. Plants Biotechnology. 22 (2). Pp 119-125.