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Effects of Nutrient Media and Cytokinins on in *vitro* Axillary Bud and Shoot Proliferation in *Bambusa tulda* Roxb

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ABSTRACT

The present study was conducted to determine the effects of different culture media and cytokinins and different doses of MS media and glutamine in the vitro axillary bud proliferation in *Bambusa tulda*. The nodal were collected from a healthy tree of *Bambusa tulda* plantation in Tropical Forest Research Institute (TFRI), Jabalpur (M.P.). Different steps were used to surface sterilizer the nodal segments the different chemicals are used for sterilization. Number of shoots formation was highest in MS medium supplemented with TDZ and shoot length was highest in B5 medium supplemented with TDZ. Maximum no. of shoots were obtained on full strength of MS medium the maximum shoot length were obtained on ½ strength of ms medium and no. Of leaves was observed on ½ strength of MS medium supplemented with glutamine.

Key word *MS media, TFRI, TDZ, B5 medium.*

Bambusa tulda is a symbodial bamboo species, grows well in humid tropical and subtropical regions of the Indian sub continent with a long period of vegetative growth followed by seedling and death of the clamp. It produces clumps up to 10 to 15 m height and 4 to 8 cm dia. It is a tall, sturdy and quick growing bamboo suitable for the production of high quality paper and furniture (Upeti *et al.,* 2001). It is reported that the succulent shoots of *Bambusa tulda* are rich in phytosterols and the fermented shoots used for the production of sterol drugs (Srivastava, 1990).

This species contained high reserve of organic matter, nitrogen, calcium, potassium and phosphorus under the soils where it grows (Qureshi *et al.,* 1969). The maturation of the tree species affects the potentiality of auxiliary buds and it is reported that the success of clonal multiplication from adult culm is restricted by many factors (Lin *et al.,* 1998). However, the reports on clonal multiplication from adult bamboos are very limited. (Chaturvedi *et al.,* 2009). Also propagation by vegetative means is difficult on account of fewer and bulky propagules and season specificity. So, tissue culture is the only convenient method for large scale production. In the present research, an attempt was made to report for mass multiplication of *Bambusa tulda*, an economically important bamboo species. The protocol developed here is successful for commercial production the species through auxiliary bud culture. Survival rate was recorded 100 per cent after plantation in the field. This type of commercial production of bamboo is the first attempt in the North Eastern part of India through tissue culture process. The survival rate is 100 per cent also first record in this type of study.

MATERIALS AND METHODS

The nodal were collected from a healthy tree of *Bambusa tulda* present in bamboo demonstration plantation in TFRI, Jabalpur. Axillary buds were washed under tap water for 10 to 15 min. Buds quickly dip in 70% ethanol for 2-3 min. Washed thoroughly with 5% cetrimide solution for 5 min. and then rinsed with running tap water. Soaked in 0.2% bavistin solution for 5 min. washed in double distilled water 3-4 times. These steps were carried out in the media preparation room. This step onwards carried out in laminar airflow hood. Quick dip in ethyl alcohol given for 1 min.by proper shaking with hand. Disinfected with the sterilizing agent 0.1 % Mercuric Chloride ($HgCl_2$) for 10 min. Sterilized explants was thoroughly washed with sterile double distilled water 3-4 times.

Inoculation/sub culturing

The inoculation of plant material and other

Table 1. Effects of different media and different cytokinins and their interactions on sprouting (%) after 7 and 14 days

MEDIA (BM)	CYTOKININS 3mg^{-1} (CS)											
	TDZ		KINETIN		BA		ADS		ZEATIN		MEAN	
	7	14	7	14	7	14	7	14	7	14	7	14
MS	100.0	100.0	100.0	100.0	93.33	100.0	80.00	86.67	86.67	86.67	92.00	94.67
SH	93.33	93.33	73.33	86.67	80.00	86.67	86.67	86.67	66.67	86.67	80.00	88.00
NITSCH	86.67	86.67	100.0	100.0	100.0	100.0	86.67	86.67	86.67	86.67	92.00	94.67
WPM	86.67	86.67	86.67	86.67	93.33	93.33	66.67	86.67	53.33	80.00	77.33	88.00
B5	80.00	93.33	93.33	93.33	80.00	86.67	93.33	100.0	93.33	100.0	87.99	94.67
MEAN	89.33	93.33	90.67	93.33	89.33	93.33	82.67	90.67	77.33	89.33		

Table 2. Effects of different media and different cytokinins and their interactions on number of shoots per nodal segment after 7 and 14 days

MEDIA (BM)	CYTOKININS 3mg^{-1} (CS)											
	TDZ		KINETIN		BA		ADS		ZEATIN		MEAN	
	7	14	7	14	7	14	7	14	7	14	7	14
MS	1.00	1.00	1.00	1.00	0.93	0.93	0.80	0.86	0.86	0.86	0.92	0.93
SH	0.93	0.93	0.73	0.86	0.80	0.86	0.86	0.86	0.66	0.86	0.80	0.88
NITSCH	0.86	0.86	1.00	1.00	1.00	1.00	0.86	0.86	0.86	0.86	0.92	0.94
WPM	0.86	0.86	0.86	0.86	0.93	0.93	0.66	0.86	0.53	0.80	0.77	0.88
B5	0.80	0.93	0.93	0.93	0.80	0.86	0.93	1.00	0.93	1.00	0.88	0.94
MEAN	0.89	0.93	0.90	0.93	0.89	0.91	0.82	0.92	0.77	0.89		

Table 3. Effects of different media and different cytokinins and their interactions on shoot length after 14 days

MEDIA(BM)	CYTOKININS 3 mgl^{-1} (CS)					
	TDZ	KINETIN	BA	ADS	ZEATIN	MEAN
MS	2.13	1.73	2.33	1.66	2.13	2.00
SH	2.33	1.40	1.80	1.73	1.66	1.78
NITSCH	1.86	1.93	1.93	1.93	2.26	1.98
WPM	2.06	1.53	1.93	1.20	1.20	1.58
B5	1.66	2.33	1.93	2.20	2.40	2.10
MEAN	2.01	1.78)	1.98	1.74	1.93	

subculture operations were carried out in laminar air flow cabinet under strict aseptic conditions. The floor of the cabinet was first swabbed with cotton dipped in 70% alcohol and all the required paraphernalia that is media, forceps, scalpels, spirit lamp, match lamp, match box, petridishes, flasks containing sterile distilled water, beaker etc., were kept inside. Later the laminar air flow cabinet was UV sterilized for duration of 45 min. Aseptic manipulations was initiated only after 10 min. After switching off the UV light forceps, scalpels and other instruments used for aseptic operation was sterilized by dipping in 90% ethanol followed by red hot flaming & cooling. The mouth of culture vessels was sterilized to kill all microorganisms sticking to the rim of the culture flasks.

Table 4. Effect of different strengths of MS medium, different concentrations of glutamine and their interactions on number of shoot per nodal segment of *Bambusa tulda* after 10 and 20 days inoculation

	GLUTAMINE mgl^{-1} (GL)							
MS MEDIA (BM)	**100**		**150**		**200**		**MEAN**	
	10	**20**	**10**	**20**	**10**	**20**	**10**	**20**
FULL MS	2.267	2.66	2.46	2.93	2.33	3.13	2.35	2.91
I/2 MS	3.000	3.33	2.400	2.53	1.40	1.60	2.26	2.48
I/4MS	1.867	2.06	1.733	2.00	1.66	1.86	1.75	1.97
MEAN	2.37	2.68	2.20	2.48	1.80	2.20		

Table 5. Effect of different strengths of MS medium, different concentrations of glutamine and their interactions on shoot length of *Bambusa tulda* after 10 and 20 days inoculation

	GLUTAMINE mgl^{-1} (GL)							
MS MEDIA(BM)	**100**		**150**		**200**		**MEAN**	
	10	**20**	**10**	**20**	**10**	**20**	**10**	**20**
FULL MS	2.26	3.60	2.16	3.00	2.06	2.80	2.16	3.13
I/2 MS	3.20	3.60	3.40	4.16	3.26	3.80	3.28	3.85
I/4MS	400	4.93	2.46	3.06	2.23	3.46	2.90	3.82
MEAN	3.15	4.04	2.67	3.41	2.52	3.35		

The first experiment was conducted to study the effect of different culture media and cytokinins on sprouting (%) in the axillary buds of *Bambusa tulda.* The second experiment was conducted to investigate the effect of different strengths of media and different concentrations of glutamine and their interactions on shoot multiplication *in Bambusa tulda.*

Axillary bud break was achieved in 7-10 days in all the aseptic cultures. The number of proliferating shoots ranged from 2 to 5. Five types of basal nutrient media viz., MS, SH, Nitsch, WPM, B_5 and five types of cytokinins viz., TDZ, Kinetin, BA, Ads and Zeatin were investigated for their effect on bud break (sprouting), shoot proliferation and shoot development.

Culture Conditions

The cultures were kept in culture room for further observation under controlled set of environmental conditions. They were incubated at 25±2^{0}C with 16 hr photoperiod provided by cool white fluorescent tubes of 40 watts.

RESULTS AND DISCUSSIONS

Effect of culture medium and cytokinins (sprouting, no. of shoots, shoot length)

After 7 days of inoculation the maximum sprouting in axillary buds (92.00 %) was obtained on MS medium. The maximum no. of shoots was obtained on MS medium (0.92). Effects of cytokinins after inoculation of 7 days maximum sprouting% was observed on culture medium supplemented with 3mg^{-1} kinetin, TDZ, BA, adenine sulphate. After 7 and 14 days No. of shoots was non-significant and shoot length after 14 days was non-significant. (Table 1, 2 and 3)

Effect of glutamine and MS strength (no. of shoots, shoot length, no. of leaves)

After 10 of inoculation the maximum no. of shoots were obtained on full strength of MS medium (2.35 the maximum shoot length (3.28cm) were obtained on ½ strength of ms medium and no. of leaves was observed on ½ strength of MS medium (2.48).Effects of glutamine after 10 and 20 days

Table 6. Effect of different strengths of MS medium, different concentrations of glutamine and their interactions on no. Of leaves of *Bambusa tulda* after 10 and 20 days inoculation

	GLUTAMINE mgl^{-1} (GL)							
MS MEDIA (BM)	**100**		**150**		**200**		**MEAN**	
	10	**20**	**10**	**20**	**10**	**20**	**10**	**20**
FULL MS	1.66	2.73	2.13	3.20	2.13	2.80	1.97	2.91
I/2 MS	2.93	2.93	2.73	3.26	1.80	2.26	2.28	2.82
I/4MS	1.60	4.20	1.66	2.20	1.66	2.26	1.64	3.00
MEAN	20.6	3.28	2.17	2.88	1.86	2.55		

of inoculation the maximum no. of shoots were obtained on medium supplemented with 100 mg^{-1} glutamine (2.37) ad after 20 days the maximum no. of shoots were obtained on medium supplemented with 100 mg^{-1} glutamine (2.68).and the effects of shoots length was found non-significant (Table 4, 5 and 6).

We can conclude that for *in vitro* axillary bud proliferation and shoot multiplication in *Bambusa tulda*, MS medium is the best nutrient medium and glutamine improved the health of cultures. The culture medium had a significant effect on sprouting % after 7 and 14 days of inoculation. For all the shoot growth parameters, MS medium clearly emerged out as the best nutrient medium. The individual effect of cytokinins was found to be non-significant for all the 3 shoot parameters at 7 and 14 days of inoculation. Almost equal number of shoots was obtained on different cytokinins. Maximum number of shoots was obtained on full strength MS medium at 10 and 20 days after inoculation. Different doses of glutamine also had significant effect on number of shoots and maximum shoots were obtained on 100 mg^{-1}.

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