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Effect of Cytokinin and Auxins on Meristem Culture of *Bambusa Arundinacea*

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Abstract

The effect of some plant growth hormones on the nodal explant culture were of bamboo has been studied with the view towards the multipurpose use of bamboo. Single nodal explant isolated from field grown plant of Bambusa arundinacea, when cultured on Murashige and Skoog's (MS) medium containing different concentration and combinations of BAP and Kin produced multiple shoots. Out of the various concentration of BAP used, the medium supplemented with 2 µM BAP and 5 µM Kinetin resulted in profuse shoot formation with increase rhizomatous portion. And when the explant were inoculated in the MS media containing the combination of BAP (0-2 µM) with Kin (0-2µM) the best result was observed with the Kin0µM + BAP1µM, among all the various combinations of theses both hormones were used. After the second sub culturing the plant was inoculated in the MS media containing the various concentrations of IBA and IAA with each BAP and Kinetin were also used, among the all the trials for IAA+Kin the greatest no of responding plant was found in the media containing IAA1.5µM + Kin 0µM and in the case of IBA+Kin the best results was obtained with the MS media containing IBA1µM+Kin0µM in the sense of no. of responding explant for rooting. The no of responding plant for rooting in the MS media containing the combination of BAP+IBA and BAP+IAA, the best result was obtained with the media containing IAA1.5 µM+BAP0 µM and IBA1.5µM+BAP0µM .

Key words: Plant tissue culture, M.S. Media, Plant growth hormones, Meristem.

Introduction

Bamboo plants distributed from tropical to temperate zones of Southeast Asia and they provide useful resources for local economies. The clums of bamboos have a variety of usages. They can be used as materials for house construction, daily sundry goods, agricultural and fisheries tools, and crafting materials. Young shoots are important as food materials as well [1,2]. As bamboos are fast-growing plants, recently they are considered as a prime renewable resource for biomass

production. Furthermore, the importance of bamboo forests as a potential modulator of global environment has been proposed [3]. Therefore, development of new utilities for bamboo plant is highly recommended. For these reasons, reconstruction methods of bamboo forests for stable supply of materials and analysis of metabolites of bamboo plants for production of new functional chemicals are essential. The common multiplication methods for bamboos are by rhizome planting, culm cutting and seedling cultivations [4,5]. Regarding endogenous metabolites, such as antioxidant, prooxidant, antibacterial, or aroma-active compounds, direct chemical analyses have been carried out in different parts of a bamboo plant [6,7,8]. By adapting the latest plant biotechnologies, new utilities of bamboos could progress beyond the current level. Tissue culture is one essential technique to micropropagate regenerated plant tissues and it is also a pre-requisite for genetic improvement through the use of different transformation strategies. At present, reports dealing with bamboo tissue culture are described mainly for the genus Bambusa [9,10,11,12] and Dendrocalamus [13,14,15,16]. Little information is available for the genus Phyllostachys. Phyllostachys pubescens Mazel ex Houz de Lehie (mouso-bamboo), P. bambusoides Sieb. Et Zucc (madake-bamboo), and P. nigra Munro var. Henonis (hachiku-bamboo) are the three major bamboo species found in Japan [17]. In order to find new utilities for the Japanese bamboos, tissue culture methods need to be established. The purpose of this study is to establish an efficient cell culture protocol for P. nigra and to reveal morphological and physiological characteristics of the cultured cells for further manipulations of the cell culture protocol of this potentially functional bamboo species.

Materials and Methods

Bamboo shoots of bambusa arundinacea were collected in mid-May 2009.Young bamboo shoots 2–3 cm in length was selected as explant materials. Culm-sheaths were removed, and then washed in water with several drops of a detergent for 15 min. After soaking, the shoots were surface sterilized first with 70% ethyl alcohol for 5 min followed by a 0.1%$HgCl_2$ for 9 min. After sterilization, they were rinsed 3 times with sterile distilled water and used as explants. Subsequently both the ends were trimmed and segment cultured on Murashige and skoog's (1962) [18] medium (20mL) with out any Plant growth substances by placing them vertically in conical flask (250mL). The $_p$H of the medium was adjusted to 5.8. All the cultures were maintained at 25±1°C in a culture room with 14/10 h day/night cycle. All laboratory experiment was carried out in Plant Tissue Culture Laboratory of Bansal College of Pharmacy, Kokta, Anand Nagar, Bhopal

After 3-4 weeks of incubation the axillary buds sprouted from the nodal explant; these buds (2-2.5cm long) were excised from the mother stumps and cultured on MS medium supplemented with different concentration of BAP and Kin. The buds produced small cluster of 3-5 multiple shoots with a rhizomatous portion, hereafter called propagules. The shoots were individually cut about 2cm above the base and discarded, while the propagules, now referred to as shoot cut was separated and further cultured for multiplication. For rooting these propagules(with shoot cut) were separated again and placed in ½ MS media containing IBA and IAA at different concentration for 10 days followed by placing in medium without IBA and IAA for 20 days. The plantlets were removed from the flask, thoroughly washed with water, transferred to plastic cup (6×6×7cm) containing 250g of autoclaved soil and placed under greenhouse conditions for

15 days. Following another 15 days, they were shifted to polythene bags (16×10cm) containing equal proportion of soil and farm yard manure (1:1) and transferred to a nethouse.

Results and Discussion

The sprouted axillary buds on the nodal explants were excised and transferred on MS medium containing different concentrations of BAP (0-5 µM) and Kinetin (0-5 µM). Subsequently within the next 3-4 weeks, differentiation was observed and multiple shoots were formed. Out of the various concentration of BAP used, the medium supplemented with 2 µM BAP and 5 µM Kinetin resulted in profuse shoot formation with increase rhizomatous portion (Table1 and 2). And when the explant were inoculated in the MS media containing the combination of BAP (0-2 µM) with Kin (0-2µM) the best result was observed with the Kin0µM + BAP1µM, among all the various combinations of theses both hormones were used (Table3). After the second sub culturing the plant was inoculated in the MS media containing the various concentrations of IBA and IAA with each BAP and Kinetin were also used, among the all the trials for IAA+Kin the greatest no of responding plant was found in the media containing IAA1.5µM + Kin 0µM(Table4) and in the case of IBA+Kin the best results was obtained with the MS media containing IBA1µM+Kin0µM (Table5) in the sense of no. of responding explant for rooting. The no of responding plant for rooting in the MS media containing the combination of BAP+IBA and BAP+IAA, the best result was obtained with the media containing IAA1.5 µM+BAP0 µM and IBA1.5µM+BAP0µM (Table6 and 7) .

Table 1: Effect of BAP added to the MS medium on shoots multiplication and explant responding

SN	Group	BAP(µM)	No. of responding explant(mean±SD)	No. of multiplying shoots(mean±SD)
1	Gp1	0	2.05 ±0.9*	3.55±1.7*
2	Gp2	0.5	1.7±0.5	3.6±2.5
3	Gp3	1	1.45±0.5	2.8±1.8
4	Gp4	1.5	2.15±1.0	2.8±2.3
5	Gp5	2	2.05±1.0	4.9±2.1
6	Gp6	2.5	1.75±0.8	2.3±1.3
7	Gp7	3	1.65±1.0	3.45±1.7
8	Gp8	3.5	1.65±0.8	2.2±1.0
9	Gp9	4.5	2.15±1.0	2.1±0.7
10	Gp10	5	1.7±0.9	1.8±0.8

(For responding explant)

Gp 6 Gp 3 Gp 8 Gp 2 Gp 10 Gp 5 Gp 1 Gp 4 Gp 9 Gp 7

(For multiplying shoots)

Gp 10 Gp 9 Gp 8 Gp 6 Gp 3 Gp 4 Gp 7 Gp 1 Gp 2 Gp 5

These are the graphical representation of Tukey multiple comparison test. At the 0.05 significance level, the means of any two groups underscored by the same line are not significantly different.

Table 2: Effect of Kin added to the MS medium on shoots multiplication and explant responding

SN	Group	Kin(µM)	No. of responding explant	No. of multiplying shoots
1	Gp1	0	1.5±1.1*	2.4±0.8*
2	Gp2	0.5	1.55±0.5	1.85±1.6
3	Gp3	1	2.05±0.9	1.9±0.9
4	Gp4	1.5	1.7±0.8	1.5±0.8
5	Gp5	2	2 ±1.0	2.3±1.8
6	Gp6	2.5	2.55±1.2	2±0.1
7	Gp7	3	1.95±0.8	1.45±1.1
8	Gp8	3.5	2.1±0.8	2.05 ±.0.23
9	Gp9	4.5	2.2±1.0	1.8±0.8
10	Gp10	5	2.4±1.1	2.6±1.6

(For responding explant)
Gp Gp Gp Gp Gp Gp Gp Gp Gp Gp
1 2 4 7 5 3 8 9 10 6

(For multiplying shoots)
Gp Gp Gp Gp Gp Gp Gp Gp Gp Gp
7 4 9 2 3 6 8 5 1 10

For description refer table 1.

Table3: Effect of Kin + BAP added to the MS medium on shoots multiplication and explant responding

SN	Group	Kin(µM) + BAP(µM)	No. of responding explant	No. of multiplying shoots
1	Gp1	0+0	1.4±1.4*	1.25±0.91*
2	Gp2	0+0.5	1.5±1.2	1.5±1.1
3	Gp3	0+1	3±1.7	2.7±1.3
4	Gp4	0+1.5	3.55±1.7	2.4±1.2
5	Gp5	0+2	2.25±1.5	1.7±1.2
6	Gp6	0.5+0	2.4±1.6	1.55±1.0
7	Gp7	1+0	2.25±1.7	1.7±1.4
8	Gp8	1.5+0	2.45±1.1	1.7±1.2
9	Gp9	2+0	1.7±1.6	1.9±1.2

(For responding explant)
Gp Gp Gp Gp Gp Gp Gp Gp Gp
1 2 6 5 7 8 9 4 3

(For multiplying shoots)
Gp Gp Gp Gp Gp Gp Gp Gp Gp
5 1 3 2 7 9 6 4 8

For description refer table 1.

Table 4: Effect of IAA + Kin added to the MS medium on explant responding for rooting

SN	Group	IAA(µM) + Kin(µM)	No. of plant responding for rooting
1	Gp1	0+0	1.1±1*
2	Gp2	0+0.5	1.5±1.2
3	Gp3	0+1	2.2±1.0
4	Gp4	0+1.5	1.5±1.0
5	Gp5	0+2	1.4±1.2
6	Gp6	0.5+0	2.1±0.8
7	Gp7	1+0	2±0.8
8	Gp8	1.5+0	2.4±1.1
9	Gp9	2+0	1.3±1.3

(For responding plant)

Gp Gp Gp Gp Gp Gp Gp Gp Gp
1 9 5 2 4 7 6 3 8

For description refer table 1.

Table 5: Effect of IBA + Kin added to the MS medium on explant responding for rooting

SN	Group	IBA(µM) + Kin(µM)	No. of plant responding for rooting
1	Gp1	0+0	1.25±1.2*
2	Gp2	0+0.5	1.6±0.6
3	Gp3	0+1	1.7±0.6
4	Gp4	0+1.5	1.7±0.6
5	Gp5	0+2	2.25±1.1
6	Gp6	0.5+0	1.55±1.2
7	Gp7	1+0	2.6±0.9
8	Gp8	1.5+0	1.45±1.1
9	Gp9	2+0	1.75±1.4

(For responding plant)

Gp Gp Gp Gp Gp Gp Gp Gp Gp
1 8 6 2 3 4 9 5 7

For description refer table 1.

Table 6: Effect of BAP + IAA added to the MS medium on explant responding for rooting

SN	Group	IAA(µM) + BAP(µM)	No. of plant responding for rooting
1	Gp1	0+0	1.2±1.1*
2	Gp2	0+0.5	*2.±1.4*
3	Gp3	0+1	*2.4±0.9*
4	Gp4	0+1.5	*1.6±1.4*
5	Gp5	0+2	*1.3±1.1*
6	Gp6	0.5+0	*0.8±0.8*
7	Gp7	1+0	*1.25±0.1*
8	Gp8	1.5+0	*3.15±1.1*
9	Gp9	2+0	*0.9±0.9*

(For responding plant)

Gp 6 Gp 9 Gp 1 Gp 7 Gp 5 Gp 4 Gp 2 Gp 3 Gp 8

For description refer table 1.

Table 7: Effect of BAP + IBA added to the MS medium on explant responding for rooting

SN	Group	BAP(µM) + IBA(µM)	No. of plant responding for rooting
1	Gp1	0+0	1±0.97*
2	Gp2	0+0.5	1.2±1.3
3	Gp3	0+1	1.8±1.2
4	Gp4	0+1.5	2.45±1.1
5	Gp5	0+2	1.9±1.1
6	Gp6	0.5+0	1.95±1.05
7	Gp7	1+0	1.6±1.3
8	Gp8	1.5+0	2.4±1.2
9	Gp9	2+0	1.1±1.07

(For responding plant)

Gp 1 Gp 9 Gp 2 Gp 7 Gp 3 Gp 5 Gp 6 Gp 8 Gp 4

For description refer table 1.

Conclusion

The present study has proved that, with the use of proper concentration of plant growth hormones the meristem culture of bambusa arundinacea can be established. The effect of various combinations of auxins and cytokinins has been tried in various concentrations. The best result of effect of same auxins and cytokinins was studied and the result mentioned in the result and discussion.

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