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In-vitro Studies on Mass Propagation of *Bambusa balcooa* Roxb.

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Abstract: *Bambusa balcooa is a clumping bamboo, ornamental plant of Indian origin. It is popular in Vietnam as food, and can be used as short life temporary constructions and have a variety of usages. The demand of it is increasing enormously and micro propagation promises to cope up with the forecasted shortage. In a short period of time large number of bamboos can be produced in the laboratory via tissue culture method, starting from elite selected genotypes. The treatment of nodal explants with mercuric chloride and sodium hypochlorite for 5 minutes resulted in the highest survival (90%) of explant with minimal microbial infection (5%). Nodal explants were culture on MS medium supplemented with different concentration of BAP and Kinetin. Explants cultured on MS media fortified with BAP (5ppm/lit.) and Kn (4ppm/lit.) showed highest percentage of shoot proliferation after 7 to 10 days. MS medium supplemented with 5 ppm/lit. of BAP and 4 ppm/lit. of Kn was found to be superior to other hormone combinations with regard to proliferation (up to 90%). Amongst different combinations of BAP and IBA used, MS medium supplemented with 2 ppm/lit. BAP and 2 ppm/lit. IBA was found to be the best for root induction in Bambusa balcooa. Visual observations of cultures indicated that higher concentration of BAP (2 ppm/lit.) and IBA (2 ppm/lit.) induces shoot induction. After hardening, rooted plantlets were successfully transferred to the soil in polythene sleeves with over 75% survivability and recorded normal growth and propagation.*

Keywords: ***Micropropagation; Bambusa balcooa; BAP; Kinetin; Root induction; Shoot induction; Auxin; Cytokinin***

Introduction

Bamboo is an ornamental plant, mainly for garden use in Europe. Besides, two different pathways for valorization of biotechnological research have emerged: (1) in the tropics bamboo is a very important plant, providing livelihood for over 500 million people and providing housing and shelter for over 1 billion people; (2) "Bamboo for Europe", an EU-funded research project has allowed to develop the potential of bamboo as an agricultural plant in Europe (Gielis and Oprins, 2000) with possible applications in wood industry (Van Acker et al., 2000) and as energy crop. So, any biotechnological developments for the improvement of ornamentals will also be useful on a much larger scale.

This article focuses specifically on the development of micro propagation systems, on the use of molecular markers as part of quality control and on research on flowering in tissue culture of bamboo. The search for one single method for large scale production for all bamboos remains thus highly desirable. Micropropagation is an excellent method to achieve this aim. In a short period of time large numbers of bamboos can be produced in the laboratory, starting from elite selected genotypes, which can be transported by air easily to any place in the world. Moreover tissue cultured plants are generally very vigorous growers and disease free (Gielis and Oprins, 1998).

For bamboo different propagation techniques are available, such as seed propagation, clump division, rhizome and culm cuttings (Banik, 1994 and Banik, 1995). But these methods suffer from serious drawbacks for large or mass scale propagation. For mass scale propagation (> 500 000 plants per year) classical techniques are largely insufficient and inefficient, and tissue culture is the only viable method. Indeed, the order of magnitude of the demand for bamboo planting materials indicates that micropropagation will inevitably be necessary for mass scale propagation (Subramanlam, 1994 and Gielis, 1999).

For tissue culture of bamboo the use of starting material (seeds or adult plants) and the choice of the propagation method are crucial (Gielis, 1999). In vitro propagation was done from nodal explants from field grown culms of B. balcooa to induce multiple shoots (Pratibha & Sharma, 2013). A unique study on *B. bamboo* of tropical region was done with mass multiplication protocol using explants from arid region. The protocol achieved multiplication rate of 3.5-fold with overall survival rate of 74.66% using vermicompost (Patel et. al., 2015).

Material and Methods

Among the notable media used are those of Murashige & Skoog, 1962.The medium was augmented with different concentrations and combinations of cytokinins (BAP & KINETIN) to see their effect on morphogenesis of the cultured explants.

Murashige and Skoog medium, 1962 is used in present project of pH 5.7 to 5.8. For solidification of the medium, agar powder (Tissue culture grade, agar-agar type) at the rate of 0.8% w/v was added to warm solution and then, boiled for proper dissolving and melting of agar powder. Other organic additives included in culture media are KNO3 (1.9 gm/L), NH4NO3 (1.6 gm/L), Myoinositol (100 mg/L) & Sucrose (30 gm/L).

Factors affecting growth of plant material in cultures

A constant temperature is to be maintained during the culturing if tissues in laboratory for their uniform growth. An optimal temperature of 25 (+/- 5) 0C favors a satisfactory growth of explants in culture. Optimal humidity inside culture laboratory was 60-70%. Increase in humidity beyond 85% makes cotton plugs wet which may facilitate bacterial contamination of cultures. Low humidity results in drying up of medium. In vitro growing shoots derive their nourishment from the medium and act as heterotrophs. The duration of photoperiod was optimal for 14-18 hours depending upon the nature of culture. Explants cultured on solid medium acquire sufficient cotton plugs. In liquid cultures continuous agitation of the medium provides sufficient aeration to the culture explants/tissues. Plant cells in culture require an acidic pH i.e. of 5.7-5.8 is optimal.

In case of *B. balcooa* we take internodes for the in vitro micro propagation. First of all it was washed with detergent under running water for at least 1 hour. The mouth of the flask was covered with apiece of muslin cloth and then, kept in running water to remove the microbial load and dust particles adhering to the surface of the explant. For surface sterilization two distinct strategies were employed as indicated below. Explants were first sterilized with sodium hypochlorite for 5 minutes and then washed with autoclaved water for 5 times & then washed with mercuric chloride for 5 minutes and then washed with autoclaved water for 5 times.

Innoculation: The explants were then, cultured on MS medium supplemented with different concentration of BAP & Kn.

Table 1: Different Combination of BAP& Kn used for Micropropagation

S.No.	BAP (ml\lit.)	Kn (ml\lit.)
1.	5	5
2.	6	4
3.	7	3
4.	5	4
5.	6	3
6.	8	6
7.	9	7

The cultures were incubated in culture room and provided with a temperature of 24-250C maintained by automatic control devices.

Results

Data presented in Table 2 show significant differences among the treatment tried.

Table 2: Effect of Various surface sterilization treatments

Treatment No.	%age of survival	Shoot initiation
T-1	40%	1 – 2

T-2	90%	3 – 4

For survival %age = (survival / Total no. of explants) x 100
For T-1 = (8 / 20) x 100 = 40%
For T-2 = (18 / 20) x 100 = 90%

Survival Percentage of Nodal Explants: The cultures infected with fungus and bacteria did not survive for long period and turned whitish and yellow. On the other hand, those free from infection remain green and considered as survival cultures. Highest survival (85%) was recorded when explants were first sterilized with sodium hypochlorite for 5 minutes and then washed with autoclaved water for 5 times & then washed with mercuric chloride for 5 minutes and then washed with autoclaved water for 5 times.

The treatment with sodium hypochlorite for 5 minutes & the washing with mercuric chloride for 5 minutes reduce the microbial infection from 3 to 5 %.

Shoot Initiation: Nodal explants were cultured on MS medium supplemented with different concentrations of BAP (5, 6, 7, 5, 6, 8, 9 ml/lit.) and Kinetin (5, 4, 3, 4, 3, 6, 7 ml/lit.). Explants were showed shoot initiation after 5 days.

Contamination: The treatment with sodium hypochlorite for 5 minutes and washing with mercuric chloride for 5 minutes reduced the microbial infection from 30 to 35 %.

Culturing of Explants: The explants were then, cultured on MS medium supplemented with different concentration of BAP and Kinetin.

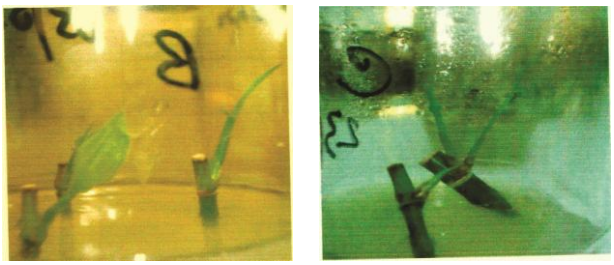
Table 3: *In vitro* culture of *Bambusa balcooa* on MS medium supplemented with BAP and Kinetin for shoot initiation.

S.No	BAP (ppm/lit.)	Kn (ppm/lit.)	%age of Response
C-1	5	3	65
C-2	7	5	60
C-3	-	6	55
C-4	9	-	50

Table 4: *In vitro* culture of *Bambusa balcooa* on MS medium supplemented with BAP and Kinetin for shoot proliferation.

S.No	BAP (ppm/lit.)	Kn (ppm/lit.)	%age of Response
C-5	6	4	75
C-6	6	3	80
C-7	5	4	90

Shoot Initiation: The nodal segments gets swollen after three days and the small originating shoot segment were observed. Then the explants showed shoot initiation after five days.



(a) **(b)**

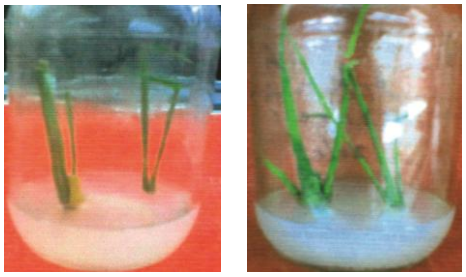
Figure 1: Shoot initiation in nodal explants of Bambusa balcooa on MS medium supplemented with (a) BAP and (b) Kinetin after 1 week.

Figure 2: Shoot initiation in nodal explants of Bambusa balcooa on MS medium supplemented with BAP and Kinetin after 10 days.

Shoot Proliferation: Shoot proliferation was observed after 2 weeks of inoculation which was cultured on MS media having BAP and Kinetin.

Table 5: Explants cultured on MS media having different concentration of BAP and Kinetin.

S.No	BAP (ppm/lit.)	Kn (ppm/lit.)	%age of response
C-5	6	4	75
C-6	5	4	90
C-7	6	3	80



(3a) ***(3b)***

Figure 3a: Shoot initiation in nodal explants of Bambusa balcooa on MS medium supplemented with BAP and Kinetin after 2 weeks.

Figure 3b: Multiple shoot proliferation in nodal explants of Bambusa balcooa on MS medium supplemented with BAP and Kinetin after 4 weeks.

Root Induction: Proliferated shoots were transferred to the rooting media having different concentration of BAP (2 ppm/lit.) and IBA (2 ppm/lit). The explant cultured on the media gets swollen after 2 to 3 days and the small hairy roots were seen.

Table. 6: In vitro culture of Bambusa balcooa on MS media supplemented with BAP and IBA

S.No.	*BAP (ppm/lit.)*	*IBA (ppm/lit.)*	*%age of Response*
R-1	2	2	80
R-2	2	-	50

%age of Response = (Survival rate / No. of Explant used) x 100

Conclusion

The treatment of nodal explants with mercuric chloride and sodium hypochlorite treatment for 5 minutes resulted in the highest survival (90%) of explant with minimal microbial infection (5%). Nodal explants were culture on MS medium supplemented with different concentration of BAP and Kinetin. Explants cultured on MS medium supplemented with BAP (5ppm/lit.) and Kn (4ppm/lit.) showed highest percentage of shoot proliferation after 7 to 10 days. MS medium fortified with 5 ppm/lit. of BAP and 4 ppm/lit. of Kinetin was found to be superior to other hormone combinations with regard to proliferation (up to 90%). Amongst different combinations of BAP and IBA used, MS medium supplemented with 2 ppm/lit. BAP and 2 ppm/lit. IBA was found to be the best for root induction in Bambusa balcooa. Visual observations of cultures indicated that higher

concentration BAP (2 ppm/lit.) and IBA (2 ppm/lit.) induces shoot induction.

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