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EFFECT OF KINETIN CONCENTRATION ON AXILLARY SHOOT BUD PROLIFERATION OF *BAMBUSA VULGARIS*

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ABSTRACT

Bambusa vulgaris is a widely cultivated and fastest growing woody species of Poaceae family that plays an important role in day to-day life of million people. As this species does not produce seeds after sparse flowering so vegetative propagation is an exclusive alternative that makes progeny available, so a few low cost propagation trials were conducted for the clonal propagation of these species by inter-nodal branch cuttings. These cuttings were then treated with different concentration of Kinetin (2 mg/l, 3 mg/l, 4 mg/l, 5 mg/l, 6 mg/l) and kept in plant growth chamber to assess shoot bud proliferation. These explants were kept under observation for 4 weeks and then sub-cultured with MS-Media for shoot proliferation. Then these sub-cultured plantlets were acclimatized and regularly cultured with Hoagland Media at specific time intervals. However, no growth was observed when explants were treated with antibacterial (Streptomycin sulphate, 0.0015 gms/l) and antifungal (Bavistin, 0.2%) treatment. The length of the longest shoot varied dependent of branch cutting types and concentrations of Kinetin solutions. This study concluded that inter nodal branch cuttings are able to develop shoots under greenhouse conditions. Cost effective and high quality plantlets were obtained in minimum time through this process of micro propagation.

KEYWORDS: *Bambusa vulgaris*, Micro Propagation, Explants, MS-Media, Plant Growth Regulators.

INTRODUCTION

Bamboo (*Bambusa vulgaris*) that is also known as Green Gold of 21st century in many Asian countries[1] is a part of 12 subfamilies of grass family Poaceae and is being divided into three tribes which contains almost 1500 species of it: Arundinarieae comprising of 546 species of temperate woody bamboos, Bambuseae comprising of 812 species of tropical woody bamboos and Olyreae comprising of 124 species of herbaceous bamboos.[2] Almost 136 species of bamboo are present in India.[3] Geographically, the latitudinal distribution of bamboo varies from 47˚S to 50˚ 30'N and an altitudinal distribution varies from sea level to 4300 mt.[2] Apart from India, China and Burma, bamboos are also found in other Asian countries like Thailand, Vietnam, Bhutan, Bangladesh and Laos.[1] A fully grown bamboo can attain a height of 20-30m whereas most of them are medium in height. Mostly they are grown in a warm climate having enough moisture and fertile soil except a few species that grow below -20°C.[4]

*Bambusa vulgar*is being a rhizomatous plant[5] can propagate both vegetatively through rhizomes and sexually through seeds.[1] But vegetative propagation is not beneficial as plants developed will be of same age as of their explant and will die at the same time of the explant after flowering and as a 100 year old plant bears flower only before culm death so sexual propagation is also a quite difficult task.[5] Although it is easy to obtain shoot tips, internodes anytime.[6] In addition to this a great damage is caused to seeds due to overgrazing of animals, overexploitation by rural and tribal communities and forest fires[7] which in result has converted hectares of fertile land into uncultivated land which brings a great concern about replenishing the bamboo.[8] Along with this, bamboo cultivation is a subject of keen interest at National and International markets due to its short rotation cycle, rapid growth and ease of handling.[9] Various methods like ground layering, air layering, culm cutting have also been successful. But a large scale establishment of bamboo using these techniques is too laborious so to overcome this problem one of the promising techniques for large scale regeneration of bamboo is plant micro propagation.[10][11] Three methods are currently used in micro propagation of bamboos according to Murashige-enhanced release of axillary buds development of advantageous shoots using organogenesis and somatic embryogenesis. Clonal fidelity can be conserved using shoot tip, nodal and axillary bud culture whereas somatic embryogenesis and organogenesis mostly generate aberrant, therefore are not recommended for clonal propagation.[12]

Bamboo can have some internodes vegetatively active for long period of time.[13] For the first time plantlets in bamboo were regenerated from zygotic embryos of *B. arundinarea*.[14] *Bambusa vulgaris* is commonly known as Buddha Bamboo and its internodes can be used as its explants.[13] *Bambusa vulgaris var.striata* also known as yellow bamboo mostly grows in full sunlight having moderate water as its requirement for good growth. It attains a height up to 8-20m with diameter of 5-10 cm. In this internodes reach upto 45 cm height and branching generally occurs from mid-culm to top having prominent nodes. For reducing cost of plantlet production MS-Media is one of the ideal liquid media solution. Multiple shoot can be induced from the MS-Media supplemented with coconut milk, kinetin and BAP. Maximum rooting is achieved from the cuttings which are taken from 1-2 year old culms[13] during carbonization process as a secondary product due to its antioxidant and antibiotic activities.[15] Split culms are used by Naga tribes of Manipur as ornament in the ear perforation.[13] It has a low calorie source of potassium which is being used for healing. The culms of bamboos stem secrete siliceous concretion called as *Banslochan* or *Tabashir* or *Tawashir*.[1] The clump forming bamboos are often used as a shield and a barrier against the storming winds, cyclones, typhoons as they can bend due to their flexibility without breaking.[2] These Bamboos being the fastest growing woody species that are able to meet the day to day basic needs of people living in south-east Asia by providing wood, paper, pulp, fodder, medicine, shelter. Bamboo is one of the expensiest ornamental and fodder crop cultivated in India and China respectively and in various other parts of the world. This crop not only help in quenching the food requirements but also plays a significant role in reforestation by liberating 35% more oxygen than other crops which in turn lowers the amount of atmospheric carbon dioxide[16] and by controlling soil erosion as its roots reaches up to 30 cm of soil surface which help in binding of the soil surface[2] and thus reducing the soil erosion by 75%.[17] Therefore, considering these applications and costing of indigenous Bamboo, an attempt was made to proliferate this species through axillary shoot bud micro propagation method.

MATERIALS AND METHODS

Growth chamber conditions

The experiments were carried out in plant tissue culture lab and an optimum temperature was maintained at 25$\pm$ 2° C in plant growth chamber.

Collection of explants

Internodal explants were selected from 5-year old yellow bamboo plants on the basis of maturity, length and diameter of internodes and growth potential. The internodal cuttings from the small branches were approximately 2cm from both the sides of the branch. The lengths and diameter of the internodes were kept uniform to reduce variations under various treatments.

Surface sterilization of explants

The nodes were then surface sterilized using 0.5% mercuric chloride.

Initiation

Murashige and skoog (MS) media was prepared with vitamins, sucrose, agar, $CaCl_2$ and different concentration of Kinetin (2 mg/l, 3 mg/l, 4 mg/l, 5 mg/l, 6 mg/l) to carry out initiation and pH was maintained at 5.7 prior to autoclaving. These cultures were stored at 25$\pm$2°C for 2-3 weeks in 16 hr photoperiod.

Shoot multiplication

This led to development of multiple sprouted buds with their elongation. These newly sprouted axillary shoots were then sub cultured with fresh multiplication media at a regular interval of 3-4 weeks for inducing more shoots.

Hardening

Plantlets were then removed from the cultured bottles *in vitro*, washed thoroughly with water and transferred to hardening trays consisting of coco peat and vermi compost in the ratio of 3:1. These plantlets were then grown in green house conditions for 3-4 weeks maintaining a temperature of 25-30°C and relative humidity of 75%.

RESULTS AND DISCUSSION

Development of internodal explants were done by using MS-Media (solid media) with different concentrations of Kinetin 2 mg/l, 3 mg/l, 4 mg/l, 5 mg/l, 6 mg/l. (Figure 1). Maximum shoot development was observed at 4 mg/l kinetin concentration in 2-3 weeks and was recorded as follows (Figure 2).

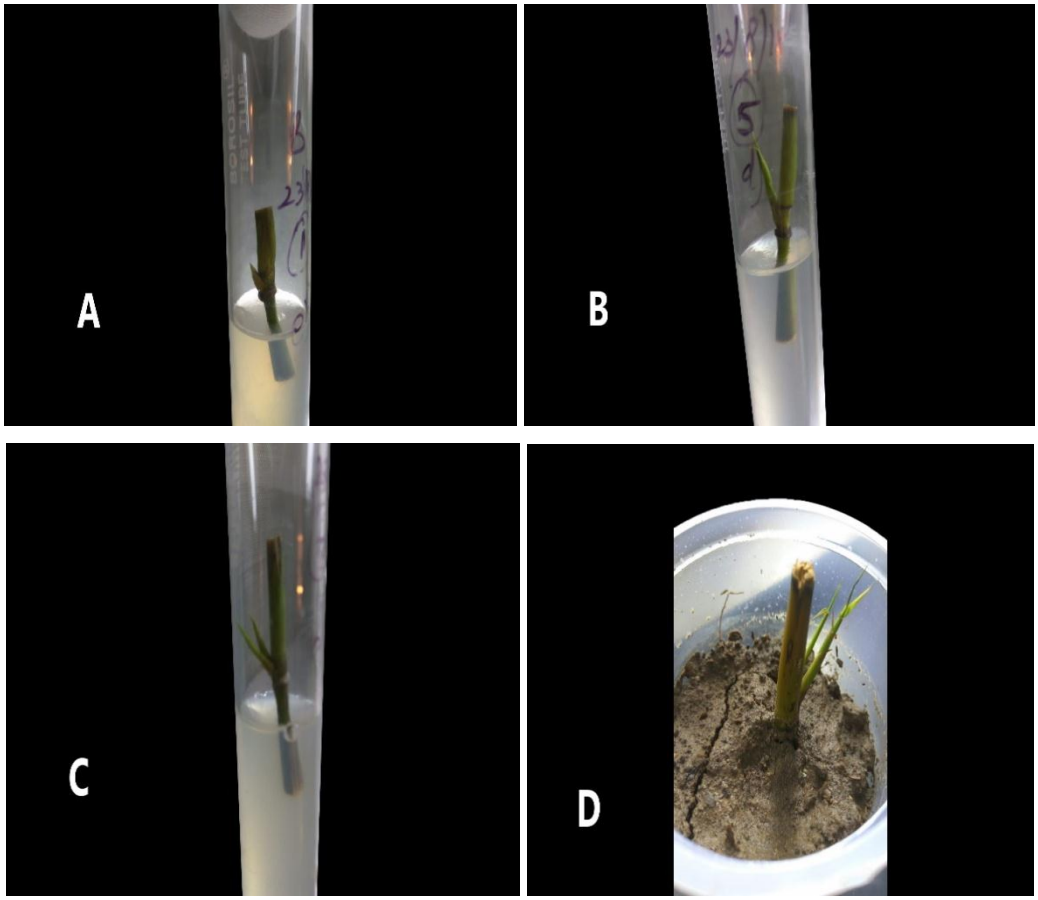

Figure 1: Plant regeneration from nodal explants of *In vitro* grown *Bambusa vulgaris.* (A) Shoot bud development on MS medium with 2.0 mg/l Kinetin, 0.5 mg/l IAA and 0.44 g/l $CaCl_2$; (B) Shoot regeneration on MS medium containing 3.0 mg/l Kinetin, 0.5 mg/l IAA and 0.44 g/l $CaCl_2$; (C) Regeneration of multiple shoots obtained on MS medium with 4.0 mg/l Kinetin, 0.5 mg/l IAA and 0.44 g/l $CaCl_2$; (D) *In vitro* regenerated bamboo plantlets growing in plastic cup. Growth Percentage of explants germinated is mentioned in detail in Table 1.

Table 1: Effect of different concentration of kinetin mediated MS medium on internodal regeneration of *Bambusa vulgaris.*

Observations	Kinetin Concentration %				
	0.2	0.3	0.4	0.5	0.6
A	12	12	12	12	12
B	9	7	10	3	8
C	75	58.3	83.3	25	66.6

A- Number of explants inoculated, B- Number of explants germinated, C-% of explants germinated.

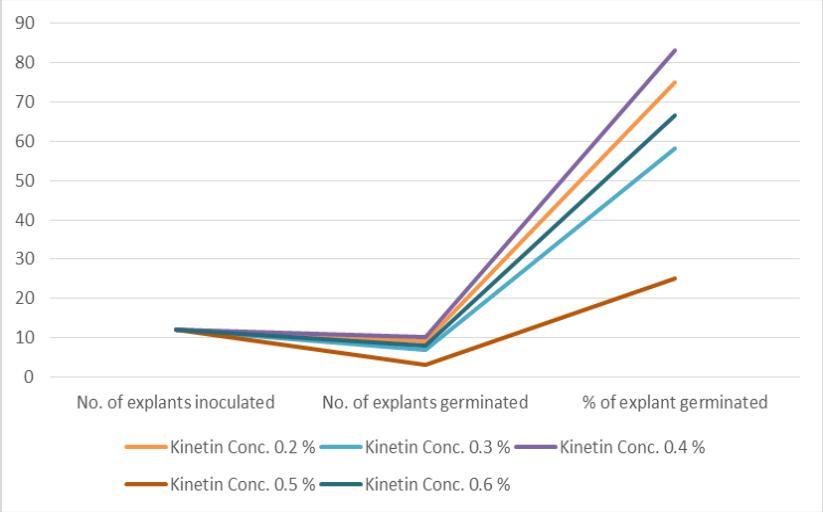

Figure 2: Different concentrations of kinetin mediated MS medium on internodal regeneration of *Bambusa vulgaris.*

As it is difficult to grow bamboo through sexual cross breeding because bamboo takes many years to blossom and then it dies. So, the regeneration of bamboos via explants such as inter nodal segments required MS-Media.[9] Bacterial contamination during *in vitro* propagation of bamboo plants is a major obstacle between establishment of new plants and successful experimentation.

Kaladhar *et al.*, (2017)[13] amended the MS-Media with BAP (0.3 mg/l) in combination with IAA, NAA and 2, 4-D respectively while this experiment was carried out without using BAP and with varied concentrations of Kinetin (Figure 3). Number of internodes regenerated on MS media with different concentrations of kinetin is mentioned in detail in Table 2.

Table 2: Regeneration from internodal explants of *Bambusa vulgaris* on MS media with distinct growth regulator concentration.

Sample No.	Kinetin Conc. (mg/l)	IAA Conc. (mg/l)	$CaCl_2$ Conc. (g/l)	No. of Internodes regenerated
Sample 1	2	0.5	0.44	10
Sample 2	3	0.5	0.44	6
Sample 3	4	0.5	0.44	11
Sample 4	5	0.5	0.44	3
Sample 5	6	0.5	0.44	9

IAA- Indole Acetic Acid, $CaCl_2$- Calcium Chloride ions.

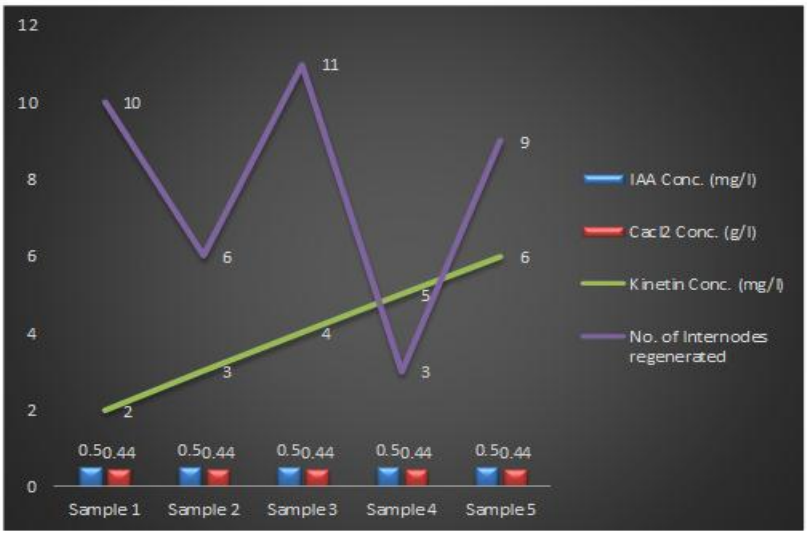

Figure 3: *In vitro* regeneration from internodal explants of *Bambusa vulgaris* on MS media with different cytokinin concentration.

Zang *et al.*, (2016)[6] developed a stable and efficient regeneration system in *D. hamiltonii* by using easily obtained shoot tips as explants which provide a useful tool for genetic and transformation in bamboo species. While in this experiment inter nodal explants for axillary shoot bud proliferation in bamboo species through micro-propagation.

A stable and efficient regeneration system is developed for *Bambusa vulgaris* by easily obtained inter nodal explants which provides a useful tool for axillary shoot bud proliferation in bamboo species through micro propagation.

Maximum axillary shoot bud proliferation was achieved in presence of kinetin at 4 mg/l. The result of the present study suggested that varied concentrations of kinetin were found to be best for induction of multiple shoots from the nodal explants of *B. vulgaris*. But at the same time the higher concentration (5 mg/l and 6 mg/l) were noticed with inhibitory effect and causing vitrification of shoots. Other concentration of kinetin proved to be less effective for axillary shoot bud proliferation.

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