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IN VITRO MICRO-PROPAGATION OF *Dendrocalamus stocksii* (Munro.) THROUGH NODAL EXPLANT

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ABSTRACT

Present investigation was carried out during 2018-19 at Tissue Culture Laboratory, Agricultural Botany Section, College of Agriculture, Nagpur with the objective to standardize micro-propagation techniques for large scale multiplication of bamboo species *Dendrocalamus stocksii*. Nodal explants collected from AICRP on Agroforestry, COA, Nagpur were used for micro-propagation. To establish aseptic cultures, the explants were disinfected with presoaking in 1% Tween 20 for 5 minutes followed by 0.2% $HgCl_2$ for 10 minutes followed by 70% alcohol for 5 minutes followed by 1% carbendezim for 10 minutes followed by 0.1% streptomycin for 15 minutes. The media combination MS + kinetin 4 mgl^{-1} + IBA 0.5 mgl^{1} was found best for shoot induction, whereas media combination MS+ 3 BAP mgl^{-1}+ 0.5 IBA mgl^{-1} resulted in getting highest number of shoots. However, very poor results were obtained for root induction, the media with 1/2MS + 5mgl^{-1} IBA proved to be best for maximum number of roots, while 1/2MS+ 6.5mgl^{-1} IBA gave maximum length of roots.

(Keywords: *Dendrocalamus stocksii, in vitro*, MS media, Growth regulators, micro-propagation)

INTRODUCTION

Bamboo is a giant grass which is one of the most fascinating and versatile group of plants known to mankind. There are 75 genera and 1250 species of the family Poaceae throughout the world.Whereas,125 species in 23 genera spread over the India (Anonymous, 2019). The genus *Dendrocalamus* has over fifty species distributed in tropical and subtropical regions of the world. It is characterized by sympodial rhizomes and large sized dense clumps. *Dendrocalamus stocksii* (Munro.) is a strong, arborescent and thorn less bamboo species. It is naturally distributed in the Central Western Ghats of Maharashtra, Karnataka, Goa and Kerala. It is commonly known as Marihal, Manga, Mes, Chiva etc. (Viswanath *et al.*, 2012). It is an extremely manageable bamboo species with a great economical and ecological importance. About 35% of *D. stocksii* is regarded as edible portion. Besides edible shoots and in handicrafts, it is a component of various agricultural implements and stakes in agricultural field. Owing to its multifarious uses and perceived importance, National Bamboo Mission (NBM) has prioritized this species for large scale cultivation in peninsular India. However, large scale cultivation is hampered by non-availability of planting stocks. Propagation of bamboo by seeds is unreliable due to long and unpredictable flowering habit and also undesirable on account of large variation found in seedling propagation. Sterility of *D. stocksii* attributed to the less quantity of pollen produced, viability of pollen, percentage of anthesis, short receptivity of stigma etc.

Therefore, in order to supplement the conventional methods, an efficient *in vitro* propagation method would offer a desirable alternative for large scale multiplication of elite genotypes. There is also an immense potential for improving species through selection and breeding programs. It will lead to a method of collective benefits of easy to raise, economic to adopt and easy to transport for selling purpose.

MATERIALS AND METHODS

The present investigation was undertaken at the Tissue Culture Laboratory of Agricultural Botany Section, College of Agriculture, Nagpur during year 2018-2019.

The healthy explants (nodal shoot segments) obtained from mature one year old healthy Culms of *D. stocksii* at AICRP on Agroforestry, COA, Nagpur were used for the present study.

After collecting explants from plant source, the sheath enclosing the bud within was carefully removed. The explants were trimmed using stainless steel secateurs

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until the length of about 3 to 4 cm. The explants were processed and surface sterilized by pre soaking in 1% Tween 20 for 5 minutes followed by 0.2% $HgCl_2$ for 10 minutes followed by 70% alcohol for 5 minutes followed by 1% carbendezim for 10 minutes followed by 0.1% streptomycin for 15 minutes. Murashige and Skoog (MS) basal medium (consisting of salts, vitamins) was used for inoculation. Different plant growth regulators (PGRs) *viz*., BAP, kinetin, IAA, IBA, NAA were added at various concentrations to MS medium before the pH of the medium was adjusted to 5.6. Media were autoclaved at 15 psi and 121°C for 20 min. Cultures at all growth stages were incubated under artificial conditions 25±2°C, 16 hours of photoperiod and 8 hours of dark period. Different PGRs were added in MS media for specific purpose i.e. for shoot induction and shoot multiplication media compositions were MS with cytokinins and auxin combinations at various concentrations in 10 treatments and 7 treatments respectively. For root induction, media composition was ½ MS media with high concentrations of auxins.

RESULTS AND DISCUSSION

Shoot induction

Ten different MS media compositions with combinations of cytokinins and auxins were used (Table 1). There were no any significant differences observed among the treatments for number of days required to bud initiation. In per cent response for shoot induction, the treatments T_3 (MS + kinetin 3mgl^{-1} + IBA 0.5 mg l^{-1}), T_5 (MS + BAP 2mg l^{-1} + IBA 0.5 mg l^{-1}), T_6 (MS + BAP 3 mg l^{-1} +IBA 0.5 mg l^{-1}), T_7 (MS + BAP 2 mg l^{-1} + NAA 0.25 mg l^{-1}) and T_8 (MS + BAP 3 mg l^{-1} + NAA 0.25 mg l^{-1}) showed highest i.e. 89.71% response for bud break among all 10 treatments. It was observed that, combination of kinetin with NAA (0.25 mg l^{-1}) showed lower response, while kinetin with IBA (0.5 mg l^{-1}) showed better results. BAP (2 mg l^{-1} and 3 mg l^{-1}) showed better response with both auxins i.e. NAA (0.25 mg l^{-1}) and IBA (0.5 mg l^{-1}). Negi *et al.* (2011) reported that MS medium supplemented with BAP (4.4µM) and kinetin (2.32µM) gelled with 0.2% gelrite yielded 80% aseptic cultures with 100% bud break. Treatment 4 (MS + kinetin 4 mg l^{-1} + IBA 0.5 mg l^{-1}) yielded maximum number of shoots explant^{-1}. It was observed that the treatments with BAP showed good results. It was also observed that the length of shoots grown in liquid media was higher than in semisolid media. The similar observation was recorded by Saini *et al.* (2016) i.e. bamboo bud proliferation and induction was better in liquid medium as compared to semisolid medium. The treatment $T_{8,}$ (MS + BAP 3 mg l^{-1} + NAA 0.25 mg l^{-1}) exhibited its superiority both in response as well as in shoot bud induction.

Shoot multiplication

The media combination MS media + 3 mg l^{-1} BAP + 0.5 mg l^{-1} IBA was found significantly superior over all other treatments for shoot emergence as it took less days i.e. 3.162 days (Table 2). In present study, it was observed that, MS media with BAP showed early shoot emergence as compared to MS media with kinetin. Choudhary *et al.* (2016) also reported BAP as an effective cytokinin for shoot multiplication. The treatments T_4 (MS + 2 mg l^{-1} BAP+ 0.25 mg l^{-1}NAA) showed 5.919 shoots and T_3 (MS+ 3 BAP mg l^{-1}+ 0.5 IBA mg l^{-1}) showed highest shoots 5.955 which was significantly superior over all remaining treatments. Same results were recorded by Yepthoni *et al*. (2019) i.e. maximum number of shoots explant^{-1} was obtained in media containing 3 mg l^{-1} BAP+0.5 mg l^{-1} IBA in ginger crop. It was observed that, BAP with concentration range of 2-3 mg l^{-1} with combination of NAA and IBA gave best results. In present study, it was also observed that shoot emergence get on increasing with subculturing. Treatments T_4 (MS + 2 mg l^{-1} BAP+ 0.25 mg l^{-1}NAA) and T_2 (MS + 2 mg l^{-1} BAP+ 0.5 mg l^{-1} IBA) expressed maximum 5.8 cm shoot length among all the treatments. In all the treatments BAP at concentration of 2-3 mg l^{-1} with both auxins i.e. NAA and IBA exhibited maximum shoot length.

Root induction

High frequency root induction is prime concern for large scale production in clonal propagation. Initial medium of shoot cultures, quality of shoots, shoot length, nutrient medium, auxin and its concentrations are important factors which influence the rooting frequency, root number, length and subsequent shoot after rooting.

Total 9 treatments were used for rooting from which only 3 treatments showed positive results (Table 3). Treatments T_2 (1/2 MS + 5 mg l^{-1} IBA) and T_3 (1/2 MS+ 6.5 mg l^{-1} IBA) were proved superior over all the treatments for root induction with average of 2.8 roots. Diab *et al.* (2008) achieved the highest percentage (70%) in full strength MS media supplemented with IBA, contrary to present study which found half strength of MS media effective. Treatment of Ms/4 media with NAA (2.5 mg l^{-1}) was found effective treatment in root induction (Sawant *et al*., 2016). It was seen that in initial days of root formation the shoots get dried but there was emergence of new shoots after root initiation. While, in case of root length, treatment T_3 (1/2 MS+ 6.5 mg l^{-1} IBA) was found superior over all the treatments. In present study, it is observed that the length of root was slow in rooting media but after sub culturing it to media like full strength of MS supplemented with 2-3 mg l^{-1} BAP gave very high impact on increasing root length.

Table 1. Treatments for *in vitro* shoot induction at various levels of growth regulators (kinetin, BAP, IBA, NAA)

Treatments		Average no. of days required to shoot induction	Response for shoot induction (in per cent)	Average no. of shoots explants^{-1}
1.	MS medium(control)	4	76.572	7
2.	MS + Kinetin 2mgl^{-1}+IBA0.5mgl^{-1}	3	80.632	7
3.	MS + kinetin 3mgl^{-1} + IBA 0.5mgl^{1}	3	89.715	9
4.	MS + kinetin 4mgl^{-1}+IBA 0.5mgl^{-1}	3	71.560	14
5.	MS + BAP 2 mgl^{-1} + IBA 0.5mgl^{-1}	3	89.715	12
6.	MS + BAP 3mgl^{-1} +IBA 0.5mgl^{-1}	3	89.715	10.5
7.	MS+ BAP 2mgl^{-1} + NAA 0.25mgl^{1}	3	89.715	10.5
8.	MS+ BAP 3mgl^{-1} + NAA 0.25mgl^{-1}	3	89.715	11.5
9.	MS + Kinetin 2mgl^{-1}+ NAA 0.25mgl^{-1}	3	71.560	7.5
10.	MS + Kinetin 3mgl^{-1} + NAA 0.25mgl^{-1}	3	71.560	9
	GM	**3.1**	**82.04**	**9.8**
	SE (m) ±	**-**	**-**	**0.316**
	CD	**-**	**-**	**0.993**
	CV	**-**	**-**	**4.564**

Table 2.Treatments for shoot multiplication at various levels of growth regulators

Treatments		Average no. of days to shoot emergence	Average no. of shoots emerged in shoot multiplication	Average length of shoots
1.	MS media (Control)	-	0.701	0.701
2.	MS + 2 mgl^{-1} BAP+ 0.5mgl^{-1} IBA	3.535	4.239	5.858
3.	MS + 3 mgl^{-1} BAP+ 0.5mgl^{-1} IBA	3.162	5.955	4.473
4.	MS + 2 mgl^{-1} BAP+ 0.25mgl^{-1}NAA	3.535	5.919	5.840
5.	MS + 3 mgl^{-1} BAP+ 0.25mgl^{-1} NAA	3.317	3.236	4.323
6.	MS + 4 mgl^{-1} KIN + 0.5mgl^{-1} IBA	3.74	2.443	4.968
7.	MS + 5 mgl^{-1} KIN + 0.5mgl^{-1} IBA	3.808	1.409	1.227
	Grand mean	**3.516**	**3.414**	**3.91**
	SE (m) ±	**0.054**	**0.128**	**0.107**
	CD (5%)	**0.169**	**0.419**	**0.402**
	CV	**2.198**	**5.101**	**4.374**

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1. T_4 (MS + kinetin 4 mg l^{-1} + IBA 0.5 mg l^{-1}) Shoot initiation treatment
2. T_3 (MS+ 3 BAP mg l^{-1} + 0.5 IBA mg l^{-1}) Shoot multiplication treatment
3 T (1/2 MS + 5 mg l^{-1} IBA) and T (1/2 MS+ 6 5 mg l^{-1} IBA) Rooting treatments

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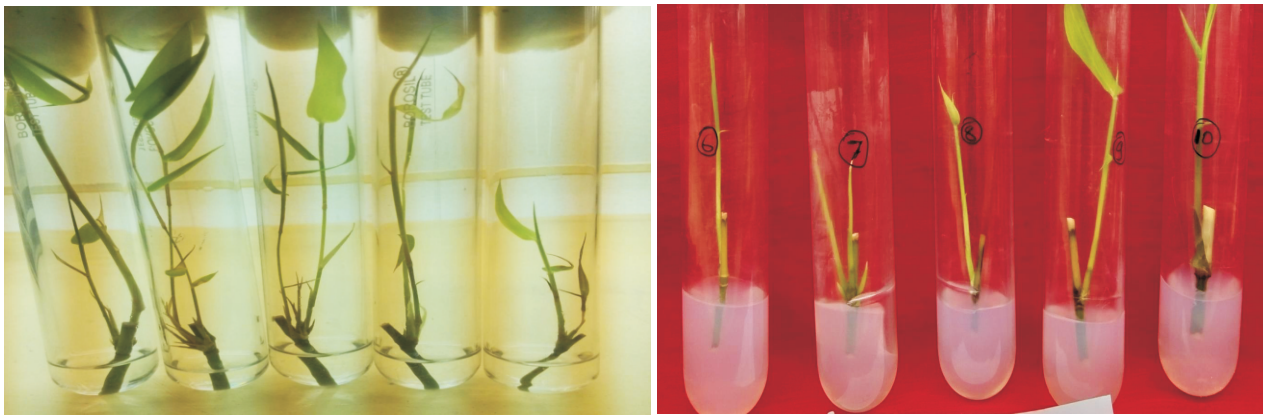

Length of shoots is maximum in liquid media than semisolid media

Well rooted plantlets of *D. stocksii*

Table 3.Treatments for in vitro root induction at various levels of growth regulators

Treatments		Per cent response in root induction (in per cent)	Average no. at root emergence	Average length of roots (in on)
1.	1/2MS (Control)	0	0	**0**
2.	1/2MS + 5mgl^{-1} IBA	20	2.8	**1.156**
3.	1/2MS + 6.5mgl^{-1} IBA	20	1.8	**1.215**
4.	1/2MS + 1mgl $^{-1}$ NAA	0	0	**0**
5.	1/2MS + 1.5 mgl^{-1} NAA	20	1.3	**1.066**
6.	1/2MS + 2 mgl^{-1} NAA	0	0	**0**
7.	1/2MS + 2.5mgl^{-1} IBA + 2.5 mgl^{-1} NAA	0	0	**0**
8.	1/2MS + 2.5mgl^{-1} NAA + 25 mgl^{-1} Ascorbic acid	0	0	**0**
9.	1/2MS + 2.5mgI^{-1} BAP+5.0 mgl^{-1} IAA	0	0	**0**

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