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In Vitro propagation Technique for Bamboo Species of Western Ghats

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ABSTRACT

Present investigation was carried out in the Plant Biotechnology centre, Dapoli during 2008-2013 with the objective of standardization of regeneration protocol for large scale multiplication of bamboo species viz., Kondyamesh (*Pseudoxytenanthera stocksii* (Munro) Naithani, Manvel (*Dendrocalamus strictus* (Roxb) Nees., Kalak (*Bambusa arundinacea* (Retz.) Willd) and Bass mesh (*Bambusa nutans*) Axillary buds and apical buds collected from field grown clump were used for micropropagation .To establish aseptic cultures, the explants were disinfected with presoaking in 0.2% Bavistin and 0.2% Streptomycin for 1 hr. followed by 0.1% $HgCl_2$ for 10 min. (Washing with sterile double distilled water 3-4 times after each step) obtained highest percentage of aseptic culture in cultivars viz., Kondyamesh (40), Bass mesh (38), Manvel (30) and Kalak (25). The media combination of MS + 1 mgl^{-1} BAP + 0.5 mgl^{-1} NAA + 80 mgl^{-1} Ads +50 mgl^{-1} Ascorbic acid + 25 mgl^{-1} Citric acid + 25 mgl^{-1} Cystein (Liquid) was the best for establishment of Manvel (40%), Kondyamesh (33.3%), Bass mesh (36%) and Kalak (29.3%) gelled with 0.8% agar and using 3% sucrose as a carbon source. Sub-culturing of shoots at every 2 weeks on same fresh multiplication medium resulted a consistent proliferation rate of bamboo cultivars with 14-15 shoots in Bass mesh, 6-7 shoots in Kondyamesh and 5-6 shoots in Kalak and 7-8 shoots in Manvel. Excised propagules of 3-4 shoots were inoculated on ¼ MS medium supplemented with high concentration of auxin (NAA) for successful rooting. The liquid medium ¼ MS + 2.5 mgl^{-1} NAA + 25 mgl^{-1} Ascorbic acid recorded rooting within 15-20 days period and the number of roots per explant varied among cultivars viz.,Kondyamesh (2-3), Bass mesh (1-2), Manvel (4-5) and Kalak (1-2). Plantlets grown in vitro were hardened under green house condition containing autoclaved mixture of soil: cocopeat: vermicompost in 2:1:1 proportion and cultures exhibited 80 per cent normal growth.

INTRODUCTION

The consumption of bamboo is for paper, board, rayon and scaffolding by the industries and many other uses. The continuous increase in the installed capacity of bamboo based industrial units for paper and board has put an enormous pressure on bamboo forests (Dhurga 2013). Survival of the forests is dependent upon the local people who also happen to be sufferers. Forests, in India, being in the state list of subjects for long time, and now in the concurrent list for the last two decades, different states have diverse provisions for the use of bamboo, which is mainly the allocation to industries and local artisans and other people. In the species *Dendrocalamus strictus* seed retain their viability for a period of about one year only (Gupta and Sood,1978).Viability of seeds under natural conditions is very short (Lakshmi et.al 2014). Propagation through seed is difficult due to unreliable flowering habit at an interval of 30-100 years. In proper storage conditions the viable period of bamboo species *viz, B. nutans* is 3 months, *Bambusa arundinacea* 6 months, *D. strictus* 24 months and B. *tulda* 3 months (Ahlawat et al. 2002).

The conventional vegetative propagation practiced with rhizome and culm cutting as an alternate method has proved to be only of limited value. There are major constraints for availability of such seed material for commercial cultivation. Hence, for fulfilling the demand of farmers for the seed material, the improvement in the bamboo tissue culture technique seems to be one of the urgent solutions. Therefore, the present study was undertaken to standardize in vitro protocol for large scale production of different bamboo species in Konkan region of Maharashtra.

MATERIALS AND METHODS

The present investigation was undertaken at Plant Biotechnology Centre, College of Agriculture, Dapoli, Ratnagiri, Maharashtra. Four bamboo species *viz.,* Kondyamesh (*Pseudoxytenanthera stocksii* (Munro) Naithani, Manvel (*Dendrocalamus strictus* (Roxb) Nees., Kalak (*Bambusa arundinacea* (Retz.) Willd) and Bass mesh (*Bambusa nutans*) established in the bamboo forest College of Forestry, Dapoli were used for present study.

The nodal segment and apical buds as explants were selected from seedling or mature bamboo of these four cultivars. The leaf sheaths covering the nodal segments containing axillary buds and apical buds (4-5cm) were carefully removed and wiped with 70 per cent (v/v) ethanol using sterilized cotton. Cleaning of the explants was carried out by using tween20 with running tap water. Then the different treatments given in table 1 were followed and the explants were washed for about 3-4 times with sterile double distilled waterand placed on different combinations of solid and liquid MS medium supplemented with 30 per cent sucrose. The cultures incubate for 14 hrs in dark and 10 hrs light at 20-25 ^{0}C for about 15 days for establishment. The established cultures were subcultured after 2 weeks on the same medium for proliferation. The cultures were healthy forming more shoots after second sub-culturing and they were separated and transferred to rooting medium. Observations such as percentage of aseptic culture, average number of shoots per explant and number of roots per shoot were recorded during sterilization, establishment, proliferation and rooting of the cultures.

RESULTS AND DISCUSSION

Surface sterilization

The leaf sheaths covering the nodal segments containing axillary buds and apical buds (5–7cm) were carefully removed and wiped with 70 per cent (v/v) ethanol using sterilized cotton. The explants were surface sterilized with different eight treatments (Table 1). The treatments *viz.,* T4 *i.e.* presoaking in 0.2 per cent Bavistin and 0.2 per cent Streptomycin for 1 hr. followed by 0.1 per cent $HgCl_2$ for 10 min (washing with steriledouble distilled water3-4 times after each step) obtained highest percentage of aseptic culture in Kondyamesh (40),Bass mesh (38),Manvel(30) and Kalak (25),followed by T5 i.e. presoaking in 0.2 per cent Bavistin and 0.2 per cent Streptomycin for 1 hr. followed by 5 per cent Sodium hypochlorite for 15min. (washing with sterile double distilled water3-4 times after each step) resulted in forming aseptic culture in Kondyamesh (36%), Bass mesh (32%), Manvel (28%) and Kalak (22%).

Table 1. Response of surface sterilization on percentage of aseptic explants

Tr. No.	Treatments details	Percentage of aseptic culture			
		KM	BM	MAN	KAL
T1	0.1% Ridomil overnight + 0.1% Streptomycin for 1 hr., 0.1% $HgCl_2$ - 10 min. Sterile DDW wash 3-4 times.	20	12	16	14
T2	0.1% Ridomil + 0.1% Streptomycin 4 hrs., 5% Sodium hypochorite - 15 min. Sterile DDW wash 3-4 times.	16	18	14	12
T3	0.1% Ridomil + 0.1% streptomycin 4 hrs followed by 5% Sodium hypochlorite 15 min followed by 0.1% $HgCl_2$ 10 min. Sterile DDW wash 3-4 times.	15	20	25	23
T4	Presoaking in 0.2% Bavistin and 0.2% Streptomycin for 1 hrs. followed by 5% Sodium hypochlorite- 15 Sterile DDW wash 3-4 times.	36	32	28	22
T5	Presoaking in 0.2% Bavistin and 0.2% Streptomycin for 1 hrs. followed by 0.1% $HgCl_2$ - 10 min. Sterile DDW wash 3-4 times.	40	38	30	25
T6	Copper sulphate 2000 ppm 1hr. + 0.1% Streptocycline 5 hrs. followed by 1% $HgCl_2$ 10 min.	11	9	12	8
T7	Copper sulphate 2000 ppm 1hr. + 0.1% Streptocycline 5 hrs. followed by 0.1% Cetrimide 30 min followed by 0.1% $HgCl_2$ 10 min. washing 3-4 times with sterile DDW.	12	16	18	20
T8	0.1 % calcium hypochlorite 45 min + 0.05 % sodium hypochlorite 20 min + 0.1% Streptocycline 5 hrs. + 0.1% Cetrimide 30 min +0.1% $HgCl_2$ 10 min washing 3-4 times with sterile DDW.	16	18	24	25

KM- Kondya mesh, BM-Bass mesh, MAN-Manvel, KAL-Kalak

Establishment and proliferation of shoots

Result presented in Table 2 indicated that two combinations media *viz.*, MS + 2.5 mgl^{-1} BAP + 0.5 mgl^{-1} NAA + 50 mgl^{-1} Ascorbic acid + 25mgl^{-1} Citric acid + 25 mgl^{-1} Cystein (Liquid) and MS + 1mgl^{-1} BAP + 0.5mgl^{-1} KIN (Liquid) gave better establishment and proliferation of nodal and apical bud explants. The media combination of MS + 1 mgl^{-1} BAP + 0.5 mgl^{-1} NAA + 80 mgl^{-1} Ads +50 mgl^{-1}Ascorbic acid + 25 mgl^{-1} Citric acid + 25 mgl^{-1} Cystein (Liquid) was the best for establishment of Manvel (40%), Kondyamesh (33.3%), Bass mesh (36%) and Kalak (29.3%) and they produced on an average 14-15 shoots in Bass mesh, 6-7 shoots inKondyamesh and 5-6 shoots in Kalak and 7-8 shoots in Manvel cultivars. Such information is also recorded by several scientists on several bamboo species (Sexsena1990; Arya and Sharma 1998; Agnihotri and Nandi, 2009; Divya and Saxena 2011; Rathore and Raivishankar 2005; Thiruvengadam et.al. 2011; Shirin and Rana 2007; Pandey and Singh 2012).

Rooting

Nine different treatments (Table 3) using ¼

strength of MS media combinationswere imposed for root induction in four bamboo species. Rooting was achieved by aseptically transferring multiplied shoots to a ¼ MS strength liquid medium prepared with 3 per cent sucrose, which was supplemented with different combinations of growth regulators .The medium with ¼ MS + 2.5 mgl^{-1} NAA + 25 mgl^{-1} Ascorbic acid recorded more number of roots per shoots within 15-20 days period and they were 2-3 roots in Kondyamesh 1-2 roots in Bass mesh, 4-5 roots in Manvel and 1-2 roots in Kalak. This treatment is followed by ¼ MS + 2.5 mgl^{-1} NAA + 25 mgl^{-1} Ascorbic acid +34 mgl^{-1} NaH_2PO_4. Plantlets were transferred from MS medium to green house conditions for hardening and the potting mixture contains autoclaved mixture of soil: cocopeat: vermicompost in 2:1:1 proportion and that resulted in more than 80per cent plant success. Such studies have been conducted in bamboo species (Ravikumar et al.1998; Chowdhury et al. 2004; Devi et al. 2012; Arya et al. (2012) and Mudoiet al. 2013).

Table 2. Response of percent establishment and number of shoots on various media combinations

Sr. No.	Media combinations	Percent establishment				Average number of shoots / explant			
		KM	BM	MAN	KAL	KM	BM	MAN	KAL
1	MS + 2 mgl^{-1} BAP + 80 mgl^{-1} Ads (Solid)	14.0	12.0	9.0	24.0	2-3	2-3	2-3	2
2	MS + 5 mgl^{-1} BAP +2 mgl^{-1} IAA + 80 mgl^{-1} Ads (Solid)	9.3	8.0	16.0	18.6	1	2	1	2
3	MS + 1 mgl^{-1} BAP + 0.25 mgl^{-1} NAA + 80 mgl^{-1} Ads (Solid)	5.3	2.4	4.3	6.6	1	1	2	2
4	MS + 1 mgl^{-1} KIN + 1.5 mgl^{-1} NAA + 60g Sucrose + 80 mgl^{-1} Ads (Solid)	18.6	20.0	16.6	28.0	1	1	2	1
5	MS + 1 mgl^{-1} BAP + 0.5 mgl^{-1}KIN (Liquid)	26.6	30.6	40.0	28.0	5-6	5-6	6-7	5-6
6	MS + 1 mgl^{-1}BAP + 0.5 mgl^{-1} NAA + 80 mgl^{-1} Ads +50 mgl^{-1}Ascorbic acid + 25 mgl^{-1} Citric acid + 25 mgl^{-1} Cystein (Liquid)	33.3	36.0	40.0	29.3	6-7	14-15	7-8	5-6
7	MS + 2.5 mgl^{-1} BAP + 0.5 mgl^{-1} NAA + 50 mgl^{-1} Ascorbic acid + 25 mgl^{-1} Citric acid + 25 mgl^{-1} Cystein (Liquid)	21	34.5	19.7	15.1	4-6	10-12	4-5	5-6
8	MS + 3mgl^{-1} BAP + 1 mgl^{-1}NAA + 50 mgl^{-1}Ascorbic acid + 25mgl^{-1} Citric acid + 25 mgl^{-1} Cystein (Liquid)	11.7	32.7	12	13.5	8-10	4-5	8-9	10-11

KM- Kondya mesh, BM-Bass mesh, MAN-Manvel, KAL-Kalak.

Table 3. Response of rooting to various media combination in bamboo

Sr. No.	Media combination	No. of roots/shoot KM	BM	MAN	KAL
1	¼ MS + 2.5 mgl^{-1}NAA + 25 mgl^{-1} Ascorbic acid	2-3	1-2	4-5	1-2
2	¼ MS + 2.5 mgl^{-1}NAA + 25 mgl^{-1}Ascorbic acid + 34 mgl^{-1} NaH_2PO_4	4-5	NR	4-5	NR
3	¼ MS + 3.0 mgl^{-1}NAA + 25 mgl^{-1} Ascorbic acid + 34 mgl^{-1} NaH_2PO_4	NR	NR	NR	NR
4	¼ MS + 1 mgl^{-1} IBA + 20 mgl^{-1} Ascorbic acid	NR	NR	NR	NR
5	¼ MS + 2 mgl^{-1} IBA + 20 mgl^{-1} Ascorbic acid	NR	NR	NR	NR
6	¼ MS + 1 mgl^{-1} IBA + 25 mgl^{-1} Ascorbic acid	NR	NR	NR	NR
7	¼ MS + 2 mgl^{-1} IBA + 25 mgl^{-1} Ascorbic acid	NR	NR	NR	NR
8	¼ MS + 1 mgl^{-1} IAA	NR	NR	NR	NR
9	¼ MS + 2 mgl^{-1} IAA	NR	NR	NR	NR

NR- Not responding, KM- Kondya mesh, BM-Bass mesh, MAN-Manvel, KAL-Kalak.

CONCLUSION

In the present study, *in vitro* propagation techniques have been standardized for four bamboo species and it may be used for mass multiplication of bamboo cultivars that can help to increase the economic status of the farmers.

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