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Standardization of protocol for shoot multiplication of jasmine (*Jasminum sambac* (L) Aiton)

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ABSTRACT

The present investigation was carried out on *Jasminum sambac* (L) Aiton belonging to family Oleaceae. The different vegetative parts viz nodal explants, shoot apices, stems and leaves were used to standardize the protocol in vitro. The explants were excised from field grown mature plants and thereafter planted on variously supplemented Murashige and Skoog's (MS) medium for multiple shoot proliferation and callus induction. Early bud break (25-26 days) and maximum percentage of bud break (83.7) was observed in *J sambac* when MS medium was supplemented with BAP (2.0 mg/l), Kn (1.0 mg/l) and Ads (50 mg/l). Maximum percentage of multiple shoots was observed on MS medium fortified with 2 mg/l BAP, 1.0 mg/l Kn and 50 mg/l Ads. Callus induction from leaf and meristem explants was achieved using different concentrations of auxins and cytokinins. Maximum percentage of cultures showing callus proliferation from leaf explants was observed in the medium containing MS basal salts supplemented with 3.0 mg/l BAP and 2.0 mg/l IAA in leaf explants. The percentage of cultures showing callus was similar in the medium containing MS basal salts supplemented with 5.0 mg/l BAP and 2.0 mg/l NAA in leaf explants. However white and friable callus was obtained in the medium containing MS basal salts supplemented with 2.0 mg/l BAP, 2.0 mg/l Kn and 2.0 mg/l IAA from meristem explants.

Keywords: Bud break; callus induction; MS medium; *Jasminum sambac*; shoot proliferation

INTRODUCTION

Among commercial loose flowers, jasmine (*Jasminum sambac*) of the family Oleaceae and native of India is endowed with large spectrum of commercial potentialities in perfumery and essential oil sector (Ambasta 1986, Anon 1976). The species is highly variable possibly a result of spontaneous mutation, natural hybridization and autopolyploidy. Only a few varieties are propagated by seed in the wild. Cultivated *J sambac* generally does not bear seeds and as a result it is difficult

to develop new varieties through conventional breeding. The only way to develop new cultivars is either through somatic mutations or gene manipulation. Therefore it is essential to develop an efficient protocol for in vitro culture of *J sambac*. Moreover as it does not bear seeds the plant is reproduced solely by cuttings and layering and these methods of propagation are dependent on the season. Layering involves more time and restricts the number of plants propagated from a bush. It can also be propagated by cuttings. However long term cutting/layering causes varietal degeneration, resistance weakness and declining of flower production (Cai et al 2007).

In vitro culture presents itself as an attractive tool for mass multiplication of important fragrant plant species and for the production of secondary metabolites. There are few reports on regeneration of jasmine employing tissue culture techniques (Jonard 1989, Khoder et al 1979). The present study examines the efficiency of nodal buds in forming multiple shoots either with agar solidified medium or agar free liquid medium.

MATERIAL and METHODS

Plant material and explant preparation

The experiment was conducted at Agricultural Biotechnology Department, OUAT, Bhubaneswar during the year 2013-14. Stem apices and leaves were used as source of explants. The shoot materials, either apical or axillary buds were first washed for 15 minutes in running tap water, cut into 1.5-2 cm pieces and then treated with 0.1 per cent carbendazim solution for 15 minutes followed by thorough washing (5-6 times) with distilled water. Surface sterilization was carried out with 0.1 per cent HgCl2 (w/v) for 10 minutes followed by washing 3 times with sterile distilled water.

Culture media and culture conditions

For shoot initiation medium, each test tube (150 x 25 mm) with 10-15 ml of Murashige and Skoog (MS medium supplemented with 30% sucrose and various combinations of BAP (1.0-6.0 mg/l), NAA (0.5-2 mg/l) and Ads (0-50 mg/l) was filled. The medium contained 3 per cent (w/v) sucrose and 0.7-0.8 per cent (w/v) agar. For callus culture, medium was supplemented with various concentration of BA (1-5 mg/l) or IAA (1-2 mg/l) or NAA (1-2 mg/l). The pH was adjusted to 5.7 with 1N KOH or 1N HCL before autoclaving at 1.05 kg/cm^2, 121°C for 20 minutes. The explants (shoot tip) were cut into pieces (3-4 cm) each containing one or two axillary buds and inoculated in the fortified medium. The leaf pieces were placed to previously prepared slants. The cultures were incubated in growth chamber (25 ± 2°C) under cool white flurorescent lamps providing 3000 lux light intensity. The photoperiod was adjusted to 16/8 h light and dark cycle. However for callus culture

from leaf, the cultures were maintained under dark condition till sub-culturing. Each treatment was represented by 20 explants and the experiment was repeated three times.

RESULTS and DISCUSSION

Effect of growth regulators on bud break

Bud break was achieved on MS medium supplemented with various concentrations of BAP, Kn, IAA and Ads. BAP and Kn favoured bud break among the different hormones tested. Adenine sulphate helped in inducing bud break at low concentration in combination with BAP and Kn. The auxins were less effective in inducing bud break than cytokinins. Early bud break (25-26 days) was initiated in *J sambac* when MS medium was supplemented with BAP (2 mg/l), Kn (1 mg/l) and Ads (50 mg/l). The maximum percentage (83.7) of bud break in *J sambac* was on MS medium having BAP (2 mg/l), Kn (1 mg/l), Ads (50 mg/l) and 3 per cent sucrose (Table 1).

Depending on the type and concentration of growth regulators used the number of days required to bud break varied between 25 to 47 days. Among all growth regulators tested the medium containing BAP (2.0 mg/l), Kn (1.0 mg/l) and Ads (50.0 mg/l) induced bud sprouting within 25-26 days of culture of *J sambac*. BAP was reported to be in general the most effective cytokinin for meristem, shoot-tip and axillary bud culture of various species (Cai et al 2007, Bhattacharya and Bhattacharya 1997).

Effect of plant growth regulators on shoot multiplication

The experiment was designed to study the effect of different concentrations and combinations of cytokinins (BA and Kinetin) and Auxins (IAA) on shoot initiation and elongation. The shoots showed differential rate of elongation; media devoid of growth regulator did not respond. Maximum percentage of multiple shoots (88.4) was observed in MS medium supplemented with BAP (2.0 mg/l), Kn (1.0 mg/l) and Ads (50 mg/l) within 8 weeks of culture. Induction of multiple shoot was better on MS medium supplemented with BAP + Kn than BAP alone. IAA was less effective in inducing multiple shoot in combination with BAP and Ads. The average number of shoots per culture was the maximum (8.7) in MS medium supplemented with BAP (2.0 mg/l), Kn (1.0 mg/l) and Ads (50 mg/l) (Table 2).

The results indicate that rate of shoot multiplication of *J sambac* declined as the concentration of BAP increased from 2.0 to 3.0 mg/l. This may be due to the ionic concentration and balance between different nutrient ions in the culture medium which is crucial for optimization of shoot multiplication of the plant.

Table 1. Effect of auxin and cytokinins on bud break of *Jasminum samabc* (L) Aiton grown in MS medium after 8 weeks of culture

MS + growth regulators (mg/l)				Days to bud break	Percentage of bud break (mean ± SE)*
Kn	BAP	IAA	Ads		
0	0	0	0	0	0
0	1.0	0.1	50	34-36	4.8 ± 0.4
0	2.0	0.1	50	35-37	13.3 ± 0.7
0	3.0	0.1	50	36-38	21.6 ± 0.5
1.0	0	0.1	50	35-38	10.0 ± 2.4
2.0	0	0.1	50	33-36	12.0 ± 1.3
3.0	0	0.1	50	44-47	15.6 ± 1.2
1.0	1.0	0	50	26-28	76.6 ± 1.4
1.0	2.0	0	50	25-26	83.7 ± 1.8
1.0	3.0	0	50	25-27	77.6 ± 1.3
2.0	1.0	0	50	31-33	75.0 ± 1.1
2.0	2.0	0	50	37-39	73.3 ± 1.4
2.0	3.0	0	50	32-34	76.6 ± 1.7
3.0	1.0	0	50	38-40	61.6 ± 1.4
3.0	2.0	0	50	40-42	62.4 ± 1.5
3.0	3.0	0	50	36-38	54.8 ± 1.6

*20 cultures per treatment repeated thrice

Table 2. Effect of auxin and cytokinins on shoot multiplication of *Jasminum sambac* (L) Aiton after 8 weeks of culture

MS + growth regulators (mg/l)				Percentage of explants developed multiple shoot (mean ± SE)*	# shoots/culture (mean ± SE)*
Kn	BAP	IAA	Ads		
0	0	0	0	0	0
0	1.0	0.25	50	14.2 ± 0.6	0.8 ± 0.06
0	2.0	0.25	50	23.3 ± 0.8	1.3 ± 0.07
0	3.0	0.25	50	31.6 ± 1.5	2.1 ± 0.05
1.0	1.0	0	50	76.2 ± 1.2	6.6 ± 0.4
1.0	2.0	0	50	88.4 ± 1.7	8.7 ± 0.8
1.0	3.0	0	50	81.6 ± 1.1	5.6 ± 0.3
2.0	1.0	0	50	79.0 ± 1.0	5.0 ± 0.2
2.0	2.0	0	50	78.4 ± 1.1	3.3 ± 0.04
2.0	3.0	0	50	80.2 ± 1.2	4.6 ± 0.07
3.0	1.0	0	50	66.4 ± 0.9	2.6 ± 0.08
3.0	2.0	0	50	67.6 ± 0.9	2.4 ± 0.04
3.0	3.0	0	50	64.8 ± 0.8	1.8 ± 0.09

Similar observations were made on *J sambac* by Cai et al (2007) and Sun et al (2009). The present study indicated that inclusion of IAA in the medium either with BAP or Kn did not favour multiplication and growth of the micro-shoots. The cytokinin requirement for shoot multiplication was essential and two cytokinins (BAP and Kn) favoured shoot multiplication. However Cai et al (2007) reported that MS medium in combination with BA (2.0 mg/l) + NAA (1.0 mg/l) and MS medium in combination with BA (1.5 mg/l) + NAA (0.3 mg/l) + GA_3 (0.5 mg/l) favoured axillary bud initiation and axillary bud proliferation respectively.

Effect of liquid medium on shoot multiplication

The effect of liquid medium on shoot multiplication was studied in comparison to agar media (0.8%). Twenty millilitre of liquid medium was taken in each test tube and absorbent cotton/filter paper bridges were inserted in the tubes prior to autoclaving. The absorbent cotton/filter paper bridges helps the explants to remain in proper position. Induction of multiple shoot was better on MS liquid medium supplemented with BAP + Kn than BAP alone. Maximum percentage of multiple shoots (90.5) was observed in MS liquid medium supplemented with BAP (2.0 mg/l), Kn (1.0 mg/l) and Ads (50 mg/l). The average number of shoots per culture was the maximum (8.9) in MS liquid medium supplemented with BAP (2.0 mg/l), Kn (1.0 mg/l) and Ads (50 mg/l) (Table 3).

The rate of shoot multiplication in liquid medium (medium devoid of gelling agent) was better than the agar gelled medium. This may be due to easier and efficient translocation of nutrients in the liquid medium than agar gelled medium. Similar results were reported in other species of jasmine (Dainty et al 1985).

Effect of media supplement on callus induction and regeneration

Proliferation in leaf explants and callus induction were achieved by using MS medium supplemented with different auxins (IAA or NAA) and cytokinin (BAP) alone or in combinations. Calli were initiated from leaf explants on the MS basal medium supplemented with BAP + NAA or BAP + IAA within 13 days of inoculation. The initial calli were small, globular and pale yellow in colour which developed on the surface of the explant and subsequently spread over the entire explant. The maximum percentage (86.7) of cultures showing callus in leaf explants was observed in MS medium supplemented with BAP (3.0 mg/l) and IAA (2.0 mg/l) which was at par with the MS medium supplemented with BAP (5.0 mg/l) and NAA (2.0 mg/l). The differential response could be due to the varying concentrations of growth regulators used in the media. Similar observations have been reported in *J grandiflorum* (Gomathi et al 2007) and in *J sambac* (Yulia et al 2012).

However Induction of callus and its proliferation by using meristem explants of *J sambac* was achieved on the MS basal

Table 3. Effect of Liquid media on shoot multiplication of *Jasminum sambac* (L) Aiton cultured on MS basal medium supplemented with 1.0 mg/l Kn , 2.0 mg/l BAP, 50 mg/l Ads after 8 weeks of culture.

MS + growth regulators (mg/l)				Percentage of explants developed multiple shoot (mean ± SE)*		# shoots/culture (mean ± SE)*	
Kn	BAP	IAA	Ads	Agar media (0.8%)	Liquid media	Agar media (0.8%)	Liquid media
0	0	0	0	0	0	0	0
0	1.0	0.25	50	12.5 ± 0.7	14.8 ± 0.9	0.6 ± 0.07	1.1 ± 0.3
0	2.0	0.25	50	21.5 ± 1.2	25.6 ± 1.5	1.4 ± 0.08	1.8 ± 0.6
0	3.0	0.25	50	32.9 ± 1.9	36.7 ± 1.8	2.3 ± 0.07	2.9 ± 0.8
1.0	1.0	0	50	73.6 ± 1.8	75.2 ± 1.4	5.6 ± 0.6	6.2 ± 0.9
1.0	2.0	0	50	86.9 ± 1.9	90.5 ± 1.2	8.6 ± 0.7	8.9 ± 0.9
1.0	3.0	0	50	79.1 ± 1.4	82.2 ± 1.9	4.9 ± 0.5	5.1 ± 0.7
2.0	1.0	0	50	76.2 ± 1.1	78.3 ± 1.6	4.6 ± 0.7	4.8 ± 0.3
2.0	2.0	0	50	75.6 ±1.3	77.1 ± 1.9	3.0 ± 0.6	3.3 ± 0.4
2.0	3.0	0	50	78.9 ± 1.5	79.6 ± 1.2	4.1 ± 0.5	4.8 ± 0.4
3.0	1.0	0	50	68.6 ± 1.8	71.6 ± 1.5	2.2 ± 0.06	2.6 ± 0.09
3.0	2.0	0	50	65.2 ± 1.7	67.1 ± 1.2	2.0 ± 0.03	2.4 ± 0.07
3.0	3.0	0	50	61.7 ± 1.5	62.9 ± 1.3	1.5 ± 0.07	1.9 ± 0.05

*20 cultures per treatment repeated thrice

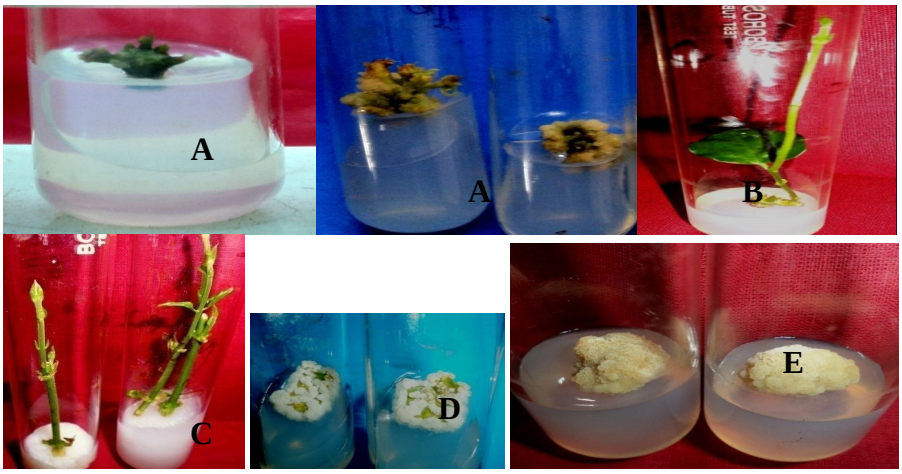

Fig 1. In vitro propagation of *Jasminum sambac* A. Enlarged axillary buds at the nodes after 2 weeks of cultur, B. Development of single axillary shoot from nodal explants on MS medium supplemented with MS medium having BAP (2 mg/l), Kn (1 mg/l), Ads (50 mg/l) and 3% sucrose after 8 weeks of culture, C. Development of multiple shoots from nodal explants on liquid medium supplemented with BAP (2 mg/l), Kn (1 mg/l), Ads (50 mg/l) and 3% sucrose after 8 weeks of culture, D. Induction of callus from leaf explants, E. induction of callus from meristem explants

Table 4. Effect of growth regulator on callus induction in meristem explants and leaf explants of *Jasminum sambac* (L) Aiton cultured after 8 weeks of culture

Meristem callus				Leaf callus			
MS + growth regulators (mg/l)			Percentage of cultures showing callus	MS + growth regulators (mg/l)			Percentage of cultures showing callus
BAP	Kn	IAA		BAP	IAA	NAA	
0	0	0	0	0	0	0	0
1.0	1.0	1.0	32.5 ± 1.4	1.0	1.0	0	12.3 ± 0.4
2.0	1.0	1.0	38.4 ± 1.2	2.0	1.0	0	18.4 ± 0.6
3.0	1.0	1.0	27.3 ± 0.8	3.0	1.0	0	37.6 ± 0.7
1.0	2.0	1.0	27.7 ± 0.3	4.0	1.0	0	27.7 ± 0.3
2.0	2.0	1.0	42.6 ± 1.2	5.0	1.0	0	22.6 ± 0.6
3.0	2.0	1.0	29.5 ± 0.7	6.0	1.0	0	23.5 ± 0.8
1.0	3.0	1.0	36.6 ± 0.9	1.0	2.0	0	33.5 ± 0.4
2.0	3.0	1.0	52.7 ± 0.7	2.0	2.0	0	56.7 ± 0.5
3.0	3.0	1.0	36.6 ± 0.8	3.0	2.0	0	86.7 ± 1.8
1.0	1.0	2.0	22.7 ± 0.4	4.0	2.0	0	52.3 ± 0.4
2.0	1.0	2.0	39.4 ± 0.8	5.0	2.0	0	38.4 ± 0.6
3.0	1.0	2.0	42.2 ± 0.9	6.0	2.0	0	34.2 ± 0.7
1.0	2.0	2.0	67.8 ± 1.4	1.0	3.0	0	27.8 ± 0.4
2.0	2.0	2.0	89.6 ± 1.5	2.0	3.0	0	39.6 ± 0.5
3.0	2.0	2.0	55.1 ± 0.8	3.0	3.0	0	52.1 ± 0.9
1.0	3.0	2.0	35.7 ± 0.7	4.0	3.0	0	46.7 ± 0.8
2.0	3.0	2.0	45.3 ± 0.8	5.0	3.0	0	47.3 ± 0.8

medium supplemented with BAP, Kn and IAA within 21 days of inoculation which were small, globular and white in colour. Maximum callus growth was obtained in the medim containing MS basal salts supplemented with 2.0 mg/l each of BAP, Kn and IAA (Table 4).

The calli were subsequently transferred to different MS media supplemented with various concentrations of cytokinins and auxins for plant regeneration. However the shoot bud like structures were also achieved in medium having MS medium supplemented with 3.0 mg/l kinetin or BAP (2-3mg/l) in combination with IAA or NAA (0.5-3.0 mg/l). Beasley and Pijut (2013) reported the plant regeneration from leaf and stem explants of *Fraxinus nigra* by using the medium of 2.5 mg/l BAP and 0.5 mg/l NAA.

CONCLUSION

High frequency plant regeneration of *J sambac* through in vitro techniques was attempted by manipulation of nutrient medium and culture conditions. Apical and axillary meristems were used as explant

sources for shoot multiplication. It was observed that the shoot multiplication in liquid medium was found to be better than agar gelled medium.

Callus induction in leaf and meristem explants was achieved using different concentrations of auxins and cytokinins. Further work is necessary to achieve the plant regeneration from proliferated calli to sustain the commercial propagation.

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