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Effect of different plant hormones on the callus induction of *Jasminum sambac*

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Abstract

Jasminum sambac is one of the important flowering plants of India and the plant is valuable for its pharmaceutical properties. The present study describes the callus induction of the plant *J. sambac* (Jasmine: node, internode and leaf) under the influence of different plants hormones like 2, 4-D, kinetin and ThiDiaZuron (TDZ) on the growth of callus. Internodes were proved to be the best explants for culture, which were grown on MS basal medium with different concentration of various growth regulators. The standard plant tissue culture protocol for callus culture and/or micropropagation was adopted. The highest efficiency of callus formation was observed in the medium the containing concentration of 2.0 mg/l 2, 4-D alone.

Keywords: *Jasminum sambacs*, tissue culture, micropropagation, plant growth regulators, callus

Introduction

Floriculture is a discipline of horticulture concerned with the cultivation of flowering and ornamental plants for gardens and also, comprising the floral industry. Jasmine is one of the important groups of flowering plants cultivated on a commercial scale and is highly esteemed for its attractive and fragrant flowers. The Jasmine belongs to the family Oleaceae, the term Jasmine is derived from Arabic word 'Jessamine' (Bailey, 1947) and also on Old Persian name 'Yesmyn' meaning fragrance. The genus jasmine comprises of about 300 species which are dispersed throughout the warmer parts of Europe, Asia, Africa and Pacific region (Bhattacharjee, 1980).

In India, Jasmines are cultivated throughout the country. However, the largest area under Jasmine flower production is in Tamil Nadu followed by Karnataka. Tamil Nadu is the leading producer of jasmine in the country with an annual production of 77247 tonnes from the cultivated area of 9360 ha. The major jasmine producing districts of Tamil Nadu are Dindigul, Salem, Madurai, Tirunelveli, Virudhunagar, Trichy, etc.

The best-known species among jasmines are Jasminum sambc Ait. and several varieties of this species viz., Arabian or Tuscan. Jasmine (Grand duke of Tuscassy, Motia, Mogra, Malligae and Kodai Mullai (Bhattacharjee, 1980) that are commercially cultivated. The species has several synonyms viz., *J. fragrans*, *J. sambac* Rorb. Mogram sambac Laik. etc. *Jasminum* is also a medicinally important plant. The roots along with the leaf of *J. sambac* are useful in opthalmopathy. It is also used for treating skin diseases, ulcer and fever. The flowers are bitter, acrid, refridgerant, alexipharmic, ophthalmic, purgative and lactifuge. The dried leaves, soaked in water are made into a poultice which is used to treat ulcers. The root is a purgative, expectorant, anthelmintic, intoxicant and cures headache, paralysis, and rheumatism (Kirtikar, 1935).

Vegetative propagated plants are mostly sterile and heterozygous (Glemin *et al*., 2006). Improvement of such plants using conventional technique is difficult. Hence they may be improved through in vitro methods. Hutchinson *et al*., (1992); Britto *et al*., 2009; and Mahesh *et al*., 2010; 2011, pointed out that tissue culture technique can be utilized for the improvement of ornamental characteristics. The variability associated with tissue culture has provided a pool

of variation upon which selection pressure was imposed to isolate unique forms of clones. The present study was undertaken to study the effects of different hormones on callus induction potential of various plant parts (node, internode and leaf) of *J. sambac*.

Materials and Methods

Plant propagules of *Jasminum sambac* were collected from the green house of Centre for Biodiversity and Biotechnology, St. Xavier's College (Autonomous), Palayamkottai. Different parts of Jasminum (node, internode and leaf) were used for callus induction. The explants of 7-8 cm in length were collected from the mother plants. The stem was defoliated and cut in to 4 cm length with 3-4 nodes. Before inoculation the explants were cut in the size of node (0.5 cm long), internodes (1.0 cm long) and leaf segments (0.5-0.7 cm). All these explants were washed with tap water twice and then treated with 5% tween-20 solution for 5 min for surface sterilization. It is and then disinfested with 70% alcohol (v/v) for one minute in a laminar air flow chamber followed by immersion in $HgCl_2$ (0.1% w/v aqueous) for 5-10 min for node, internode segments and 1min for leaf segments. Then the explants were washed thoroughly with sterilized distilled water. Then they were transferred into the callus induction medium.

Explants were cultured in MS media supplemented with different combination and concentration of hormones. Murashige and Skoog, 1962 agar medium supplemented with 3% sucrose and different concentrations and combinations of plant growth regulators (PGRs) were used. (2, 4-Dichlorophenoxyacetic acid (2, 4-D; 0.5–3.0 mg/l), 6-furfurylaminopurine (Kinetin; 0.1–1.2 mg/l) and ThiDiaZuron (TDZ; 0.1-1.2 mg/$^{-l}$). The culture tubes were incubated at a controlled temperature of 25^{o}C µmol m^{-2s-1} light under 16 hrs of daily illumination provided by cool white florescent tubes approximately at 1500-2000 lux. The humidity was also maintained at 70-80 % in the culture room and observations were recorded.

Results and Discussion

The MS basal medium supplemented with various hormones were used to study the callus induction responses. Internode explants of *J. sambac* were cultured on MS media containing different concentrations of 2,4-D alone and 2,4-D in combinations with Kinetin or TDZ. Data were analyzed after two weeks of culture and the results showed that there was a wide range of variations in the days to callus initiation, percentage of explants that developed callus, callus texture, callus color and degree of callus formation depending on the culture media formulations (Table1). Callus initiation on cut ends of in vitro cultured explants could be observed in all hormone combinations after 7 - 14 days.

Table 1: Effect of plant hormones on callus induction in *J. sambac* (SC- Success of Callogenesis)

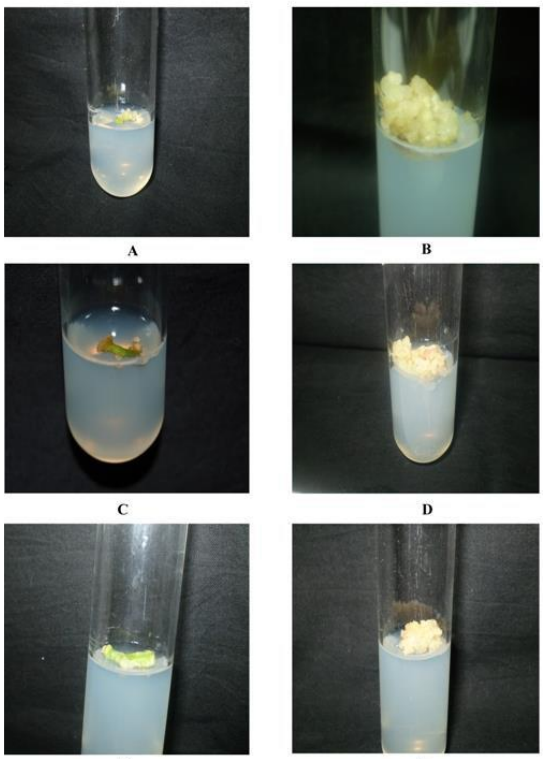
Medium	Node (SC %)	Internode (SC %)	Leaf (SC %)
T_0	00. ±00	00±00	00±00
T_1	45.4 ±0.4	71.4± 0.5	32.5± 0.5
T_2	63.3± 0.4	72.3± 0.2	38.2± 0.2
T_3	77.5 ±0.4	83.5± 0.4	46.5± 0.5
T_4	86.8 ±0.2	89.3± 0.4	48.4± 0.4
T_5	76.4± 0.4	82.2± 0.2	50.5± 0.4
T_6	70.3± 0.4	80.2± 0.3	38.2± 00
T_7	46.3± 0.3	58.3± 0.4	22.4± 0.4
T_8	56.6± 0.5	67.4± 0.4	20.4± 0.4
T_9	63.4± 0.3	71.5± 0.3	21.4± 0.3
T_{10}	69.3± 0.4	76.4± 0.4	25.4± 0.4
T_{11}	72.3± 0.3	70.5± 0.5	22.2± 0.2
T_{12}	70.0± 0.1	69.4± 0.4	20.3± 0.4
T_{13}	48.3± 0.3	27.3± 0.3	22.1± 0.2
T_{14}	53.3± 0.4	39.2± 0.2	28.4± 0.4
T_{15}	64.4 ±0.4	58.4 ±0.4	27.2± 0.2
T_{16}	68.3± 0.3	63.5 ±0.4	26.2± 0.2
T_{17}	70.6± 0.4	68.4± 0.4	24.4± 0.4
T_{18}	73.4± 0.6	71.1± 0.2	22.2± 0.2

However, the explants cultured on MS medium without growth regulators did not produce any callus. These results are in support of the results obtained by Fiegert *et al*., (2000), Jayasree *et al*. (2001) and Yasmin *et al*., (2003). Among all the growth regulators used, 2,4-D was found to be the most effective growth regulator ever when used alone or in combinations with cytokinins (BA). Castillo et al. (1998) reported that auxin 2,4-D by itself or in combination with cytokinin has been widely used to enhance callus induction and maintenance. Moreover, many researchers observed 2,4- D as the best auxin for callus induction as common as in monocot and even in dicot plants (Mamun *et al*., 1996).

Within the different concentrations of 2,4-D when used alone, the highest percentage (89.3) for friable yellow callus formation from the internode explant was recorded in the MS medium

supplemented with 2.0 mg/l (Table -1 and Plate 1). This result is in agreement with Shirin *et al*., (2007) who used 2,4-D for callus induction from internodal and leaf explants obtained from four potato cultivars including Diamant and found that among all concentrations and combinations 2,4-D at 2.0 mg/l was found to be the most effective auxin concentration for callus induction in all potato cultivars.

Explants and their callus of *J.sambac*



A - Leaf, B - Leaf callus (60 days old), C - Node, D - Node callus (60 days old), E - Internode, F - Internode callus (60 days old).

In this investigation it was observed that 2,4-D alone was produced from the friable green callus in a shorter period (Shortened by one week) than when used in combination with Kinetin or TDZ. After sufficient callus induction, the explants were initiated subsequently through organogenesis and then were sub-cultured on MS medium supplemented with different concentrations of BAP and TDZ.

Conclusion

In conclusion, the system established in the present study for the tissue culture of *J. sambac* get enough callus and plant regeneration efficiency to perform transgenic operation. Moreover, as the potentiality of shoot multiplication from callus continued for a long time, regeneration may be characterized by somaclonal variation and can improve to traits for agronomic importance.

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