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Callus induction in the leaf segments of *Jasminum angustifolium*

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Abstract

Jasminum angustifolium commonly called Wild jasmine in English and Kattumullai in Tamil and belongs to the family Oleaceae. The explants were finally rinsed four - five times with sterile distilled water and inoculated on MS (Murashige and Skoog, 1962) supplemented with various concentrations of growth regulators. The interaction of the auxin (NAA) with the cytokinin (BAP) in the concentrations tested were not effective to obtain callus in the leaf explants of *J. angustifolium*. However, in the presence of 0.5, 1.0, 1.5, 2.0 and 2.5 mg L-1 NAA and in the absence of BAP, root emission was observed in the explants. This also happened when they were inoculated in the presence of 2.0 and 3.0 mg L-1 NAA with 1.0 mg L-1 BAP. The highest callus formation in the leaf explants of *J. angustifolium was* observed with the isolated addition of 2, 4- D to the culture medium. There was no callus formation in the absence of this growth regulator. The interaction of the auxins NAA and 2,4-D with the cytokinin BAP, in the tested concentrations, are not enough for callus production in the leaf explants of *J. angustifolium.* The presence of 2,4-D is essential for callus induction in the leaf explants of this species. When used by itself in the culture medium, TDZ is not effective in inducing callogenesis in the leaf explants of *J. angustifolium*. Maximum production of callus in the leaf explants of *J. angustifolium* is obtained when they are cultured on MS medium, supplemented with 2.5 or 3.0 mg L-1 2,4-D.

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

Introduction

Jasmine is a one of the important flower of India, where most people have a love for the fragrant flowers. *Jasminum angustifolium* commonly called Wild jasmine in English and Kattumullai in Tamil and belongs to the family Oleaceae (Sridhar, 2006). These plant roots were used for ringworm, ophthalmopathy, ulcerative stomatitis, leprosy, pruritus and wounds. The leaves were used as an emetic in cases of poisoning, hepatoprotective (Warrier et al., 1995), antitumor activity (Raju et al., 2010) and against Dalton's Ascitic Lymphoma (Joshi et al., 2008).

J. angustifolium are propagated vegetatively and are mostly sterile and heterozygous (Glemin et al., 2006). In commercial cultivation unimproved wild derived saplings were used for decades. So they should be improved. Hence it is easy to improve this through tissue culture. The present study describes the procedure for the callus induction and culture of *J. angustifolium* following standard plant tissue culture protocol using leaf segments on growth regulators and studying their effect on callus induction.

Material and Methods

Leaf explants of *J. angustifolium* were collected from botanical garden at Kodaikanal, Tamil Nadu. After trimming of the larger leaves, explants were washed under running tap water followed by treatment with a surfactant, Tween 20 (5% v/v) for 10 min. The explants were further treated with 70% ethanol for 10-15 sec, followed by 5-10 min in double distilled water, surface sterilization was done with mercuric chloride (0.1% w/v) solution for 2-3 min. Then the explants were finally rinsed four - five times with sterile distilled water and inoculated on MS (Murashige and Skoog, 1962) supplemented with various concentrations of growth regulators. Sucrose (30 g) and 0.8% agar were used for the subtraction. After adjusting the pH (5.8), the

medium was autoclaved at 121ºC for 15 minutes. The cultures were given illumination by white fluorescent light with an intensity of 50 µmol $m^{-2}s^{-1}$ and maintained at 25 ± 2ºC under 16: 8 light and dark regimes. All treatments were repeated at least three times with 30 replicates and data were subjected to statistical analysis. The effect on callus induction was studied with different concentrations and their combinations of 2, 4-dichlorophenoxyacetic acid (2, 4-D; 0.5–3.0 mg/l), Naphthaleneacetic acid (NAA; 0.5–3.0 mg/l), benzylaminopurine (BAP; 0.1–1.2mg/l), thidiazuron (TDZ; 0.1-1.2 mg/l) and indole acetic acid (IAA;0.5 -3.0 mg/l) proved the best.

Photo-1: Natural habit

Results and Discussion

The interaction of the auxin (NAA) with the cytokinin (BAP) in the concentrations tested were not effective to obtain callus in the leaf explants of *J. angustifolium*. However, in the presence of 0.5, 1.0, 1.5, 2.0 and 2.5 mg L-1 NAA and in the absence of BAP, root emission was observed in the explants. This also happened when they were inoculated in the presence of 2.0 and 3.0 mg L-1 NAA with 1.0 mg L-1 BAP. The highest callus formation in the leaf explants of *J. angustifolium was* observed with the isolated addition of 2, 4- D to the culture medium. There was no callus formation in the absence of this growth regulator.

TDZ was effective only in the callogenesis, when used in the concentration of 1.0 mg L-1, associated with 2,4-D, at the concentration of 2.5 mg L-1. Table 1 shows that 2,4-D at the concentrations of 2.5 and 3.0 mg L-1 promoted the highest callus fresh matter, followed by the concentrations of 1.5 and 1.0 mg L-1. The lowest callus fresh matter was observed with the use of 0.5 mg L-1 2,4-D and with the combination of 2.0 mg L-1 2,4-D and 0.2 mg L-1 TDZ. Based on the fact that the concentrations of 2,4-D at 3.0 and 2.5mg L -1 produced results that did not differ statistically from each other, we suggest the use of this growth regulator at 2.5 mg L-1 for callus induction and highest formation of fresh mass (0.880 g) in the leaf segments of *J. angustifolium*

Table -1: Callus fresh matter obtained from *J. angustifolium* leaf segments of inoculated in MS medium, supplemented with 2,4-D, BAP and TDZ.

No	Treatments	Average of callus fresh matter (g)*
T_5	2.5 mg L-1 2,4-D	0.880 a
T_9	3.0 mg L-1 2,4-D	0.825 a
T_{10}	2.0 mg L-1 2,4-D	0.644 b
T_2	1.0 mg L-1 2,4-D	0.570 b
T_3	1.5 mg L-1 2,4-D+ 1.0 mg L TDZ	0.383 c
T_{14}	2.5 mg L-1 2,4-D+1.0 mg L TDZ	0. 365 c
T_{16}	3.0 mg L-1 2,4-D + 1.5 mg L BAP	0.282 c
T_8	1.0 mg L-1 2,4-D	0.197 c
T_{17}	1.0 mg L-1 2,4-D + 0.5 mg L BAP	0.172 c
T_{11}	1.0 mg L-1 2,4-D + 0.5 mg L TDZ	0.127 d
T_{19}	1.0 mg L -1 2,4-D + 1 mg L BAP	0.141 d
T_{15}	2.0 mg L-1 2,4-D + 0.5 mg L BAP	0.107 e

* Same letters in the column do not significantly differ at the 5% level of probability using Tukey test.

Callus culture of *J. angustifolium*

Concerning the effect of combination of 2,4- D with BAP, it was observed that the concentrations of 2,4-D at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg L^{-1} and the combination of 2.0 mg L^{-1} 2,4-D with 1.0 mg L^{-1} BAP promoted high weight variability, whereas the results from the other treatments remained at a similar level. Similar results were achieved by Santos *et al*., 2005; Soares *et al*., 2003; John De Britto *et al.*, 2009;

and Mahesh *et al*., 2010; 2011; 2012 and all recommended 2,4-D at a concentration of 2.5 mg L-1 for the highest production of callus in *Salix humboldtiana* Willd. In general, the results found in this work indicated that the combination of 2,4-D with BAP produced the lowest callus fresh mass in the leaf explants of *J. angustifolium*.

Conclusions

The interaction of the auxins NAA and 2,4-D with the cytokinin BAP, in the tested concentrations, are not enough for callus production in the leaf explants of *J. angustifolium.* The presence of 2,4-D is essential for callus induction in the leaf explants of this species. When used by itself in the culture medium, TDZ is not effective in inducing callogenesis in the leaf explants of *J. angustifolium*. Maximum production of callus in the leaf explants of *J. angustifolium* is obtained when they are cultured on MS medium, supplemented with 2.5 or 3.0 mg L-1 2,4-D.

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