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IN VITRO CALLUS INDUCTION OF *SALVIA SANTOLINIFOLIA* (BOISS)

Tour Jan[1], Beena Naqvi[2] and Raiha Qadri[1]

[1]*Department of Botany, University of Karachi, Karachi-75270, Pakistan*
[2]*PCSIR Laboratories Complex, Karachi, Karachi-75270, Pakistan*
*****Corresponding author email: tour_jan@yahoo.com

ABSTRACT

An efficient callus induction protocol through nodes, internodes and leaves explants were established in *Salvia santolinifolia*, a medicinally important herb belonging to the family Lamiaceae. Calli were initiated on Murashige and Skoog (1962) (MS) medium supplemented with various concentrations of Napthalenacetic acid (NAA) (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) and Indole-3-acetic acid (IAA) (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) and BA (0.5). Maximum fresh weight (5.25 ± 2.39 g) of callus was achieved on MS medium containing 1.5 mg/l of NAA from leaf explants. It was observed that the combination of cytokinins and auxin suppressed callus induction.

Key word: Lamiaceae, *Salvia santolinifolia*, nodes, internodes, leaves, callus induction.

INTRODUCTION

Salvia santolinifolia Boiss. is a member of family Lamiaceae. Many species belong to this family are known for their medicinal values (Kirtikar *et al.,* 1991). The medicinal value of Lamiaceae species appear to be due to its antimicrobial activity and essential oil (Nadir *et al.,* 2013), a novel antioxidant rosmarinic acid (Kintzios *et al.,* 1999), an antitumour activity (Ginda and Kakisawa, 1990), a range of bi and tricyclic diterpense isolated from these species show antifeendant and antifungal properties (Esquivel *et al*., 1985). An unusually large number of useful secondary metabolites such as essential oils, terpenoid compounds and phenolic derivatives have been isolated from *Salvia* spp. which are of great value in the pharmacopoeias throughout the world (Banthrope *et al*., 1989; Luis *et al.,* 1992; Ulubelen and Topou, 1992).

There is, however, an increasing interest in the development of efficient protocols for the tissue culture and micropropagation of certain *Salvia* species (Arikat *et al*., 2004; Gostin, 2008). In addition, plant secondary metabolite production can be improved by the in vitro induction of morphogenesis (Wu *et al*., 2003). In spite of the fact that considerable progress has been made in the field of the in vitro production of various secondary metabolites, such as rosmarinic acid and cryptotanshinon, the application of biotechnological methods for the propagation of this species is rather limited.

The present study focuses on the optimization of efficient protocol for induction of callus of *Salvia santolinifolia.* The effects of auxins (NAA, IAA and 2,4-D) and combination of auxin (NAA) with cytokinin (BA) were tested for callus induction at various concentrations.

MATERIAL AND METHODS

Axillary branches (7-10 cm long) were excised from the selected plant. For the isolation of nodes and internodes, shoot apex and leaves was removed from the axillary branches. The plants were at least 1 year old, fully mature and growing in natural habitat.

Excised young shoots were first kept under running tap water for 10-20 min. Shoots were surface sterilized with 0.05% aqueous solution of Mercuric chloride containing few drops of Tween-20 in 300 ml for 10-15 mins. followed by 3-4 times rinsing with sterile distilled water prior to inoculation.

Finally explants were cut to desired size and inoculated on Murishage and Skoog (1962) (MS) medium supplemented with NAA (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l), IAA (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l), 2,4-D (0.5, 1.0, 2.0 and 2.5 mg/l), sugar (3%) and agar (agar-agar Mikrobiologie, Merck, U.S.A.) (0.6%). The effect of BA (0.5 mg/l) in combination with NAA (1.0, 1.5 and 2.0 mg/l) was also tested in MS medium. pH was adjusted to 5.5 to 5.55 and autoclaved at 121 °C at a pressure of 15 psi for 20 min. All cultures were maintained at 26 ± 2 C°, under a light regime of 16 hrs day and 8 hrs nights.

RESULTS

Surface sterilized explants (nodes, internodes and leaves) were inoculated on MS medium supplemented with NAA (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l), IAA (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) 2,4-D (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) and

BA (0.5 mg/l) for the induction of callus (Table 1). The formation of callus initiated at the portion of nodes and internodes explant that was direct in contact with the media at all concentrations of NAA and IAA. Whereas, in leaves explants the formation of callus occurred at the entire edges under the influence of auxins (NAA and IAA).

Among the different concentrations of NAA, intermediate concentration (1.5 mg/l) induced maximum amount of callus (fresh weight) from leaves (5.25±2.39 g), followed by nodes (4.91±2.69 g) and internodes (4.25±2.44 g) respectively (Table 1).

Morphologically, the calli formed were yellowish, loose and friable in appearance in the existence of NAA in nodes, internodes and leaves explants (Fig. 1).

In the presence of different concentrations of IAA maximum amount of callus (fresh weight) was obtained from leaves explants (4.91±3.64 gram) at 2.5 mg/l followed by internodes (4.59±3.75 g) and nodes (4.20±3.19 g) at 1.5 mg/l of IAA respectively (Table 1). Low level (0.5 mg/l) of IAA produced less amount of callus (fresh weight) from all the three explants (nodes, internodes and leaves) (Table 1).

Morphologically, the calli formed from nodes, internodes and leaves explants were yellowish-green, loose and friable in appearance at all concentrations of IAA. There was no morphogenetic response of any explant (Nodes, internodes and leaves) on medium without growth regulators and all the explants died within 7-10 days of inoculation (Table 1).

The types of auxins (NAA and IAA) and their concentration significantly effect the induction of roots morphogenesis. The formation of roots occurred after 7-12 days of inoculation from the surface of calli produced from nodes, internodes and leaves explants. The number of roots produced under the influence of different concentrations of NAA and IAA from the three ex-plants (nodes, internodes and leaves) was different (Table 1).

The maximum number of roots (2-9) growth was observed in leaves callus at 2.5 mg/l of NAA while at 0.5 mg/l of NAA minimum (1-3) numbers of roots were formed (Table 1). The callus induced from nodal explants produced better numbers of roots (2-6) at 1.0 mg/l of NAA as compared to root produced (1-3) on 2.0 mg/l of NAA (Table 1). However, in the internodal callus roots growth was 1-5 on NAA at 2.5 mg/l whereas no root growth was observed at 0.5 and 1.0 mg/l of NAA (Table 1).

Root morphogenesis from callus of nodes, internodes and leaves explants in the presence of different concentrations of IAA showed that maximum numbers of roots (2-8) were obtained at 0.5 mg/l of IAA in nodal callus followed by and leaves callus (2-6) at 1.5 mg/l of IAA (Table 1). Root morphogenesis did not occurred from the internodal callus at any concentration of IAA used (Table 1).

There was no effect of 2,4-D at any concentrations on explants (nodes, internodes and leaves) (Table 1). All the three explants remained green for 7-10 days of inoculation and gradually turned brown and died.

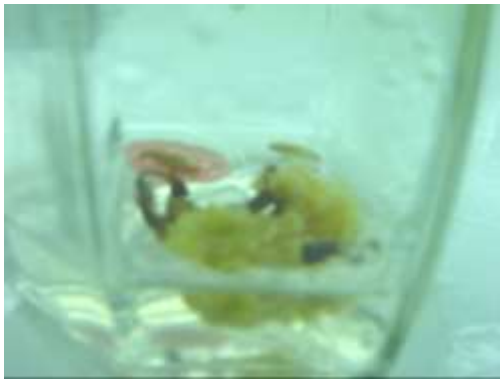

Fig. 1. Callus induced from leaves explants of *Salvia santolinifolia* at 1.5 mg/l of NAA on MS medium

The effect of auxin (NAA) in combination with cytokinin (BA) on the induction of callus from node, internode and leaf

At all combination of NAA and BA callus formation occurred from nodes, internodes and leaves explants (Table 2). At the higher level of NAA (2.0 mg/l) and low level of BA (0.5 mg/l) maximum amount of callus (fresh weight) was produces in all three explants (2.93±1.74 g in leaf explants), (2.82±1.64 g from internodes) and (2.67±1.08 g from nodes) (Table 2). However, when NAA concentration was reduced and BA concentration was kept constant less amount of callus was produced from all explants (Table 2).

Morphologically, the calli formed were yellowish-white, loose and friable in appearance. Moreover, the combined effect of auxin and cytokinin (NAA and BA) inhibited roots formation from calli of all explants (node, internode and leaf) (Table 2).

Table 1. The induction of callus from nodes, internodes and leaves under the influence of different concentrations of NAA, IAA and 2, 4-D with in 30 days culture.

Growth Regulator	Concentrations (mg/l)	Mean fresh weight (g) of callus ±SE			Range of roots formation		
		Node	Internode	Leaf	Node	Internode	Leaf
NAA	0.5	2.99±2.04	4.25±2.44	3.08±1.74	1-6	0.0	1-3
	1.0	4.12±2.35	3.56±1.57	4.33±2.43	2-6	0.0	1-3
	1.5	4.91±2.69	**4.53±2.54**	**5.25±2.39**	1-5	1-3	1-4
	2.0	**5.18±2.66**	4.17±2.64	5.16±3.25	1-3	1-5	1-5
	2.5	4.94±2.14	3.98±1.81	4.34±1.86	1-4	1-3	2-9
IAA	0.5	3.9±2.03	3.66±1.78	3.96±1.38	2-8	0	1-3
	1.0	3.68±2.45	4.46±2.64	3.80±1.74	1-7	0	1-5
	1.5	**4.20±3.19**	**4.59±3.75**	4.68±2.99	1-3	0	2-6
	2.0	3.85±1.59	3.70±2.55	4.85±2.58	1-4	0	1-5
	2.5	3.96±1.58	3.72±2.05	**4.91±3.64**	1-4	0	1-4
2,4-D	0.5	0.0	0.0	0.0	--	--	--
	1.0	0.0	0.0	0.0	--	--	--
	2.0	0.0	0.0	0.0	--	--	--
	2.5	0.0	0.0	0.0	--	--	--
Control (Without GR)	0	0	0	0	--	--	--

SE: Standard Error; GR: Growth Regulators

Table 2. The effect of NAA and BA on the induction of callus from node, internode and leaf of *Salvia santolinifolia*.

Growth Regulator	Concentrations (mg/l)	Mean fresh weight (g) of callus ±SE		
		Node	Internode	Leaf
NAA+BA	1.0±0.5	1.82±1.12	2.31±1.71	2.83±1.54
	1.5±0.5	2.43±1.63	1.95±1.57	2.46±1.44
	2.0±0.5	**2.67±1.08**	**2.82±1.64**	**2.93±1.74**

SE: Standard Error

DISCUSSION

The optimization of efficient protocol for induction of callus of *Salvia santolinifolia* using three different explants leaves, nodal and inter-nodal segments was studied. Variable amounts of callus were formed under the influence of auxins (NAA, IAA and 2,4-D) and combination of auxin (NAA) with cytokinin (BA) at various concentrations. The in vitro response strongly depends on the explant and the growth regulator concentrations used (Bueno *et al*., 2010). Although various species of *Salvia* have already been tissue cultured by scientists around the world (Wang and Wu 2010; Mederos-Molina, 2004; Gostin, 2008; Karam *et al*., 2003; Arikat *et al*., 2004) but there were no results reported on *Salvia santolinifolia* tissue culture.

When an assessment was made among explants it was found that leaf explant responded the best in term of callus induction and maximum amount of calli were recorded from leaves under the influence of NAA (1.5 mg/l). Callus produced from nodes, internodes and leaves of *Salvia santolinifia* was yellowish-white, soft, and friable and was looking healthy. The results of Mederos-Molina, (2004) are in support that different concentrations of NAA in *Salvia canariencis* stimulated better callus induction. Whereas our results are in contrast with Bueno *et al*., (2010), they reported that only nodal explants respond better out of young leaves, cotyledons and uninodal explants from *Salvia hispanica*. It was found that IAA also induced callus from node, internode and leaf explant at all concentration but amount of calli was slightly less than the amount of calli induced with influence of NAA.

The addition of BA to NAA containing media did not enhance callus growth but retarded growth of the callus against the control in primary cultures and a complete inhibition of root morphogenesis were observed in all combination of NAA plus BA. It was previously reported that higher concentrations of BA increased the shoots formation (Bueno *et al*., 2010). Other reports show that the combination of cytokinins with auxins has favorable effects on the initiation of callus. Wu *et al*. (2003) achieved callus proliferation on MS medium containing NAA and BA. Fatima *et al*., (2009) has enhanced callus growth by the addition of BA and Kin to auxin containing medium. According to Gostin (2008), the supply of both, kinetin and NAA also stimulated the direct rooting of the shoots in S. officinalis. NAA in the presence of BA enhanced callus formation in *Salvia officinalis* (Ioja-Baldura *et al*., 2010). From the stem segments of *Ephedra alata* callus was initiated on MS medium containing a combination of 2, 4-D

and Kinetin (Hegazi and El-lamey, 2011). Karam *et al*., (2003) produced callus from the leaves of *Salvia fruticosa* in the presence of thidiazuran and IAA. Results showed that 2, 4-D did not support callus induction and roots formation as did the control (hormone free) medium.

It is clear from our results that along with callus induction, roots morphogenesis also occurred on NAA and IAA containing media. Best roots morphogenesis occurred from nodes and leaves explants at all concentration of NAA and IAA. Internodes explants showed poor morphogenetic response on media supplemented with NAA whereas there was no response observed on media supplemented with IAA. It was reported that high rooting percentage in *S. fruticosa* cultured was found in MS medium with IBA (Arikat *et al*., 2004). Various *in vitro* cultures of *Salvia miltiorrhiza* have been established, including cell suspension, adventitious root, and hairy root cultures, (Wang and Wu, 2010).

This study concluded that not only explants but the correct concentration and combination of growth regulator played a vital role in callus induction and root proliferation in *Salvia santolinifia*.

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