Pak. J. Bot., 46(1): 325-328, 2014.

IN VITRO REGENERATION OF *SALVIA SANTOLINIFOLIA*

TOUR JAN* AND KHALIDA KHATOON

Department of Botany, University of Karachi, Karachi-75270, Pakistan.
**Corresponding author email: tour_jan@yahoo.com*

Abstract

In vitro shoots of *Salvia santolinifolia* were produced under the influence of different types of cytokinins supplementation by nodal segments on MS media. Excised young nodal segments of *Salvia santolinifolia* obtained from adult field-grown plants, successfully regenerated plant lets through organogenesis. Addition of BA at 1.0, 2.0 and 3.0 mg/l produced maximum number and length of shoots. The multiplication of shoots was always slow in primary cultures and increased during subculture. Regenerated shoots produced roots on transfer to medium containing 2.5 mg/l of IBA. Plant lets thus obtained were grown in sterile soil and sand mixture (1:1).

Introduction

Salvia is immensely important genus of the family Lamiaceae as far as its medicinal significance is concerned. Among species of the genus, *Salvia cavaleriei* is used for the treatment of dysentery, haemoptysis, boils and fall injuries (Zhang & Li, 1994). *Salvia apiana* called white sage, is used as a diaphoretic and diuretic and externally as a skin wash. The leaves are burnt as an aromatic smudge (Dentali & Hoffmann, 1990). Extract of the intact plant of *Salvia santolinifolia* showed anticancer activities (Amirghofran *et al*., 2010).

Literature survey on the *In vitro* propagation of *Salvia* species shows that different media MS (Murashige & Skoog, 1962), NN (Nitsch & Nitsch, 1969) and B5 (Gomborg *et al*., 1968) have been successfully used for the regeneration of shoots and plants from a variety of explants which are often apical buds excised from shoots of field grown plants (Olszowsky & Furmanowa, 1990) and *In vitro* produced plants (Arikat *et al*., 2004), leaves from whole plants (Kintzios *et al*., 1999), petioles and lamina excised from *In vitro* produced plants (Morimoto *et al*., 1994; Skala & Wysokinska, 2004; Karam *et al*., 2003). Nodal segments with axillary buds excised from mature field grown plants (Misic *et al*., 2006), from *In vitro* produced plants (Santos-Gomes *et al*., 2002; Arikat *et al*., 2004), and from stem segments without axillary buds excised from *In vitro* produced plants (Tawfik & Mohamed, 2005) and cotyledons from immature zygotic embryos (Liu *et al*., 2000) have been frequently used.

Information on the *In vitro* propagation of *Salvia santolinifolia* is lacking, it is therefore, in the first instance the investigation was undertaken to develop a protocol for the micropropagation of this species.

Material and Methods

Excised young shoots were first kept under running tap water for 5 mins and then were surface sterilized with 0.05% aqueous mercuric chloride solution containing 3-6 drops of Tween-20 in 200 ml of disinfecting solution for 12-15 mins. Sterilized shoots were rinsed 3-4 times with sterile distilled water perior to inoculation.

First three nodal segments below stem tip and the last three nodes of the axillary branches were used as explants for shoot regeneration. For the isolation of nodes from stem tip, shoot apex was removed from the axillary branches and 1-1.5 cm long nodal segments were excised and placed horizontally on the surface of medium.

MS (Murashige & Skoog, 1962) medium containing 3% sucrose was used. Growth hormones were always added before sterilizing the medium. pH of the medium was adjusted to 5.5 to 5.55 with KOH or HCl and 0.6% agar (agar-agar Mikrobiologie, Merck, U.S.A.) was used as solidifying agent. Cultures were maintained at 26±2°C, under a light regime of 16 hrs day and 8 hrs nights. Light was provided from cool white fluorescent tubes.

For the growth and multiplication of shoots the nodal segments were cultured on MS medium containing a range of concentrations of cytokinins alone or in combination with NAA. The total number of new shoots produced and the length of individual shoot were recorded. First subculture was made after 30 days. For root induction individual shoots regenerated on BA (2.0 and 3.0 mg/l) containing medium were excised and cultured on rooting media which contained a range of concentration of IBA, NAA, IAA and Phloroglucinol and also on hormone free medium.

Results

Culture establishment: First, second and third nodes excised from the base of axillary branches did not respond. Almost all young nodal segments (1-3 nodes) excised below the stem tip exhibited axillary shoots formation on BA (2.0 and 3.0 mg/l) supplemented medium within first week of culture (Table 1).

Shoot proliferation: All young nodes (1st-3rd node) below the stem tip formed shoots on all concentrations of the cytokinins used [BA (0.2, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l), 2iP (0.3, 1.0 2.0, 3.0, 4.0 and 5.0 mg/l) and Kin (0.2, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l)]. The shoots induced on various concentrations of BA and 2iP were healthier as compared to the ones induced on Kinetin supplemented medium. Shoots produced under the influence of Kinetin were short (less then 0.5 cm in length) and their emergence was also delayed for 2-4 days as compared to BA and 2iP induced cultures (Table 2). No greater difference was observed between BA and 2iP treated cultures as regards induction of shoots and their length. In 21-25 day old cultures some new shoots appeared. The first formed shoots elongated and their tips necrosed on medium supplemented with 2iP. Very little callus formation occurred in all treatments. However greater number and highest length of shoots were obtained (data not shown) during subculture on media containing BA, 2iP and Kin (2.0, 3.0 and 3.0 mg/l). Highest rooting of *Salvia santolinifolia* were obtained with 2.5 mg/l of IBA (Fig. 1) and longest roots were obtained (data not shown) with 3.5 mg/l of IBA.

Table 1. The effect of different concentrations of BA on the initiation of shoots from first three and last three nodes of the axillary branches of *Salvia santolinifolia* grown for 30 days in culture.

Explants	Hormone	Concentration (mg/l)	Mean no. of shoots ± SD	Mean length of shoots (cm) ± SD
	BM	0.0	0.0	0.0
First three nodes (below stem tip) of the axillary branches	BA			
		2.0	1.45±0.97	3.34±2.94
		3.0	1.8±0.75	3.14±2.23
Last three nodes of the axillary branches	BA			
		2.0	0.0	0.0
		3.0	0.0	0.0
	BM	0.0	0.0	0.0

BM: Basal Medium

Table 2. The effect of different concentrations of cytokinins on shoot regeneration from nodal explants of *Salvia santolinifolia*, cultured for 30 days.

Hormones	Concentration (mg/l)	% Response	Mean no. of shoots ± SD	Mean length of shoots (cm) ± SD	Callus formation
BA					
	1.0	57.14	1.5 ± 1.06	2.7 ± 1.02	±
	2.0	72.72	1.4 ± 0.9	4.3 ± 2.94	±
	3.0	90.47	1.8 ± 0.7	3.1 ± 2.23	±
	4.0	71.42	0.97 ± 0.8	2.5 ± 1.34	±
	5.0	85.71	0.6 ± 0.07	1.85 ± 1.19	±
2iP					
	2.0	42.85	1.3 ± 1.05	3.75 ± 2.34	±
	3.0	42.85	0.6 ± 0.25	3.6 ± 1.36	±
	4.0	57.14	1.5 ± 1.06	2.83 ± 1.77	±
	5.0	71.42	0.97 ± 0.6	2.5 ± 1.28	±
Kin					
	2.0	57.14	1.0 ± 0.97	0.4 ± 0.27	±
	3.0	42.85	1.33 ± 0.97	0.3 ± 0.13	±
	4.0	85.71	0.91 ± 0.66	0.5 ± 0.35	±
	5.0	71.42	0.82 ± 0.57	0.27 ± 0.17	±
BM	--	0.0	0.0	0.0	0.0

(±): Very little; (--): No callus; BM: Basal Medium

Fig. 1. Rooting of regenerated shoots of *Salvia santolinifolia*, after 10 days of culture on MS medium containing IBA (2.5 mg/l).

The influence of the addition of NAA to the cytokinin containing media on shoot multiplication: Results from Table 3 show that the addition of NAA to the cytokinin supplemented media completely inhibited the formation of shoots and enhanced callus growth in several treatments. The induced calli were yellowish-white and soft in nature. A complete inhibition of callus formation was observed in treatments containing BA+NAA (4.0+1.0 mg/l) and 2iP+NAA (2.0+1.0 mg/l). Maximum callus formation occurred in medium lacking cytokinin, however, in media containing both auxin and cytokinin equimolar concentrations of the two hormones stimulated good callus growth.

Discussion

The type of cytokinin used influenced very much the extent of shoot morphogenesis from nodal segments in *Salvia santolinifolia*. Shoot induction and multiplication occurred in the presence of all cytokinins tested (Table 2), however, an enhanced multiplication of shoots was observed on subculture in the presence of 2.0 and 3.0 mg/l of BA. The number of shoots produced under the influence of various cytokinins was variable. BA produced a greater number of shoots than either with 2iP or Kinetin (Table 2). Many investigators have reported a similar effect of BA on the induction and multiplication of shoots in other *Salvia* species. Arikat *et al*., (2004) obtained large number of shoots with an increased length from nodal explants of *Salvia fruticosa* cultured on MS medium containing BA (0.16 mg/l). A favourable effect of BA has also been reported by Misic *et al*., (2006) on the promotion of growth of axillary buds in *Salvia branchyodon*. *Salvia chamelaegnea* also produced shoots on medium containing 1.0 mg/l BA (Huang & Standen, 2002). Similar results have been reported by Jusaitis (1995) and Nobre *et al*., (2000) in other plant species.

There are still other species of *Salvia* which require a combination of BA and an auxin for shoot induction and multiplication (Skala & Wysokinska (2004), Santos-Gomes *et al*., (2002) and Liu *et al*., (2000). Shoots tip explants of *Salvia nemorosa* exhibited best shoot regeneration in the presence of BA and IAA (Skala & Wysokinska, 2004). A combination of 2, 4-D and BA favoured formation of shoots of maximum number and length in large percentage of explants of *Salvia officinalis* (Santtos-Gomes *et al*., 2002). Shoot regeneration and their multiplication in *Salvia seclarea* also required an auxin (IAA or NAA) and BA (Liu *et al*., (2000). In *Salvia santolinifolia* the addition of auxin to the cytokinin containing media completely inhibited shoot formation and favoured callus development from nodal explants.

In *Salvia santolinifolia* shoot induction and proliferation occurred rapidly in the presence of 2iP alone but tips of the older and longer shoots became necrotic when reached to a height of 6-7.5 cm or above. Healthy shoots were formed in large number only on BA supplemented medium. It is therefore, BA was selected as shoot multiplication hormone. On the contrary cultures produced under the influence of NAA inhibited shoots formation even when combined with BA or 2iP (Table 3). A similar response with NAA has been demonstrated in *Ilex dumas* and *I. paraguariensis* (Luna *et al*., 2003 and Sansberro *et al*., 1999).

IBA is the sole hormone that produced roots in the *In vitro* produced shoots of *salvia santolinifolia*. Roots regeneration on *In vitro* induced shoots by IBA treatment has previously been reported for several plants species, including *Salvia blancoana* and *Salvia valentine* (Cuenca & Amo-Marco, 2000). The regenerated shoots of *Salvia fruticosa* were cultured on medium supplemented with IBA (0.65 mg/l) exhibited the highest rooting percentage compared to those cultured with IAA or NAA (Arikat *et al*., 2004).

Table 3. The influence of the addition of NAA in cytokinins containing shoot multiplication medium on the formation of shoots from nodal segments of *Salvia santolinifolia* grown in culture for 28 days.

Hormones	Concentration (mg/l)	% Response	Mean no. of shoots	Extent of callus formation
BA + NAA				
	1.0 + 1.0	28.57	0.0	+++
	2.0 + 1.0	28.57	0.0	+
	4.0 + 1.0	42.85	0.0	--
2iP + NAA				
	0.2 + 1.0	57.14	0.0	+
	1.0 + 1.0	57.14	0.0	+++
	2.0 + 1.0	28.57	0.0	--
Kin + NAA				
	0.2 + 1.0	42.85	0.0	++
	1.0 + 1.0	42.85		+++
	2.0 + 1.0	42.85	0.0	+++
NAA				
	1.0	42.85	0.0	++++

(+): Visible callus growth; (++): Small callus growth; (+++): Good callus growth; (++++): Excellent callus growth; (--): No callus

References

Arikat, A., F.M. Jawad, N.S. Karam and R.A. Shibli. 2004. Miropropagation and accumulation of essential oil in wild sage (*Salvia fruticosa* Mill.). *Sci. Hort*., 100: 193-202.

Cuenca, S. and J.B. Amo-maro. 2000. *In vitro* propagation of two Spanish endemic species of *Salvia* through bud proliferation. *In vitro Cell. Dev. Biol. Pl*., 36: 225-229.

Gamborg, O.L., R.A. Miller and K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res*., 50: 151-158.

Huang, L.D. and V.J. Standen. 2002. *Salvia chamelaeagnea* can be micropropagated and its callus induced to produce rosmarinic acid. *S. Afr. J. Bot*., 68(2): 177-180.

Jusaitis, M. 1995. *In vitro* propagation of *Phebolium equestre* and *Phebolium hellebrandii* (Rutaceae). *In vitro Cell Dev. Biol. Pl*., 31: 140-143.

Kintzios, S.A. Nikolaou and M. Skoula. 1999. Somatic embryogenesis and *In vitro* rosmarinic acid accumulation in *Salvia officinilis* and *Salvia fruticosa* leaf callus cultures. *Pl. Cell Rep*., 18: 462-466.

Liu, W., C.E. Chilcott, R.C. Reich and G.M. Hellmann. 2000. Regeneration of *Salvia sclarea* via organogenesis. *In vitro Cell. Dev. Biol. Pl.*, 36: 201-206.

Luna, C., P. Sansberro, L. Mroginski and J. Tarrago. 2003. Micropropagation of *Ilex dumosa* (Aquifoliaceae) from nodal segments in a tissue culture system. *Biocell*, 27(2): 205-212.

Misic, D., D. Grubisic and R. Konjevic. 2006. Micropropagation of *Salvia brachyodon* through nodal explants. *Biologia Plantarum*, 50(3): 473-476.

Morimoto, S., Y. Goto and Y. Shoyama. 1994. Production of lithospermic acid and rosmarinic acid in callus tissue and regeneration plantlets of *Salvia miltiorrhiza. J. Nat. Prod*., 5(6): 817-823.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with *tobacco* tissue cultures. *Physiol. Pl*., 15: 473-497.

Nitsch, J.P. and C. Nitsch. 1969. Haploid plants from pollen grains. *Sci.*, 163: 85-87.

Nobre, J., C. Santos and A. Romano. 2000. Micropropagation of the Mediterranean species *Viburnum tinus*. *Pl. Cell, Tiss. Org. Cult.*, 60: 75-78.

Olszowska, O. and M. Furmanowa. 1990. Micropropagation of *Salvia officinalis* by shoot buds. *Planta Med*., 56: 637.

Sansberro, P., H. Rey, L. Mroginski and M. Collavino. 1999. *In vitro* plant regeneration of Ilex paraguariensis (Aquifoliaceae). *In vitro Cell. Dev. Biol. Pl*., 34: 401-402.

Santos-Gomase, P.C.S., R.M. Seabr, P.B. Andrade and M.F. Ferreira. 2002. Phenolic antioxidant compounds produced by *In vitro* shoots sage (*Salvia officinalis* L.). *Pl. Sci*., 162: 981-987.

Skala, H. Wysokinska. 2004. *In vitro* regeneration of *Salvia nemorosa* L. from shoots tips and leaf explants. *In vitro Cell. Dev. Biol. Pl.*, 40(6): 596-602.

Tawfik, A.A. and M.F. Mohamed. 2005. Organogenic response of Salvia officinals L. to dark preconditioning thidiazuron and benzyladenine. *PGRSA Quarterly*, 33(4): 125-133.

(Received for publication 20 March 2012)