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Callus culture of *Spilanthes paniculata* (DC) Jansen- An Aromatic Medicinal plant

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Abstract: ***Spilanthes paniculata (DC) Jansen is an important medicinal plant with rich source of therapeutic and medicinal constituents of family Asteraceae. Callus culture of Spilanthes paniculata (DC) Jansen was established in Murashige and Skoog's media augmented with various concentration/combination of growth regulators by using leaf, nodal and internodal explants. Maximum callogenic response i.e. 84.62 % from internodal explants was shown at BAP 1.75 mg/l, 74 % was shown at BAP 1.5 mg/l from nodal explants and 75 % from leaf explants at 2, 4-D 1 mg/l.***

Keywords: Asteraceae, Callus, BAP, 2,4-D, *Spilanthes paniculata*.

1. Introduction

There is a need to develop callus culture of various plant species which can be used for the extraction of various phytochemicals. *In-vitro* cultures yield much more concentration of phytochemicals as compared to *in-vivo*. *Spilanthes paniculata* (DC) Jansen- an important medicinal plant with rich source of therapeutic and medicinal constituents of family Asteraceae is commonly known as toothache plant. The plant occurs throughout India, ascending upto 1700m often occurs as a weed in rice fields, is an erect usually pubescent annual herb with 1-2 inch long ovate crenate leaves. *Spilanthes paniculata* has been used as a traditional medicine for toothache, rheumatism and fever. The flower heads are chewed to relieve the toothache and other mouth related troubles. Leaves are used externally for treatment of skin diseases (Agharkar, 1991). The raw leaves of *Spilanthes paniculata* are used as flavouring for salads, soups and meats in Brazil and India. Whole plant is used in treatment of dysentery (Verma et al., 1993). Root decoction is used as purgative. Leaf decoction is used as diuretic and lithotriptic. The bioactive compounds from *Spilanthes paniculata* possess antilarvicidal activity (Ramsewak et al., 1999). It exhibits analgesic, strong larvicidal activity on *Anopheles stephensi* Liston, *Anopheles culicifacies*, antimicrobial and cytotoxic activity (Pandey and Agrawal, 2007). The plant is also used in the traditional system of medicine for the treatment of various disease complications including infections of throat and gums, paralysis of tongue, a popular remedy for stammering in children and as diuretic (The wealth of India, 2004). Spilanthes paniculata has been well documented for its use as spices, antiseptic, anti-bacterial, anti-fungal, anti-malarial and as remedy for toothache, flu, cough, rabies and tuberculosis (Burkill, et al., 1966).

The present work aims to develop an effective high frequency method for the production of large amount of from various explants of *Spilanthes paniculata* that can serve as a source of active componds.

2. Material and Methods

The experimental plant material for the present study was collected from Melghat Tiger Reserve forest, Amravati (Maharashtra) in the month of August 2011. The plants were maintained in the Departmental garden. Murashige and Skoog's (MS) media (1962) was chosen as a basal media throughout the experimentation. Growth regulators employed throughout the study were BAP, NAA and 2,4-D. Various types of explants used were leaf, nodal and internodal. The explants were washed thoroughly first with running tap water and then with distilled water. The various explants were surface sterilized with 70% alcohol for nearly two minutes. Then immersed in 0. 1% mercuric chloride (HgCl2) for 3 minute, followed by rinsing with sterile double distilled water for 3-4 times. Then the explants were soaked by placing them on sterile filter papers and then inoculated on MS medium augmented with different concentrations and combinations of auxins and cytokinins to induce callogenesis in them.

All the standard physical conditions were provided to cultures in-vitro. The cultures were kept at 16 hours light period with $20 \pm 2\ ^0$C temperature and 70% humidity,

3. Results and Discussion

Various types of explants (leaf, nodal and internodal) on culturing into MS media substituted with different concentrations of plant hormones showed varied initiation. Leaf explants showed callogenic initiation after 10 days of inoculation whereas nodal and intermodal explants showed initiation after 15 days of inoculation into MS media supplemented with varying combination/concentration of growth regulators. At 1 mg/l BAP callogenic response from leaf explant was 66.66 % (Fig. 1A). Whitish callus with callogenic response of 48.66 % was observed from leaf explants at 0.75 mg/l NAA (Fig. 1B). Maximum callogenic response i.e. 75 % from leaf explants was shown at 2, 4-D 1 mg/l (Fig. 1C). At this concentration the colour of callus was yellowish (Table 1). Callogenesis was noticed in *Spilanthes acmella* Murr. when the media was fortified with various concentrations of auxins and cytokinins, highest percentage of callus induction (80%) was observed in the media

supplemented with 3.0 mg/l of 2, 4-D. Among cytokinins the medium supplemented with 3.0 mg/l BAP supported highest (70%) percent of callus induction. Among auxins, the media fortified with 2, 4-D (2.0 mg /1) showed better callus growth as compared to other combinations at all the three stages. Among cytokinins, callus growth was better in media containing 2.0 mg/1 BAP. Callus so produced was greenish white and fragile (Kuldeep and Narender, 2010).

Table 1: Callogenic response from leaf explants at different concentration of growth regulators.

Sr. No.	Growth Hormone	Conc. (mg/l)	Response (%)	Callus colour
1	BAP	1.00	66.66	Yellowish
		1.50	50	Light brown
		2.50	58.33	Light green
2	NAA	0.50	58.32	Greenish
		0.50	50	Light brown
		0.75	48.66	Whitish
3	2,4-D	1.00	75	Yellowish
		1.25	44.62	Light brown
		1.50	41.66	Greenish

Maximum callogenic response i.e. 74 % (Fig. 1D) from nodal explants was shown at BAP 1.5 mg/l. At this concentration the colour of callus was found as light yellowish (Table 2). Among 2,4-D, maximum callogenic response (72.32 %) was observed At 0.75 mg/l. At this concentration, dark brown callus was observed (Fig. 1 E). Young explants exhibited better response as these are physiologically and biochemically more active as well as they have less rigid cell wall (Mishra and Bhatnagar, 1995). In case of *Fraxinus angustifolia*, BAP was found to be superior than 2, 4-D (Perez-Parson et al., 1994). Similar observations have also been reported in *Asparagus officinalis* (Ha et al., 2008) and *Elaeagnus angustifolia* (Zeng et al., 2009). To achieve maximum callus induction, a defined auxincytokinin ratio was required, as also advocated by Latto et al., (2006) in *Chlorophytum arundinacem*, Junaid et al. (2007) in *Catharanthus roseus* and Burbulis et al., (2008) in *Brassica napus*. A combined effect of cytokinins (BAP and Kn) and auxins (IAA, NAA and 2, 4-D) was also studied for callus formation. The media supplemented with BAP (2.0 mg/1) + 2, 4-D (1.0 mg/1) supported ninety per cent callus induction after 12 days of inoculation.

Table 2: Callogenic response from nodal explants at different concentration of growth regulators.

Sr. No.	Growth Hormone	Conc. (mg/l)	Response (%)	Callus colour
1	BAP	1.50	74	Light yellowish
		2.00	56.88	Yellowish Friable
		2.50	40	Light green Friable
2	2, 4-D	0.75	72.32	Dark brown
		1.00	58.62	Light brown
		1.50	38.54	Green

Each value is the mean of three replicate.

Maximum callogenic response i.e. 84.62 % (Fig. 1F) from internodal explants was shown at BAP 1.75 mg/l. At this concentration the colour of callus was observed as white yellowish (Table 3). The axillary buds of *Spilanthes acmella* L.,cultured on MS medium supplemented with high concentration of IBA (6-10 mg/l) formed callus (Ang and Chan, 2003). According to Eellarova and Kimakova (1999), higher concentration of BA could induce the formation of callus tissue that also caused the chromosomal instability of the regenerated plants.

The results showed that lower concentration of growth regulators favored the callus formation with respect to higher concentration of growth regulators which lowered the callogenic response. Various explants (leaves, internodes, roots, cotyledons, petioles of cotyledons, etc.) of *Spilanthes paniculata* (DC.) Jansen were cultured on MS media supplemented with different auxins (e.g.: 2, 4-D, IAA, IBA, NAA) at concentration 2 mg/l. Different types of calli with varied amounts were obtained. The callusing responses from cotyledonary and leaf explants in MS medium supplemented 2, 4-D (3-4 mg/l) were 75% and 80%, respectively (Sheela et al., 2008).

Table 3: Callogenic response from internodal explants at different concentration of growth regulators

Sr. No.	Growth Hormone	Conc. (mg/l)	Response (%)	Callus colour
1	BAP	1.50	75	Dark green
		1.75	84.62	White yellowish
		2.0	48	Brown Friable
2	NAA	1.00	50	Light green
		1.25	58.66	Dark brown
		2.0	72	green

Each value is the mean of three replicate.

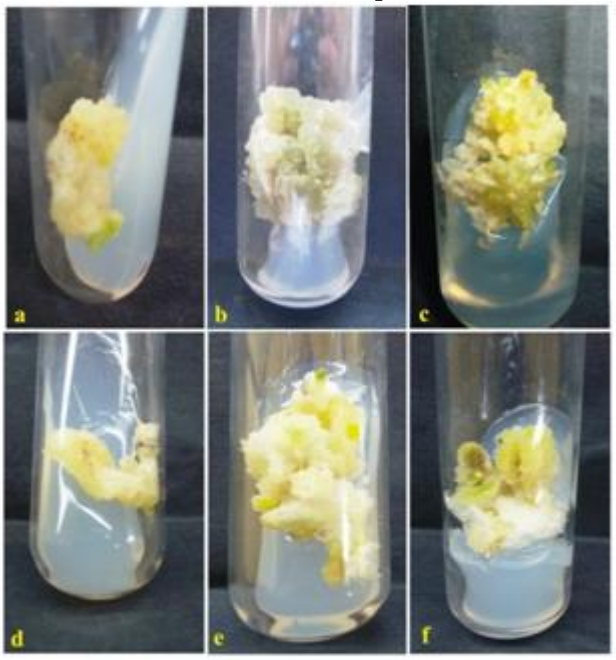

Figure 1: Callus culture of *Spilanthes paniculata* (DC) Jansen.
a: Callus formation from leaf explants at 1 mg/l BAP.
b: Callus formation from leaf explants at 0.75 mg/l NAA.
c: Callus formation from leaf explants at 1 mg/l 2,4-D.
d: Callus formation from nodal explants at 1.5 mg/l BAP.
e: Callus formation from nodal explants at 0.75 mg/l 2,4-D.
f: Callus formation from internodal explants at 1.75 mg/l BAP

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