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IN VITRO MICROPROPAGATION STUDIES IN SPILANTHES ACMELLA MURR

Botany

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ABSTRACT

Tissue culture technique is proving useful for the multiplication of medicinal plants and others plants *in vitro*. Manipulation of tissue culture medium with various growth regulators enables required response in the stage of multiplication. This has boosted the speed not only for multiplication of useful plants but for extraction of valuable secondary metabolites from in vitro cultures as well. During the present piece f work efforts have been made to multiply shoots of medicinal plant *Spilanthes acmella in vitro.* Successful protocol for micropropagation was established using shoot tip as explants.

KEYWORDS

Spilanthes acmella, Multiplication, in vitro

INTRODUCTION:

Spilanthes acmella Murr. (Family: Asteraceae) commonly Known as Akarkara or toothache plant. This plant is widely distributed in the tropical and sub-tropical regions including America, North Australia, Africa, Malaya, Borneo, India and Sir Lanka (Jansen, 1981). The plant is a rich source of Alkamide spilanthol, which is supposed to be responsible for most of its medicinal properties. It is traditional reported for its uses as spice antiseptic, anti-bacterial, anti-fungal, and as remedy for toothache, stomatitis, throat, complaints, and tuberculosis. *Spilanthes acmella* is conventionally propagated through seeds which lose their viability within a short period of time. Dependence on season and slow germination rates are some of the other major limiting factors in conventional propagation (Patil et al. 2006) In order to produce high value secondary metabolites such as scopoletin, a constant source of plant material is required which could be utilized as a ready stock to meet the demand of the pharmaceutical industries for the production of bioactive compounds. In this context, in vitro approaches have proved handy in establishing plants that are genetically uniform and enriched in selected characters.

The flowers and leaves of this plant and traditional medicine have been used to treat many diseases including for stammering toothache, stomatitis, and throat, complaints. It has potent diuretic activity and the ability to dissolve urinary calculi. It exhibits antimalarial properties as well (Burkill, 1966; Singh, 1995; Ramsewak *et al., 1999;* Pandy and Agrawal, 2009*).* Due to these medicinal values, the plant is being over-exploited in recent years. In addition, the efficiency of reproduction is also found to be less due to its low seed germination and viability and lack of vegetative propagation methods.

2. MATERIALS AND METHODS

Collection of plant material:

The Nodal segments of *Spilanthes acmella* (1.0-1.5cm) were excised from the plants growing in garden of Botany Department, Dr. Babasaheb Ambedkar Marathwada University.

Sterilization of explant & preparation of culture:

The explants were washed with liquid detergent under running tap water to remove dust particles. The explants were then treated with 0.1% (w/v) mercuric chloride for 3-5 minutes under aseptic conditions. After this these explants were then thoroughly washed 4-5 times with sterilized double distilled water to remove the traces of mercuric chloride. The nodal segments were inoculated on MS medium supplemented with various concentrations (0.5-٣,٠ mg/l) of auxins (IAA, NAA, 2, 4-D and IBA) and cytokinins (BAP and Kin) alone and in various combinations for shoot regeneration. The cultures were incubated at a temperature of 25±2ºC and a photoperiod of 16hrs light (intensity of 2000 lux) and 8hrs of dark. The in vitro developed single/multiple shoots (2.5 – 3.0 cm long) were excised and implanted in culture tubes containing full and half strength MS medium fortified BAP and NAA under aseptic conditions for rooting.

Media preparation and culture conditions:

Media (Murashige & Skog.1962) preparation and cultures were supplemented with plant growth regulators and gelled with 3% (w/v) sucrose and 0.8% agar was used throughout the study. The pH of medium was adjusted to 5.8 prior to autoclaving for 20 minutes at 121˚ C and 15 psi. The cultures were maintained at 25±2˚C under 16/8 light/dark cycle with the light intensity of 3000 lux. Different plant growth regulators like Benzyl amino purine (BAP), Indole-3-acetic acid (IAA), kinetin (kin) were employed for regeneration.

Shoot Multiplication:

When the regenerated shoots from apical shoot tip auxiliary shoots from nodal explants attained a length of 2 -3 cm, they were excised and inoculated onto ½MS media. The media was supplemented with various combinations of BAP, IAA and kin to determine best hormonal concentration for multiplication and growth for each explants, tow subculture cycles were performed with 18 days interval and the data were recorded during subculture and after 30 days of second culture.

RESULTS AND DISCUSSION:

Within 5 weeks, an average of 2.3 to 2.6 shoots were formed from each axillary bud of *Spilanthes acmella* when they were cultured on MS supplemented with 0.5, 1.0, 2.0 and 3. mg l-1 of BAP. The addition of IAA, as low as 2 mg l-1, into MS medium containing Kn , however, did not show significant influence on multiple shoots formation from the axillary bud explants (Table 1). This observation suggested that the induction of multiple shoots formation of *S. acmella* depended only on the presence of kin in the culture medium. The axillary buds cultured on basic MS medium without any growth regulator produced single shoot with complete root system (Fig. 1). All the multiple shoots formed in MS media supplemented with kin + IAA 0.5, 1.0 ,2.0, 3.0 mg/l respectively formed small clusters, and accompanied with swelling and callus growth without any root system (Fig.2)**.**

3.1 Direct organogenesis

Table1. Effect of cytokinins and Auxins supplemented individually and in various combinations on nodal segments of *Spilantes acmella* .

Auxins/ cytokinins (mg/l)	Concentration of growth regulators (mg/l)	No. of explants responded	Response %	Number of shoots (Mean+SE)	Shoot Length (cm) (Mean ± SE)
Control				1.0 ± 0.00	1.2 ± 0.48
BAP	0.5	5	25	3.4 ± 0.53	0.17±2.5
	1.0	7	35	4.3 ± 0.08	0.27±2.8
	2.0	16	75	2.7 ± 0.70	0.17±2.5
	3.0	18	90	2.0 ± 0.57	2.0 ± 0.17

+MS+Kin IAA	1.0±1.0 1.0±2.0 3.0 +1.0	5 10 09	26 30 38	2.4 ± 0.53 2.3 ± 0.74 2.7 ± 0.75	2.5 ± 0.15 2.2 ± 0.23 1.8 ± 0.36
Kin+ IBA	2.0±1.0 3.0±1.0	16 18	75 40	0.40±2.8 0.7±2.3	0.10±20 0.70±25
+ MS IAA+ BAP	0.5±05 0.5±1.0 0.5±1.5 2.0 +0.5 0.5+3.0 0.5+5.0	6 13 14 14 17 15	30 65 70 70 85 75	2.2 ± 0.48 2.5 ± 0.82 4.0 ± 0.28 0.4±1.0 0.80±2.0 2.0±4.10	1.3 ± 0.19 1.5 ± 0.25 1.9 ± 0.12 2.0±0.40 3.0±20.0 0.19±0.10

CONCLUSION:

Results obtained from our previous study revealed that, after 4 weeks of initial culture, nodal explants cultured on MS medium with BAP (1.0 mg/l(developed maximum number of multiple shoots. Effectiveness of BAP in inducing bud break was also observed in many other plant species

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