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Spilanthes acmella Murr. An important endangered medicinal plant and its conservation through Tissue culture techniques

Nelofar Gulam Nabi and Mukta Shrivastava

Department of Botany Govt., MLB Girls P.G. College Bhopal-462003 (India)
nelofarnabi2@gmail.com ; mukta.shrivastava07@gmail.com.

Abstract

Spilanthes acmella Murr. belongs to the Genus: *Spilanthes*, Family: Asteraceae (Compositae). Its natural population has declined very fast due to indiscriminate and illegal collections and destruction of its habitats as a result it is included in the endangered list of plants. It's *in vitro* protocol techniques are currently unavailable to help growers to meet the demand of the plant for cultivation and pharmaceutical industry.

The present study deals with rapid and efficient protocol development for *in vitro* propagation of *Spilanthes acmella* Murr. Shoot induction on Murashige and Skoog medium supplemented with various auxins and cytokinins individually and in various combinations has been achieved by using axillary and apical meristems.MS medium fortified with 1.0 mg/l BAP and 0.1 mg/l NAA was found to be effective individually.

Key words: *Spilanthes acmella*, medicinal plant, conservation, Tissue culture, Growth regulators.

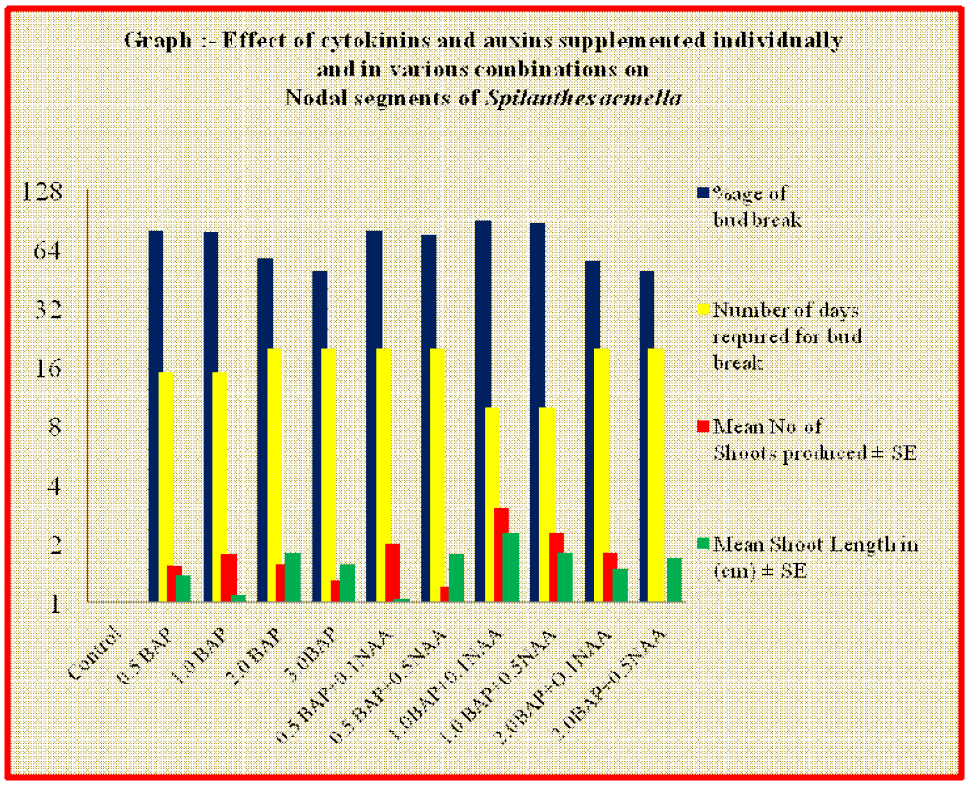
S*pilanthes acmella* Murr. belongs to the genus *Spilanthes*, family Asteraceae (Compositae). It is an herb found all around the world and widely distributed throughout the tropics and subtropics. It is native to the tropics of Brazil, and is grown as an ornamental (and as a medicinal) in various parts of the world. In India, it is confined to South India Commonly it is known as Toothache plant or Eyeball plant. The name Eyeball plant should be obvious to anyone who is familiar with the plant's flowers, which are yellow and gradually turn to dark red in the centre. The active constituent spilanthol chiefly present in leaves and flower heads, and produce analgesic activity used to numb toothache. It is also used as a defensive medicine for scurvy and stimulates digestion. Besides these medicinal uses, the flower heads have been used as a spice for appetizers by the Japanese. Chhattisgarh and Jharkhand[1]. It has potent diuretic activity and the ability to dissolve urinary calculi. It exhibits antimalarial properties as well[2]. Spilanthol, the most active antiseptic alkaloid extracted from this plant, is found effective at extremely low concentrations against blood parasites, and indeed is a poison to most invertebrates while remaining harmless to warm-blooded creatures. It is

Table-1. Effect of cytokinins and auxins supplemented individually and in various combinations on Nodal segments of *Spilanthes acmella*.

MS+Auxins/ cytokinins(mg/l)	%age of bud break	Number of days required for bud break	Mean No of Shoots produced ± SE	Mean Shoot Length in (cm) ± SE
Control	0	0	0	0
0.5 BAP	80	15	1.55±0.01	1.38±0.08
1.0 BAP	79	15	1.78±0.01	1.10±0.35
2.0 BAP	58	20	1.57±0.13	1.80±0.42
3.0BAP	50	20	1.30±0.05	1.58±0.13
0.5 BAP+0.1NAA	80	20	2.00±0.46	1.05±0.31
0.5 BAP+0.5NAA	76	20	1.21±0.02	1.78±0.01
1.0BAP+0.1NAA	90	10	3.04±0.15	2.27±0.10
1.0 BAP+0.5NAA	87	10	2.27±0.10	1.80±0.11
2.0BAP+O.1NAA	56	20	1.80±0.42	1.50±. 0.09
2.0BAP+0.5NAA	50	20	1.01±0.04	1.70±0.08
3.0 BAP+0.1NAA	50	20	1.27±0.10	1.30±0.19

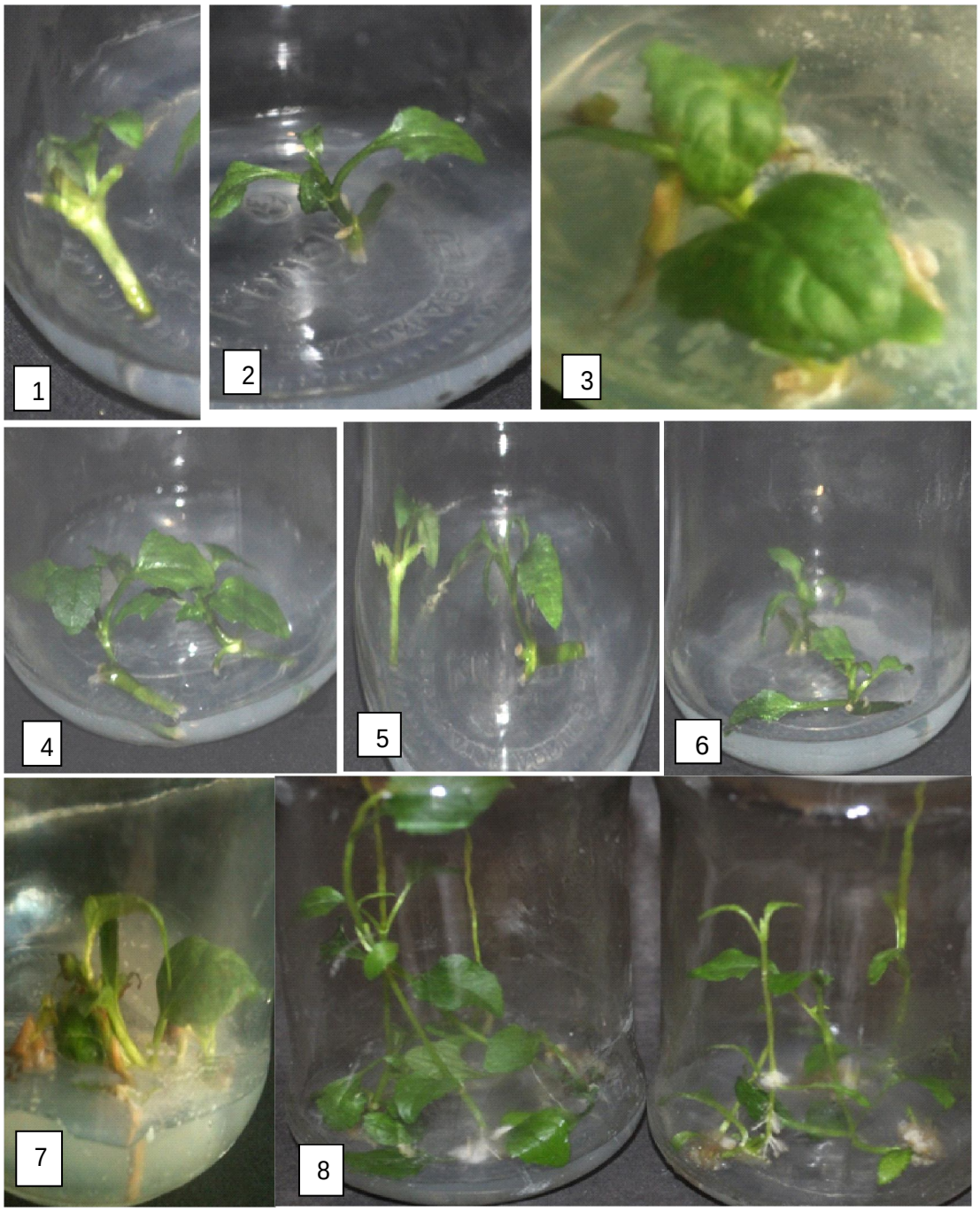
further recommended as a cure for dysentery and rheumatism, and to enhance the immune system. It exhibits general immunomodulator properties when used internally, boosting production of leukocytes and antiviral interferon, as well as promoting phagocytosis. It stimulates wound healing, protects the individual from cold and flu[1]. The leaves are also used to treat bacterial and fungal skin diseases. 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. Thus the present study has been designed to develop a reliable and reproducible protocol of this important endangered plant which could be used for mass multiplication of this plant species to meet the increasing requirement of the pharmaceutical industry as well as for the conservation of germplasm.

Nodal segments (1.0-1.5cm) were excised from the plants growing in polyhouse of Botany Department OF GOVT. M.L.B College Bhopal. All 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 for3-5 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 of BAP (1.0-3.0 mg/l) alone or with combination of auxins (0.1-0.5 mg/l) NAA, in various combinations for shoot induction and 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.Visual observations like callus induction, growth of callus, number of days taken for bud break, percentage of bud break and number of shoots regenerated per explants were recorded regularly. A mean of 20 replicates was taken per treatments.

A combined effect of different cytokinins (BAP) and auxins (NAA) in various combinations was also studied. The medium with BAP (1.0 mg/l) + NAA (0.1mg/l) showed maximum (90%) bud break after 10 days of inoculation. Supplementation of NAA with BAP did not make much difference. In case of BAP supplemented media, the medium with BAP (0.5 mg/l) showed eighty percent bud break after 15 days of inoculation with 2.3 shoots per explants (Table-1.). The combination of NAA with cytokinins (BAP) promoted shoot formation in various plant species as observed by Yasmeen and Rao (2005), Guo *et al*. (2007) and Jawahar *et al.*, (2008). The medium supplemented with BAP (1.0 mg/l) + NAA (0.1 mg/l) supported maximum number of shoots (3.0) per explant and responded best among all media tried in combination.



1,2 shoot induction in 0.5 BAP,4-7 2-3 shoots in 1.0 mg/l BAP+0.1mg/l NAA, 8- shoot induction after 10 days in 1.0 mg/l BAP+0.1mg/l NAA

The plant holds great promise as a commonly available medicinal plant and it is indeed no surprise that the plant is referred to in the Indian traditional circles. From the available literature on various aspects of the plant -traditional to biochemical and ethnobotanical to pharmacological and micro propagation however there are many gaps which need to be filled by concurrent researchers in different disciplines. One must make the best use of the naturally available resources which provide valuable raw material for advanced research. The present study deals with rapid and efficient protocol development for *in vitro* propagation of ***Spilanthes acmella*** Murr. Shoot induction on Murashige and Skoog medium supplemented with various auxins and cytokinins individually and in various combinations has been achieved by using axillary and apical meristems.MS medium fortified with 1.0 mg/l BAP and 0.1 mg/l NAA was found to be effective individually.

Thus the present study has been designed to develop a reliable and reproducible protocol of this important endangered plant which could be used for mass multiplication of this plant species to meet the increasing requirement of the pharmaceutical industry as well as for the conservation of germplasm.

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