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In Vitro Multiplication Studies in Spilanthes Acmella L. (Murr.)

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Abstract: ***During the present investigation, protocolforin vitro shoot multiplication of medicinal plant Spilanthes acmella L.(Murr.)was developed using axillary buds. The axillary buds explants were aseptically inoculatedon Murashige and Skoog (MS) with various concentration and combinations of growth hormones such as auxins and cytokinins. Multiple shoot formation wasrecorded on MS medium fortified with 1.0 mg /L of IAA along with various concentration of BAP. Rate of regeneration was higher using 1.0 mg /L of IAA along with 3.0 mg/L of BAP with stem node as an explant.***

Keyword: *Spilanthes acmella* ,Shoot , Multiplication .

1. Introduction

*Spilanthes acmella*Murr., a perennial herb (Family Asteraceae), is renowned for its medicinal and insecticidal properties. It is widely grown in the tropics and subtropics, and can be found in damp pastures, at swamp margins, onrocks near the sea and as a weed of roadsides.*Spilanthes acmella*is used for more diseases such as antitoothach, antibacterial, anti-inflammatory antimicrobial and antifungal properties (Veena Joshi et al 2013).Leaves and Flowers of the plant have a pungent taste and usedin spice for appetizers and as folk medicine for toothache, stomatitis and throat complaints. 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 in vertebrates while remaining harm less to warm-blooded creatures.

Due to its 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 (Singh and Chaturvedi, 2010).During the last few years, considerable efforts have been mode for *in vitro* plant regeneration of this threatened medicinal herb using Murashige and Skoog (1962) media supplemented with various concentrations of auxins and cytokinins.

2. Material and Methods

Preparation of explants

The explants were collected from young healthy plantlets of *Spilanthes acmella*, growing in botanical garden, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University Aurangabad. All explants were washed with running tap water for 5 minutes.After removing the leaves, thenodal segments werewashed with ethanol 70% (v/v) for 1 minute, followed by treatment with 0.1% (w/v) mercuric chloride for 3-5 minutes under aseptic conditions ad rinsing three times with double distilled water. Finally explants were used for aseptic inoculation.

Tissue culture media

Freshly prepared stocks of Murashige and Skoog (1962) were used to prepare the medium. The MS was fortified with different concentrations of Auxins and Cytokinins. Sucrose was added in medium (30 g/lit) and was gelled with 7.5 gm agar.The pH of the media was adjusted to 5.8 with 0.1 N NaOH or 0.1 N HCL before autoclaving at 1.06 kg/ cm -2 and 121^{0}C for 15 min.

Aseptic Inoculation

After surface sterilization explants were trimmedand aseptically inoculated on MS in a laminar air flow cabinet. After aseptic inoculation the culture were maintained at 25±2°C temperature with 50 -60% relative humidity and a 16/8 hours (light/dark) photoperiod provided with diffuse light (1,600 lux).

3. Result and Discussion

Initiation of shoots was recorded after one week. After 5 weeks of inoculation, an average of 3 to 10 shoots were emerged out from each axillary bud on MS, supplemented with 0.5, 1.0, 1.5, 2.0, 3.0 and 5.0 mg l-1 of BAP. The level of IAA was low (0.5 mg l-1)but it has shown significant influence on shoot multiplication from the axillary bud explants (Table 1).Concentration IAA 0.5 mg/L with BAP 3.0mg/L gave the highest i.e. 3 to10 shoots per culture in MS. No results were recorded with1.5mg/L BAP.

Table 1: Effects of Auxin and cytokinins on multiplication in *Spilanthes acmella*

Explant	Growth hormones (mg/L)		Shoot length	% of shoot inductuion
	IAA	**BAP**		
Nodal Segments do	0.5	0.5	0	0
		1.0	1.0± 0.645	0.1%
		1.5	5.5± 0.641	40%
		2.0	0± 0	0
		3.0	8.4± 1.779	50%
		5.0	6.25± 0.646	4%
	1.0	0.5	7.5± 0.713	20%
	1.0	1.0	0	0
	1.0	1.5	0	0

	1.0	2.0	0	0
	1.0	3.0	0	0
	1.0	5.0	5±0.735	40%
	1.5	0.5	0	0
	1.5	1.0	0	0
	1.5	5.0	0	0

Adventitious root formation was recorded on multiplied shoots after 25 days. This may be due to moist conditions in the culture jar. Our results are similar to the results recorded in*Aegilmarmalos*(Jamdhade and Pandhure ,2016) who obtained maximum number of shoots in the medium fortified with 5.0 mg/l BAP and 2.0 mg/l NAA (Plate.1).

Plate 1: Effect of Growth hormones on multiple shoot formation in *Spilanthes acmella*

4. Conclusion

The result presented here indicates that *in vitro* regeneration of complete plantlets is possible using nodal segments as an explant. Ayurvedic preparations have great demand across the globe. Peoples are rushing towards safer medicines now a day. Increasing demand of medicinal plants could not supply sufficient plant material for the extraction of ayurvedic medicines. In vitro studies are suitable option for multiplication of rootstock of plant material and to fulfill the need of the peoples.

5. Acknowledgment

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