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IN VITRO CALLUS INDUCTION STUDIES ON*IPOMOEASEPIARIA* AN IMPORTANT MEDICINAL PLANT

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ABSTRACT: The objective of this study was to develop in vitro callus induction protocol for *Ipomoeasepiaria*. The*Ipomoeasepiaria* is an important medicinal climbing plant belonging to the family Convolvulaceae mainly used for the various skin diseases. Callus of the leaves and nodal segment were cultured on Murashige and Skoog (MS) medium supplemented with BAP (6-benzyl amino purine) Kinetin and IAA (Indole acetic acid). Callus initiation was observed in all concentration with varied mass. Highest percentage of callus (90%) induction observe in combination of BAP (1.0mg/l) Kin (1.0mg/l) IAA(0.5mg/l) for explants leaves, node respectively. The experimental result of calli was shown as brownish white, friable and ascribed meristematic nature. The protocol in the study might be useful for the production of disease free and healthy plant materials.
Key words: *Ipomoeasepiaria*, *In vitro* callus induction, BAP, Kin, IAA.

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INTRODUCTION

Ipomoeais the largest genus in the flowering plant family Convolvulaceae with over 500 species. It is a large and diverse group with common name including morning glories. Purple heart glory is a slender vine 3 -5 m long heart shaped leaves are 2-6, 2-5 cm on smooth. Leaves smooth heart shaped or arrow shaped flower white to pink-purple funnel shaped flower occur in stalked cymes. The plant is having cooling and rejuvenating effect. This is climbing plant this climber with a slightly thickened or tuberous perennial root.In traditional practices it is mainly used in the treatment of women sterility and pediatric diseases *Ipomoea sepiaria* is used in the treatment ulcers and considered as a goof antidote to arsenic and also reported to have antiviral property. It is useful in vitiated conditions of pitta, burning sensation, psychic disorders, strangury, hyperdipsia and general debility [1]. In flolklore practices this herb is known as a good antidote to arsenic poisoning, uterine tonic, aphrodisiac and anti-ulcer drug. It is reported to be used in burning sensation, strangury, general debility and sterility in women. The literatures further specify the used of root is case of diabetes and constipation. In one of the Ayurvedic texts Basavarajeeyam (18 century) it is mentioned that the root power in the dose of 1 teaspoon is administered with rice water for leucorrhoea [2]. In traditional practice the juice of the leaf is installed in the right nostril during 2nd and 3rd months of ante-natal period to the pregnant women for begetting male progeny known as ‘pumsavana karma mentioned in Ayurvedic classics [3].

MATERIAL METHODS

Plant material

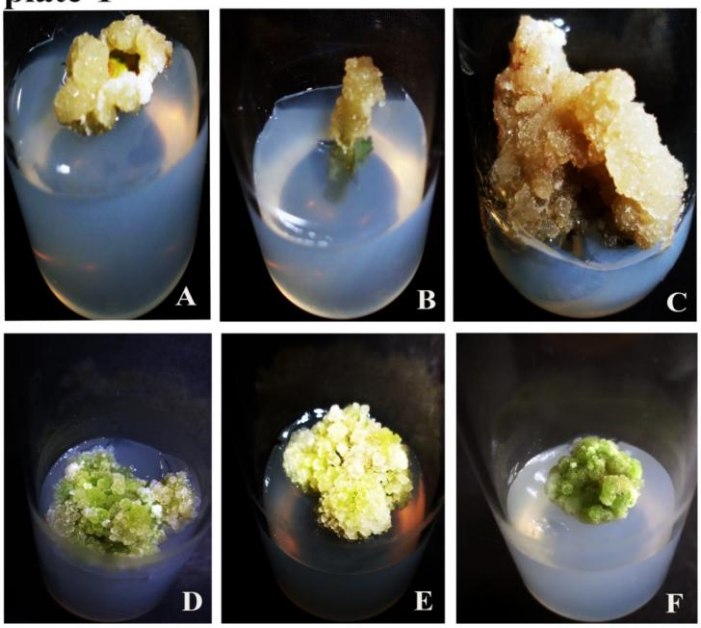
Healthy plants of *Ipomoeasepiaria* were collected from RMS colony Tiruchirappalli district, Tamilnadu. The collected explants were washed thoroughly with running tap water for 30 minutes. Then the explants were rinsed with 1% salonsolution containing 6-8 drops of tween 20 for 15 minutes and again washed with double distilled water to remove the traces of detergent solution. Next, the explants rinsed with 0.1% (w/v) mercuric chloride solution for 3-4 min.Then the explants washed with 70% alcohol for 30 seconds followed by washing again washed withdistilled water for 6-8 time and the explants were placed inside the laminar air flow chamber. Then the explants were placed in sterile petriplates for inoculation. The sterilized leaves were used as explant source for in vitro culturing. medium with various combinations of BAP, Ki and IAA were used for callus induction. The MS medium consists of macronutrients, micronutrients, iron source and vitamins supplemented with sucrose (15g) as a carbon source and agar (0.8mg) as a solidifying agent. BAP Benzyl amino purine and Kinetin, and three IAA Indole-3- acetic acid, in different concentrations were also supplemented in to the MS medium for callus induction. The BAP concentrations were constant (3.0 mg/l). Ki concentrations were ranged from 1.0 mg/IAA 0.5 mg/l. Callus cultures were maintained on solid MS medium and sub cultured with frequent intervals and used for further studies.

RESULTS AND DISCUSSION

Callus Induction

The callus formation depends on explantsource and influenced by type of growth regulator in the concentration and combinations in the growth medium. For callus induction (3-5 mm in length) nodal cuttings and leaves were cultured on MS medium supplemented with various concentrations of BAP, Ki, and IAA. The callus growth was initiated in about 8 days and rapid growth followed for majority of culture. The best response in leaf (90-100%) was observed on MS medium supplemented with (++++) BAP 1.0mg/l and Ki 1.0mg/l, with IAA 0.5mg/l were callus production from leaf cuttings respectively Brown hard. The highest response (80%) in callus formation was observed in basal media, supplemented with 1.5mg/l of NAA and 0.5 mg/l Kinetin [4]. Higher concentration of 2,4-D (3mg/l) was more effective for callus induction on terminal buds flower explants [5]. The combination of NAA (96+0.96%) 0.2mg/l with 0.2mg/l of BAP gave green hard callus [6]. Form the different concentration of hormones in leaf explant (+++) BAP 2mg/l and Ki 1 mg/l with IAA 0.5mg/l (70-100%) was induced the callus color Light green soft. The leaf explant is better for induction and growth of leaf callus (++) BAP 3mg/l and Ki 1mg/l with IAA 0.5mg/l (60-100%) color of callus white with brown. Medium containing low response for leaf explant was observed on (+) BAP 4 mg/l and Ki 1 mg/l with IAA 0.5 mg/l (40-100%) color of callus Green hard. At the highest concentration low for leaf explant in BAP 5 mg/l and Ki 1 mg/l with IAA 0.5 mg/l callus induction was not supportive for callus induction. The highest growth of callus formation in node BAP (+++) 1 mg/l and 1 mg/l with 0.5 mg/l (70-100%) gave color of callus soft brown. The minimum response in node explant (++) was observed on MS medium supplemented BAP 2 mg/l and Ki 1 mg/l with 0.5 mg/l (50-100%) result in color of callus white soft.The node explant of lowest result in term of percentage of callus induction (+) BAP 3 mg/l and Ki 1 mg/l with IAA 0.5 mg/l (20-100%) color of callus light whitehard. The highest concentration for node explant in medium is not rest in BAP 4 mg/l, Ki 1 mg/l with 0.5 mg/l and BAP 5 mg/l Ki 1 mg/l with 0.5 mg/l. The comparatively, leaf cultures response better then nodal cuttings culture in term of percent cultures production callus. Callus produced from leaves of *Ipomeaturpethum* in MS+2BAP medium [7]. In order to induce callus differentiation, various concentration of ABA and 2,4-D (0.5mg/l)were assed to the basic medium SPM [8].

plate-1



A-Callus from leaf explant after 20 days
B-Callus from node explant after 18 days
C-Callus from leaf explant after 45 days
D-Callus from leaf explant after 27 days
E-Callus from leaf explant after 32 days
F-Callus from leaf explant after 22 days

Table-1: Effect of different concentration of BAP, Ki alone with IAA on Callus proliferation from leaf explants

Explant leaf	BAPmg/l	Ki mg/l	IAAmg/l	Degree Of Callus Formation	% Of Callus Response	Nature Of Callus
	1	1	0.5	++++	90-100	Brown hard
	2	1	o.5	+++	70-100	Light green Soft
	3	1	0.5	++	50-100	White with brown soft
	4	1	0.5	+	40-100	Green hard
	5	1	0.5	-	-	-
Node	1	1	0.5	+++	70-100	Brown soft callus
	2	1	0.5	++	50-100	Light white callus soft
	3	1	0.5	+	20-100	Light white callushard
	4	1	0.5	-	-	-
	5	1	0.5	-	-	-

Note: No callus (-), Small callus (+), Moderate callus (++), Quite massive callus (+++), Very quiet massive callus (++++).

CONCLUSION

An efficient callus induction protocol for *Ipomoea sepiaria* has been developed for these findings. The treatment of BAP1.0mg/l, Ki 1 mg/l and, IAA 0.5 mg/l is good for maximum callus induction. Therefore the results reported in the study would be an important step towards the development of good quality and quantity of callus to provide successful platform for regeneration, and genetic transformation.

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