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IN VITRO CALLOGENESIS OF *SOLIDAGO VIRGAUREA* L. IN COMBINED PLANT GROWTH REGULATORS

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ABSTRACT

Callus induction was established from different explants of Solidago virgaurea L. in combined plant growth regulators. The different explants such as nodes, internodes and leaves were cultured on MS medium fortified with various concentrations and combinations of auxins and cytokinins (2, 4-D, BAP, NAA, IAA, IBA, Kn and Zeatin). Plant Growth Regulators in the combinational concentration IBA (4.5mgl^{-1}) and BAP (0.5mgl^{-1}) influenced the callus initiation from nodal explants. IBA (4.0mgl^{-1}) and BAP (1.0mgl^{-1}), IBA (3.5mgl^{-1}) and BAP (1.5mgl^{-1}) were responsible for the callus induction from internodal and leaves explants respectively.

Key words: *Callus, Medicinal, Plant Growth Regulators, Solidago virgaurea*

Abbreviations: *IAA - Indole-3-Acetic acid, IBA - Indole-3-Butyric acid, Kn -Kinetin, MS-Murashige and Skoog, NAA - α-Naphthalene-Acetic acid*

INTRODUCTION

Medicinal plants are an important source of chemical compounds for the traditional medicine. About 80% of the population living in developing countries still use traditional medicines derived from plants for their primary health care. Due to the short supply of plant derived compounds indiscriminate exploitation of the natural resources in rise without proper care for cultivation resulting to endangered conditions of important medicinal plant species[1]. The *in vitro* propagated medicinal plants furnish a ready source of uniform, sterile and compatible plant material for biochemical characterization and identification of active constituents[2]. Plant tissue culture techniques have been increasingly applied to many medicinal plants in particular for mass propagation, conservation of germplasm, study and production of bioactive compounds, and for genetic improvement. Medicinal plants have vast genetic diversity which is a valuable source of agronomic genes of interest for the future. Large scale plant tissue culture is found to be an attractive alternative approach to the traditional methods of plantations, as it offers a controlled supply of biochemicals independent of plant availability and more consistent product quality[3]. Minimal growth of tissue in culture has been used to store plant materials from a wide variety of species. *In vitro* propagation techniques may help in the conservation of biodiversity of locally used medicinal plants[4].

In view of the present circumstances, biotechnological intervention is highly called for in order to address callogenesis, a significant method to produce elite clones and their secondary metabolites of valuable genotypes produced without any seasonal constraints, would be of special advantage in this respect[5].

Solidago virgaurea L, is a safe and gentle remedy for a number of disorders. In particular, it is a valuable astringent remedy treating wounds and bleeding[6]. The plant contains saponins that are antifungal and act specifically against the *Candida* fungus which is the cause of vaginal and oral thrush[7] rutin which is used to treat capillary fragility and phenolic glycosides which are anti-inflammatory[8]. And the leaves are antihelmintic, antiseptic, aromatic, carminative and stimulant[9]. In the present study, a reliable protocol has

been developed for large scale propagation of this important medicinal plant using callus proliferation from various explants (node, internode and leaf) in the combined plant growth regulators is described.

MATERIAL AND METHODS

Various explants (node, internode and leaves) of *Solidago virgaurea* L. were collected from the medicinal Garden, St. Xavier's college, Palayamkottai-02. The explants were thoroughly washed in running tap water for 30min. followed by the treatment in 1% Labolene, a neutral detergent (Qualigens, India) for 3min. and finally rinsed with the distilled water for 4-5 times to remove the surface micro flora. The washed explants were surface sterilized with 0.1% (w/v) aqueous mercuric chloride ($HgCl_2$) for 3 min. and the chemical sterilent was removed by rinsing the materials with sterilized and cooled distilled water 4-5 times. The explants were aseptically excised into transverse segments of 1cm length with the help of a sterilized blade and were inoculated on to the culture media.

The media consisted of MS basal constituents[10] with 3% sucrose and 0.8% bacteriological grade agar (Hi-media, India). Growth regulators tested individually and combined at the various concentration range of each of 0.5 to 5.0mg/l, 2, 4-D, IAA, IBA, NAA, BAP, Kin and Zeatin. The pH of the medium was adjusted between 5 .6 to 5 .8 prior to autoclave. About 20ml of medium was dispensed into sterilized culture tubes and were autoclaved at 121°C at 15-PSI (1.06 kg/cm^2) pressure for 15 min. The various explants were inoculated on the culture media and were incubated at 25± 2°C, 16h photoperiod, provided by cool white fluorescent tubes (Philips, India:3000Lux) and 60-65 % relative humidity. The callus induction was observed at the end of second weeks of incubation and subcultured immediately.

OBSERVATIONS AND DISCUSSION

In the present investigation, various explants cultured on MS media supplemented with BAP in combinations with IAA, IBA, NAA, 2, 4-D, Kin and Zeatin. Among the hormone combinations, callus formation potentiality was the highest in 4.5mg/1 IBA and 0.5mg/1 BAP for nodal explants (Table 1; Fig 1b). And internodal callus induction was noticed in 4.0mg/l IBA and 1.0mg/1 BAP (Table 1; Fig ld).

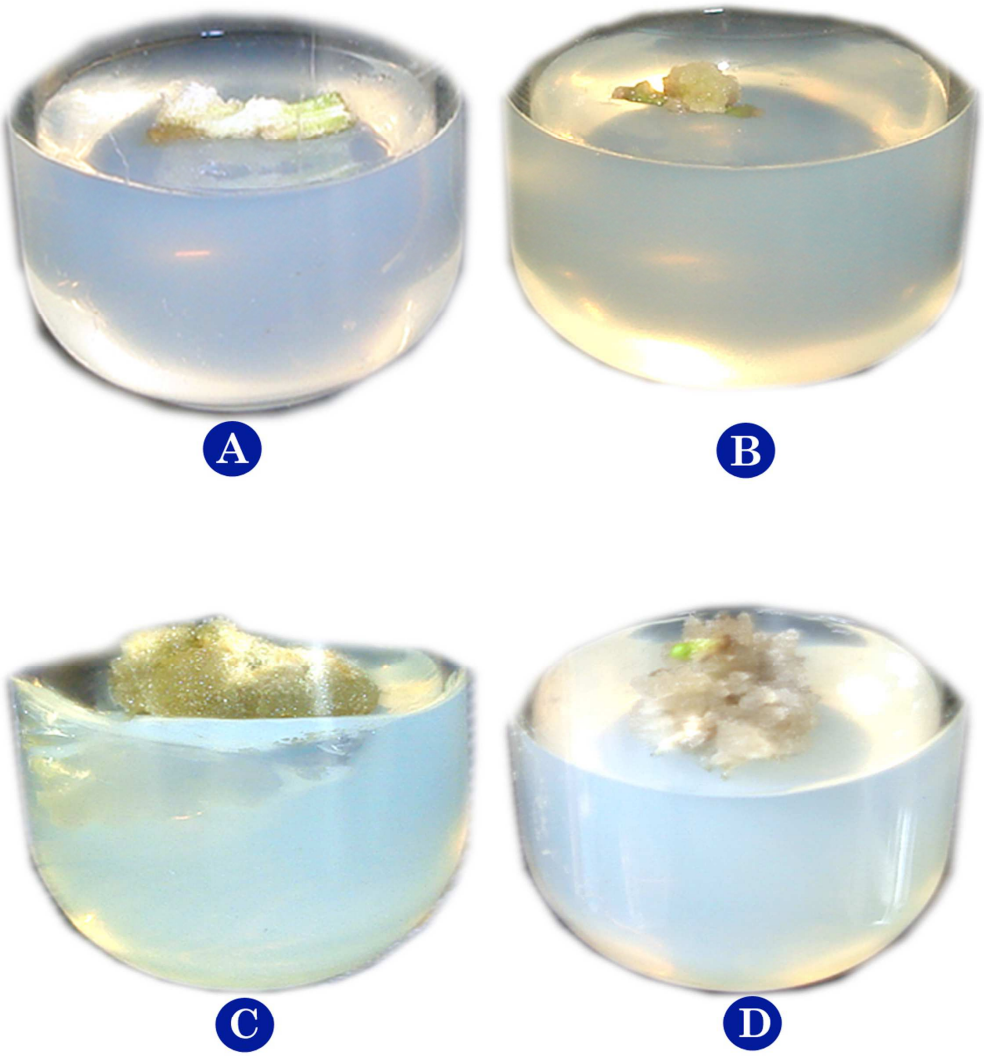
Table – 1

Effect of different concentrations of BAP and IBA for callus induction from various explants

Explants	Hormone Concentration (mg/l)		Callus Induction
	BAP	IBA	
Node	0.5	4.5	++++
	1.0	4.0	++
	1.5	3.5	++
	2.0	3.0	No response
	2.5	2.5	No response
	3.0	2.0	No response
Inter node	0.5	4.5	++
	1.0	4.0	++++
	1.5	3.5	++
	2.0	3.0	No response
	2.5	2.5	No response
	3.0	2.0	No response

\+ : Low range of callus formation
++ : Moderate range of callus formation
++++ : High range of callus formation

Fig - 1
Callus induction from various explants of *Solidago virgaurea* L.



A - Callus initiation

B - Nodal callus induction on MS + 4.5mg/L IBA & 0.5mg/L BAP.

C - Inter nodal callus induction on MS + 4.0mg/L IBA & 1.0mg/L BAP.

D - Leaf callus induction on MS + 3.5mg/L IBA & 1.5mg/L BAP.

Minimum days (10) required for callus initiation from all the tested explants. Induction of callus from explants is an important step for successful plant regeneration. Three types of explants viz. node, internode and leaves were used. Explants were cultured on MS medium supplemented with different combinations of plant growth regulators. Assessment on callus induction was studied through days to callus initiation. The explants varied significantly the BAP and IBA hormone concentrations.

The purpose of this study was to develop *in vitro* callogenesis from different explants of *Solidago virgaurea* in the combined plant growth regulators. In the present work *in vitro* callogenesis was established a rapid and reproducible method. Callus induction was successfully initialed from *Vigna unguiculata* using BAP[11]. This result is in confirmation with previous report[12] in which callus formation from internode was obatained on MS medium supplemented with 4.0mg/l IBA in *Datura metal*. Callus induction was proliferated from leaf explants of *Coleus forskohlii* using MS medium fortified with 1.5mg/l BAP and 3.5mg/l IBA[13].

CONCLUSIONS

The present *in vitro* callus induction from different explants of *Solidago virgaurea* L. in combined plant growth regulators was successfully derived from various concentrations of BAP and IBA. The paper described a prime protocol for the large scale production of callus that may be utilized for the cultivation practices and large scale production of plantlets of *Solidago virgaurea* L.

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