

In Vitro characteristics of callus induction of *Bryonia Laciniosa*— A medicinal plant

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Abstract – The present study deals with the In Vitro Callus induction of *Bryonia Laciniosa* which is an important medicinal plant which belongs to the family Cucurbitaceae. It is widely used for inflammation, cough, flatulence and skin diseases. The efficient in vitro Callus induction of *Bryonia Laciniosa* was achieved from internode and leaf explants on MS medium with different concentrations and combinations of 2,4-D and Kin. The highest rate of Callus induction was observed from the leaf explants on MS medium with 2,4-D (3 mg/l) + Kin (1 mg/l). The regenerated callus were transferred in to half strength MS medium fortified 2,4-D for Callus elongation. The elongated Callus was successfully the current study showed efficient in vitro Callus induction capabilities of *Bryonia Laciniosa*.

Keywords- In Vitro Callus induction, MS medium, 2,4-D and Kin.

INTRODUCTION

Bryonia lacinosa syn *Bryonopsis lacinosa* (Cucurbitaceae) plant is distributed throughout India and locally known as “Shivlingi” and “Gargumaru.” It is an annual climber with bright red fruits and is reported to be highly medicinal [1]. Locally in India its seeds are being used for promoting conception in women. Plant as a whole is bitter, tonic and mild laxative. Its leaves are used on inflammations. Roots are given against asthma and promotes conception. In India, Gond and Bharia tribes of the Patalkot Valley, Madhya Pradesh worship this plant. According to them, this herb is a boon for the childless parents. The herbal healers (Bhumkas) prepare certain combination of herb and prescribe it to the needed person. *B. laciniosa* is widely employed as a herbal drug for the treatment of gastrointestinal, respiratory, rheumatic and metabolic disorders, as well as for liver and infectious diseases [2]. The leaves, roots and seeds extracts of the plant were studied on various health problems in women like infertility and menstrual disorders. The whole plant of *Bryonia laciniosa* is recommended traditionally for inflammation, controlling fever, inducing diuresis and as a tonic. Plant is also used

against snake-bite (part not specified). From leaves a bitter principle, bryonin, has been reported [3]. *Bryonia alba* is well established homeopathic medicine, while *B. laciniosa* is being used as trivial medicine since long in India, but not much work has been done on the plant except fatty acids from fruit, sugars and a glucomannan from seeds [4] have been reported.

The present investigation was carried out to develop a simple and efficient protocol for callus induction of *bryonia laciniosa*. This is an alternative and cost effective method to conserve the medicinal plant.

MATERIALS AND METHODS

A. Plant Material

Bryonia laciniosa plants were collected from Bhilai region Dist- Durg Chattisgarh, India and were successfully planted in St. Thomas College herbal garden for further use.

The plant specimens are maintained in the Department of Microbiology and Biotechnology, St. Thomas College. For the initial experiments, healthy internodal and leaf explants were collected from two months old plant.

B. Selection and Surface Sterilization of Explants

After selection of nodal segment and leaves as ideal explants for our experimentation, we have chosen them for further studies on the effect of growth hormones 2,4-Dichlorophenoxyacetic acid (2, 4-D) and Kinetin(Kin). All explants were first washed under running tap water for 30 min and then washed distilled water and transferred to Laminar air flow chamber. The explants were surface sterilized with 0.1 % (w/v) HgCl₂ solution for 4 min, and then washed with sterilized distilled water. The 70% ethanol was added and waits for 5 min and then explants were washed with sterilized distilled water for 5-7 times. Now the explants were cut to the required size and inoculated onto culture medium. All the explants were placed horizontally on the medium, and the leaves were placed with their dorsal side in contact with the medium.

C. Culture Medium and Conditions

The culture medium used for the explants selection was MS medium [5] supplemented with 3% (w/v) sucrose

and pH was adjusted to 5.8 with 1N NaOH or HCl before addition of 0.8% (w/v) agar (Hi media, India) and enriched with varying concentrations of 2,4-D and 2,4-D in combination with Kin were used further to determine the optimum growth regulator levels. The concentrations tested for 2, 4- D (0.5-5.0 mg/l) and Kin (0.5-2.0mg/l).Molten media were dispensed into test tubes (Borosil, India) (25×150mm; 10 ml) and closed with non-absorbent cotton plugs and media were autoclaved at 104 kpa and 1210C for 20 min. The cultures were maintained at $25\pm2^{\circ}\text{C}$ under a 16 hour photoperiod of $35\mu\text{mol m}^{-2} \text{s}^{-1}$ irradiance provided by cool white fluorescent light with 55-65% relative humidity. For hardening off, 7 to 8 weeks old rooted shoot lets were removed from the culture flasks. After freeing the agar with the running water they were transferred into small polythene bags containing sterilized cow dung, sand and red soil (1: 1: 1) and kept in a mist house. After acclimation in the mist house for 2 months, they were transferred to green house.

RESULTS AND DISCUSSION

Internode and leaf explants were inoculated on MS basal medium with supplemented with various concentrations and combination of 2,4-D (0.5-5.0mg/l) & Kin (0.5-2.0 mg/l) were used for culture initiation and Callus induction. The intermodal explants were showed the best Callus induction response on 2,4-D (3 mg/l) + Kin (1.0mg/l) (Table. 1) (Fig. 1. A & B), The similar results has also suggested by [6] on *Aloe barbadensis* in 2, 4-D 3 mg/l+Kin 1.0 mg/l through nodal explants. These findings are in agreement with who observed in other plant species *Glinus lotoides* [7]. The highest number of multiple Callus was recorded from leaf explants on different concentration and combination of plant growth regulators like 2,4-D (3.0mg/l) + Kin (1.0mg/l) showed the best response (Table. 1) (Fig. 3 A,B &C).The similar results were obtained for *Tecomella undulata* where 2,4-D (3.0mg/l) + Kin (1.0mg/l) stimulated number of multiple Callus through leaf explants[8] (Patel, 2013)and other plants like *Momordica dioica* [9],*plumbago zeylanica* [10]. After 4 weeks the elongated Callus from intermodal and leaf explants were transferred to the Shoots induction medium containing half strength MS basal medium with IBA (1.0 mg/l The *in vitro* multiple calluses were transferred from culture medium successfully acclimatized in a cow dung, sand and red soil (1: 1) in the greenhouse with natural photoperiod conditions. The *in vitro* regeneration of medicinal plant *bryonia laciniosa* revealed that the tissue culture showed good response in proliferation of multiple calluses in MS medium by supplementing with BAP, NAA & IBA. These present studywas to establish reliable regeneration protocol for *bryonia laciniosa*, which can be used foreasier cultivation, propagation and plant genetic studies. In this present investigation has also opened new researchers for genetic manipulation of *bryonia laciniosa* for disease, pestresistance or enhancing

secondary metabolites, using a rapid regeneration protocol.

Table1. Effect of auxin and cytokinin on explants response, Callus initiation and growth of callus.

Concentration of growth regulator		Explants response		Callus initiation	
2,4-D	Kin	Internode	leaf	Internode	Leaf
0	0				
0.5		62	65	32	36
1.0		61	62	32	35
2.0		60	65	28	30
3.0		52	64	35	44
4.0		60	61	28	34
5.0		60	60	25	29
	0.5	58	60	24	29
	1.0	62	65	35	41
	1.5	57	62	32	39
	2.0	54	59	29	34
2.0	1.0	67	68	57	66
3.0	1.0	75	80	68	75
4.0	1.0	69	74	55	64

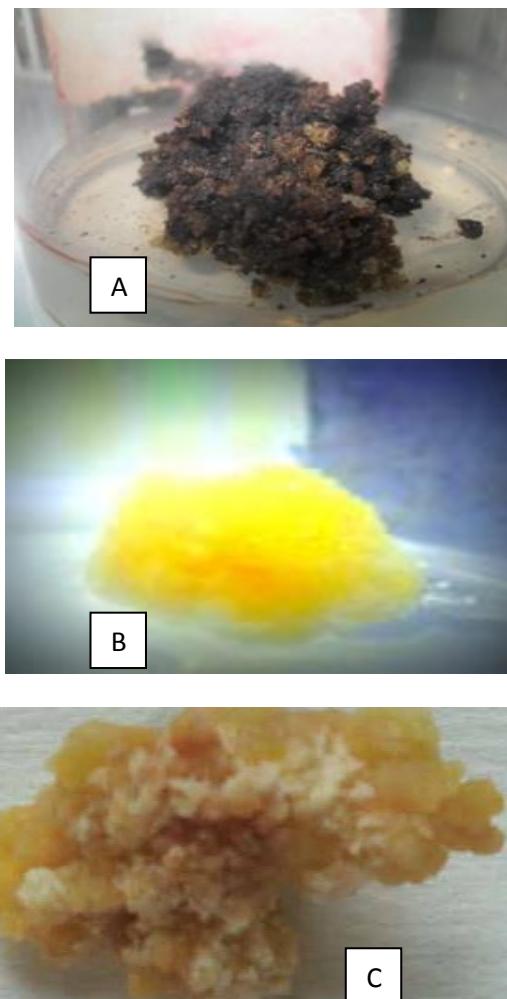


Fig. 1- Callus initiation from leaf (A-C)

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