DIRECT RHIZOGENESIS, PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF BRYONIA LACINIOSA

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Abstract - Efficient rhizogensis was established for Bryonia laciniosa using leaf derived calli. The maximum morphogenic callus induction rate (80%) was observed from leaf explant by culturing in MS medium supplemented with 2.0 mg/l IAA + 2.0mg/l IBA. Calli size and fresh weight increased substancially through subculturing. The highest percentage of root induction (70%) and highest mean number of roots (12.33) per culture were obtained with 2.0 mg/l IAA + 2.0 mg/l IBA. Phytochemicals like alkaloids, tannins, flavonoids, saponins and glycosides were also found present. The study indicates the presence of nutritional and chemical components that are beneficial in addition to the numerous medicinal values of the plant. Antibacterial effect of Bryonia laciniosa was evaluated on bacterial strains like Bacillus cereus, Staphylococcus aureus, Pesudomonas Enterobacter aerogenes, Escherichia coli and Klebsiella pneumoniae. The solvents used for the extraction of plants were water and methanol. The in vitro antibacterial activity was performed by agar disc diffusion. The most susceptible bacteria was K. pneumoniae. The extracts of callus and plant material could not inhibit any of the bacterial strains investigated**.**

Keywords - Rhizogenesis, Antimicrobial, Bryonia Laciniosa, Phytochemical Analysis

I.INTRODUCTION

Interest in plant tissue culture so far has been greatest in the area of rapid clonal propagation of selected species of crops and ornamental plants however the most conspicuous advantages of micropropagation are reflected in the rapid multiplication of medicinal plants which grows slowly or which cannot be stored by conventional methods.

Cultivation of medicinal plants especially high value medicinal plants is creating new dimension in the field of agriculture. Indian herbal industry is at blooming stage; however, cultivation of medicinal plant is not so easy. It is challenging task because less is known about their seed biology. Due to its therapeutic activity and diversified uses, demand of B. lacinosa is increasing in Indian and international market. Its seeds have poor germination percentage (5-11%), low viability and long dormancy period. Due to large scale and indiscriminate collection of its parts for gainful trade and insufficient attempts either to allow its replenishment or its cultivation. There is a greater need for commercial cultivation of this species (Kirtikar,1987; Rai, 1989).

II. DETAILS EXPERIMENTAL

2.1 Collection, Identification of Plants and Explant Sources

The Bryonia laciniosa plants were collected from Bhilai area, Durg District and Identified. Young leaves were collected, washed under running tap water for at least 10-15 min, followed by soaking in 5% (v/v) detergent solution (Teepol Qualigen, India) for 5 min. After thorough washing in sterilized distilled water, the explants were surface sterilized with freshly prepared 0.1% (w/v) aqueous mercuric chloride solution for 3 min. Followed by repeated washing with sterile distilled water, the explants were cut in to 0.5-1 cm segments then inoculated on MS medium (Murashige and Skoog, 1962).

2.2 Culture Conditions

The callus culture medium consisted on MS basal medium supplemented with sucrose 3% (w/v). The pH of the media was adjusted to 5.7 using 0.1N NaOH or 0.1N HCl prior to adding 0.8% agar (Hi-media, Mumbai). Medium was dispensed in 20 ml aliquots in to culture tube (25x150 mm), which were covered with an aluminum foil. Media were steam sterilized at 121ºC and 1.05 kg cm-2 s-1- for 20 min.

2.3 Callus Induction and rhizogenesis

Callus cultures were established from different explants of Bryonia laciniosa grown aseptically as described above. Callus cultures were maintained at 27^0C in darkness. For root differentiation, the callus tissues that formed on the initial explants were transferred to the root induction medium and cultured under dark conditions, 25 ± 2ºC and 60% relative humidity. Every four week, the callus tissues were transferred to fresh medium for callus maintenance and root differentiation. All tests were repeated at least thrice using newly established cultures. Culture medium supplemented with different concentrations of 2,4-D, IAA, IBA and BAP with 3% (w/v) sucrose tested individually for root initiation.

2.4 Phytochemical screening

The leaves were washed with distilled water, oven dried at 80^oC for 2h to obtain a constant weight. The samples were then finely ground and stored in plastic containers at ambient temperature.

Five grams of each sample was placed into two conical flasks containing 100 ml water and ethanol respectively. The mixtures were covered and allowed to soak for 3h, after which they were filtered. The preparations were covered and labeled. Khandelueal (2001) quantitative methods were used for the analyses. The phytochemical screening for the

presence of alkaloids, tannins, flavonoids, saponins, and carbohydrates were carried out according to the methods of Trease and Evans (2002), and Williams et al. (2005), Odebiyi & Sofovora (1978) and Banso et al.(1976).

2.5 Anti-microbial activity of extract

The antimicrobial assay was performed by methods viz. agar disc diffusion method. The molten Mueller Hinton agar was inoculated with 100 µl of the inoculum (1 x 108 cfu/ml) and poured into the Petri plate (Hi-media). For agar disc diffusion method, the disc (0.7 cm) (Hi-Media) was saturated with 100 µl of the test compound, allowed to dry and was introduced on the upper layer of the seeded agar plate. 100 µl of the test compound was introduced into the well. The plates were incubated overnight at 37°C. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain, controls were maintained where pure solvents were used instead of the extract. The result was obtained by measuring the zone diameter. The experiment was done three times and the mean values are presented.

III. RESULTS AND DISCUSSION

PGR 's	Concentration	Callus growth	Root induction
2,4-D	0.5 mg/ml	++	40%
IBA	3mg/ml	+++	55%
2,4-D+IBA	0.5 +3 mg/ml	+++	57%
IBA + BAP	1+2 mg/ml	++++	60%
BAP +2,4-D	1+ 1.5mg/ml	++	35%
BAP+IAA	2+2 mg/ml	+++	55%
BAP + IAA	4+4mg/ml	++++	67%
IBA+ kinetin	3+1mg/ml	++	40%
IAA+IBA	2+2 mg/ml	++++	70%
BAP	0.5 mg/ml	++	40%

Table 1: Effect of auxins and cytokines on callus and root induction

The effect of various concentrations of auxins individually and in combination with cytokinins was tested on MS basal medium for induction of callus from leaf of Bryonia laciniosa.
The callus Initiation started from leaf on 5th day in 2 mg/l IBA with 2 mg//lAA gives 70 per cent callus induction frequencies respectively.

Auxin or cytokinins either alone or in combinations were efficient for the induction of callus depending onthe varied concentrations of the growth regulators, callus was observed at the cut end of leaf explant. As reported in Withania somnifera (2006).It is clear that leaf explant proved to be best in term of callus induction. Similar kind of results was observed in Bryonia laciniosa (2011) .

However, in higher concentration of auxin, the percentage of root induction is slightly decreased in callus between 0.5-2.0 mg/l range beyond 2.0 mg/l the induction percent of callus frequency was poor and non significant (Table-1).

Table 2 shows the results of the phytochemical studies on aqueous and methanolic extracts of Bryonia laciniosa leaves. In the table (+++) sign indicates the presence of a particular constituent in high concentration, (++) indicates presence in moderate concentration, while (+) indicates it in low concentration. (-) indicates absence of a constituents as reported by Prarthana (2014).

Locally in India seeds of bryonia laciniosa are being used for promoting conception in women. It was thought that seeds may be containing some hormone like substance or substances that may help in the secretion of hormones in the human system or may be possessing substance which may be having strengthening effect on uterine muscle. Ayurvedic literature survey indicated that the use of entire plant is bitter tonic, hepatoprotective, antipyretic, laxative and used to correct the metabolic abnormalities. Plant is also used against snake-bite(Singh and Malviya, 2006). Its leaves are used on inflammation (Sivakumar,et al.,2004).

TESTS	LEAFEXTRACT		CALLUSEXTRACT	
	Aqueous	Methanol	Aqueous	Methanol
Carbohydrate	--	+	-	+
Tannins	+	-	+	-
Glycosides	++	++	++	++
Saponins	+	++	+	++
Resins	-	-	-	-
Phlobatannins	+	-	+	-
Flavanoides	+++	+++	+++	+++
Alkaloids	++++	++++	++++	++++
Sterols	++	-	++	-

Table 2: Phytochemical analysis of Leaf and Callus of Bryonia laciniosa

Fruits are recommended in liver disease, leucoderma, inflammation and abdominal disease Fruits are used as blood purifier. Root extract is having diuretic, hepatoprotective and kidney stone removing properties. The antibacterial activity of Bryonia laciniosa leaf and callus extract of both solvent (aq./meth.) against Bacillus cereus, Staphylococcus aureus, Pseudomonas aerogenes, Escherichia coli and Klebsiella pneumoniae is shown in **Table 3**. The aqueous Extract of callus showed considerable more activity than methanol extract. Maximum antibacterial activity of callus was shown against Klebsiella pneumoniae. Neither the extract was able to inhibit the K. pneumonia. Similar results were also reported by Venkatesan et al., Prescott et al. and Stainer et al. who reported diseases such as pneumonia, urinary and respiratory tract infection.

Bacterial strains	Effect of extract (in MM)		Effect of callus	
	Aqueous	Methanol	Aqueous	Methanol
Bacillus cereus	11	09	13	08
Staphylococcus aureus	12	11	14	06
Pesudomonas aerogenes	16	14	18	12
Escherichia coli	09	--	12	---
Klebsiella pneumoniae	15	16	18	10
Gentamycin (100ug/disc)	22	19	24	18

Table 3 :Zzone of inhibition (mm) of bacterial growth on aqueous and methanol extract of bryonia by disc diffusion method

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