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Rauwolfia serpentina: Protocol optimization for *callus induction* from *leaf explants*

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Abstract:- *Rauwolfia serpentina* L Benth Kurz, common name Sarpgandha is a small perennial medicinal shrub of family Apocynaceae. It has a vast array of secondary metabolites, which have a number of commercial applications. The present investigation is an effort to developing an efficient protocol for callus induction using various explants of the same. Callus induction was obtained both from leaf as well as stem explants. It is noteworthy that in the present investigation callus formation from seed explants was also observed in MS basal media (Plate 2). Two different combinations of hormones *i.e.*2, 4-D + BAP, IAA + BAP, IBA+BAP & Kinetin+NAA with different concentrations were tried for callus induction. In the present research work BAP alone induced callusing in both the explants *i.e.* leaf and stem, which was 91.54% & 88.83% respectively

Keywords:- *Rauwolfia serpentina, medicinal plant, micro-propagation, phytohormone, tissue culture.*

Abbreviation: *BAP—6 Benzyl amino purine, NAA—Napthaleneacetic acid, MS—Murashige and Skoog, IBA—Indolebutaric acid.*

I. INTRODUCTION

Rauwolfia serpentina L Benth Kurz, common name Sarpgandha is a small perennial medicinal shrub of family Apocynaceae. The maximum height of the plant is up to sixty cm. Their roots are tuberous with pale brown cork. The leaves of plant are in whorls of three, elliptic to lanceolate or ovate, bright green above and below pale green and thin. Flowers are in irregular corymbose cyme, white, often tinged with violet.

The flowering time is from March to May in Indian condition. Its fruit are Drupe, single or didymous and shining black. It is an important plant of Indian subcontinent and South East Asian Countries. It is medicinally famous plant in Ayurveda, Siddha, Unani and western systems of medicines (Ajay *et al.*, 2011).

It is widely distributed in the tropical part of the Himalayas, the Indian peninsula, Sri Lanka, Burma, and Indonesia. The plant is indigenous to India, Bangladesh and other regions of Asia and found to grow in the wild in many places around the country (Ghani, 1998).

The root extract of this plant is very useful in disorders of gastro intestinal tract viz., diarrhea, dysentery, cholera and colic (Quresh *et al.*, 2009). Mixed with other plant extracts, they have been used in the treatment of cholera, colic and fever. In particular, phenolic phytochemicals have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammatory activities (Arts, *et al.*, 2005&Scalbert, *et al*., 2005). Leaves are used in removal of opacities of cornea (Sarika *et al*., 2012). The plant is extensively used in treatment of insanity and snake bite (Kokate *et al*., 2003).

The drugs obtained from this plant are useful in mental disease, epilepsy, anxiety, excitement, schizophrenia, insomnia and insanity sleeplessness and several other ailments (Ojha & Mishra, 1985; Ghani., 1998). The plant product has also been used as febrifuge and stimulant to uterine contraction for insomnia and most of all for insanity (Vakil, 1949). Reserpine was used to cure the high blood pressure or hypertension and its complications, stroke, and the diseases related with nervous system (Achmad, 1987). Reserpine is also use as tranquilizer (Kokate *et al.*, 2003).

II. MATERIALS AND METHODS

Mature explants of *Rauwolfia serpentina* were obtained from different regions of Hazaribag. The plant materials were taxonomically identified with the help of flora (Haines, 1921). Plant subjected to cultures are very sensitive to toxic chemicals contained in the medium. Therefore, chemicals of highest grade of purity were used in culture media to avoid contamination as well as accumulation of toxic chemicals. Culture media in glass containers were sealed using aluminium foil or plastic closures and sterilized by autoclaving at 15 lb Pa and121^{o} C for 15 to 20 min after addition of requisite amounts of phytohormones. Prior to any aseptic transfer the LAF cabinet was swapped with 70% alcohol. It was then exposed to UV light for 15 min. The instruments such as scissors, forceps, scalpels etc. were dipped in alcohol first and sterilized by flaming before their use. Required MS medium supplemented by various hormonal combination were used for callus induction.

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III. RESULT AND DISCUSSION

Callus induction was obtained from leaf explants. It is noteworthy that in the present investigation callus formation from seed explants was also observed in MS basal media (picture). Two different combinations of hormones *i.e.*2, 4-D + BAP, IAA + BAP, IBA+BAP & Kinetin+NAA with different concentrations were tried for callus induction.

The effect of BAP on callusing alone was also investigated. Bhatt *et al.,* (2008) has reported that BAP alone did not induce callus either from leaf or stem explants but in the present research work BAP alone induced callusing in the explants *i.e.* leaf, which was 91.54% (Table).

The highest callus induction in the leaf explantswas88.10% in the medium containing 0.125 mg/l IBA & 1 mg/l BAP. Combination of 2,4-D & BAP at concentration of 0.125 mg/l & 1.5 mg/l showed 86.71% callus response from leaf. However, Bhatt *et al.*,(2008) obtained best response in media containing 0.125mg/l 2,4-D and 1mg/l BAP from leaf explants utilizing same combination of plant growth regulators but their best result were at a lower concentration of BAP which was 1.0mg/l.

Kinetin (2mg/l) with NAA (1.5mg/l) &IAA (0.2mg/l) with BAP (1 mg/l) were found to play a prominent role in callus induction with 90.97%& 88.55% response respectively (Fig). BA and NAA were the best hormones for callus induction as reported by a number of researchers (Biswas *et al.*, 2007). BAP alone at concentration of 5.0 mg /l showed the highest frequency of callus induction which was 91.54% for leaf explants (Table:).

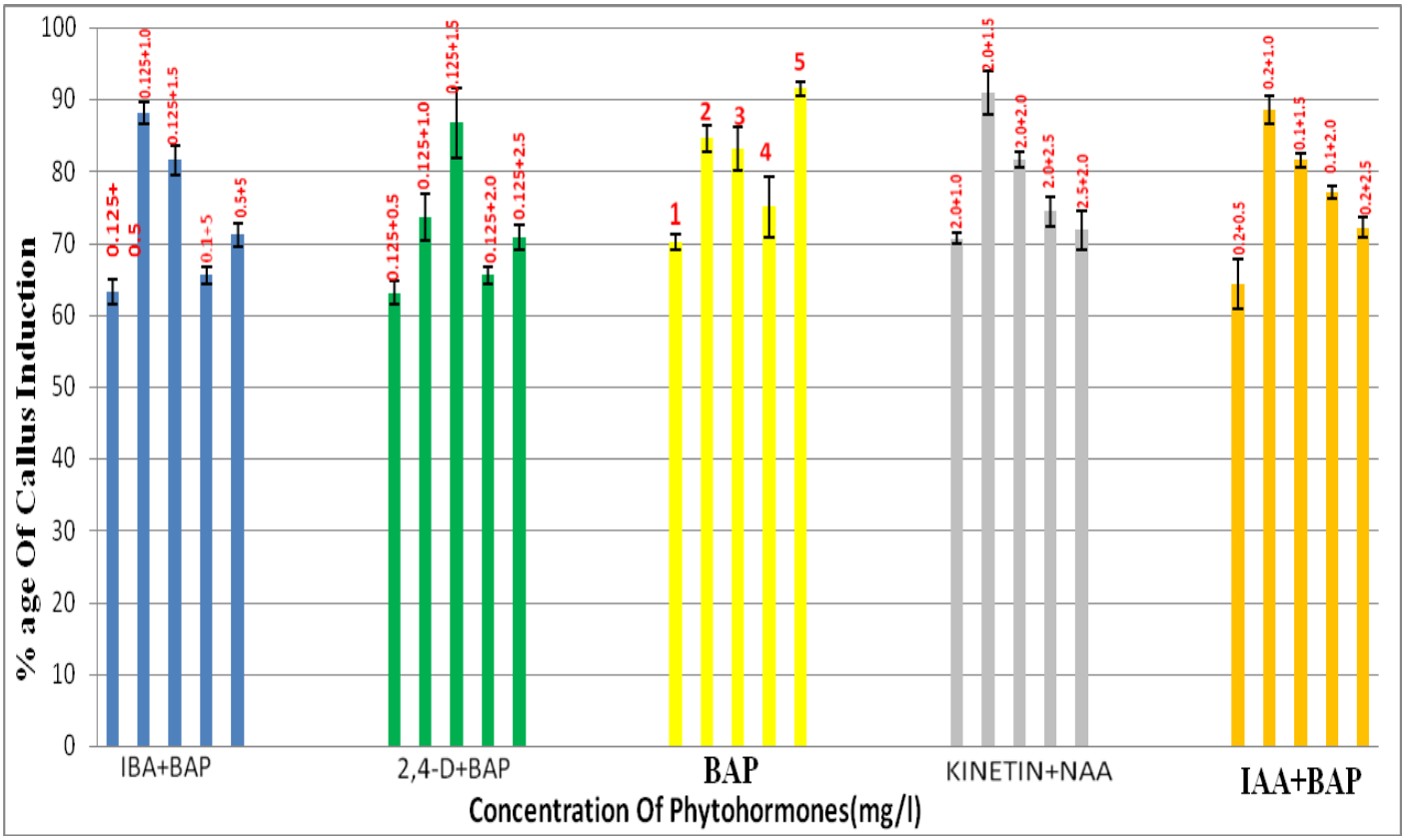

Fig. 1: Column graph showing % age of callus induction utilizing different combination and concentration of phytohormones from leaf explants of *Rauwolfia serpentina.*

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Sl.No	PGR (mg/l)	Concentration (mg/l)	% Age response of germination			Avg. %	S.D
			1st	2nd	3rd		
1.	IBA+BAP	0.125+0.5	66.25	64.85	62.4	63.14	1.73
		0.125+1.0	**88.52**	**86.45**	**89.35**	**88.10**	**1.49**
		0.125+1.5	81.54	83.65	79.54	81.57	2.05
		0.1+5.0	65.21	66.89	64.56	65.55	1.20
		0.5+5.0	72.54	71.65	69.23	71.14	1.71
2.	2,4-D+BAP	0.125+0.5	61.21	64.2	63.7	63.03	1.60
		0.125+1.0	76.62	74.21	70.14	73.65	3.27
		0.125+1.5	**81.24**	**88.56**	**90.33**	**86.71**	**4.81**
		0.125+2.0	65.21	66.89	64.56	65.55	1.20
		0.125+2.5	70.21	69.45	72.68	70.78	1.68
3.	BAP	1.0	69.21	71.23	70.1	70.18	1.01
		2.0	86.54	84.24	82.98	84.58	1.80
		3.0	83.67	85.79	79.87	83.11	2.99
		4.0	70.79	75.32	79.21	75.10	4.21
		5.0	**90.54**	**92.54**	**91.54**	**91.54**	**1.0**
4.	KINETIN+NAA	2.0+1.0	69.87	70.9	71.25	70.67	70.7
		2.0+1.5	**90.21**	**94.3**	**88.41**	**90.97**	**3.01**
		2.0+2.0	80.45	82.65	81.56	81.55	1.10
		2.0+2.5	76.58	72.4	74.26	74.41	2.09
		2.5+2.0	69.56	71.14	74.86	71.85	2.72
5.	IAA+BAP	0.2+0.5	67.2	65.22	60.54	64.32	3.42
		0.2+1.0	**88.65**	**86.54**	**90.48**	**88.55**	**1.97**
		0.1+1.5	82.51	80.65	81.54	81.56	0.93
		0.1+2.0	77.45	77.84	76.12	77.13	0.90
		0.2+2.5	70.58	73.24	72.65	72.15	1.39

Table :1 Callus induction (%) from leaf explants on MS media supplemented with various phytohormonal combinations & concentrations for *Rauwolfia serpentina.*

(a)

(b)

(c) **(d)**

Picture:2 (a), (b), (c) & (d) Stages of callus formation from leaf explants in *Rauwolfia serpentina*

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