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The Influence of Different Hormone Concentration and Combination on Callus Induction and Regeneration of *Rauwolfia serpentina* L. Benth

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Abstract: The influence of media composition on callus induction and subsequent regeneration of *Rauwolfia serpentina* L. Benth has been studied. High frequency (96.43%) callus induction was obtained when nodal segments from *in vitro* raised shoots were cultured on MS medium supplemented with 0.5 mg L^{-1} BA and 2.0 mg L^{-1} NAA. The callus differentiated into adventitious shoots when it was subcultured on MS medium supplemented with 2.0 mg L^{-1} BA with 0.2 mg L^{-1} NAA. Regenerated shoots were best rooted on half-strength MS medium with 1.0 mg L^{-1} each of IBA and IAA.

Key words: Propagation, callus, regeneration, indole alkaloids, endangered

INTRODUCTION

An endangered woody shrub *Rauwolfia serpentina* L. Benth belongs to the Apocynaceae family holds the tremendous potentialities for massive propagation and isolation of indole alkaloids through *in vitro* culture. Alkaloids of this plant have a great medicinal importance to treat cardiovascular diseases (Anitha and Kumari, 2006), hypertension (Von Poser *et al*., 1990), arrhythmia (Kirillova *et al*., 2001), various psychiatric diseases (Bhatara *et al*., 1997; Kirtikar and Basu, 1993), breast cancer (Stanford *et al*., 1986), human promyelocytic leukemia (Itoh *et al*., 2005) like complicated diseases.

Explant of an alkaloid producing plant, cultured *in vitro*, has been found to retain the capacity to synthesis alkaloids identical to that in the intact plant (Yoshimatsu and Shimomura, 1991). Sometimes, high yield of secondary metabolites is observed in tissue grown as callus masses produced during differentiation (Benavides and Caso, 1993; Maheshwari *et al*., 2007). Beside this callus culture facilitates optimization of alkaloids production (Yamamoto and Yamada, 1986; Premjet *et al*., 2002; Anitha and Kumari, 2006) and subsequent isolation (Kirillova *et al*., 2001).

As propagation by means of seeds is very much difficult due to low germination percentage (Salma *et al*., 2008) and still now traditional Unani and Ayurvedic practitioners are dependent on crude extract of roots of this plants; *in vitro* propagation scheme is utmost necessary to keep pace with its demand.

We here reported the influence of media composition on effective callus induction to facilitate modern approaches of alkaloids production as well as regeneration for the conservation of the threatened genotype.

MATERIALS AND METHODS

The experiment was carried out in the year 2007 in the Plant Tissue Culture Laboratory, Biological Research Division, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhanmondi, Dhaka, Bangladesh.

Nodal explants of *R. serpentina* from actively field grown plants were cleaned thoroughly under a continuous stream of running tap water for 30 min. After that the explants were washed with detergent and kept under running tap water for 15 min. Then treated with 1% Savlon and washed in running tap water for 30 min. Explants were surface sterilized with 50% (v/v) ethanol (1 min) and followed by 0.1% (w/v) $HgCl_2$ (3 min). Finally the explants were washed thoroughly three times with autoclaved double distilled water.

The nodal explants were then cut into appropriate size (6-8 mm) and cultured on MS (Murashige and Skoog,

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1962) medium containing 1.5 mg L^{-1} BA and 0.2 mg L^{-1} NAA. Nodal pieces (6-8 mm) taken from 6-week-old *in vitro* shoots were used to initiate callus culture. In this study we report the influence of plant growth regulators such as BA and NAA for effective callus and shoot induction. Regenerated shoots were rooted on half strength MS medium with IBA and IAA.

Media were adjusted to pH 5.8 before addition of 0.6% agar and autoclaved at 121°C for 15 min. All cultures were incubated at 25±1°C with a photoperiod of 16 h at 3000 lux light intensity of cool white fluorescent light.

Weekly visual observation of culture was made. All experiments were repeated twice with at least 18 cultures per treatment and data were taken after 4-6 weeks of culture.

RESULTS AND DISCUSSION

Nodal segments from 3-4 years old field grown plants produced multiple shoots within two weeks of culture. Within seven to fifteen days of culture callus formed at the cut surface of nodal pieces of *in vitro* raised shoots, when grown on MS medium supplemented with 0.5 and 1.0 mg L^{-1} BA in combination with 0.2-2.5 mg L^{-1} NAA. Maximum (96%) callus induction was observed on MS medium fortified with 0.5 mg L^{-1} BA and 2.0 mg L^{-1} NAA after two successive subcultures. In this combination yellowish green calli were developed (Fig. 1a, b). Percentages of callus induction, degree of callusing and type of callus have been shown in Table 1.

BA and NAA was the best hormones for callus induction as reported by a number of researchers (Polanco *et al*., 1988; Biswas *et al*., 2007). According to Dixon and Gonzales (1994), inclusion of an auxin and cytokinin will be necessary for callus growth and somewhat higher auxin concentration may be required for callus initiation. There were significant differences in callus induction frequencies and degree of callusing. Frequency of callusing increased with increasing concentration of auxin. However, when the auxin concentration surpassed 2.0 mg L^{-1} decreased level of callus induction was observed.

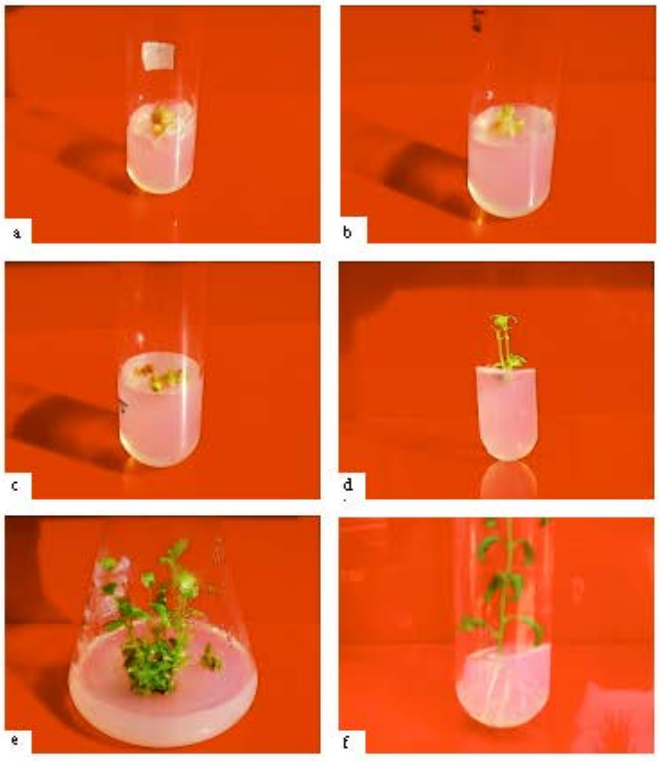

Fig. 1: Callus induction and regeneration of *Rauwolfia serpentina* L: (a) and (b) Induction of callus on MS medium supplemented with 0.5 mg L^{-1} BA + 2.0 mg L^{-1} NAA, (c) Shoot morphogenesis from callus on MS medium supplemented with 2.0 mg L^{-1} BA + 0.2 mg L^{-1} NAA, (d) Regenerated shoots in test tube on media same as Fig. (c), (e) Regenerated shoots in culture flask on media same as Fig. (c) and (f) Development of adventitious roots on half strength MS medium supplemented with 1.0 mg L^{-1} IAA + 1.0 mg L^{-1} IBA

Table 1: Effect of different concentrations and combinations of BA and NAA on callus induction

MS medium+hormones (BA+NAA) mg L^{-1}	% of callus induction	*Degree of callus	Type of callus
0.5+0.2	54.12	++	Yellowish green
1.0+0.2	42.18	+	
0.5+0.5	66.32	++	
1.0+0.5	64.32	++	
0.5+1.0	72.28	++	
1.0+1.0	69.24	++	
0.5+1.5	84.52	+++	
1.0+1.5	82.36	+++	
0.5+2.0	96.43	+++	
1.0+2.0	94.18	+++	
0.5+2.5	87.36	+++	
1.0+2.5	85.24	+++	

* + = Similar size of explant, ++ = Twice size of explant, +++ = Thrice size of explant

Table 2: Shoot organogenesis from callus cultures of *R. serpentina*

MS medium+hormones (BA+NAA) mg L^{-1}	Response (%)	No. of shoots ($\bar{X}$±SE)
1.0+0.1	55	2.5±0.10
1.0+0.2	45	2.1±0.10
1.5+0.1	65	2.7±0.09
1.5+0.2	65	2.4±0.09
2.0+0.1	75	2.9±0.06
2.0+0.2	80	3.1±0.06
2.5+0.1	75	2.8±0.07
2.5+0.2	70	2.8±0.08

Table 3: Effect of different concentrations of IBA and IAA in half strength MS medium on root formation in regenerated shoots

Concentration (mg L^{-1}) IBA+IAA	% of root formation	No. of total roots/culture ($\bar{X}$±SE)	Average length of roots/culture in cm ($\bar{X}$±SE)
0.1+0.1	35	2.01±0.06	1.54±0.07
0.5+0.5	65	3.43±0.08	3.00±0.04
1.0+1.0	80	5.01±0.05	4.38±0.07
1.5+1.5	50	3.0±0.06	2.62±0.08

Percentage of response for different growth regulator combination on shoot bud differentiation from callus was observed. The highest shoot morphogenesis (Fig. 1c) and regeneration (Fig. 1d, e) was obtained when callus were subcultured on MS medium supplemented with 2.0 mg L^{-1} BA and 0.2 mg L^{-1} NAA (Table 2). Though Kn with IAA and other media composition were used for regeneration purposes, BA and NAA were reported by many researchers (Sinha and Roy, 2002; Huda *et al*., 2003). Shoot regeneration via a callus phase was the way to induce somaclonal variation and thus pave the way for improvement of the species (Thorpe *et al*., 1991) and such indirect organogenesis have been described in many medicinal plant species including *Asparagus cooperi* (Ghosh and Sen, 1989), *Plumbago zeylanica* (Das and Rout, 2002), *Holostea ada-kodein* (Martin, 2002), *Rotula aquatica* (Martin, 2003), *Gloriosa superba* (Sivakumar *et al*., 2003), *Phellodendron amurense* (Azad *et al*., 2005).

Rooting experiments were conducted in half-strength MS medium supplemented with IAA and IBA. Root induction and elongation (Fig. 1f) was found to be more prominent in the medium containing 1.0 mg L^{-1} each of IAA and IBA resulted in 80% root initiation (Table 3). Roots elongated up to 4-5 cm within 15 days of incubation period.

The effectiveness of half-strength MS basal medium supplemented with auxin on root induction has been reported in many medicinal plants (Huda *et al*., 2003; Mederos-Molina, 2004; Ahmed *et al*., 2005). Root induction of *R. serpentina* was reported on half-strength MS medium supplemented with IAA or IBA alone (Ahmed *et al*., 2005) with an efficacy around 73%. But this study shown a higher root induction efficacy of 80% on half-strength MS medium supplemented with IAA and IBA combination. The root lengths were varied in all media concentrations.

R. serpentina is a medicinally important endangered herb. Propagation through tissue culture of this plant has been described earlier but most of them have focused on mass propagation by means of stimulating axillary shoot growth (Mathur *et al*., 1993; Roy *et al*., 1994). For the production of alkaloid identical to the mother plant (Yoshimatsu and Shimomura, 1991), high yield of secondary metabolites (Benavides and Caso, 1993; Maheshwari *et al*., 2007) and optimization of alkaloid production (Yamamoto and Yamada, 1986; Anitha and Kumari, 2006; Premjet *et al*., 2002) and subsequent isolation (Kirillova *et al*., 2001) effective callus mass production is necessary. Though some studies have been reported induction of callus and regeneration of plants from callus culture of *R. serpentina* (Sarker *et al*., 1996) but callus induction efficacy and the number of regenerated plantlets were not so high. So this study will be fruitful to make up the demand of this plant for commercial utilization and production of alkaloids as well as will help in the conservation of such threatened genotype.

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