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A novel protocol for micropropagation of *Rauvolfia serpentina* : In low concentration of growth regulators with sucrose and phenolic acid

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SUMMARY

A novel *in vitro* propagation system for *Rauvolfia serpentina* (Apocynaceae family), a medicinally important plant has been developed. 200 alkaloids have been isolated from the plant. In this study *in vitro* plantlets were directly regenerated from the apical segments in MS medium supplemented with different concentration of IBA (0.125-0.5 mg/l) in combination with BAP (0.5-2.0 mg/l), which are far below the limits reported by other workers making this process cost effective. For multiplication of shoots MS media supplemented with BA (0.1 mg/l) and different concentration (0-50 g/l) of sucrose. Use of sucrose especially the higher concentration has never been attempted before and gave very positive results. The elongated and multiple shoot primordial sub-cultured on to rooting media using two-step pulse treatment method using IBA (25-100 μM) and phenolic acid (1%). Rooted plantlets were successfully transferred to the field, after acclimatization in the net house.

Key words : *Rauvolfia serpentina*, Micro propagation, Regeneration. Nodal segment, Growth regulator.

Rauvolfia serpentina (Indian snake root), an important medicinal shrub, belongs to the Apocynaceae family. *Rauvolfia* root is bitter, acrid, laxative anthelmic, diuretic and sedative .The alkaloids are classified in to 3 groups, *viz.*, reserpine, ajmaline and serpentine groups.

Reserpine group comprising reserpine, rescinnamine, deserpine group etc. Ajmaline group comprising ajmaline, ajmalicine, ajmalinine, isoajmaline etc. Serpentine group comprising serpentine, alsotonine etc. (Husain, 1993; Iyengar, 1985). The root is a sedative and is used to control high blood pressure and certain forms of insanity (Ghani, 1998). In Ayurveda it is also used for the treatment of insomnia, epilepsy, asthma, acute stomachache and painful delivery. It is used in snake bite, insect stings and mental disorders. Reserpine is a potent hypotensive and tranquillizer but its prolonged usage stimulates prolactine release and cause breast cancer. The juice of leaves is used as a remedy for the removal of opacities of cornea. (Baksha *et al*., 2007). The present communication report is use of low concentration of growth regulators, sucrose and phenolic acid for regeneration of *Rauvolfia serpentina*.

MATERIALS AND METHODS

The methods of plant tissue culture were the standard method as described in plant cell, tissue and organ culture fundamental methods (Gamborg and Phillips, 2004). The explants used for the *in vitro* propagation of *Rauvolfia* were nodal pieces of 0.7-0.8 cm collected from 3-4 month old mature plants growing in medicinal plants nursery of Bhopal. The explants were washed thoroughly first under running tap water for 30 min to remove adherent particles, then treated with a liquid detergent for 20 min followed by washing in tap water and rinsed 5 times with double distilled water with 1 % savlon and finally treated with $HgCl_2$ (0.1%) for 6 minute in a laminar flow cabinet and washed four times with autoclaved double distilled water to remove any trace of $HgCl_2$ solution.

Culture media and incubation:

The basal medium used in all experiments was Murashige and Skoog (1962) mineral formulation (MS) containing standard salts, vitamins, 3% (w/v) sucrose, $CaCl_2$(0.44 gm) and 0.8% (w/v) agar. MS medium was used for induction of shoots and MS half media was used for induction of roots. The pH of the medium was adjusted to 5.8 before gelling with agar and autoclaved at 121°C at 15 lbs pressure for 20 min. The surface sterilized explants were inoculated on the above medium under aseptic conditions.

Shoot initiation and multiplication media:

For shoot induction, apical segment explants were placed on MS medium with 6-benzyladenine (BA) at different concentrations (0.5, 1.0, 1.5, 2.0 mg/l) either singly or in combination with indole-3-butyric acid (IBA) at different concentrations (0.125, 0.25, 0.37, 0.5 mg/l).

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