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Mass Propagation of *Rauwolfia serpentina* L. Benth

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Abstract: A protocol for mass propagation through axillary bud proliferation was established for *Rauwolfia serpentina* L. Benth. (Apocynaceae). MS medium supplemented with 1.5 mg L^{-1} BA and 0.2 mg L^{-1} NAA elicited the maximum number of shoots (4 multiple shoots) from nodal explants. These adventitious shoots were best rooted on half strength MS medium supplemented with 1.0 mg L^{-1} each of IBA and IAA. The *in vitro* raised plants were acclimatized in glass house and successfully transplanted to field condition with almost 95% survival.

Key words: Mass propagation, adventitious, heterogeneous, indole alkaloids, microcutting

INTRODUCTION

Rauwolfia serpentina L. Benth. is a medicinally important perennial herb belongs to the family Apocynaceae. The plant is indigenous to Bangladesh, India and other tropical region of the world (Roy *et al*., 1994). *R. serpentina* contains some 50 indole alkaloids and most of the total alkaloid content present mainly in root bark (Klyushnichenko *et al*., 1995). Among all the alkaloids reserpine, yohimbine, serpentine, deserpidine, ajmalicine, ajmaline, etc. are used to treat hypertension (Von Poser *et al*., 1990) and breast cancer (Stanford *et al*., 1986). Roots are used in traditional medicine as a valuable remedy for many complex diseases e.g., high blood pressure, insomnia, anxiety, excitement, schizophrenia, insanity, epilepsy, hypochondria and other disorders of the central nervous system (Bhatara *et al*., 1997; Kirtikar and Basu, 1993; Dastur, 1988). The root extract of this plant is also used to hasten the expulsion of the fetus, to treat painful affection of bowels, diarrhoea (Tona *et al*., 1999), dysentery, cholera and colic (Ghani, 1998).

For centuries, root of *R. serpentina* has been used in the traditional Unani and Ayurvedic medicine (Andrew and Chevallier, 1996). But during the middle of 20th century the importance of its major alkaloid reserpine has attracted much attention in the field of allopathic medicine as a remedy for hypertension (Von Poser *et al*., 1990; Vakil, 1949), insomnia and schizophrenia (Bhatara and Gupta, 1997; Bleuler and Stoll, 1955).

Mass scale collection of this plant from natural habitat by the pharmaceutical industries as well as local ayurvedic and unani practitioners is leading to a depletion of this plant resource. Propagation by means of seeds to replenish the exhausting supply might prove ultimately even unwise, since of its poor seed viability and very low germination percentages (25-50%) that may be ascribed largely to the presence of cinamic acid derivatives in the seeds (Mitra, 1976). However, alkaloid content might get reduced in successive progenies through adverse gene recombination (Anonymous, 1950).

In vitro propagation studies of different plant species have shown that the technique may be a solution for rapid propagation of such selected useful plant species and subsequent exploitation (Bonga and Durjan, 1987). It also has been found that explant of an alkaloid producing plant, cultured *in vitro* retain the capacity to synthesis alkaloids to that in the intact plant (Sarker *et al*., 1996). On the other hand *in vitro* micropropagation has a number of advantages over sexual propagation (Abbott, 1978). In sexual method superior genotypes may lost through recombination but micropropagation can preserve superior gene combinations practically unaltered. *In vitro* propagation of *R. serpentine* has been reported by many researchers as Ahmad *et al*. (2002), Sarker *et al*. (1996), Roy *et al*. (1994) and Mathur *et al*. (1993). The special purpose of current study was to identify most suitable media supporting competence necessary for large-scale propagation scheme to replenish the exhausting supply and to conserve the threatened species.

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MATERIALS AND METHODS

The experiment was carried out in the Plant Tissue Culture Laboratory, Biological Research Division, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhanmondi, Dhaka, Bangladesh in the year 2007. Nodal segments containing buds of 3-4 years old plants were collected from the medicinal plant garden of BCSIR.

For surface sterilization, explants were cut into small pieces and cleaned thoroughly under a continuous stream of running tap water for 30 min. After that the segments 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. The explants were then taken under laminar airflow cabinet and surface sterilized with a 0.1% $HgCl_2$ for 5 min followed by their washing three times with autoclaved double distill water. Explants of approximately 1 to 2 cm in length were cut and inoculated aseptically onto MS (Murashige and Skoog, 1962) media supplemented with different concentrations and combinations of auxin and cytokinine. The pH of the medium were adjusted to 5.8±0.05 before adding agar and the media were autoclaved at 1.1 kg cm^{-1} for 20 min at 121°C. Cultures were incubated at 25±1°C with a photoperiod of 16 h at 3000 lux light intensity of cool white fluorescent light.

All cultures were initiated in 150×25 mm^2 glass tube containing 15-20 mL of medium. The cultures were regularly subcultured on fresh medium at 4 weeks intervals in glass tubes or 100 mL flasks. Observations were recorded every 5 days following inoculation and subculturing. For inducing adventitious shoots and their development, nodal segments containing buds were cultured on MS medium supplement with 0.5-2.0 mg L^{-1} BAP with NAA (0.2, 0.5) mg L^{-1}. Auxiliary shoots produced within four weeks of culture were isolated and regularly subcultured on fresh medium at four-week intervals. In rooting experiments proliferated shoot of approximately 2-4 cm length were rescued aseptically from culture vessels and transferred to freshly prepared half strength MS macro and micro-nutrients contained in glass tube. IBA and IAA (0.1-1.5) mg L^{-1} were used as supplementary with the media for root induction. Culture tubes containing rooted plantlets were kept in a room of normal temperature (30±2°C) and normal daylight for 7 days. Plantlets were then taken out from the culture tubes and washed carefully under running tap water for complete removal of media. Then the plantlets were transplanted to small plastic pots containing garden soil and compost in a ratio of 2:1. The pots were immediately covered with polythene bag to prevent desiccation. After the hardening period the plantlets were transferred to field condition (Fig. 1).

Fig. 1: Mass Propagation of *Rauwolfia serpentina* from shoot node derived culture: (a) Formation of Multiple Shoots in test tube on MS media supplemented with BA 1.5 mg L^{-1} + 0.5 mg L^{-1}. (b) Shoot multiplication in flask on media same as Fig. (a). (c) Development of adventitious roots on half strength MS medium supplemented with IAA 1.0 mg L^{-1} + IBA 1.0 mg L^{-1}. (d) Transplantation of plantlets into plastic pots

Observations were recorded every week following inoculation and subculturing. 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

Shoot induction was observed on all the used concentrations and combinations of BA and NAA. MS supplemented with very low level of BA with NAA exhibited low percentage of shoot formation and the response for average shoot length was also very poor. Multiple shoots emerged from the nodal explants within two weeks of incubation. Among different concentrations of growth hormone tested, 1.5 mg L^{-1} BA and 0.2 mg L^{-1} NAA induced 90% shoot formation and elicited the maximum number of shoots (4 multiple shoots) from nodal explants (Table 1). These shoots attained a height of 4-6 cm within four weeks of additional culture. There were significant differences in shoot formation frequencies, number of shoots per culture and length of shoots per culture.

Rooting experiments were conducted in half-strength MS supplemented with IAA and IBA. Root induction was found to be more prominent in the medium containing 1 mg L^{-1} each of IAA and IBA resulted in 80% root initiation (Table 2). Roots elongated up to 4-5 cm within 15 days of incubation period.

The rooted plantlets were successfully transferred to hardening and well established in field condition (Fig. 1). The survival rate was 95% and plant showed normal growth with similar phenotype of mother plants.

Multiple shoot formation from nodal segment was reported using higher concentration of growth hormone (Verma *et al*., 2002; Selvakumar *et al*., 2001; Sudha and Seeni, 1996). The present study exemplifies a positive modification of shoot induction and multiplication efficacy on MS basal media supplemented with very low concentrations of auxin with cytokinin.

Although Kn is reported to promote shoot bud initiation in nodal explants of many plant species, BA is the most efficient cytokinin for the axillary bud initiation and subsequent proliferation (Hamdy and Hattori, 2006; He *et al*., 2005; Baskaran and Jayabalan, 2005; Gupta *et al*., 2001). Addition of auxin at low concentration significantly enhanced the growth of culture and elongation of proliferated shoots (Hu and Wang, 1983). Manipulation of cytokinin (BA) and auxin (NAA) concentration was found to be very important for effective mass scale propagation. Excision and culture of the nodal segments from *in vitro* derived shoots facilitated the development of increased number of shoots.

Table 1: Effect of different concentrations and combinations of BA and NAA on shoot proliferation

Concentration (mg L^{-1}) BA+NAA	Shoot formation (%)	No. of total shoots/explant	Average length of shoots/culture
0.5+0.2	45	1.70±0.15	1.91±0.10
0.5+0.5	55	2.09±0.16	2.03±0.09
1.0+0.2	65	3.23±0.22	3.45±0.12
1.0+0.5	60	2.67±0.30	3.15±0.09
1.5+0.2	90	3.89±0.15	4.08±0.12
1.5+0.5	70	3.53±0.13	3.71±0.20
2.0+0.2	80	3.40±0.17	3.49±0.12
2.0+0.5	65	3.00±0.15	3.25±0.06

Table 2: Effect of different concentrations of IBA and IAA in half strength MS medium on root formation in regenerated shoots

Concentration (mgL^{-1}) IBA+IAA	Root formation (%)	No. of total roots/culture	Average length of roots/culture
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	3.00±0.06	2.62±0.08

The effectiveness of half-strength MS basal medium supplemented with auxins on root induction has been reported in many medicinal plants (Ahamed *et al*., 2005; Mederos-Molina, 2004; Huda *et al*., 2003). Root induction was reported on half strength MS supplemented with IAA and IBA alone (Ahamed *et al*., 2005; Vesperinas, 1998) with an afficacy around 73%. But this study shows a higher root induction efficacy of 80% on half strength MS supplemented with IAA and IBA combination. The root lengths were varied in all media concentrations.

The ultimate success of *in vitro* propagation lies in successful establishment of plants in the soil. 80-85% survival of plantlets were recorded in the experiments of previous workers (Sudha and Seeni, 1996; Roy *et al*., 1994). The high survival rate in the present study indicates that this procedure could be easily adapted for large-scale propagation. As *R. serpentina* holds the tremendous potentialities for massive propagation towards the commercialization of many invaluable indole alkaloids this present study will be fruitful for this novel application and will help in the conservation study of such threatened species.

REFERENCES

Abbott, A.J., 1978. Practice and promise of micropropagation of woody species. Acta Hort., 79 (1): 113-127.

Ahamed, A.B.A., T. Gouthaman, A.S. Rao and M.V. Rao, 2005. Micropropagation of *Phyla nodiflora* (L.) Green: An important medicinal plant. IJB., 3 (3): 186-190.

Ahmad, S., M.N. Amin, M.A.K. Azad and M.A. Mosaddik, 2002. Micropropagation and plant regeneration of *R. serpentina* by tissue culture technique. Pak. J. Biol. Sci., 5 (1): 75-79.

Andrew and Chevallier, 1996. The Encyclopedia of Medicinal Plants. 1st Edn. DK Publishing Inc., New York, USA., pp: 259.

Anonymous, 1950. The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products. Raw Materials Vol. 8. Publication and Information Directorate, CSIR, New Delhi, pp: 376-391.

Baskaran, P. and N. Jayabalan, 2005. An efficient micropropagation system for *Eclipta alba*. A valuable medicinal herb. *In vitro* Cell Dev. Biol., 41 (4): 532-539.

Bhatara, V.S. and S. Gupta, 1997. History of *Rauwolfia serpentina*. Can. J. Psychiatry, 42 (7): 777-778.

Bhatara, V.S., J.N. Sharma, S. Gupta and Y.K. Gupta, 1997. Images in psychiatry. *Rauwolfia serpentina*: The first herbal antipsychotic. Am. J. Psychiatry, 154 (7): 894.

Bleuler, M. and W.A. Stoll, 1955. Clinical use of reserpine in psychiatry: Comparison with chlorpromazine. Ann. New York Acad. Sci., 61 (1): 167-173.

Bonga, J.M. and D.J. Durjan, 1987. Cell and Tissue Culture in Forestry. Vols. 1, 2, 3. Martinus Nijhoff Publ., Dordrecht.

Dastur, J.F., 1988. Medicinal Plants of India and Pakistan. D.B. Taraporevala and Sons, New Delhi, pp: 141-142.

Ghani, A., 1998. Monographs In: Medical Plants of Bangladesh: Chemical Constituents and Uses. 2nd Edn. Asiatic Soc. Bangladesh, pp: 276.

Gupta, S.K., S.P.S. Khanuja and S. Kumar, 2001. *In vitro* propagation of *Lippia alba*. Curr. Sci., 81 (2): 206-210.

Hamdy, M.A.A. and K. Hattori, 2006. *In vitro* micropropagation of (*Vicia faba* L.) cultivars Waza Soramame and Cairo 241 by nodal explants proliferation and somatic embryogenesis. Biotechnology, 5 (1): 32-37.

He, M.L., S.Y. Qi and L.J. Hu, 2005. Rapid *in vitro* propagation of medicinally important *Aquilaria agallocha*. Zhejiang Univ. Sci. B, 6 (8): 849-852.

Hu, C.Y. and P.J. Wang, 1983. Meristem, Shoot Tip and Bud Culture. In: Handbook of Plant Cell Culture, Evans, D.A., W.R. Sharp, P.V. Ammirato and Y. Yamada (Eds.). Macmillan Publ. Co., New York, pp: 177-227.

Huda, S., R. Islam, M.A. Bari and M. Asaduzzaman, 2003. Shoot differentiation from cotyledon derived callus of chickpea (*Cicer arietinum* L.). Plant Tissue Cult., 13 (1): 53-59.

Kirtikar, K.R. and B.D. Basu, 1993. Indian Medicinal Plants. Vol. 2. Dehra Dun Publishers, Culcutta, India, pp: 289.

Klyushnichenko, V.E., S.A. Yakimov, T.P. Tuzova, Ya. V. Syagailo, I.N. Kuzovkina, A.N. Wulfson and A.I. Miroshnikov, 1995. Determination of indole alkaloids from *R. serpentina* and *R. vomitoria* by high-performance liquid chromatography and high-performance thin-layer chromatography. J. Chromatogr. A, 704 (2): 357-362.

Mathur, A., P.S. Ahuza and A.K. Mathur, 1993. Micropropagation of *Panax quinquefolium*, *R. serpentina* and Some Other Medicinal and Aromatic Plants of India. In: Adapted Techniques for Commercial Crops of the Tropics, Quynh, N.T. and N.V. Uyen Agricultural Publishing House, Ho Chi Minh City, pp: 155-173.

Mederos-Molina, S., 2004. *In vitro* callus induction and plants from stem and petiole explants of *Salvia canariensis* L. Plant Tissue Cult., 14 (2): 167-172.

Mitra, G.C., 1976. Studies on the formation of viable and nonviable seeds in *R. serpentine*. Ind. J. Exp. Biol., 14 (1): 54.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 15 (3): 473-497.

Roy, S.K., M.Z. Hossain and M.S. Islam, 1994. Mass propagation of *Rauwolfia serpentina* by *in vitro* shoot tip culture. Plant Tissue Cult., 4 (2): 69-75.

Sarker, K.P., A. Islam, R. Islam, A. Hoque and O.I. Joarder, 1996. Plant regeneration of *Rauwolfia serpentina* by organogenesis from callus cultures. Plant Tissue Cult., 6 (1): 63-65.

Selvakumar, V., P.R. Anbudurai and T. Balakumar, 2001. *In vitro* propagation of the medicinal plant *Plumbago zeylanica* L. through nodal explants. *In vitro* Cell Dev. Biol., 37 (2): 280-284.

Stanford, J.L., E.J. Martin, L.A. Brinton and R.N. Hoover, 1986. Rauwolfia use and breast cancer: A case-control study. J. Natl. Cancer Inst., 76 (5): 817-822.

Sudha, C.G. and S. Seeni, 1996. *In vitro* propagation of Rauwolfia micrantha, a rare medicinal plant. Plant Cell Tissue Organ Cult., 44 (3): 243-248.

Tona, L., K. Kambu, K. Mesia, K. Cimanga, S. Apers, T. De Bruyne, L. Pieters, J. Totte and A.J. Vlietinck, 1999. Biological screening of traditional preparations from some medicinal plants used as antidiarrhoeal in Kinshasa, Congo. Phytomedicine, 6 (1): 59-66.

Vakil, R.J., 1949. A clinical trial of *Rauwolfia serpentina* in essential hypertension. Br. Heart J., 11 (4): 350-355.

Verma, P.C., D. Singh, L. Rahman, M.M. Gupta and S. Banerjee, 2002. *In vitro* studies in *Plumbago zeylanica*: Rapid micropropagation and establishment of higher plumbagin yielding hairy root cultures. J. Plant Physiol., 159 (5): 547-552.

Vesperinas, E.S., 1998. *In vitro* root induction in hypocotyls and plumule explants of *Helianthus annuus*. Environ. Exp. Bot., 39 (3): 271-277.

Von Poser, G., H.H. Andrade, K.V. Da Silva, A.T. Henriques and J.A. Henriques, 1990. Genotoxic, mutagenic and recombinogenic effects of rauwolfia alkaloids. Mutat. Res., 232 (1): 37-43.