Bangladesh J. Sci. Ind. Res. **42**(1), 37-44, 2007

In vitro Rapid Clonal Propagation of *Rauvolfia serpentina* (Linn.) Benth

R. Baksha,[a] Miskat Ara Akhter Jahan,[b] Rahima Khatun and John Liton Munshi[b]

[a]Biotechnology Division, BRRI, Gazipur -1701 and [b]Biological Research Division, BCSIR Laboratories, Dhaka-1205, Bangladesh

Abstract

A protocol was developed for *in vitro* clonal propagation of *Rauvolfia serpentina* through direct regeneration from shoot tip explants. Multiple shoots (eight shoots per explant) induction were obtained on MS medium supplemented with 4.0 mg/l BAP and 0.5 mg/l NAA within 10-15 days. The elongated shoots rooted well in half strength MS medium with 0.5 mg/l NAA. The *in vitro* raised plantlets were acclimatized in glass house and successfully transplanted to field condition with 80 % survival. The results indicated that large scale commercial micropropagation of *Rauvolfia serpentina* is technically feasible.

Introduction

Rauvolfia serpentina (Linn.) Benth, an important medicinal plant under shrub, belongs to the Apocynaceae family. The plant is indigenous to Bangladesh, India and other regions of Asia and found to grow in the wild in many places around the country (Ghani, 1998). Few drugs have attracted such a world wide attention as the roots of *Rauvolfia serpentina*. For centuries, the drug *Rauvolfia* has been used in the Ayurvedic system of medicine in India. It is only during the last decade that the importance of the drug and of its major alkaloid reserpine has been recognized in the allopathic system in the treatment of hypertension and as a sedative or tranquilizing agent (The wealth of India, 1950). Its roots contain 50 indole alkaloids including the therapeutically important reserpine, deserpidine, rescinnamine and yohimbine. This herbal plant is used as medicine for high blood pressure, insomnia, anxiety and other disorders of the central epilepsy (Ghani, 1998). Major part of the commercial supply of the drug used in U.S.A. and European countries originates from India, Pakistan, Cylon, Burma and Thailand, India being a major supplier. India which holds almost a world monopoly has been threatened with the depletion of wild resource of the plant with the increasing

demand. Despite their wide geographical distribution and edaphic tolerance, *Rauvolfia* species have not lent themselves to easy cultivation due to various factors which influence their propagation, growth and development and also their alkaloid content. At present all supplies of *Rauvolfia serpentina* roots are furnished by wild plants. Since supplies from wild sources are limited, it may not be possible to maintain a sustained and steady supply at the present rate of exploitation (1950).

Propagation by direct sowing of seeds in the field has not been found successful. Sun-dried and stored seeds generally gave a low rate of germination and seeds stored more than 7-8 months practically did not germinate. The germination percentage of seed is very poor and variable (25-50 %) and is often as low as 10 percent. Propagation by means of seeds might prove ultimately even unwise, since variation in alkaloidal yield is apparently genetically controlled and might get reduced in successive progenies through adverse gene recombination (1950).

At present, *Rauvolfia* reserves are becoming increasingly exhausted while the requirements of pharmaceutical industry in raunatine, reserpine, ajmaline etc. can not be met. *In vitro* propagation studies of different plant species has shown that this technique may be a solution for rapid propagation of such selected useful plant species and subsequent exploitation (Bonga, 1987; Boro 1998). *In vitro* regeneration of *Rauvolfia* have been reported by many authors (Butenka, 1964; Mitra and Kaul, 1964; Vollosovich and Butenka, 1970; Kukreja *et. al.* 1989 and Roy *et. al.* 1994). The present study was undertaken to develop a more efficient protocol for rapid *in vitro* multiplication of *Rauvolfia serpentina* using shoot tip explant as an initial plant material.

Materials and Methods

The experiment was conducted at Biological Research Division in Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh during the period of 2004. The apical shoots with leaf primordia of two nodes (2-4 mm long) were collected from the field grown three months old of *Rauvolfia serpentina* and washed thoroughly under running tap water. For surface sterilization the shoot tips were then treated with 1 % savlon for 10 minutes with constant shaking. Then these were washed four times with distilled water. Finally the shoot tips were treated with 0.1 % $HgCl_2$ solution for 10 minutes under laminar air flow cabinet and washed three times with double distilled water to remove any trace of $HgCl_2$. After surface sterilization the shoot tips of 2-4 mm long were excised and placed on MS (Murashige and Skoog, 1962) medium supplemented with different combinations of

auxin and cytokinin to identify appropriate combinations for multiple shoot regeneration of *Rauvolfia serpentina*. Media consisted of 3 % sucrose, 0.6 % agar, pH was adjusted to 5.7 before addition of agar and autoclaved at 120^{O} C for 15 minutes. All cultures were inoculated in 250 ml flasks containing 150 ml medium. Explants were kept at 25 ± 2^{O} C under 16 hours photoperiod regime at 2000 lux light intensity of cool white fluorescent light. Elongated healthy micro shoots were then excised and implanted to the rooting medium consisting of half strength MS supplemented with IBA and NAA in different concentrations. After initiation of root the plantlets were carefully separated from the medium to avoid damaging and washed with tap water to remove the agar adhering to them. The plantlets were then transplanted to polythene bags containing a mixture of soil and sand (2:1). The plantlets were kept in a glass house for one week with normal light and temperature. New leaves emerged from the plantlets after 10-15 days in the glass house; they were then placed in the normal environment for 1h and assessed for signs of wilting. The exposure time was increased daily until the plants were fully acclimatized. When the plantlets were established fully under normal environmental conditions, they were transferred into the field for their normal growth. The cultures were regularly subcultured on fresh medium at four weeks intervals and observations were recorded. Each experiment consisted of 25 replicates and repeated thrice. The means and standard errors of the results were also calculated.

Results and Discussion

Shoot tip explants from field grown three months old mature plants of *Rauvolfia serpentina* were cultured on MS medium supplemented with various concentrations of BAP and NAA in combination (Table I). Initiation of multiple shoot regeneration and

Table I. Effect of different concentrations and combinations of auxins and cytokinins on multiple shoot formation in *Rauvolfia serpentina*

Growth regulators (mg/l)	% of explant producing shoot	Number of shoots / explant	Mean length of shoot(cm)
BAP + NAA			
0.5 + 0.5	55	2.2 ± 0.81	1.5 ± 0.31
1.0 + 0.5	60	3.2± 0.02	1.8 ± 0.50
2.0 + 0.5	75	4.5± 0.52	2.5± 0.2
4.0 + 0.5	85	7.5 ± 0.50	4.0 ± 0.16
5.0 + 0.5	50	2.0 ± 0.52	1.5 ± 0.22

Each treatment consist of 25 replicates.

elongation of shoot primordia started after two weeks of culture (Fig. 1). The best and rapid multiple shoot potentiality was observed on MS medium, supplemented with

Fig.1. Development of auxiliary shoots and elongation of shoot buds from shoot tip explant after two weeks of culture

BAP 4.0 mg/l + NAA 0.5 mg/l, in which highest percentage (85) of response were observed with 7-8 multiple shoots per culture, within four weeks (Fig. 2). The response of explants were also observed on, BAP (0.5, 1.0 and 2.0 mg/l) with NAA 0.5 mg/l supplemented media. The percentage of response on the above concentrations were 55, 60 and 75 with 2.2, 3.2 and 4.5 multiple shoots per explant respectively. However, increased rate of BAP (5.0 mg/l) in the medium resulted in low percentage of response 50, over growth of leaves and shortening of internodes (Fig. 3). Cytokinins have been defined as substances that stimulate cell divisions in

Fig.2. Development and multiplication of auxiliary shoots on MS containing BAP 4.0 mg/l + NAA 0.5 mg/l after four weeks of culture

plants and interact with auxin in determining the direction of cell differentiation (Wareing and Phillips, 1981). Effectiveness of BAP + NAA for *in vitro* shoot regeneration and multiplication from shoot tip cultures were

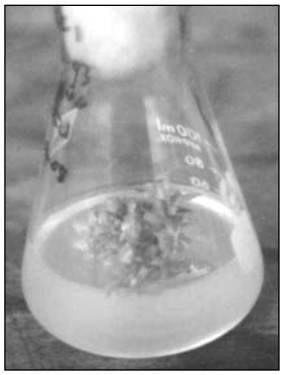

Fig.3. Over growth of leaves and shortening of internodes on MS containing BAP 5.0 mg/l after four weeks of culture

also reported in several other plants (Litz and Conver, 1978; Tokuhara and Mii, 1993; Liao *et. al.* 2004). Genotype, tissue type and developmental stage may all be determining factors in the comparative ability to respond to auxin or cytokinin. Rapid multiple shoot proliferation throughout the year was observed when axillary buds of regenerated shoots were subcultured individually in the same nutrient medium. Average of 20 cultures showed 35 shoots per flask (Fig. 4) when subcultured on the same medium every eight weeks. For root induction individual regenerated healthy shoots (5-6 cm long)

Fig.4. Development and multiplication of auxiliary shoots on MS containing BAP 4.0 mg/l + NAA 0.5 mg/l after eight weeks of culture

were excised and transferred on half strength MS medium supplemented with 0.5 to 2.0 mg/l of IBA and NAA. Rooting like many herbaceous crop plants is a minor problem (Hu and Wang, 1983), in *Rauvolfia* almost 100 % rooting was attained in medium containing NAA (0.5 mg/l) and sucrose (30 gm/l) within 10-15 days (Table II) (Fig. 5). Root formation was not observed when shoots were cultured on a medium lacking auxin. The superiority of NAA over other auxins has also been reported for other plant species such as *Caphaelis ipecacuanha* (Jha and Jha, 1989), *Plantago ovata* (Wakhlu and Barna, 1989), *Rehum emodi* (Lal and Ahuja, 1989). The plantlets with well developed roots and shoots were transferred to small polythene bags containing a mixture of soil and sand (2:1) and then acclimatized gradually to the normal environment. Then the plants were finally transferred to soil after two weeks. *In vitro* raised plantlet cannot survive when directly placed in a glass house

Table II. Effect of different auxins in half strength MS medium on rooting of micro shoots in *Rauvolfia serpentina*

Auxin (mg/l)	Response (%)	Average no. of roots/shoot
NAA		
0.5	100	3.5 ± 0.31
1.0	70	2.8 ± 0.50
2.0	55	1.5 ± 0.15
IBA		
0.5	85	2.5 ± 0.45
1.0	65	1.8 ± 0.50
2.0	45	1.5 ± 0.25

Each treatment consist of 25 replicates

Fig.5. Adventitious healthy root formation of regenerated shoots on MS containing NAA 5.0 mg/l after 10-15 days of culture

or field. Acclimatization is necessary because tissue culture raised plantlets are not suited for *in vivo* conditions. The physiological and anatomical characteristics of micropropagated plantlets necessitate that they should be gradually acclimatized to the environment in the green house or field (Hazarika, 2003). The survival of the plantlets under field condition depend not only on the proper environmental conditions but also on the proper growth and development conditions of the regenerated plants (Baksha *et. al*, 2003). Almost 80 % of the regenerated plants survived and showed a vigorous growth without any morphological variation.

For successful micropropagation axillary buds or shoot tip cultures are preferred as pre existing meristems, are capable of developing into shoots with clonal fidelity. It is believed that only clones derived from meristem, shoot tip and bud cultures are generally phenotypically homogenous thereby indicating stability (Hu and Wang, 1983). 1In the present investigation, a high frequency and rapid shoot induction was observed in *Rauvolfia serpentina* through shoot tip explants with BAP (4.0 mg/l) and combination of NAA (0.5 mg/l). For root induction NAA (0.5 mg/l) was found to be ideal growth regulator. For commercial exploitation this method for rapid micropropagation of *Rauvolfia serpentina* (a valuable medicinal plant) is reliable and promising.

Reference

Baksha, R. Alam, R. Mannan, Sk.A. and Rahman, A.B.M.M. (2003) Acclimatization of micropropagated sugarcane (*Saccharum officinarum*) plants for transplantation under field condition. *Pak Sugar Journal*. **28**(5): 2-5.

Bonga, J.M. and Durjan, D.J. (1987) Cell and tissue culture in forestry. Vols. 1,2,3, Martinus Nijhoff Publ., Dordrecht.

Boro, P.S. Sharma Deka, A.C. and Kalita, M.C. (1998) Clonal propagation of *Alternanthera sessilis* : A biopharmaceutically potent

herbal medicinal plant. *J. Phytol. Res.* **11**: 103-106.

Butenka, R.G. (1964) Isolated tissue culture and physiology of plant morphogenesis. Nauka, Moscow.

Ghani, A. (1998) Medicinal plants of Bangladesh. Chemical constituents and uses. Asiatic Society of Bangladesh, Ed. 2nd pp. 36.

Hazarika, B.N. (2003) Acclimatization of tissue cultured plants. *Current Science.* **85**(12): 1704-1712.

Hu, C.Y. and Wang, P.J. (1983) Meristem, shoot tip and bud cultures. *In*: Handbook of Plant Cell Culture, Evans, D.A, W. R. Sharp, P.V. Ammirato and Y. Yamada (Eds). Vol.1; 177-227. MacMillan Publishing Company, New York.

Jha, S. and Jha, T.B. (1989) Micropropaga-tion of *Caphaelis ipecacuanha*. *Plant Cell Rep*.**8**: 437-439.

Kukreja, A.K. Mathur, A.K. Ahuja, P.S. and Thakur R.S. (1989) Tissue Culture and Biotechnology of Medicinal and Aromatic Plants. ICSIR, Lucknow, India.

Lal, N. and Ahuja, P.S. (1989) Propagation of Indian rhubarb (*Rheum emodi* Wall.) using shoot tip and leaf explants culture. *Plant Cell Rep.* **8**: 439-496.

Liao, Z. Chen,M. Tan, F. Sun, X. and Tang, K. (2004) Micropropagation of endangered Chinese aloe. *Plant Cell Tissue and Organ Culture.* **7641**: 83-86.

Litz, R.E. and Conver, R.A. (1978) *In vitro* propagation of sweet potato (*Ipomoea batata* L. Poir.) *Hort. Sci.* **13**: 659-660.

Mitra, G.C. and Kaul, K.N. (1964) *In vitro* culture of root and stem callus of *Rauwolfia serpentina* Benth. for reserpine. *Indian J. Exp Biol* **2**: 49-51.

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **15**: 443-477.

Roy, S.K. Hossain, M.Z. and Islam, M.S. (1994) Mass Propagation of *Rauvolfia serpentina* by *in vitro* Shoot Tip Culture. *Plant Tissue Cult.* **4** (2): 69-75.

The Wealth of India : A Dictionary of Indian Raw Materials and Industrial Products. Raw Materials Vol.VIII, Publication and Information Directorate, CSIR, New Delhi, (1950) P: 376-391.

Tokuhara, K. and Mii, M. (1993) Micropropagation of *Phalaenopsis* sp. and *Doritaenopsis* sp. by culturing shoot tip of flower stalk buds. *Plant Cell Rep.* **13**: 7-11.

Vollosovich, A.G. and Butenka, R.G. (1970) Tissue culture of *Rauwolfia serpentina* as a resource of alkaloids. In: Culture of isolated organs, tissues and cells of plant. 253-257. Butenka, R.G. (Ed) Nauka, Moscow.

Wakhlu, A.K. and Barna, K.S. (1989) Callus initiation, growth and plant regeneration in *Plantago ovata* Forsk.cv.Gl-2, *Plant Cell, Tissue and Organ Cult.* **17**: 235-241.

Wareing, P.F. and Phillips I.D.J. (1981) The control of Growth and differentiation in plants. Pergamon Prees. New York. Ed. 3rd.

Received : February 22, 2006;
Accepted : February 11, 2007