Journal of Biological Research-Thessaloniki **13**: 85 – 92, 2010
J. Biol. Res.-Thessalon. is available online at http://www.jbr.gr
Indexed in: WoS (Web of Science, ISI Thomson), SCOPUS, CAS (Chemical Abstracts Service) and DOAJ (Directory of Open Access Journals)

In vitro cloning of *Rauvolfia serpentina* (L.) Benth. var. CIM-Sheel and evaluation of its field performance

Yogeshwar MISHRA*, Gufran USMANI and A.K. MANDAL

Tropical Forest Research Institute, P.O.-R.F.R.C., Mandla Road, Jabalpur, 482021 (M.P.), India

Received: 9 February 2009 Accepted after revision: 11 June 2009

A protocol was developed for *in vitro* clonal propagation of *Rauvolfia serpentina* (L.) Benth. var. CIM-Sheel through direct regeneration from nodal segment explants. The maximum response (98.33%) of bud out-growth was obtained on MS (Murashige & Skoog, 1962) medium supplemented with 1 μM BA (6-benzyladenine) + 1 μM NAA (α-naphthalene acetic acid) with highest axillary shoot number (7.66). Single shoots cultured on MS medium supplemented with 10 μM BA alone produced maximum number of multiple shoots (9.16) within 20-25 days. The elongated shoots, pulse-treated with 50 μM NAA for 24 h, rooted *ex vitro* successfully within 30 days. The plantlets were maintained in polythene bags for 1½ to 2 months in shadehouse irrigated every other day and successfully transplanted to field conditions with 90% survival rate after six months of transfer. Twelve to thirteen month-old micropropagated plants displayed uniform flowering, fruiting, seed germination, morphological growth characteristics and root yield.

Key words: *ex vitro* rooting, flowering, microshoots, seed germination, shoot multiplication.

Abbreviations: BA – 6-benzyladenine; IBA – indole 3-butyric acid; MS – Murashige and Skoog (Murashige & Skoog); NAA– α-naphthalene acetic acid.

INTRODUCTION

Rauvolfia serpentina (L.) Benth. is an important medicinal plant, which belongs to the Apocynaceae family, and is indigenous to India and other tropical countries of Asia. Only a few drugs have attracted such a worldwide attention as the roots of *Rauvolfia serpentina*. The roots contain 50 indole alkaloids including the therapeutically important reserpine, deserpidine, rescinnamine and yohimbine. According to Ayurveda, the roots and the whole plant are used for the treatment of cardiovascular disorder, snake bite, rheumatism, hypertension, insanity, epilepsy and hypochondria (Kirtikar & Basu, 1993). India, which holds almost a world monopoly, has been threatened with the depletion of wild resource of the plant with 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 alkaloidal content. At present, all supplies of *Rauvolfia serpentina* roots are furnished from natural resources, which are declining due to overexploitation by tribal and local collectors (Rajendra & D'Souza, 1999). This has led to listing of this species as "endangered" by the International Union for Conservation of Nature and Natural Resources (IUCN) (Jain *et al.*, 2003). Thus, there have been various attempts to develop *in vitro* propagation techniques for rapid and mass multiplication and conservation of this plant. High frequency regeneration from various explants of *R. serpentina* has been reported. Sehrawat *et al.* (2002) and Ahmad *et al.* (2002) found shoot apices as suitable explants for shoot induction cultured on MS medium supplemented with different concentrations of BA and NAA. Singh & Guru (2007) used excised shoot tips, and nodal segment explants and reported a higher number of shoots from excised shoot tip explants cultured on MS medium enriched with BA and NAA. Nodal segments are an important tissue source for micropropagation and plants raised from them are comparatively more resistant to genetic variation (Pierik,

* Corresponding author: tel.: +91 761 4044009, fax: +91 761 2840484, e-mail: m_yogiraj@yahoo.com

1991). Morphogenesis from nodal segment explants with axillary buds in *R. serpentina* has also been reported (Kataria & Sekhawat, 2005). These reports emphasized the development/refinement of the existing protocols from wild or natural sources of *R. serpentina*. The present communication deals with the genetically improved variety CIM-Sheel of *R. serpentina* with high root yield (FW 331 g/plant, DW 185 g/plant) and maximum reserpine content (0.035%) in 24 month-old crop (Gupta *et al.*, 2005). The development of new improved varieties increases the value and marketability of the crop. Furthermore, the application of breeding programs for certain endangered species can remove the threat to their survival out in nature, as in the case of *R. serpentina*. Keeping in view this fact, critical analysis of the earlier protocols necessitated the formulation of a well documented, reproducible and economical *in vitro* propagation method for genetically improved varieties. To our knowledge this is the first report on a microrpopagation/regeneration system from nodal segment explants of the CIM-Sheel variety and an evaluation of the field performance of micropropagated plants of *R. serpentina*, in general.

MATERIALS AND METHODS

Explants and culture establishment

Roots of *Rauvolfia serpentina* (L.) Benth. var. CIM-Sheel were collected from CIMAP (Central Institute of Medicinal and Aromatic Plants), Lukcnow (Uttar Pradesh, India). The roots, grown in a sand bed, started shooting after 15 days and the explants were collected from 30 day-old shoots. The explants, used to initiate aseptic cultures, were single nodal segments of 1.0-1.5 cm in length comprising unsprouted axillary buds. They were collected and washed thoroughly under running tap water. For surface sterilization, the nodal segments were then treated with 1% (v/v) solution of cetrimide (ICI Ltd., Kolkata, India) for 10 min under constant shaking. Then they were washed four times with distilled water. Finally, the explants were treated with 0.1% mercuric chloride ($HgCl_2$) solution for 2 min in a laminar air flow cabinet and washed three times with double-distilled water to remove traces of $HgCl_2$. After trimming both ends, single explants were placed aseptically in culture tubes containing MS semi-solid medium supplemented with different combinations of BA (0.0, 0.1, 1.0 and 10 μM) and NAA (0.0, 0.1, 1.0 μM) to identify appropriate combinations for multiple shoot regeneration.

Shoot proliferation

Sprouted axillary shoots were excised and maintained for 4 weeks on MS semi-solid medium supplemented with the combination of bioregulators found more suitable for maximum bud sprouting, i.e., 1 μM BA+ 1 μM NAA. Then, elongated (> 2.5 cm long) healthy micro shoots were planted, singly, in media supplemented with BA (0.0, 0.01, 0.1 or 10.0 μM) and NAA (0.0, 0.01, 0.1, 1.0 or 10.0 μM) in various combinations, to screen out the most effective combination for shoot proliferation. The experiment was conducted according to a two-way factorial design, with BA and NAA the two factors at various levels.

Rooting

Microshoots (> 2.5 cm in length) harvested from the fourth subculture onwards were used for *ex vitro* rooting. Microshoots were thoroughly washed in running tap water. After blotting, the cut ends of their bases were dipped in a 50 μM IBA (indole 3-butyric acid) or a NAA solution, prepared in 50% ethyl alcohol, for 0, 8, 12 and 24 hrs. The microshoots were transferred to root trainers comprising 25 cells of 150 cc each (Neevedita Plastic Industries, Nagpur, India), filled with autoclaved sand. The planted root trainers were shifted to plastic trays filled with half strength of iron free MS salts, covered with perforated transparent polythene sheet and maintained at 25 ± 2 °C under 16h photoperiod. After an acclimatization period of 2-3 weeks when the plants were completely weaned, they were moved to a shadehouse. One-month old plantlets were transplanted in polythene bags containing soil: sand: farmyard manure (1:1:1) in the shadehouse conditions for 1½ to 2 months receiving irrigation every other day and sprays of ½ MS solution once every 15 days.

Flowering, fruiting, seed germination and growth characteristics of micropropagated plants

The micropropagated plants were maintained in 50% shade under field conditions. Flowering, fruiting, seed characteristics, growth and dry root yield of ten randomly selected plants were recorded after 12 to 13 months. Growth was measured in terms of total length of the shoot up to the tip and mean width of the shoot determined at basal, middle and upper first axillary branching leaves of the stem using a vernier scale. Mean length of the fruit and fresh weight of the depulped seeds blotted on filter paper were also de-

termined. Seeds collected from mature fruits were scarified with sand paper and treated overnight with 300 ppm gibberelic acid. The treated seeds were inoculated in root trainers filled with sand and seed germination data was recorded after 6 weeks. The freshly collected root samples from 10 selected plants were thoroughly washed with running tap water so as to remove adhered soil form root surface. The root samples obtained so were cut in to 2.5-3 cm pieces and dried in shade for one month and dry root weight was measured.

Culture conditions and analysis of data

For culture establishment and shoot multiplication experiments, the medium contained 3% (w/v) sucrose as carbon source and 0.8% (w/v) agar (Loba Chemie Ltd., India) as gelling agent. The pH of the medium was adjusted to 5.8 using 0.1 N NaOH or 0.1 N HCl before autoclaving for 15 min at 1.06 kg cm^{-2} (121 °C). For bud induction, the nodal segments were inoculated in test tubes (25 × 125 mm, Borosil, India) containing 10 ml of semi-solid medium and shoots were cultured in 400 ml glass jars containing 50 ml of semi-solid medium for the *in vitro* shoot multiplication experiment. All culture vials were covered with plastic caps. The cultures were incubated at a temperature of 25 ± 2 °C under 16 hrs illumination with fluorescent light (approx. 45 μmol s^{-2}). Each experiment was conducted twice and ten replications per treatment were taken. The data for bud sprouting, multiple shoot induction and shoot multiplication were recorded after four weeks of inoculation. All experiments were conducted in complete randomized design (CRD) and data were analyzed by two-way analysis of variance employing Duncan's multiple range test for means comparisons at $p = 0.05$ using SYSTAT-12 statistical package (SYSTAT analysis software, 1982, version 12, USA). The interactions of the treatments in culture establishment shoot multiplication and *ex vitro* rooting experiments were studied in their factorial combination.

RESULTS

Induction of multiple shoots

The axillary buds on the nodal cuttings showed a visible growth after five days in culture and most of them grew into shoots within 25 days. The data on bud sprouting and number of shoots effected by the treatment of BA and NAA alone or in all possible combinations are shown in Table 1. There were significant differences among the different concentrations of BA and NAA and their interactions in bud sprouting response and shoot number. Among different concentrations of BA, 1.0 μM was found to be significantly more effective in sprouting response (61.66%) and shoot number (4.5) as compared to the control (27.21% sprouting; 2.16 shoot number) and on par with responses to 0.1 μM and 10 μM BA. Similarly, 1.0 μM NAA caused maximum sprouting response (65.41%) and shoot number (4.91) as compared to the control (32.91 % sprouting; 2.37 shoot number) and 0.1 μM NAA (39.16 % sprouting; 2.62 shoot number). Highest values of bud sprouting percentage (98.33 %) and

TABLE 1. Effects of BA, NAA and their interactions on sprouting percentage and shoot number in *Rauvolfia serpentina* L. Benth. var. CIM-Sheel bud culture

NAA	BA (μM)				
(μM)	0.0	0.1	1.0	10	Mean
			a. Sprouting percentage		
0.0	21.66^{f}	26.66^{e}	35^{d}	48.33^{c}	32.91^{i}
0.1	21.66^{f}	41.66^{c}	51.66^{b}	41.66^{c}	39.16^{h}
1.0	38.33^{c}	61.66^{b}	98.33^{a}	63.33^{b}	65.41^{g}
Mean	27.21^{m}	43.32^{l}	61.66^{j}	42.10^{k}	
			b. Shoot number		
0.0	1.83^{c}	2.33^{c}	2.33^{c}	3.0^{b}	2.37^{e}
0.1	1.83^{c}	2.5^{c}	3.5^{b}	2.83^{b}	2.66^{e}
1.0	2.83^{b}	4.66^{b}	7.66^{a}	4.5^{b}	4.91^{d}
Mean	2.16^{h}	3.16^{g}	4.50^{f}	3.44^{g}	

Values sharing the same superscript letter are not significantly different ($p < 0.05$)

number of shoots (7.66) were recorded on medium supplemented with 1.0 μM BA + 1.0 μM NAA and were found to be significantly ($p \leq 0.05$) higher than other combinations (Table 1a, b; Fig. 1A).

Shoot proliferation

The data pertaining to the effects of BA and NAA alone or in all possible combinations on shoot multiplication in a two-way factorial experiment are presented in Table 2. Single shoots cultured on different auxin-cytokinin supplemented media produced multiple shoots. A significant effect of different levels of BA on the rate of shoot multiplication was noticed. The maximum rate of shoot multiplication (4.16) was obtained on the medium supplemented with 10 μM BA, which was statistically higher than the shoot multiplication rate obtained with other concentrations of BA. Among different concentrations of NAA, 0.01 μM produced significantly more shoots (3.65) than any other concentrations, which was statistically on par with the shoot number obtained with the control (3.39). In interaction studies, 10 μM BA alone was found sufficient to produce maximum number of shoots (9.16) and interactions of BA and NAA did not favour shoot multiplication, which were significantly lower than BA alone (Table 2a; Fig. 1B). In contrast to shoot multiplication, a significant increase in shoot length was caused by BA and NAA interaction. The maximum shoot length (9.4 cm) was obtained with 10 μM BA + 0.1 μM NAA, which was statistically higher than the shoot length obtained with other combinations. However, this combination was statistically on par with the shoot length (8.3 cm) obtained on 10 μM BA + 0 .01 μM NAA (Table 2b).

Ex vitro rooting

In vitro developed microshoots planted in root trainers filled with autoclaved sand (Fig. 1C) produced new leaves within ten days. Microshoots surviving one month after planting were assumed to be rooted and the rooting percentage for each treatment was estimated. Data revealed a significant effect of auxins and duration of pulse treatments (Table 3). Treating microshoots with 50 μM NAA for 24 h resulted in 100% rooting (Fig. 1D), which was significantly higher than other durations and hormone (IBA). The plants, maintained in polythene bags for 1½ to 2 months, showed vigorous growth under shadehouse conditions (Fig. 1E). Almost 90% of the regenerated plants survived and flowered without any morphological variation within 12-13 months of their transfer to the field (Fig. 1F).

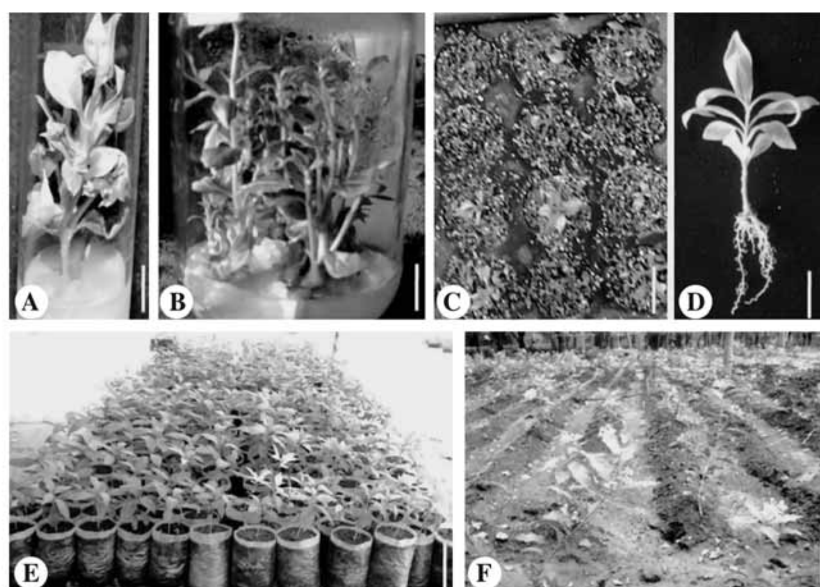

FIG. 1. *In vitro* micropropagation system for cloning of *Rauvolfia serpentina*: (A) initiation of shoot culture on MS medium supplemented with 1 μM BA + 1μM NAA (bar = 0.5 cm); (B) shoot multiplication on MS medium supplemented with 10 μM BA (bar = 0.5 cm); (C) shootlets treated with 50 μM NAA and transferred to root trainers filled with autoclaved sand for *ex vitro* rooting; (D) one month-old *ex vitro* rooted plantlets (bar = 0.7 cm); (E) 1½ to 2 month-old plantlets maintained under shadehouse conditions (bar = 6 cm); (F) growth of plantlets after 12-13 months of transfer in field conditions (bar = 20 cm).

TABLE 2. Effect of BA, NAA and their interactions on shoot multiplication and shoot length in *Rauvolfia serpentina* L. Benth. var. CIM-Sheel

NAA	BA (μM)					
(μM)	0.0	0.01	0.1	1.0	10	Mean
			a. Number of new shoots/shoot			
0.0	1.49 c	1.33 c	2.16 c	2.33 c	9.16 a	3.39 d
.01	3.33 c	1.66 c	2.49 c	4.15c	6.66 b	3.65 d
0.1	1c	2.16 c	1.83 c	1.83 c	3.49 c	2.06 e
1.0	1.16 c	1 c	1 c	1.5 c	1.33 c	1.19 e
10	0.49 c	1.66 c	0.99 c	0.5 c	0.16 c	0.76 f
Mean	1.49 h	1.66 h	1.66 h	2.06 h	4.16 g	
			b. Shoot length (cm)			
0	3.24 b	3.91 a	3.81 a	4.65 a	5.53 a	4.23 f
.01	3.58 b	3.91 a	5.41 a	8.3 a	6.52 a	5.54 e
0.1	5.58 a	4.08 a	5.74 a	9.4 a	5.39 a	6.04 e
1.0	4.66 a	3.15 b	4.33 a	3.81 a	2.33 c	3.65 g
10	0.5 d	3.94 b	2.33 c	2.66 b	0.66 d	2.02 h
Mean	3.46 j	3.80 j	4.32 j	5.76 i	4.10 j	

Values sharing the same superscript letter are not significantly different ($p < 0.05$)

TABLE 3. Effect of pulse treatment of NAA and IBA on *ex vitro* rooting in microshoots of *Rauvolfia serpentina* L. Benth. var. CIM-Sheel

Duration of pulse treatment (hrs)	NAA (50 μM)	IBA (50 μM)	Mean
	% Rooting		
0	–	–	–
8	30 c	13.33 e	21.67 h
12	76.66 b	16.66 d	46.66 g
24	100 a	40 c	70 f
Mean	51.67 i	17.5 j	

Values sharing the same superscript letter are not significantly different ($p < 0.05$)

TABLE 4. Flowering, fruiting and seed characteristics of the micropropagated plants of *Rauvolfia serpentina* L. Benth. var. CIM-Sheel after 12-13 months of plantation in the field

Shoot length (cm)	Mean width of shoot (cm)	No. of flower/ plant	No. of fruits/ Plant (%)	Mean length of fruit (cm)	Mean weight of seed (mg)	Seed germination (%) after 6 weeks	Dry root yield/ plant (g)
63.71	1.28 ± 0.18	28	20.5 (73.15)	0.984± 0.04	43.2 ±1.75	65.1	158±15

Value in parentheses indicates percentage of flowers producing fruits

Flowering, fruiting, seed germination and growth characteristics of micropropagated plants

From the perusal of data presented in Table 4, it was observed that the micropropagated plants grown under 50% shade conditions were almost uniform in growth with an average shoot length (63.71 cm) and width measurements (1.28 cm). No aberrations and growth defects were observed in the plants. After 12-13 months, the plants flowered, with the period between flower bud emergence and fruit ripening varying from 25 to 30 days. The flowering and fruiting characteristics of the randomly selected clonal plants were uniform and an average of 28 flowers and 20.5 fruits produced per plant. In all of the plants, approximately 73% of the flowers produced fruits of an average size of 0.98 cm. The seeds of uniform weight (43.2 mg) collected from these resulted in 65% germination within 6 weeks. An average of 158 g dry root yield was obtained in 10 randomly selected plants.

DISCUSSION

Induction of multiple shoots

In the present investigation, MS medium supplemented with BA alone produced shoots in all of the explants. By supplementing the medium with NAA both bud sprouting and induction of multiple shoots per explant were increased which shows that auxin (NAA) also plays a significant role in multiple shoot induction and contradicts the earlier report of Sarma *et al.* (1999). The enhanced rate of multiple shoot induction in cultures supplemented with BA and NAA may be attributed to increased rate of cell division and production of several primordial outgrowths induced by cytokinin (BA) in the axillary meristematic zone of the explant tissues. The combined effect of BA and NAA on axillary bud proliferation from nodal explants of *R. serpentina* is well documented (Baksha *et al.*, 2007; Singh & Guru, 2007; Salma *et al.*, 2008). Baksha *et al.* (2007) and Singh & Guru (2007) obtained 85% bud sprouting with 7.5 shoots and 86% bud sprouting with 5 shoots, respectively, on MS medium supplemented with BA and NAA. Salma *et al*. (2008) obtained 90% bud sprouting with only 4 shoots. In the present study, the combined effect of BA and NAA on bud sprouting (98.33%) and shoot number/explants (7.66) was higher than these published reports, which speaks to the strength of results of the present investigation. Further, shorter period (4 weeks) for axillary shoot proliferation from nodal explants in our study is also comparable with 8 weeks and 6 weeks period reported by Baksha *et al.* (2007) and Salma *et al*. (2008), respectively. These variable responses may be attributed to the specific age, physiological condition and genotype of the donor plants from which the nodal explants were excised. In the present study we collected the explants from 30 day-old plants of a recently developed variety, i.e. CIM-Sheel of *R. serpentina*.

Shoot proliferation and shoot length

The effectiveness of BA + NAA on *in vitro* shoot regeneration in *R. serpentina* has been observed in previous reports (Mehatre *et al.*, 2004; Baksha *et al.*, 2007; Salma *et al*., 2008). In contrast to these reports, in the present study 10 μM BA alone was found sufficient to produce more shoots and the interactions of BA and NAA did not favour shoot proliferation, which was significantly lower than that with BA alone. Purohit *et al.* (1994) also found BA alone sufficient to produce a high number of shoots in safed musli (*Chlorophytum borivilianum*). It appears that explants possess poor ability to utilize NAA from the medium. As compared to longer shoot regeneration period (6-8 weeks) and additional supply of NAA in the culture medium (Baksha *et al*., 2007; Salma *et al*., 2008), our protocol with shorter shoot regeneration period (4 weeks) and only one phytohormone (10 μM BA) is more suitable and economical.

In contrast to shoot proliferation, BA alone did not increase shoot length, which was significantly enhanced by the interactions between BA and NAA. In earlier reports too, the shoot length in *R. serpentina* was maximized with the combined action of BA and NAA, whereas BA alone or its interactions with other hormones significantly decreased shoot length (Baksha *et al.*, 2007; Singh & Guru 2007).

Ex vitro rooting

In the present investigation the microshoots, pulse-treated with 50 μM NAA for 24 h achieved with 100% *ex vitro* rooting in pure sand. In accordance with the present findings, Kataria & Shekhawat (2005) and Pant & Joshi (2008) also reported 98% and 86.6% *ex vitro* rooting of *in vitro* raised shoots treated with NAA, respectively. *Ex vitro* rooting is attractive and advantageous in a micropropagation method as it is cost effective with simultaneous rooting and harden-

ing of the plants (Nemeth, 1986). Furthermore, such plants give a high field survival percentage due to less infection and require little post-transplant care (Moncousin, 1991). Earlier, *in vitro* rooting has been attempted in *R. serpentina*, which prolonged the period for production of rooted, hardened and acclimatized plantlets (Ahmed *et al.*, 2002; Baksha *et al.*, 2007). Ahmed *et al.* (2002) induced *in vitro* rooting within 4-5 weeks and another one month for hardening and acclimatization of the plantlets. Baksha *et al.* (2007) also reported *in vitro* rooting and hardening in 15 days each and also encountered acclimatization problem of the plantlets. Thus, in the present study, 100% *ex vitro* rooting was achieved, omitting the need for additional steps of *in vitro* rooting and hardening. This will lead to the shortening of the propagation period and reduction of cost and, consequently, development of a more efficient and cost effective protocol.

Conclusion

In conclusion, we developed an efficient method for *in vitro* propagation of *R. serpentina* using nodal segment explants collected form 30 day-old plants. Improvement over previous protocols was achieved for culture establishment (Baksha *et al.*, 2007; Singh & Guru, 2007), shoot multiplication (Mehatre *et al.,* 2004; Baksha *et al.,* 2007; Salma *et al*., 2008) and *ex vitro* rooting (Kataria & Shekhawat, 2005; Pant & Joshi, 2008), which reduced the cost, number of steps and time period required for micropropagation of *R. serpentina*. Thus, this method is economical and promising for commercial exploitation and cloning of a selected variety of *R. serpentina*, i.e. CIM-Sheel, reported herein.

ACKNOWLEDGEMENTS

Financial support from the National Medicinal Plants Board, Ministry of Health and Family Welfare, New Delhi (Sanction No. GO/MP/-07/2006) is gratefully acknowledged.

REFERENCES

Ahmad S, Amin MN, Azad MAK, Mosaddik MA, 2002. Microrpopagation and plant regeneration of *Rauvolfia serpentina* by tissue culture technique. *Pakistan journal of biological sciences*, 5: 5-79.

Baksha R, Akhter Jahan MA, Khatun R, Munshi JL, 2007. *In vitro* rapid clonal propagation of *Rauvolfia serpentina* (Linn.) Benth. *Bangladesh journal of scientific and industrial research*, 42: 37-44.

Gupta AK, Khanuja SPS, Gupta MM, Darokar MP, Singh S, Chauhan HS, Mathavan RE, Saikia D, Shasany AK, Verma RK, 2005. A genetically improved variety of *Rauvolfia serpentina* with high alkaloid yield named "CIM-Sheel". *Journal of medicinal and aromatic plant sciences*, 27: 532-534.

Jain SP, Singh J, Singh SC, 2003. Rare and endangered medicinal and aromatic plants of Madhya Pradesh. *Journal of economic taxon botany*, 27: 925-932.

Kataria V, Sekhawat NS, 2005. Cloning of *Rauvolfia serpentina*- an endangered medicinal plant. *Journal of sustainable forestry*, 20: 53-65.

Kirtikar KR, Basu BD, 1993. *Indian Medicinal Plants*. *Vol. 2*. Dehra Dun Publishers, Calcutta, India.

Mehatre SP, Dhumale DB, Tule DS, 2004. Response of axillary buds to various culture conditions for *in-vitro* growth in Sarpagandha. *Annals of plant physiology*, 18: 136-138.

Moncousin CH, 1991. Rooting of *in vitro* cuttings. In: YPS Bajaj (ed.) *Biotechnology in Agriculture and Forestry, Vol. 17. High-Tech and Micropropagation*. Springer-Verlag, Berlin, Heidelberg: Germany.

Murashige T, Skoog F, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, 15: 473-479.

Nemeth G, 1986. Induction of rooting. In: Bajaj YPS, ed. *Biotechnology in Agriculture and Forestry*, *Vol. 1: Trees I*. Springer-Verlag, Berlin, Germany: 49-64.

Pant KK, Joshi SD, 2008. Rapid multiplication of *Rauvolfia serpentina* Benth. Ex. Kurt through tissue culture. *Scientific World*, 6: 58-62.

Pierik RLM, 1991. Commercial aspects of micropropagation. In *Horticulture – New technologies and applications* (eds Prakash JK and Pierik RLM), Dordrecht, The Netherlands.

Purohit SD, Dave A, Kukda G, 1994. Micropropagation of safed musli (*Chlorophytum borivilianum*), a rare Indian medicinal herb. *Plant cell, tissue and organ culture*, 39: 93-96.

Rajendra K, D'Souza L, 1999. *In vitro* propagation of ayurvedic plants. In: Khan IA, Khanum A, eds. *Role of Biotechnology in Medicinal and Aromatic Plants*, *Vol. II*. Ukaaz Pub., Hyderabad: 207-215.

Salma U, Rahman MSM, Islam S, Haque N, Khatun M, Jubair TA, Paul BC, 2008. Mass propagation of *Rauvolfia serpentina* L. Benth. *Pakistan journal of biological sciences*, 11: 1273-1277.

Sarma D, Sarma S, Baruah A, 1999. Microrpopagation and *in vitro* flowering of *Rauvolfia tetraphylla*. A potent source of antihypertension drugs. *Planta medica*, 65: 277-278.

Sehrawat AR, Sanjogta U, Chowdhury JB, 2002. Establishment of plantlets and evaluation of differentiated roots for alkaloids in *Rauvolfia serpentina*. *Journal of*

plant biochemistry and biotechnology, 11: 105-108.

Singh G, Guru SK, 2007. Multiple shoot induction in intact shoot tip, excised shoot tip and nodal segment explants of *Rauwolfia Serpentina. Indian journal of plant physiology*, 12: 360-365.