RAPID MULTIPLICATION OF *RAUVOLFIA SERPENTINA* BENTH. EX. KURZ THROUGH TISSUE CULTURE

Krishna K. Pant* and **Sanu D. Joshi****
*Institute of Agriculture and Animal Science, Tribhuvan University, Nepal.
**Central Department of Botany, Institute of Science and Technology, Tribhuvan University, Nepal.

Abstract: *Rauvofia serpentina Benth. ex Kurz (Apocynaceae) is a highly important medicinal plant growing in the terai belts of Nepal. This plant is facing a high threat from various kinds of poachers in the wild due to improper ways of collection as well as almost no conservation strategy. In the present study, we have identified the way for rapid in vitro multiplication of this species using tissue culture technique as an effective tool for ex situ conservation. In this study, MS medium along with BAP and NAA alone or in combinations at different concentrations have given successful results in producing callus, multiple adventitious shoots both from callus and nodes and multiple roots from both callus as well as nodes. The in vitro as well as ex vitro rooting of the micro shoots have been achieved. The rooted seedlings have been successfully acclimatized. The best media for shoot multiplication from the node and callus cultures have been identified as MS + NAA 0.5 + BAP 0.5 ppm and MS + BAP 2.0 ppm, respectively. For rooting in and ex vitro, NAA has been found to be the best among auxins. For callus induction all the auxins specially NAA 1.0 ppm and 2,4-D 2.0 ppm have been found to be the best.*

Keywords: Micropropagation; *Rauvolfia serpentina*; *In vitro* rooting; Pulse treatment and acclimatization.

Abbreviations: MS = Murashige and Skoog (1962), BAP = 6-benzylaminopurine , NAA = *a*-naphthaleneacetic acid , 2,4-D = 2, 4 Dichlorophenoxyacetic acid.

INTRODUCTION

Rauvolfia serpentina Benth. ex Kurz. belonging to the family Apocynaceae is one of those highly used medicinal plants, which had been used in Indian sub continent for more than 4000 years. This plant is listed in earliest Ayurvedic medicinal text the Charaka Samhita (c. 700 B.C.) and has been used since at least that time to treat mental illness and insomnia (Kataria and Shekhawat, 2005). It was known in folk medicine even in pre-Vedic period. In Ayurvedic medicine, it was used for treatment of snake-bite, insect stings and mental disorders. Later on, it was also reported to be used for treatment of epilepsy, diarrhea and dysentery and as a uterine stimulant to facilitate childbirth in various parts of India. It has been popular with certain tribals in Bihar (India) as mad man's medicine for several thousand years. In addition, it is used in modern system of medicine for treatment of various disorders like high blood pressure, sleeplessness, insomnia, mental disorders, fever etc (Anonymous, 2007a).

Although, a number of species of *Rauvolfia* are reported to contain medicinally important alkaloids, only two species, viz., Serpent Wood (*Rauvolfia serpentina*) and African Serpentwood (*Rauvolfia vomitoria*) are commercially known to be important and used for isolation of therapeutically important indole alkaloids. The main hypotensive alkaloid - reserpine, which is part of oleoresin fraction, was isolated by Muller and co-workers in 1952, after which the plant was adopted in modern medicine as an effective hypotensive agent and tranquilizer (Anonymous, 2007b). *Rauvolfia serpentina* is indigenous to moist deciduous forests of South East Asia including Nepal, India, Bangladesh, Burma, Sri Lanka, Malaya and Indonesia. Most of the supply of this drug is obtained from wild sources of this region. Zaire is the largest producer and exporter of the drug to Europe (Anonymous 2007b).

It is an erect evergreen, perennial under shrub of about 73 cm to 1 m in height under very favourable conditions with 2n = 22. Its roots are prominently tuberous and usually branched (0.5 to 2.5 cm in diameter). The roots go up to 40 to 60 cm deep into soil. Its roots are the parts used in medicine because they possess high alkaloid concentration. Most of the alkaloids (approx. 40 to 50 %) are contained in the root skin and the skin (Anonymous, 2008). It is found and distributed in the foot hills of the Himalayan ranges, up to the elevation of 1300 - 1400 m and almost all over the county.

Roots nearly verticle, tapering, as much as 15 cm thick at the crown and long giving a serpent-like appearance, occasionally branched or tortuous developing small fibrous roots. Roots are greenish-yellow externally and pale-yellow inside, extremely bitter in taste. Leaves borne in whorls of 3-4, deciduous, elliptic-lanceolate or obovate, pointed, green on the upper surface, pale-green underneath, 7.5 x 20 cm in size. Flowers numerous, borne in terminal or axillary, long-stocked clusters, tubular, 5-lobed, 1-3 cm long, whitish pink in colour. At the onset of fruit/seed, calyx pedecel and flowering stock become bright red. Fruits in pairs, obliquely ovate, 7.5 mm in size, purple black and bluish when ripe, with stone containing

Author for Correspondence: Krishna K. Pant, Institute of Agriculture and Animal Science, Tribhuvan University, Nepal. Email: krishna.k.pant@gmail.com

1 or 2 seeds. The plant thrives well in deep fertile soils that are rich in organic matter. It prefers slightly acidic soils (pH 4 - 6.3). Although, the plant is native of tropical humid climate, it grows in tropical and subtropical areas that are free from frost.

As mentioned above, this plant got its value due to a large number of medicinally active alkaloids in its roots. This importance is causing its excessive collection from the forests of Nepal though a few efforts have been made to conserve them in the community forests. Its indiscriminate use and poor methods of conventional propagation and conservation have led this species to be included in the list of endangered plants. *R serpentina* merit special attention for their economic value and present critical condition in the ecosystem due to unsustainable extraction (Mishra, 2008). Understanding this fact, the government of Nepal, Ministry of Forest and Soil conservation has identified this species for conservation and to study for the identification of methods to propagate and cultivate. Thus a need arises to generate an efficient protocol for cloning of *R. serpentina* in order to regenerate propagules to replenish depleting forests and meeting the demand of commercial cultivation (Kataria and Shekhawat, 2005). Since seed germination of *Rauvolfia* is highly variable. In 1955, Nair observed 15-20% seed germination, but only 10-13% plant could be developed from the germinated seeds of *R. serpentina*. According to Hedayatullah (1959), seed germination of *R. serpentina* was quite erratic, ranged from 8-48% with an average of 19%.Even the latest internet sources report the seed germination percentage to vary from 5 to 30 even when only heavy seeds are chosen for sowing purpose. Other conventional methods of propagation are time consuming; a lot of plant material is required, expensive and tedious with a relatively very small out put. Hence, tissue culture method used in this study is one of the best and reliable methods of propagation of this species. So, we believe that the result of this study will be a step forward in meeting the national conservation goal.

MATERIALS AND METHODS

The seeds of *R. serpentina* were collected from Chitwan, Nepal. To produce sterile explants for the experiment, the healthy seeds that sank in the water were selected and treated with liquid detergent for 15 minutes and was washed under running tap water for 45 minutes. After this the seeds were treated with 90% ethyl alcohol for 5 minutes and washed with distilled water. Finally, the seeds were treated with 0.2% mercuric chloride for another 5 minutes and washed with sterile water four times under the laminar air flow hood before inoculation in the hormone free Murashige and Skoog's (1962) medium (MS). The different parts of *in vitro* germinated seeds on Hormone free MS media and grown seedlings of *R. serpentina* were used as explants for the experiments to make sure that the explants have no residual effects of hormone from the previous culture conditions. Different parts like nodes, internodes, leaves and shoot tips were excised and pieces of about 0.5-1cm were inoculated in glass test tubes (150 mm X 25 mm) and jam bottles with approximately 16.5 ml of MS medium containing MS basal salts, 3% sucrose, 100 mg myo-inositol, 0.8% agar and different concentrations of NAA, indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 2,4-D, Kinetin (Kn) and BAP either singly or in combinations inside the laminar air flow cabinet. The concentration ranges for all the hormones used singly in the media were 0.1, 0.5, 1.0 and 2.0 ppm. except for 2,4-D which was 3 ppm. Similarly, in combination of NAA and BAP, NAA concentration was limited up to 1.0 ppm and BAP up to 2.0 ppm. Media were adjusted to pH 5.8 with 0.1 N KOH/HCl and autoclaved at 121^{o}C and 15 lb pressure for 20 minutes. They were cultured under illuminated condition of 16-hour photoperiod using cool white fluorescent lamps at 25^{o}C ±1^{o}C. The results were observed and recorded in every week. The multiple adventitious shoots and callus produced were sub-cultured after 8 weeks for the first time and regularly at an interval of 4-5 weeks in the same or various hormone as well as hormone free MS media for further responses. In each case a total of 6 replications were used for each treatment and the experimental trials were repeated three times. Multiple shoots obtained from the subculture of callus as well as nodes in different hormone media were detached and were either cultured *in vitro* in the rooting medium containing different concentrations of IAA , IBA and NAA or pulse treated with 100 ppm of the same hormones. The pulse treated shoots were transferred to 100% sand, 100% coco peat and 50% each coco peat and sand mixture *in vivo* at an interval of two weeks and kept inside the plastic house to maintain humidity. The *in vitro* rooted seedlings were transferred directly to 100% coco peat and 50% each coco peat and sand mixture *in vivo* at an interval of two weeks in the plastic house. Finally, the survived seedlings were transferred to 50% each sand and garden soil and kept in the shade and were regularly observed and watered at an interval of 3-5 days depending upon the moisture on the bed. *In vivo* rooting response and hardening responses were recorded. The photographs were taken by a Canon Power Shot A 450 Digital Camera (5 Mega Pixels and 3.2 × optical zoom). All the physical conditions were kept constant throughout the experiment. The callus induction, percentage and number of adventitious shoots/explant/culture, root induction and acclimatization were calculated, data were analyzed using analysis of variance and descriptive one way ANOVA (SPSS 11.5 for windows), and the mean comparison was by a least significant difference test at the 1% level of probability.

RESULTS AND DISCUSSIONS

For induction of callus all the explants, nodes, internodes, shoot tips and leaves used in the experiment were found to be effective but the internodes were less effective than the other two explants. All the explants cultured in MS hormone free media and MS supplemented with BAP or Kn at all concentrations showed either no or very little response in callus induction whereas NAA and 2,4-D alone were found to be directly proportional to callus induction up to 1 ppm and 2.0 ppm respectively (Fig. 1 and 2). IAA and IBA gave positive effects in callus induction up to 0.5 ppm above this level both showed negative effect (Sudha and Seeni 1996), and in combination of NAA and BAP, a higher NAA concentration (1 ppm) along with all most all the concentrations of BAP were found to be effective. Initially the callus development was very slow in

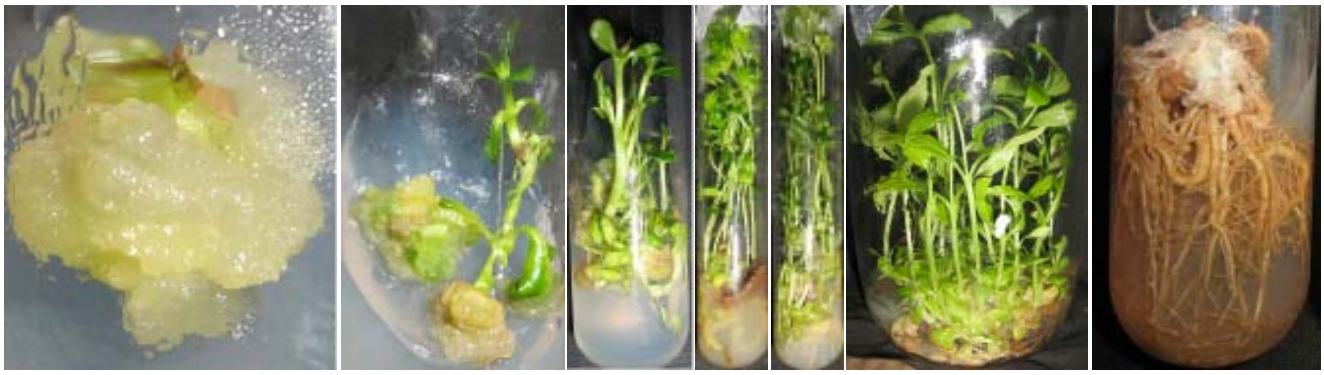

Fig. 1 Fig. 2 Fig. 3 Fig. 4 Fig. 5 Fig. 6 Fig. 7

Figure 1-7: Callus induction and shoot multiplication.

Fig. 1: Callus induced from the leaf explant in MS+2,4-D 2.0ppm. **Fig. 2:** Callus formation from the leaf and shoot as well as shoot induction at the node of the same explant in MS + NAA 1.0ppm+BAP 2.0ppm. **Fig. 3:** Multiple shoot formation form the node explants in MS + NAA 0.1 + BAP 1.0ppm. **Fig. 4:** Multiple shoot formation form the node explants sub culture in MS + NAA 0.5ppm+BAP 0.5ppm. **Fig. 5:** Multiple shoot formation from the node sub culture in MS + NAA 0.1 + BAP 0.5ppm. **Fig. 6:** Multiple shoot induction from the callus sub culture in MS + BAP 2.0ppm. **Fig. 7:** Multiple root induction from the callus sub culture in MS + NAA 0.5ppm.

Fig. 8 Fig. 9 Fig. 10 Fig. 11

Fig. 12

Figure 8-12: Root induction and acclimatization.

Fig. 8: *In vitro* rooting response of micro shoots in NAA 0.1, 0.5, 1.0 and 2.0ppm respectively from left to right. **Fig. 9:** Showing an *in vitro* rooted plant from the media containing MS + NAA 0.5 + BAP 0.5ppm. **Fig. 10:** Showing an *in vivo* (pulse treated with 100ppm NAA) rooted plant. **Fig. 11:** Rooting response of hormone free MS medium. **Fig. 12:** Acclematization: 12 weeks, 12 weeks, 6 weeks and 2 weeks respectively from left to right.

the first 1 or 2 weeks from all sources of explants (nodes, internodes and leaves). After 3 to 4 weeks they started to swell up from the cut bases and ultimately the entire explants (Fig1 and 2). Generally the callus formation was observed after 4th week of culture except a few. The callus induction initiated from the cut bases (usually lower end) and gradually the whole explants surface gave out callus which grew continuously up to 10 to 12 weeks of culture. The sub cultured callus in the same medium gave out secondary callus (usually milky white in colour) from the surface of the primary callus in the callus inducing media. The regular sub culture after every 4 to 6 weeks gave a lot of callus for further experiments. The callus sub cultured in all BAP containing media differentiated in to multiple adventitious shoots giving out an average of 7 shoots/ piece of callus in MS + BAP 2.0 ppm (Fig. 6). The media containing BAP along with lower concentration of NAA further gave multiple shoots as well as roots (Sarker et al., 1996). From the result it is obvious that cytokinins generally do not induce callus but induce multiple adventitious shoots. Auxins normally induce callus as well as roots up to an optimum concentration level in this species. Inductions of callus and plant regeneration are the most reliable tools to multiply she plants in a large scale (Sarker et al., 1996).

The leaves and internodes were found to be less effective explants than the nodes and shoot tips (Rajkarnikar et.al., 2000) for the production of multiple shoots (Sarker et al., 1996). For the induction of shoots all the auxins alone had either no or very little effect but both of the cytokinins used in the experiment alone showed positive relation with an increase in concentration up to 2.0 ppm. Sharma et al. (1999) observed the multiple shoot development on MS media with Kn (0.1-0.2 mg/l) and BAP (0.4-0.5 mg/l) in *R. tetraphylla*.

In combination of NAA and BAP, normally lower NAA and higher BAP concentration favoured the production of adventitious shoots. Sudha and Seeni (1996) reported 77% success rate with 4 shoots/ node in BA 13.2 mg and NAA 2.68 mg and in sub cultures they had reduced the concentration of both the hormones to 4.4 and 0.27 mg respectively in *R. micrantha*. The explants mainly nodes responded in 2-3 weeks of culture. They gave multiple adventitious shoots up to 4/ explant in various concentrations of BAP and NAA in MS media (Fig. 3). Ahmad et al., (2002) found that MS + 0.5 mg/l BA + 0.2 mg/l NAA as the most suitable combination for induction of callus and development of shoot buds formation but it failed to support elongation of shoot, but Rajkarnikar et.al. (2000) found

Table 1: Response of different hormones at different concentrations to the explants

Hormone Concentration in ppm	Mean±SE Callus Induction	Mean±SE Root Induction	Mean±SE Shoot Induction
Control (MS basal)	0.00	1.66±0.33	0.00
NAA 0.1	0.66±0.33	2.00±0.57*	0.00
NAA 0.5	1.66±0.33	2.00±0.57*	0.00
NAA 1.0	2.00±0.57*	2.00±0.57*	0.00
NAA 2.0	1.66±0.33	1.33±0.66	0.00
BAP 0.1	0.00	0.00	0.66±0.33
BAP 0.5	0.00	0.00	0.33±0.33
BAP 1.0	0.00	0.00	1.66±0.33
BAP 2.0	0.33±0.33	0.00	2.00±0.57*
NAA 0.1 + BAP 0.1	0.33±0.33	0.00	1.00±0.57
NAA 0.1 + BAP 0.5	0.33±0.33	0.00	1.66±0.33
NAA 0.1 + BAP 1.0	0.33±0.33	0.00	1.66±0.33
NAA 0.1 + BAP 2.0	0.33±0.33	0.33±0.33	0.33±0.33
NAA 0.5 + BAP 0.1	1.66±0.33	1.66±0.33	0.66±0.33
NAA 0.5 + BAP 0.5	0.00	1.66±0.33	2.00±0.57*
NAA 0.5 + BAP 1.0	1.66±0.33	1.66±0.33	1.00±0.57
NAA 0.5 + BAP 2.0	1.00±0.57	0.00	1.00±0.00
NAA 1.0 + BAP 0.1	2.00±0.57*	1.00±0.57	0.33±0.33
NAA 1.0 + BAP 0.5	2.00±0.57*	1.00±0.57	0.00
NAA 1.0 + BAP 1.0	1.33±0.33	1.66±0.88	0.33±0.33
NAA 1.0+ BAP 2.0	1.66±0.33	1.66±0.88	0.66±0.33
2,4-D 0.5	0.33±0.33	0.00	0.00
2,4-D 1.0	0.66±0.33	0.00	0.00
2,4-D 2.0	2.00±0.57*	0.00	0.00
2,4-D 3.0	1.00±0.57	0.00	0.00
Kn 0.5	0.33±0.33	0.00	1.66±0.33
Kn 1.0	0.00	0.00	1.66±0.33
Kn 2.0	0.00	0.00	1.33±0.66
IAA 0.1	0.66±0.33	0.66±0.33	0.33±0.33
IAA 0.5	1.66±0.33	1.66±0.88	0.33±0.33
IAA 1.0	0.66±0.33	1.00±0.57	0.33±0.33
IAA 2.0	0.66±0.33	1.00±0.57	0.33±0.33
IBA 0.1	0.33±0.33	0.00	0.33±0.33
IBA 0.5	1.00±0.57	1.00±0.57	0.33±0.33
IBA 1.0	0.00	1.00±0.57	0.33±0.33
IBA 2.0	0.00	1.00±0.57	0.33±0.33
Total	0.78±0.08	0.75±0.09	0.62±0.07

Mean Value range: 0 = no, < 1.0 = low, < 2.0 good, e^ 2.0 excellent response.

*Indicate very high significance at $p < 0.01$.

3 mg/l BAP + 0.1 mg/l NAA as the best medium for micro-shoots sprouting. So, they transferred the tiny shoots to media with 2.0 mg/l BA with 0.5mg/L NAA, where they showed excellent elongation. The adventitious shoots obtained were further sub cultured in the fresh medium containing similar hormone combination. In 3rd sub cultures, MS + NAA 0.1 + BAP 0.5 - 1.0 ppm and MS + NAA 0.5 + BAP 0.5 ppm gave 7 and 11 shoots/ explants, respectively (Fig 4 and 5). From the result it is obvious that in sub cultures number of shoot per explants generally increase up to a certain limit (3 in our case) and the concentration of cytokinin requirement for multiple adventitious shoot formation is reduced than the first culture. Rajkarnikar et al. (2000) found that BAP 1 mg/l and 2 mg/l with 0.1 mg/l of NAA showed the best result with 8-12 shoots. They further state that in higher concentration of BAP callusing occurred at the base of explants and after 7th sub-culture of micro-shoots in the best medium i.e. BAP 1 mg/l and NAA 0.1 mg/l, the multiplication rate of micro-shoots increased up to 12 -16 from one shoot and after which the shoot number did not increase. In a similar experiment; Kataria and Shekhawat (2005) had found 20-25 fold increment in multiple shoot production using BAP 10 µM and IAA 5 µM after 3-4 sub cultures.

The most effective explants for the production of roots *in vitro* were found to be either shoot tips or calii derived from all kinds of explants. For the induction of roots, both the treatments of Cytokinins alone showed no response of any kind where as MS (hormone free) medium showed positive result (Fig. 11). Most of the auxins especially NAA showed excellent effect up to 1ppm level beyond which the efficiency gradually decreased (Fig. 8). 2,4-D had no effect of any kind in root induction. In combination of NAA and BAP higher concentrations of NAA (i.e. up to 1.0 ppm) along with all concentrations of BAP gave positive results (Fig. 9). In some cases of callus culture as well as shoot cultures, thick multiple roots have been achieved which might have medicinal as well as commercial significance (Fig. 7). The experiment on callus culture and possibility of producing economically important roots of this species is under progress. In a similar *in vitro* rooting experiment, Ahmad et al. (2002) used 0.2 mg/l IBA+ 0.2 mg/l NAA combination which performed well. The *in vitro* rooted micro shoots survived and grew well in the coco peat at the first stage of acclimatization in the plastic house. These established plants performed 100% success rate even after transferring in the sand and soil mixture in shade (Fig. 12) whereas Ahmad et al. (2002) have succeeded up to 95%. Sharma et.al. (1999) observed Rooting from *in vitro* shoots on NAA containing media in *R. tetraphylla*.

The adventitious shoots obtained from callus culture as well as shoot node cultures after pulse treatment rooted well with 100 ppm. of NAA (Fig. 10) at the rate of 86.6% in sand where with 100 ppm. IAA and IBA the rooting percentage were 66.6% and 53.3% respectively. Out of all the rooted seedlings an 80.6% survived in the coco peat in the plastic house. Finally in the sand and soil mixture a 60% of the seedlings survived (Table 2). In a similar experiment by Kataria and Shekhawat (2005) found 98% success rate in rooting using 50 µM each of IBA and NAA and using half strength macrosalts in the bed, 80% of the shoots hardened similar to Sudha and Seeni (1996).

While analyzing the data statistically, ANOVA showed high level of significance between the groups in all cases. NAA 1.0 ppm either singly or in combination with a low concentration of BAP have been found to be highly significant at 1% level of significance in callus producing. Almost all the concentrations of NAA have been found to be significant at 1% level and superior to other auxins in root induction. Similarly, both the cytokinins above 0.5 ppm have been found to be significant (Table 1).

Table 2: Response of various auxins (hormones) to *in vivo* rooting and acclimatization

Bed	IAA	IBA	NAA
Sand rooting (Pulse Treatment)	10/15 : 66.6%	8/15 : 53.3%	13/15 : 86.6%
Coco peat stabilizing	25/31 : 80.6%		
Sand soil mixture in shade condition	15/25 : 60.0%		

ACKNOWLEDGEMENT

We are thankful to the Central Department of Botany for providing laboratory facilities. We are also thankful to the University Grants Commission (Nepal) for providing partial funding.

CONCLUSION

R. serpentina, a gift of nature is facing severe threat to its existence due to various reasons. It is listed in the IUCN endangered category as well as in CITES Appendix II. Understanding this fact and considering its economic as well as medicinal importance; researchers, government authorities, non-governmental agencies and all the stake holders should think and act on conserving and utilizing this species in a sustainable way before it is too late (extinction). For quick and efficient increment of its population, various propagation methods developed like our study should be utilized and local farmers/collectors should be encouraged to cultivate. Above all an awareness/education campaign about the 'importance of medicinal plants and the ways to conserve them' should be conducted throughout the country.

REFERENCES

Ahmad, S., Amin, M.N., Azad, M.A.K. and Mosaddik, M.A. 2002. Micropropagation and Plant Regeneration of *Rauvolfia serpentina* by Tissue Culture Technique. Pakistan Journal of Biological Sciences. 5(1): 75-79.

Anonymous 2007a. In "Cultivation and Processing of Selected Medicinal Plants." National Institute of Industrial Research. downloaded on 14-08-2007 from http://www.niir.org/ books/book/ zb,,103_a_0_0_a/Cultivation + and + Processing + of + Selected + Medicinal + Plants/

Anonymous 2007b. Serpent wood is an effective. downloaded on 14-08-2007 from http://www.banajata.org/a/s4.htm

Anonymous, 2008. *Rauwolfia Serpentina* (Serpagandhha). Downloaded on 10-06-2008 from http://sanwariaherbals.com/ Rauwolfia%20Serpentina.htm

Hedayatullah, S. 1959. Culture and propagation of *Rauwolfia serpentina* Benth. in East Pakistan. Pakistan J. Sci. Indust. Res. 2: 118-122.

Kataria, V. and Shekhawat, N.S. 2005. Cloning of *Rauvolfia serpentina*-An Endangered Medicinal Plant. Journal of sustainable forestry. 20(1): 53-65

Mishra, M. 2008. Harvesting practices and management of two critically endangered medicinal plants in the natural forests of central India. Downloaded on 10-06-2008 from .http://www.fao.org/ DOCREP/005/Y4496E/Y4496E33.htm.

Rajkarnikar, K. M., Sainju, H. K. and Bhatta, G. D. 2000, *In vitro* culture of *Rauwolfia serpentina* L. Benth. Ex. Kurz., Proceedings of Nepal Japan Joint Symposium 2000: 232-234.

Sarker, K. P., Islam, A.; Islam, R.; Hoque, A.; Joarder, O. I. 1996. *In vitro* propagation of *Rauvolfia serpentina* through tissue culture. Planta Med. 62(4): 358-9.

Sarker, K. P., Islam, A.; Islam, R.; Hoque, A.; Joarder, O. I. 1996. Plant Regeneration of *Rauvolfia serpentina* by Organogenesis from Callus Cultures. Plant Tissue Cult. 6(1) : 63-65.

Sharma, D., Sharma, S. and Baruah A., 1999 Micro propagation and *in vitro* flowering of *Rauvolfia tetraphylla*; a potent source of antihypertension drugs. Planta Med. 65(3): 277-8.

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

❐