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Efficient Plantlet Regeneration from Nodal Explant Culture of *Rauvolfia Micrantha* Hook. F. An Endangered Medicinally Important Plant

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Rauvolfia micrantha Hook. f. holds an important place in the biomedical industry because of how well it lowers blood pressure. This is because reserpine is found in the oleoresin part of the roots. Poor seed viability, a low rate of seed germination, and a huge amount of genetic variation make it hard to grow R. micrantha commercially in the traditional way. Using a semisolid nutrient culture medium, the present optimized protocol provides a perfect method for setting up aseptic cultures to make plantlets in an experiment. R. micrantha nodal explants were grown on MS medium with different quantities of cytokinins BAP/Kn/TDZ (0.5-10.0 mg/L) alone. Compared to all other concentrations of Kn/BAP alone, the most growth of shoot buds was seen at (8.0 mg/L) TDZ. As the concentration went above 8.0 mg/L, the ability of the cytokinins to make shoot buds grow slowly went down. On MS medium with (2.0mg/L) NAA, the shoots that had been grown in the lab made more roots. So, the plant that was grown in a lab using nodal cultures was put in pots with garden soil and put outside in the shade, where the temperature and light were just right. In glasshouse or field conditions, 90–95% of the plants that were grown in a lab survived, and 85% of the plants that were grown using this protocol looked the same. After 8 weeks in their new pots, these plants bloomed. The established protocol can be used to quickly make more of the plants that are true to type.

Key Words: *- Rauvolfia micrantha Hook. f., In vitro culture, Plant growth regulators, Plant Regeneration, Rooting, and Hardening.*

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I. Introduction:

Rauvolfia micrantha Hook. f. (Apocynaceae) is a perennial woody shrub found in the Tinnevelly and Travancore hills of the Western Ghats in southern India at a height of 600 meters. Antihypertensive-hypertensive and tranquilizer alkaloids, ajmalicine, reserpine, sarpagine, reserpine, and serpentine, are prevalent in the roots (Anonymous, 1969). In addition, it acts as an alternative for *Rauvolfia serpentine* root in business lots provided to American buyers in growing quantities (Youngken,1954). In the ancient Indian medical system (Ayurveda), *R. micrantha* is used as a replacement for *R. serpentina* to treat a number of nerve ailments, particularly in Kerala (Sahu, 1979). The plant has also been described as uncommon and unique to the Western Ghats' southern woodlands (Sahu, 1979).Different reasons, including endemic areas, restricted range, tiny populations in accessible places, and anthropogenic stresses on forestlands, have all contributed to *R. micrantha's* decline in the wild. Poor seed viability, limited germination, and scarce and delayed roots of seedlings and vegetative cuttings plague conventional multiplication. Given the pressing requirement for conservation, in vitro multiplication can be accomplished by shoot tip and nodal explant cultures (Sudha and Seeni, 1996). Plant tissue culture is a well-known biotechnological method for the fast growth of medicinal plants for commercialization, conservation (Nadeem *et al.*, 2000), and cryopreservation (Kitto, 1997). (Decruse *et al.*, 1999). The common mechanisms for the clonal proliferation of excellent medicinal plant species have been somatic embryogenesis and organogenesis (Gary and Brent, 1986).

Plant production in terms of quantity and quality of secondary metabolites has been increased in some circumstances by somatic embryogenesis (Gastaldo *et al.*, 1994). Plants in *Rauvolfia* species have been regenerated *in vitro* primarily through organogenesis (Sarker *et al.*, 1996; Sudha and Seeni, 1996; Patil and Jayanthi, 1997) and rarely via somatic embryogenesis in *Rauvolfia vomitoria* (Tremouillaux-Guiller and Chenieux, 1991) and *Rauvolfia caffra* (Tremouillaux-Guiller and Chenieux, 1991). (Upadhyay *et al.*, 1992). Generally, embryogenesis has been accomplished utilizing plant tissues such as cotyledons, hypocotyls, leaves, or internodes, but seldom roots (Vuorela *et al.*, 1993). Root segment culture organogenesis and somatic embryogenesis are very useful for genetic transformation experiments with *Agrobacterium rhizogenes*. It is also a good experimental setup for studying cell differentiation and the regulatory mechanism of totipotency in plant

cells. Furthermore, plants regenerated from root segments are thought to be genetically homogeneous (Sharma *et al.,* 1993). Root culture, according to Chaturvedi *et al.* (1981), might be utilized to preserve the germplasm of various plant species, including *R. serpentiana.*

The recent research details a micropropagation method for *R. micrantha* that involves the cultivation of nodal explants as the source of the direct creation of numerous shoots.

II. Materials And Methods

Nodal segments (1.0 cm to 2.5 cm) of R. micrantha with and without axillary buds were taken from healthy, young branches of a one-year-old plant growing in the research field of the Department of Botany at the Government Degree College for Women in Karimnagar District (T.S.). The cuttings were washed with running tap water and treated for 5 minutes with 5% teapot. They were washed well under running tap water, then sterilized on the outside with 0.1% w/v Mercuric chloride ($HgCl_2$) for 4-5 minutes, and then rinsed at least three times with sterile distilled water. Before inoculation, sterile pieces of nodes were dried on sterile filter paper.

Culture media and culture conditions:

The explants were inoculated into an MS medium that included 30 gm/L of sucrose and was fortified with different quantities of cytokinin BAP/KN/TDZ in conjunction with IAA (Table- 1). The medium was then solidified with 0.8% agar (Difcobacto) Before adding 0.8% agar to any of the media, the pH was changed to 5.8 and the containers were autoclaved at 1210 degrees Celsius under 15 pounds per square inch for 15–20 minutes. For the cultures of differentiating explants, MS+ BAP/KN/TDZ and MS+IAA+BAP /KN media were utilized. Culture tubes were kept at a temperature of 25 degrees Celsius and 20 degrees Celsius above room temperature with a photoperiod of 16 hours and white fluorescent light (40-50). After a total of six weeks of culture, the axillary shoots that had multiplied were placed into a medium for roots.

III. Results and Discussion

For successful micro-propagation, auxiliary buds are preferred explants, as they possess pre-existing meristem that is easily developed into shoots while maintaining clonal fidelity. Micropropagation using auxiliary bud culture has been reported for a large number of plant species including *Prosopis chilensis* (Caro *et al.,* 2002), *Ilex dumosa* (Luna *et al.,* 2003), *Spilanthes acmella* (Haw and Keng, 2003), *Mucuna pruriens* (Faisal *et al.,* 2006) and *Tylophora indica* (Faisal *et al.,* 2007). We, therefore, used the nodal segments explants having auxiliary bud for multiple shoot induction and proliferation

Table 1 shows the findings that were obtained from the axillary and nodal bud cultures with regard to the formation of numerous shoots and roots. When *R. micrantha* was cultivated on a variety of hormonal combinations, the results were all over the place. After the inoculation, the axillary buds began to become active within the first week, and by the second and third weeks, new shoots were clearly distinguishable with leaves and internodes. The survival rate of *R. micrantha* explants grown from nodal segments of the wild plant varied greatly depending on the season. The current observations indicate that the explants were obtained from field-grown plants at various times throughout the year in order to ascertain the most suitable time of year for the development of the culture. Explants that were obtained during the months of August and October exhibited a shorter time for sprouting and a rapid expansion of shoot buds.

Effect of BAP:

The outcomes of growing *R. micrantha* nodal buds on MS medium supplemented with BAP at concentrations ranging from (0.5 to 10.0 mg/L) are illustrated in (Table 1) and reported in (Fig-A). The medium that included BAP at a concentration of 4.0 mg/L not only generated the greatest number of shoots (6.0± 0.32 shoots/explant), but it also demonstrated the highest proportion of responsive cultures (65%). The rate of shoot bud proliferation was found to be decreasing progressively as the concentration of BAP was raised up to (6.0 mg/L), and the rate of shoot multiplication was found to be decreasing when the concentration of BAP has increased above (10.0 mg/L).

Table-I Analysis of plant growth regulators on *in vitro* multiple shoot induction from nodal explants of *R. micrantha* on MS medium with various concentrations of BAP, KIN, and TDZ after 8 weeks of culture.

Growth hormone concentration (mg / L)	% Of cultures responding	The average number of shot buds/explant (S.E) *	The average number of shot lengths (S.E) *
BAP			
0.5	46	4.0 ± 0.32	3.0 ± 0.42
1.0	50	5.2 ± 0.35	3.2 ± 0.75
2.0	60	5.4 ± 0.32	5.4 ± 0.32
4.0	65	6.0 ± 0.32	6.2 ± 0.36

6.0	54	5.8 ± 0.36	5.2 ± 0.36
8.0	40	5.5 ± 0.23	4.5 ± 0.23
10.0	38	4.5 ± 0.23	4.0 ± 0.23
KIN			
0.5	42	4.3 ± 0.25	4.0 ± 0.85
1.0	46	5.6 ± 0.32	4.8 ± 0.32
2.0	52	6.2 ± 0.32	5.2 ± 0.32
4.0	64	7.0 ± 0.35	6.0 ± 0.35
6.0	68	5.3 ± 0.32	7.3 ± 0.32
8.0	57	4.2 ± 0.23	5.2 ± 0.23
10.0	47	4.0 ± 0.23	4.2 ± 0.23
TDZ			
0.5	48	5.0 ± 0.32	5.0 ± 0.32
1.0	52	5.6 ± 0.32	5.8 ± 0.42
2.0	67	6.0 ± 0.32	6.6 ± 0.62
4.0	72	7.0 ± 0.42	7.5 ± 0.52
6.0	60	8.0 ± 0.32	8.5 ± 0.42
8.0	54	6.8 ± 0.32	6.8 ± 0.72
10.0	48	6.5 ± 0.23	6.5 ± 0.43

Effect of KIN:

The result of growing nodal buds of *R. micrantha* on MS medium supplemented with Kn at concentrations ranging from (0.5 to 10.0 mg/L) was reported. When compared to the results of the tests conducted with various concentrations, the result at (6.0 mg/L) KIN showed the highest proportion of responsive cultures (70). Whereas more shoots were regenerated from nodal explants at (6.0 mg/L) KIN (7.0± 0.35) shoots/explant) followed by (8.0 mg/L) KIN, KIN at (1.0, 2.0, and 4.0 mg/L) produced (5.6 0±.32), (6.2 ± 0.3), and (7.0±0.35) shoots/explant, respectively. Responses were obtained from 46, 52, and 64 different civilizations (Table 1).

Effect of TDZ:

The results of growing *R. micrantha* nodal explants on MS media that had been supplemented with TDZ at doses ranging from 0.5 to 10.0 mg/L were seen. These findings were observed after growing the nodal explants on MS medium. The TZD dose of 6.0 mg/L produced the largest proportion (72) of responsive cultures when compared to the other concentrations that were tested. Although a greater number of shoots were formed from nodal explants at TDZ concentrations of 4.0 mg/L (8.32 shoots/explant) than at TDZ concentrations of 8.0 mg/L (8.0) shoots/explant), respectively. The number of shoots that were generated from each implant varied from (5.6 0.3) to (7.0 0.42) when TDZ was present at doses of (0.5, 1.0, and 2.0 mg/L). The percentages of civilizations that responded were as follows: 52, 67, and 72 percent, respectively: (Table1, Fig -B).

***In Vitro*Rooting:**

The fully developed, vigorous shoots were then transplanted into MS media that had been enriched with varying amounts of NAA (ranging from 0.5 to 4.0 mg/L). There was abundant histogenesis observed on 3.0 mg/L NAA, which responded with 6.0 0.32 roots/explants in comparison to NAA concentrations of (0.5-2.0 mg/L); however, 96% of plants produced roots with 6.0 0.32 roots/explants (Table 2), indicating that this concentration of NAA was optimal for root production (Fig-E). Acclimatization After the rooted plantlets had been taken from the culture media, the agar from the roots was removed by washing them under running water from the faucet.

The plantings were then moved into poly pots that had been filled with vermiculite that had been soaked beforehand, and they were kept in a growth room that had a temperature of 28 degrees Celsius and relative humidity of 70–80 percent. After a time of three weeks, they were transferred to poly bags that contained a mixture of soil, sand, and manure in a ratio of 1: 1: 1. These bags were then placed in a shade house for a further duration of three weeks. The potting mix containing the seedlings was watered with Hogland's solution once every three days for a total of three weeks (Fig-F). On MS medium that was fortified with various doses of cytokinin (namely BAP, Kn, and TDZ), we were able to successfully regenerate shoots from nodal bud cultures. This resulted in the successful production of plants. As a role growth regulator, TDZ, at a concentration of 6.0 mg/L, caused a significantly greater number of shoots than BAP/KIN at the same concentration.

Table 2. Evaluation of the impact of MS+ NAA (1.0-4.0mg/L) on the formation of roots from micro shoots of *R. micrantha* after four weeks.

PGR concentration (mg / L)	**% Of cultures responding**	Mean number of roots/shoot **(S.E) ***	Mean root length (cm) **shot lengths (S.E) ***
NAA			
0.5	46	4.0 ± 0.32	3.0 ± 0.42
1.0	50	5.2 ± 0.35	3.2 ± 0.75
2.0	60	5.4 ± 0.32	5.4 ± 0.32
3.0	65	6.0 ± 0.32	6.2 ± 0.36
4.0	54	5.8 ± 0.36	5.2 ± 0.36

However, neither lower nor higher concentrations of BA were found to be appropriate for the generation of the greatest number of regenerated shoots. In a similar vein, it was previously revealed that BA was critical for shoot regeneration in a number of plants (Stefaan *et al.,* 1994; Faisal *et al.,* 2006,2007). Kin and 2iP were also shown to be appropriate for shoot regeneration, although the reaction was significantly less robust than that of BA. On a medium containing Kn and 2iP, similar reactions were found from the culture as well. These findings were likewise documented in studies including Mucuna pruriens (Faisal *et al.,* 2006). In addition to this, it was discovered that the number of shoots decreased when the BA concentration was increased above the ideal level. This finding is consistent with what Haw and Keng found in their earlier research (2003). Explants grown on medium containing elevated levels of BA showed shrunken nodes, vitreous leaves, and callus forming at the cut edge, especially at the proximal ends of the nodal explants. These results have also been reported in *Peganum harmala* (Saini and Jaiwal, 2000) and *Holostemma ada kodien* (Martin, 2002). It is possible that the action of accumulating auxin at the basal cut ends, increased cell proliferation, leading to the development of callus. This was notably the case when cytokinins were present. It was hypothesized by (Preece *et.al.*,1991) that the production of callus at the basal cut ends of nodal explants on cytokinin-enriched media typically took place in species with a high apical dominance.

Fig: *In vitro*Plant let regeneration from Nodal explant Culture and plant establishment of R. ***R. micrantha***a) Direct multiple shoots developed on MS + 6.0 mg/L BAP. b) Direct multiple shoots developed on MS +6.0 mg/L TDZc) Rooting of individual micro shoots on MS+NAA (3.0mg/L) Fig. f) hardening of plantlet

However, it was shown that (2.0 mg/L) TDZ in conjunction with was more effective in promoting shoot bud proliferation when compared to (0.5 mg/L) IAA + BAP (2.0mg/L). Among all hormonal combinations and concentrations that were employed, BAP may have been the only factor that successfully induced greater plantlet regeneration than any of the other hormonal treatments. BAP was found to enhance the regeneration frequency as reported by Gulati and Jaiwal (1930) and (Chandra, and Pal1995). The effectiveness of BAP on the induction of bud break and shoot proliferation has been reported in *Rotula aquatic* (Sebastian *et.al.,* 2002). BAP was found to be effective in the induction of bud break and shoot proliferation in *Rotula aquatic*. In order to achieve better rates of shoot multiplication in a number of plants, nodal explants were also utilized (Shekhawat and Galston1983). Explants of immature cotyledonary nodes were responsible for a high rate of plant regeneration in a number of different species (Tivarekar, and Eapen 2001).

The inclusion of NAA in MS medium, albeit at a lower concentration than originally intended, made rhizogenesis easier. On MS media with (3.0 mg/L) NAA, the highest number of roots (6.0 ± 0.32) and the highest frequency of root development (65%) were achieved. Within the growing chamber, regenerated plantlets that had completely formed shoots and roots were successfully hardened off for a period of four weeks on a planting substrate of choice, and they were finally planted in natural soil (Figure 1D). Exhibited any discernible changes as a result of the transition. This observation lines up with a number of prior discoveries in a similar vein (Singh *et al.,* 2006; Faisal *et al.,* 2006 and 2007).

IV. Conclusion:

In conclusion, an effective technique for the micropropagation of a significant medicinal plant, R. micrantha, were devised over the course of this research by optimizing a variety of growth regulators and medium. The nodal explants that were cultivated on a medium for woody plants that had 6.0 mg/L of TDZ added to it were found to be in the best condition. These findings could make it easier to propagate and conserve R. micrantha on a broad scale, which would result in an increase in the number of plant materials available for the manufacture of active chemicals derived from this species.

References:

[1]. Caro LA, Polci PA, Lindström LI, Echenique CV, Hernández LF (2002). Micropropagation of *Prosopis chilensis* (Mol.) Stuntz from young and mature plants. Biocell, 26: 25–33

[2]. Chandra M and Pal A, 1995. Differential responses of two cotyledons of *Vigna radiate* L. *In vitro Plant Cell Rep,* 15 - 63-67

[3]. Decise, S: Seri. S Pushpangadan, P(1999). Effect of cryopreservation in seed rotation of selected rare medicinal plants of India. *Seed Sci Techal* 27:50)-50s, 1999,

[4]. Faisal M, Ahmad N, Anis M (2007). An efficient micropropagation system for Tylophora indica: an endangered, medicinally important plant. *Plant Biotechnol. Rep.*, 1: 155-161.

[5]. Faisal M, Siddique I, Anis M (2006). *In vitro* rapid regeneration of plantlets from nodal explants of *Mucuna pruriens* a valuable medicinal plant. *Ann. Appl. Biol.*, 148: 1-6

[6]. Gary, A Tre, H. M. (1996) Establishing a micropropagation system for American Kinng (*Pans quinquefolium*). *Hart Science* 21 212-236; 1986.

[7]. Gastaldo, P., Carli, S., Profumo. P (1994). Somatic embryogenesis from stem explants of *Aesculus hippostanum*. *Plant Cell Tis. Organ Cult*. 39:97-99, 1994. ,

[8]. Gulati A and Jaiwal PK, (1930). Culture condition affecting plant generation from cotyledons of (*Vigna radiate* L.) *Plant cell Tissue Organ Cult,* 23 - 1-7.

[9]. Haw AB, Keng CL (2003). Micropropagation of *Spilanthes acmella* L., a bio-insecticide plant, through the proliferation of multiple shoots. *J. Applied Hort*., 5: 65–68.

[10]. Ilahi I, Rahim F, Jabeen M (2007). Enhanced clonal propagation and alkaloid biosynthesis in the culture *of Rauwolfia. Pak. J. Plant Sci*., 13: 45-56.

[11]. Kitti, S. 1. (1992) Commercial micropropagation HortScience 32:1012-1014; 1992.

[12]. Lee, K.; Zapata Aries, J Brantere, H.: Aura, H (1997). Histology of somatic embryo initiation and organogenesis from thine explant of Afins app: Plant Cell Tres. Organ Cult 511-8, 1997,

[13]. Luna C, Sansberro P, Mroginski L, Tarrago J (2003). Micropropagation of *Ilex dumosa* (Aquifoliaceae) from nodal segments in a tissue culture system. *Biocell*, 27: 205–212.

[14]. Martin KP (2002). Rapid propagation of *Holostemma ada-kodien* Schult., a rare medicinal plant, through axillary bud multiplication and indirect organogenesis. *Plant Cell Rep.*, 21: 112-117

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

[16]. Nadeem, M., Palni, L. M. S. Purshit, A. N: Pandey, I: Nandi, S. K (2000). Propagation and conservation of *Podophyllum henandrum* Royle an important medicinal herb, Biol. Consery, 92:121-129 2000.

[17]. Patil. V. M. Jay not, M. (1997) Micropropagation of ten species of *Rauvolfia* (Ap) Curr. Sei, 72961-065, 1997

[18]. Preece JE, Huttreman CA, Ashby WC, Roth PL (1991). Micro and cutting propagation of silver maple. I. Results with adult and juvenile propagules. J. Am. Soc. Hortic. Sci., 116: 142-148

[19]. Sahu, B. N. (1979) Taxonomy of Indian species*: Rauvolfia serpertina*, vol. II. New Delhi: Today and Tomorrow's Publishers; 1979:70-71. Samundaray, S.: Bond, G. Ra Das, P. Regeneration of plant via

[20]. Saini R, Jaiwal PK (2000). *In vitro* multiplication of *Peganum harmalaan* important medicinal plant. Indian J. *Exp. Biol.*, 38: 499-50

[21]. Sarker, K. P. Talam. A lalan, R. Hoque, A. Janler, 0, L fa (1996) in vitro propagation of *Rauvolfia serpertina* through tissue culture. Planta Med 2338-390, 1996

[22]. Sebastian DP, Benjamin S, Hariharan M, 2002. Micropropagation of *Rotula aquatic* L our – An important woody medicinal plant. Phytomorphol.52: 137-144.

[23]. Sharma, K. Yang, E C Thorps, T. A (19910 Histology of shoot bud integrity from seedling net segments of *Brassica napus* L. Ann. Hot.71-461-400 1991
[24]. Shekawat NS, Galston AW, 1983. Isolation, culture, and regeneration of moth bean, *Vigna aconitifolia* leaf protoplasts. Plant Science Letters, 32 - 43–51.
[25]. Singh AK, Sharma M, Varshney R, Agarwal SS, Bansal KC (2006). Plant regeneration from alginate to encapsulated shoot tips of *Phyllanthus amarus* Schum and Thonn, a medicinally important plant species. *In Vitro Cell. Dev. Biol. Plant*., 42: 109-113
[26]. Sudha, C. G Seeni, S.(1996) *In vivo* propagation of *Rauvolfia micrantha*, a rare medicinal plant. Plant Cell Tiss Organ Cult. $4/243-24 1996
[27]. Tivarekar S and Eapen S, (2001). High-frequency plant regeneration from immature cotyledon of mung bean, *Plant cell Tissue Organ Cult*, 66 - 227-230.
[28]. Tremullen-Guiller, L. Qenieus. J. C (1991) Somatic embryogenesis from leaf protoplasts of *Rauvolfia micrantha* shoot culture Plant Cell Report 102-105;
[29]. Upadhyay, N. Makkeychuk, A. Ya: Nikolarva, L. A Batygins, T. B Organogenesis and somatic embryogenesis in leal callus culture of *Rauvolfia caffia* J. Plant Phys. 140218-222
[30]. Youngkhen, HW Malabar *Rauvolfia,micrantha* Hd F. J. Am. Pharm Asse 43:141-143, 1954

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