

IN VITRO CLONING OF AN ENDANGERED MEDICINAL PLANT, *RAUWOLFIA SERPENTINA* (L.)

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Abstract: An efficient protocol for *in vitro* cloning of *Rauwolfia serpentina* L. was developed using leaf segment (LS), nodal segment (NS) and internodal segments (INS) as explants. The technique involves *in vitro* shoot regeneration, rooting of microshoots and transplantation of regenerated plantlets under *in vivo* condition. Sterilized explants were cultured on MS media supplemented with different auxin (IAA, IBA, NAA, & 2,4-D) and cytokinins (Kn & BAP) within a concentration range of 0.5-3.0mg/L used singly or in combination. The best shoot multiplication was obtained from nodal explants on MS medium supplemented with BAP+NAA (1.5+0.5) mg/L along with CW (5% v/v). Excellent rooting of microshoots (4-6cm) was noticed on the medium (1/2 MS salt) fortified with combination of auxins [NAA+IBA, (1+0.5)mg/L]. Compact callus which was hydrated, green and crystalline in appearance was obtained from LS and INS on medium having 1.5mg/L 2,4-D. Nodal explants were superior to internodal as well as leaf explants in response to shoot proliferation. Regenerated plantlets were transferred to pots having mixture of sand:soil:vermiculite(1:1:1) and little fungicides (Eco fungicide). The survival rate of plantlets was much promising (around 85%) and regenerated plantlets were healthy, green and morphologically identical to mother plants.

Keywords: *Rauwolfia serpentina*, Callus, Phytohormones, Multiple shoot, Conservation

REFERENCES

AAP (2009-10), National mission on medicinal plants, Mission Director- State Horticulture Mission, Bihar.

Ahmed, S.; Amin, MN.; Anjum, A. and Haque, ME.; (2002). *In vitro* antibacterial activity of *Rauwolfia serpentina* and its tissue culture. Niger. J. Nat. Prod. Med, **6**:45-49.

Bemis, DL.; Capodice, JL.; Gorroocurn, P.; Katz, AE. And Buttyan, R. (2006). Antiproliferative activity of β -carboline alkaloid enriched extract from *Rauwolfia vomitoria*. Int. J. Oncol, **29**:1065-1073.

Bhaskaran, S.; and Smith RH. (1990). Regeneration in cereal tissue culture: A review. Crop sci. **30**: 1328-1336.

Campbell, JIA.; Mortensen, A. and Molgaard, P. (2006). Tissue lipid lowering effect of a traditional Nigerian antidiabetic infusion of *Rauwolfia vomitoria* foliage and *Citrus aurantium* fruit. J. Ethnopharmacol. **104**: 379-386.

Chaudhury, A. and Qu, R. (2000). Somatic embryogenesis and plant regeneration of turf-type bermudagrass: effect of 6-Benzaldehyde in callus induction medium. *Plant cell tiss. Org.Cult.*, **60**:113-120.

Chopra, RN.; Naya, SL. and Chopra IC (1956). Glossary of Indian medicinal plant (New Delhi India:CSIR publication).

Dutta, PK.; Chaudhary, SB. and Rao, PR. (1962). Germination and chemical composition of *Rauwolfia serpentina* seeds. Indian J. Pharm, **24**:61-63.

Dwari, M.; and Chand, PK. (1996). Evaluation of explants growth regulators and culture passage for enhanced callus induction, proliferation and plant

regeneration in the tree legume *Dalbergia lenceolaria*. Phytomorphology, **46**: 123-131.

Ezeigbo, II.; Ezeja, MI.; Madubuike, KG.; Ifenkwe, DC.; Ukwani, IA.; Udeh, NE. and Akomas, SC. (2012). Antidiarrhoeal activity of leaf methanolic extract of *Rauwolfia serpentina*. Asian Pac. J. Trop. Biomed, **2**(6):430-432.

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

Gupta, AK.; Chitme, H.; Dass, SK. and Misra, N. (2006). Hepatoprotective activity of *Rauwolfia serpentina* rhizome in paracetamol intoxicated rats. J. Pharmacol. Toxicol, **1**: 82-88.

Jain, SP.; Singh, J. and Singh, SC. (2003). Rare and endangered medicinal and aromatic plants of Madhya Pradesh. J. Econ. Taxon. Bot, **27**: 925-932.

Joshi, N. and Kumar, N. (2000). *Rauwolfia*. In: Aromatic and medicinal Plants in Central Himalayas. Kumar, N. (Ed.). Defence Agricultural Research Laboratory, Pithoragarh, Uttarakhand, India.

Khanam, N. and Sharma, GK. (2014). Rapid *in vitro* propagation of *Aloe vera* L. with some growth regulators using lateral shoots as explants. World journal of Pharma and Pharmaceutical Sciences, **3**(3): 2005-2018.

Kirillova, NV.; Smirnova, MG. and Komov, VP. (2001). Sequential isolation of superoxide dismutase and ajmaline from tissue culture of *Rauwolfia serpentina* Benth. Prikl. Biokhim. Mikrobiol, **37**:181-185.

Kumar, A.; Ahmad, S. and Naseem M. (2010). *In vitro* plant regeneration from organ cultures of *Gmellina arborea* Roxb. *J.Indian bot. Soc.*, **89** (1& 2): 197-203.

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- Lakshmi-sita, G.; Chattopadhyaya, S. and Tejavathi, DH.** (1986). Plant regeneration from shoot callus of rose wood (*Dalbergia latifolia* Roxb.). *Plant cell reports*, **5**: 255.
- Mallick, SR.; Jena, RC. and Samal, KC.** (2012). Rapid *in vitro* multiplication endangered medicinal plant sarpagandha (*Rauwolfia serpentina*). *American journal of plant science*, **3**: 437-442.
- Manuchair, E.** (2002). Reserpine. In: *Pharmacodynamics basis of Herbal Medicine*. Ebadi, MS (Ed.) CRC Press, Boca Raton.
- Mitra, GC.** (1976). Studies on the Formation of Viable and Non-Viable Seeds in *Rauwolfia serpentina* Benth. *Indian Journal of Experimental Biology*, **14**(1): 54-56.
- Mukhopadhyay, S. and Sharma, AK.** (1986). Induction, maintenance and growth rate study of callus culture of *Costus speciosus* (Koen.) SM. Manna, GK. and Sinha, U;(ed), *Perspective in cytology and Genetics*. Ratravani printers, New Delhi, pp-205-211.
- Murashige, T. and Skoog, F.** (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*, **15**, 473-479.
- Narayanswamy, S.** (1977). Regeneration of plants from tissue cultures. In: Reinert, J. and Bajaj, YPS (ed) *Applied and fundamental aspect of plant cell, tissue and organ culture*. Springer-Verlag, Berlin, pp. 197-206.
- Naseem, M. and Jha, KK.** (1994). Differentiation and Regeneration in *Cleome* leaves cultured *in vitro*. *Egypt J. Bot.*, **34** (1): 37-39.
- Naseem, M. and Jha, KK.** (1997). Rapid clonal multiplication of *Cleome gynandra* DC. through tissue culture. *Phytomorphology*, **47** (4): 405-411.
- Patil, VM. and Jayanthi, M.** (1997). Micropropagation of two species of *Rauwolfia* (Apocynaceae). *Current Science*, **72**(12) : 961-965.
- Paul, D.; Paul NK. and Basu, PK.** (2008). Seed germination response of *Rauwolfia serpentina* Benth. To certain physical and chemical treatments. *Journal of Bio-sci*, **16**: 129-131.
- Qureshi, SA.; Nawaz, A.; Udani, SK. and Azmi, B.** (2009). Hypoglycaemic and hypolipidemic activities of *Rauwolfia serpentina* in Alloxan induced diabetic rats. *Intl. J. Pharmacol.*, **5**(5):323-326.
- Rao, GB.; Rao, PU.; Rao, ES.; Rao, TM.; Rao, M. and Praneeth, VSD.** (2012). Evaluation of *in vitro* antibacterial activity and antiinflammatory activity for different extracts of *Rauwolfia tetraphylla* L. root bark. *Asian Pac. J. Trop. Biomed.* **2**(10):818-821.
- Sachdev, KS.; Aiman, R. and Rajapurkar, MV.;** (1961). Antihistaminase activity of serpentina. *Br. J. Pharmacol. Chemother.*, **16**(2):146-152.
- Salma, U.; Rahman, MS.; Islam, S.; Haque, N.; Khatun, M.; Jubair, TA. and Paul, BC.** (2008). Mass propagation of *Rauwolfia serpentina* L. Benth. *Pak. J. Biol. Sci.*, **11**: 1273-1277.
- Singh, P.; Singh, A.; Shukla, KA.; Singh, L.; Pandey, V. and Naiwal, TK.** (2009). Somatic embryogenesis and *In Vitro* Regeneration of an Endangered Medicinal Plant Sarpagandha (*Rauwolfia serpentina* L.). *Researcher*, **1**(3) 46:53.
- Srivastava, A.; Tripathi, AK.; Pandey, R.; Verma, RK. and Gupta, MM.** (2006). Quantitative determination of reserpine, aimaline and aimalicine in *Rauwolfia serpentina* by reverse phase high performance liquid chromatography. *J. Chromatogr. Sci.* **44**:557-560.
- Sushila, T.; Reddy, GS. and Jyothsna, D.** (2013). Standardization of protocol for *in vitro* propagation of an endangered medicinal plant *Rauwolfia serpentina* Benth. *Academicjournals*, **7** (29): 2150-2153.
- Tran Tanh Van, M.** (1973). Direct flower neof ormation from superficial tissue of small explants of *Nicotiana tabacum* L. *Planta*, **115**: 87.
- Von, PG.; Andrade, HH.; Da, SKV.; Henriques, AT. and Henriques, JA.** (1990). Genotoxic, mutagenic and recominogenic effects of *Rauwolfia* alkaloids. *Mutat. Res.* **232**: 37-43.
- Weerakoon, SW.; Arambewela, LSR.; Premakumara, GAS. and Ratnasooriya, WD.;** (1998). Sedative activity of the crude extract of *Rauwolfia densiflora*. *Pharmaceut. Biol.* **36**(5):360-361.