Annals of Plant Sciences 7.2 (2018) pp. 1988-1991

ANNALS OF PLANT SCIENCES
ISSN: 2287-688X
OPEN ACCESS
www.annalsofplantsciences.com

Research Article

In vitro callus induction in inflorescence segments of medicinally important endangered plant *Rauwolfia serpentina* (L.) Benth. ex Kurz – a step towards *ex situ* conservation

Saranjeet Kaur
Department of Science, Cordia College, Sanghol, District- Fatehgarh Sahib, Punjab, India.

Received: 11/22/2017; **Accepted:** 1/10/2018

Abstract: The regenerative competence of inflorescence segments of *Rauwolfia serpentina* was assessed in M (Mitra *et al.*, 1976) medium and its combinations with growth adjuncts such as cytokinins [N^6-benzyladenine (6-BA - 0.5, 1.0 mg l^{-1}), furfurylaminopurine (Kn-0.5, 1.0mg l^{-1}) and auxin [α-naphthalene acetic acid (NAA - 0.5, 1.0 mg l^{-1})]. Inflorescence segments (1.00 cm in length) were used as explants. The regeneration response was obligatory to the use of growth regulators in the medium. Cytokinins favoured shoot bud mediated pathway of plantlet development and BAP favoured early shooting. The explants callused in NAA enriched medium. The callus was best maintained in NAA (0.5 mg l^{-1}) through repeated sub-culturing. The entire plant of *R. serpentina* is the target of massive commercial collections resulting into shrinking habitats. Present study is the first ever report on the successful use of inflorescence segments for *ex situ* conservation of *R. serpentina*.

Keywords: Auxin, Callus, Cytokinins, Endangered, Medicinal plant

Introduction

Rauwolfia serpentina (L.) (f = Apocynaceae) is commonly known as 'sarpagandha'. It is an evergreen erect, glabrous, perennial shrub bearing white or pink blooms in cymose inflorescences with deep red peduncles (Fig.1a).

Figure 1. A plant of *Rauwolfia serpentina* with cymose inflorescence.

The species is distributed from India to Indonesia at an altitude of 1,000 m. In India, the species is widespread in the sub-Himalayan region, the Eastern and Western Ghats(http://www.bsienvis.nic.in/CITES/R.%20serpentina). The plant harbours 200 alkaloids (approx.) and the major alkaloids are ajmaline, ajmalicine, ajmalimine, deserpidine, indobine, indibinine, reserpine, reserpiline, rescinnamidine, serpentine, serpentinine (http: faculty.iiitd.ac.in/~bagler/webservers/SerpentinaDB/). It secretes a milky liquid which contain alkaloids and secondary metabolites (Rashmi and Trivedi, 2016). The species is also used by the tribals as a folklore medicine. Since ages, the roots of *R. serpentina* are used in the traditional system of Unani and Ayurvedic medicine (cf. Salma *et al.*, 2008). The roots are extensively used as a laxative, diuretic, expectorant, febrifuge, anti-helmenthic, anti-pyretic, anti-dysenteric, also stimulates uterine contractions, and acts as an antidote to the snake venom. The juice extracted from the roots is used in the treatment of opacity of the cornea. (http://www.bsienvis.nic.in/CITES/R.%20serpentina).

The alkaloids of the species are anti-carcinogenic (Stanford *et al.*, 1986), treats human promyelocytic leukemia (Itoh *et al.*, 2005), anti- hypertensive (Von Poser *et al.*, 1990), treats cardiovascular diseases (Anitha and Kumari, 2006), arrhythmia (Kirillova *et al.*, 2001), psychiatric diseases (Bhatara *et al.*, 1997; Kirtikar and Basu, 1993) and anti-diabetic (Pathania *et al.*, 2013). Since *R. serpentina* shows broad range of medicinal use, whole plant is the target of massive commercial collections for pharmaceutical industries, scientists, researchers and traders resulting into its ever-shrinking habitats. As a consequence, the species is included in appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 2017) (https://cites.org/eng/app/appendices.php).

R. serpentina shows very low seed germination percentage due to the presence of cinnamic acid derivatives which is reported to be a strong inhibitor of seed germination (Mitra, 1976). *In vitro* culture techniques offer an indispensable means for crop improvement besides rapidly propagating disease-free plantlets within short span of time and promoting their sustainable development. It provides a viable system for the conservation of endangered aromatic and medicinal plants of economic and commercial importance (Arora and Bhojwani, 1989; Sharma *et al.*, 1991; Sudha and Seeni, 1994; Sahoo and Chand, 1998; Karuppusamy and Pullaiah, 2007; Jawahar *et al.*, 2008; Mallon *et al.*, 2010). A number of biotechnological reports are available in the literature on *in vitro* propagation of *R. serpentina* using stem-nodes as explant (Ahmad *et al.*, 2002; Sarker *et al.*, 1996; Roy *et al.*, 1994; Mathur *et al.*, 1993; Salma *et al.*, 2008; Rashmi and trivedi, 2016), but so far there is no single report on the utility of inflorescence segments as explants for raising cultures of *R. serpentina in vitro*. The explants cultured *in vitro* are known to retain the capacity to synthesize identical alkaloids to that of the *in vivo* grown *R. serepentina* (Yoshimatsu and Shimomura, 1991).

In vitro raised callus tissue possess multifaceted utility (Fig. 2a) as it yields a high contents of secondary metabolites during differentiation (Benavides and Caso, 1993; Maheshwari *et al.*, 2007). The callus retains the capacity to

*Corresponding Author:
Saranjeet Kaur
Department of Science,
Cordia College, Sanghol,
Fatehgarh Sahib, Punjab, India.
E-mail: sarana_123@rediffmail.com

synthesize alkaloid production (Yamamoto and Yamada, 1986; Prem *et al*., 2002; Anitha and Kumari, 2006). From these secondary metabolites, therapeutic drugs can be extracted without sacrificing the whole plant. Through the transformation techniques, the callus suspension cultures are used in gene delivery system in plants such as *Zea mays* (Frame *et al*., 1994) and *Oryza sativa* (Nagatani *et al*., 1997). Repeated sub-culturing of callus efficiently maintains the germplasm *in vitro* and is a useful technique to generate polyploids as well. Friable calli are reported to generate totipotent cell cultures (Brisibe *et al*., 2000). In the present scenario, sound scientific approaches are required to save the ever-declining populations of *R. serpentina*. Currently, an attempt was made to conserve the species *in vitro* using inflorescence segments as explants by inducing and maintaining the callus in the cultures, simultaneously regenerating the plantlets with a view to make the species readily available for the isolation of medicinally important bioactive compounds without collecting the species from its wild habitat. This step would definitely assist with the *ex situ* conservation and thus saving the species in its natural habitat. With this view, the present studies were planned to access the regeneration potential of inflorescence segments of *R. serpentina* and initiate callus cultures *in vitro*.

Materials and Methods

Inflorescence segments (1.00 cm long) procured from *in vivo* grown *R. serpentina* plants were used as explants. These were inoculated on Mitra *et al*., 1976 (M) medium and its combinations with growth regulators such as cytokinins [6-benzyl aminopurine (BA - 0.5,1.0 mgl^{-1}), furfurylaminopurine (Kn - 0.5,1.0 mgl^{-1}) and auxin [α-naphthalene acetic acid (NAA - 0.5,1.0 mgl^{-1})] (Hi- media, Mumbai, India). Sucrose 2% (wv^{-1}) was invariably used as carbon source. The medium was gelled with 0.9% Agar powder (Hi- media, Mumbai, India). The pH of the medium was adjusted to 5.8 after adding the growth regulators. The medium was dispensed in the test tubes (25 mm × 150 mm) and autoclaved at 121°C at pressure of 1.06 kg cm^{-2} for 15 min. Autoclaved medium was kept at 37°C to check any further contamination.

Surface sterilization

The inflorescence segments (1.00 cm long) were scrubbed with a soft brush in flowing tap water. Gently, they were again scrubbed using dish wash liquid detergent to get rid of any debris left over the surface of the segments. These were rinsed thoroughly in running tap water. Later, inside laminar airflow they were surface sterilized with 0.1% mercuric chloride ($HgCl_2$; Qualigens, Mumbai, India) in an aqueous solution containing 1-2 drops of liquid soap as a wetting agent for 3-4 minutes. The aqueous solution of mercuric chloride was decanted and segments were rinsed 2-3 times with sterilised distilled water to remove any trace of mercuric chloride left over the surface. Thereafter, these sterilised explants were placed in a petri dish and very carefully their ends on both sides were severed-off and inoculated vertically into M medium and its supplementations with various growth adjuncts.

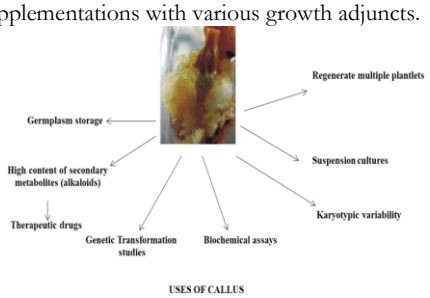

Figure 2a. Multifaceted utility of callus tissue

Callus proliferation and maintenance

For callus proliferation and maintenance, the callus obtained from inflorescence segments was subcultured in M medium supplemented with different concentrations of auxin - NAA (0.5, 1.0, 1.5, 2.0 mgl^{-1}). Sub-culturings were done after every 15 days.

Inoculations and culture conditions

The inoculations were done under aseptic conditions in a laminar airflow cabinet. The culture vessels were incubated at 25 ± 2°C under 60% - 70% relative humidity and 16/8 hrs light/dark photoperiod at 40 μ mol $m^{-2}s^{-1}$ light intensity provided by white fluorescent tubes (Fluorescent tubes, Philips India Ltd., Mumbai, India).To check the reproducibility of the protocol, the experiment was repeated twice. The result was expressed on minimum of eight replicates. The cultures were observed regularly under binocular microscope and data recorded accordingly.

Observations and statistical analysis

The experiment was designed following complete randomized design (CRD) with eight replicates per treatment. The effect of medium on percentage of regeneration, time of initiation of regeneration response, and time taken in weeks to form complete plantlets was tested applying Tukey's multiple comparison test ($P \leq 0.05$) in one way ANOVA to separate significantly different groups. The statistical analysis was performed using the SPSS (version 17) software package. (SPSS Inc., Chicago, USA).The results are expressed as mean ± SD of eight replicates.

Results

In the present experiment, culture initiation was markedly influenced by the chemical stimulus in the nutrient pool. The regeneration response in the segments was obligatory to the use of growth adjuncts in the nutrient pool (Table 1; Fig. 3a-f).

Table 1. Effect of auxin and cytokinin on *in vitro* callus induction in *Rauwolfia serpentina* in M medium

Additives	% explant responded	Initiation of response (Days)	Regeneration pathway	Development of shoots in (Days)	Development of roots in (Days)	Remarks
Basal M	0.00± 0.00	0.00± 0.00	-	0.00± 0.00	0.00± 0.00	-
BA $_{(0.5)}$	75.00± 0.10^{ab}	12.14± 0.110^{b}	Sb	7.02± 0.04^{b}	20.65± 0.10^{b}	-
BA$_{(1.0)}$	100± 0.00^{c}	11.00± 0.00^{c}	Sb	7.11± 0.03^{b}	18.33± 0.00^{a}	-
KN$_{(0.5)}$	50± 0.01^{a}	12.32± 0.20^{bc}	Sb	7.22± 0.00^{b}	20.11± 0.40^{b}	-
KN$_{(1.0)}$	100± 0.00^{c}	13.11± 0.01^{b}	Sb	6.50± 0.00^{a}	19.00± 0.00^{a}	-
NAA $_{(0.5)}$	100± 0.00^{c}	21.00± 0.90^{a}	C	28.00± 0.00^{c}	33.02± 0.50^{d}	Shoots were induced upon a shift to BAP enriched medium and initiated shoots after 28 days
NAA $_{(1.0)}$	100± 0.00^{c}	21.00± 0.75^{a}	C	28.00± 0.00^{c}	32.65± 0.00^{d}	”
NAA$_{(1.0)}$+BAP$_{(1.0)}$	100± 0.00^{c}	14.00± 0.00^{b}	C	23.01± 0.00^{d}	27.00± 0.00^{c}	

C-callus; Sb- shoot bud; concentration within parenthesis is mgl^{-1}.

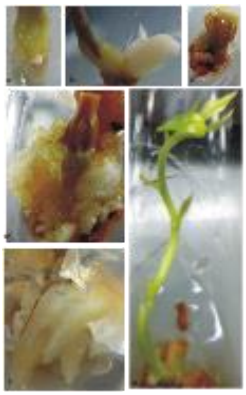

Figure 3a. Bud break in M + BAP (0.5 mg l^{-1}); b. development of shoot bud in M + BAP (1.0 mg l^{-1}); c. callusing of internal tissues in the segment in M + NAA (1.0 mg l^{-1}); d. profuse callusing in inflorescence in M + NAA (0.5 mg l^{-1}); e. plantlet development in BAP (1.0 mg l^{-1}); f. development of roots in M + BAP (1.0 mg l^{-1}).

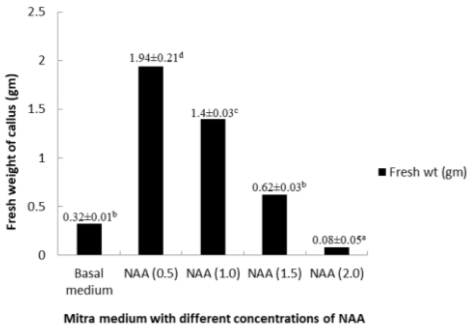

Figure 4. Effect of different concentrations of NAA on growth of the callus of *R. serpentina* on fresh weight basis.

Highlights

- The species is medicinally important, categorized as endangered, needs conservation
- Internal tissues of the inflorescence segments callused profusely in NAA
- Germplasm storage through callus induction, callus growth was maintained through repeated subcultures in low salt concentration Mitra medium
- Shoots were obtained from the explants directly without any intervening callus stage

Cytokinins (BA/Kn; 1.0 mg l^{-1}) in the medium induced regeneration response in cent per cent explants. It induced regeneration via axillary bud break within 11 and 13 days respectively (Fig. 3a). With passage of time, the buds grew in size (Fig. 3b) and developed shoot primordia and later transformed into plantlets. A low concentration of BA/Kn (0.5 mg l^{-1}) reduced the percentage of the responding explants. The shoots rooted within 18 and 19 days and subsequently formed plantlets (Fig. 3e). Rooting was never a problem in the cultures. The cultures developed creamish-white roots (Fig. 3f).

A replacement of cytokinins with auxin NAA (0.5, 1.0 mg l^{-1}) proved beneficial in inducing callus in cent per cent explants. The explants callused profusely, even the internal tissues (cortical region) at the inflorescence node region were also amenable to transform in exogenous and endogenous callus (Fig. 3c). Phenotypically, the callus was friable, very soft and off-white to green in colour (Fig. 3d). The callus tissue was severed-off the explant and sub-cultured on to the same medium composition. The callus proliferated more in NAA 0.5 mg l^{-1}. Simultaneously, to initiate the shoots in the callus, it was transferred to medium supplemented with BA medium where it developed shoots and developed into plantlets after 28 days. In order to determine best concentration of NAA for callus proliferation and maintenance, small clumps of calli, more or less of equal size, were subcultured on fresh Mitra medium supplemented with various concentrations of NAA at 0.0, 0.5; 1.0; 1.5; 2.0 mg l^{-1}. The growth of the callus was calculated in terms of fresh weight basis. The maximum weight of callus i.e 1.94 mg was obtained on NAA 0.5 mg l^{-1} (Fig. 4)

Discussion

Presently, the regeneration potential of the inflorescence segments of *R serpentina* was tested in M medium and its combinations with growth adjuncts. The nature of regeneration pathway, time taken to initiate the cultures and their development into plantlets were markedly influenced by chemical stimulus in the nutrient pool. In this experiment, the efficacy of M medium is tested. Earlier workers mostly used MS medium, which is a more complex medium consisting of high salt concentration, for initiation and multiplication of *in vitro* cultures of *R. serpentina* using stem node as explants (Mathur *et al.,* 1993; Shah *et al*., 2003; Rashmi and trivedi, 2016). In this experiment, the positive response of the inflorescence segments in M medium suggests that the species has wide nutritional amplitude and it appears that a simple defined M medium is also fully capable of initiating the regeneration response in the explants. The response in the inflorescence segments was found obligatory to the use of growth adjuncts in the nutrient pool. Literature studies reveal that the segments require a stimulus by adding certain concentration of plant growth regulators for initiation of response as earlier also observed in *R. serpentina* in MS medium (Shah *et al*., 2003; Rashmi and Trivedi, 2016).

Cytokinins (BAP/Kn) favoured direct shoot bud mediated regeneration; no callus was induced. The results are in accord with similar earlier findings in *R. serpentina* (Murashige *et al*., 1974; Rashmi and Trivedi, 2016). A variety of plant growth regulators are reported to induce callus in the *in vitro* cultures. Presently, auxin individually (NAA) and with BAP promoted callus mediated regeneration in the explants. The explants callused profusely. The callus was cream coloured, friable and embryogenic in nature similar to those reported earlier by Roja and Heble (1996), where auxin efficiently promoted callusing and shoot initiation in the explants along with cytokinins. A perusal of literature reveals that generally the lower concentration of NAA and a high BAP concentration favoured the shoot formation in *Rauvolfia micrantha* (Sudha and Seeni, 1996). The combination of BAP and NAA was found most suitable for shoot initiation in *R. serpentina* (Ahmad *et al*., 2002; Rashmi and Trivedi, 2016). A subcultured callus tissue retains the ability to induce adventitious shoots. The shoot differentiated in the callus clumps when subjected to BAP treatment in M medium. The results are similar to those obtained in *Chlorophytum borivilianum* (Nakasa *et al*., 2016).

Presently, the callus was maintained in Mitra medium which is a medium with low salt concentration and among the various concentrations of NAA tested; the concentration of 0.5 mg l^{-1} proved best for the callus growth in the cultures. Our results are in contrast to the earlier reports made by Smith (1992), where the callus

required a high salt concentration for its proliferations. Although, similar to Smith (1992) the regeneration capacity of friable callus was maintained best by repeated sub-culturing on the medium with same nutrient composition i.e. NAA 0.5 mg l^{-1}. From the above discussed results it can be concluded that the explants of *R. serpentina* can be successfully conserved *in vitro* and simultaneously plantlets can also be obtained by manipulating the regeneration pathway through the application of auxin and cytokinin selectively. Through repeated sub-culture method callus can be conserved using a low salt concentration medium i.e. M medium supplemented with NAA (0.5 mg l^{-1}).

References

1. Ahmad S, M N Amin, M A K Azad and M A Mosaddik. "Microprpagation and Plant regeneration of *Rauwolfia serpentina* by tissue culture technique." *Pakistan Journal of Biological Sciences* 5(2002): 75-79.
2. Anitha S and B D R Kumari. "Stimulation of reserpine biosynthesis in the callus of *Rauwolfia tetraphyla* L. by precursor feeding." *African Journal of Biotechnology* 5(2006): 659-661.
3. Arora R and S S Bhojwani. "*In vitro* propagation and low temperature storage of *Saussurea* lappa C.B. Clarke-an endangered medicinal plant." *Plant Cell Reports* 8(1989): 44-47.
4. Benavides M P and O H Caso. "Plant regeneration and thiophin formation in tissue cultures of *Tagetes mendocina*." *Plant Cell Tissue and Organ Culture* 35(1993): 211-215.
5. Bhatara V S, J N Sharma, S Gupta and Y K Gupta. "*Rauwolfia serpentina*: The first antipsychotic." *American Journal of Psychiatry* 154(1997): 894-896.
6. Brisibe E A, A Gajdosova, A Olesen and S B Andersen. "Cytodifferentiation and transformation of embryogenic callus lines derived from anther culture of wheat." *Journal of Experimental Botany* 51(2000): 187-196.
7. CITES (2017) (https://cites.org/eng/app/appendices.php).
8. Frame B R, P R Drayton, S V Bagnall, C J Lewnau, W P Bullock, H M Wilson, J M Dunwell, J A Thompson and K Wang. "Production of fertile transgenic maize plants by silicon carbide whisker-mediated transformation." *The Plant Journal* 6(1994): 941–948.
9. Itoh A, T Kumashiro, M Yamaguchi, N Nagakura, Y Mizushina,T Nishi and T Tanahashi. "Indole alkaloids and other constituents of *Rauwolfia serpentina*." *Journal of National Proceeding* 68(2005): 848-852.
10. Jawahar M, A V P Kartikeyan, D Vijai, M Mahajan, S Ravipaul and M Jeyseelan. "*In vitro* plant regeneration from different explants of *Cardiospermum helicacabum* L." *International Journal of Biological and Chemical Sciences* 2(2008):14-20.
11. Karuppusamy S and T Pullaiah. "*In vitro* shoot multiplication of *Bupleurum distichophyllum* Wight. A native medicinal plant of southern India." *Plant Tissue. Culture & Biotechnology* 17(2007): 115-124.
12. Kirillova N V, M G Smirnova and V P Komov. "Sequential isolation of superoxide dismutase and ajmaline from tissue culture of *Rauwolfia serpentina* Benth." *Prikladnaia Biokhimiia Microbiologiia*, 32(2001):181-185.
13. Kirtikar K R and B D Basu. "Indian medicinal plants" Bishen Singh Mahendra Pal Singh, Publishers, Dehradun, India 2(1993), pp.289.
14. Maheshwari P, B Songora,S Kumar, P Jain, K Srivastava and A Kumar. "Alkaloid production in *Vernonia cinerea*: callus, cell suspension and root cultures." *Journal of Biotechnology* 2(2007): 1026-1032.
15. Mallon R, R J Oubina and M Gonzalez. "*In vitro* propagation of the endangered plant *Centaurea ultreiae*: assessment of genetic stability by cytological studies, flow cytometry and RAPD analysis." *Plant Cell Tissue and Organ Culture* 101(2010): 31-39.
16. Mathur A, P S Ahuja and A K Mathur "Micropropagation of *Panax quiquefolium*, *Rauwolfia serpentina* and some other medicinal and aromatic Plants of India." In: Adapted Propagation Techniques for Commercial Crops of the Tropics. (eds. Quynh N T and N V Hyen) *Agriculture Publishing House*, 1993, Hochi Minh, Vietnam.
17. Mitra G C, R N Prasad and A R Chowdhury. "Inorganic salts and differentiation of protocorms in seed callus of an orchid and correlated changes in its free amino acid content." *Indian Journal of Experimental Biology* 14(1976): 350–351.
18. Murashige T, M Serpa and J B Jones. "Clonal multiplication of *Gerbera* through tissue culture." *Horticultural Science* 2(1974): 170-180.
19. Nagatani N, S Takumi, M Tomiyama, T Shimada and E Tamiya. "Semi-real time imaging of the expression of a maize polyubiquitin promoter-GFP gene in transgenic rice." *Plant Sciences* 124(1997): 49-56.
20. Nakasha J J, U R Sinniah, N Kemat and K S Mallappa. "Induction, subculture cycle, and regeneration of callus in safed musli (*Chlorophytum borivilianum*) using different types of phytohormones." *Pharmacognosy Magazine* 12*(2016)*: 460–464.
21. Pathania S, V Randhawa and G Bagler. "Prospecting for novel plant-derived molecules of *Rauwolfia serpentina* as inhibitors of aldose reductase, a potent drug target for diabetes and its complications." *PLoS ONE* 8(2013): 613-627.
22. Prem D, I Kazutaka and T Sanro. "Stimulation of the production of podophyllotoxin by biogenetic precursors and an elicitor in *Juniperus chinensis* stem-derived callus cultures." *Pakistan Journal of Biological Sciences* 5(2002): 313-316.
23. Rashmi R and M P Trivedi. "Rapid *in vitro* regeneration of an endangered medicinal plant sarpagandha (*Rauwolfia serpentina* L.)" *European Journal of Pharmaceutical and Medical Research* 3(2016): 276-284.
24. Roja G and M R Heble. "Indole alkaloids in clonal propagales of *Rauwolfia serpentina* Benth. Ex. Kurz." *Plant Cell Tissue and Organ Culture* 44(1996): 111-115.
25. Roy S K, M Z Hossain and M S Islam. "Mass propagation of *Rauwolfia serpentina* by *in vitro* shoot tip culture" *Plant Tissue Culture* 4(1994): 69-75.
26. Sahoo Y and P K Chand. "Micropropagation of *Vitex negundo* L. a woody aromatic medicinal shrub through high frequency of axillary shoot proliferation." *Plant Cell Reports* 18(1998): 301-307.
27. Salma U, M S M Rahman, S Islam, N Haque, M Khatun, T A Jubair and B C Paul. "Mass Propagation of *Rauwolfia serpentina* L. Benth." *Pakistan Journal of Biological Sciences* 11(2008): 1273-1277.
28. Sarker K P, A Islam, R Islam, A Hoque and O I Joarder. "*In vitro* propagation of *Rauwolfia serpentina* through tissue culture." *Planta Medica* 62(1996):358-359.
29. Shah M I, M Jabeen and I Hahi. "*In vitro* callus induction, its proliferation and regeneration in seed explants of wheat (*Triticum vulgare* L.) var. Lu-26S." *Pakistan Journal of Botany* 35(2003): 209-217.
30. Sharma N, K P S Chandel and V K Srivastava. "*In vitro* propagation of *Coleus forskohlii* Briq. – a threatened medicinal plant." *Plant Cell Reports* 10(1991): 67-70.
31. Smith, R H. "Plant Tissue Culture Techniques and Experiments." Academic Press, INC., California. 1992, pp. 171.
32. Stanford J L, E J Martin, L A Brintin and R N Hoover. "*Rauwolfia* use and breast cancer. A case-control study." *Journal of National Cancer Institute* 76 (1986):817-822.
33. Sudha CG and S Seeni. "*In vitro* multiplication of *Adhatoda beddomei* C.B. Clarke- A rare medicinal plant." *Plant Cell Reports* 13(1994): 2003-2007.
34. Sudha C G and S Seeni. "*In vitro* propagation of *Rauwolfia serpentina*, a rare medicinal plant." *Plant Cell Tissue and Organ Culture* 44(1996): 243-248.
35. Von Poser G, H H Andrade, K V Da Silva and J A Henriques. "Genotoxic, mutagenic and recombinogenic effects of *Rauwolfia alkaloids*." *Mutation Research* 232(1990): 37-43.
36. Yamamoto O and Y Yamada. "Production of reserpine and its optimization in cultured *Rauwolfia serpantina* cells." *Plant Cell Reports* 5(1986): 50-53.
37. Yoshimatsu K and K Shimomura. "Efficient shoot formation on internodal segments and alkaloid formation in the regenerates of *Cephaelisipeca cuanha* A. Richard." *Plant Cell Reports* 9(1991): 567-570.

Cite this article as:
Saranjeet Kaur. *In vitro* callus induction in inflorescence segments of medicinally important endangered plant *Rauwolfia serpentina* (L.) Benth. ex Kurz – a step towards ex situ conservation. *Annals of Plant Sciences 7.2 (2018) pp. 1988-1991.*
http://dx.doi.org/10.21746/aps.2018.7.2.1

Source of support: Nil; **Conflict of interest:** Nil