International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 12, Issue 1-2025

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2025.12.01.004

Study of phytohormones effect on micropropagation and callus induction in *Rauwolfia serpentina*

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Abstract

Objective: The present study aimed to investigate the impact of varying concentrations of BAP and IAA on callus induction in *R. serpentina*. Two hormone combinations, BAP + IAA, with different concentrations, were applied to induce callus formation.
Method: The newly emerging leaves were used as explants, which were transferred to MS medium along with various combinations of growth regulators for callus regeneration.
Result: The least callusing was observed with BAP (1.0 mg/l) + IAA (0.5 mg/l) and BAP (2.5 mg/l) + IAA (0.4 mg/l) treatments in MS medium. Moderate callusing was noted with BAP (2.0 mg/l) + IAA (0.5 mg/l), while good callusing was observed with BAP (1.5 mg/l) + IAA (0.5 mg/l) in MS medium. Very good callusing occurred with BAP (2.5 mg/l) + IAA (0.5 mg/l) treatment.
Conclusion: The study concludes that higher concentrations of BAP, particularly in combination with a constant IAA concentration, significantly enhance callus induction in *R. serpentine.* Additionally, varying hormone combinations exhibit differential impacts on callusing, with BAP (2.5 mg/l) + IAA (0.5 mg/l) treatment showing the most favorable results.

Keywords: *Rauwolfia serpentina,* callus induction, BAP, IAA, MS medium, shoot induction

1. Introduction

Medicinal plants have been the subject of man's curiosity and purpose for treatment of chronic diseases since time immemorial. Herbs are now in great demand, because of their proven efficacy and little or no side effects.

The genus Rauwolfia was named in honour of sixteenth century traveler and botanist Leonard *Rauwolfia serpentina* refers to long tapering snake like roots.

The dried root of *Rauwolfia serpentina* Benth. Ex Kurz, commonly known as Rauvolfia root, serpentine or serpentine root, in Sanskrit as Sarpagandha, and in Hindi chandrabhaga or Chota – chand, is one of the most important crude drugs used in modern medicine. The roots of the plant were used for centuries by the Ayurvedic and Unani systems of medicine in India for various types of ailments, ranging from disorders of the central nervous system such as maniacal behavior, insanity, snake bite, schizophrenia, epilepsy and insomnia to intestinal disorders, childbirth and opacity of the cornea. The roots of this shrub have been used in ayurvedic medicines from ancient time of Indian medical therapy **(Singh *et al.,* 2015)**. The roots of this plant have been used for centuries in ayurvedic medicines under the name sarpagandha and nakuli for the treatment of mental disorders. It has been stated that the drug is useful in mental disease, epilepsy, sleeplessness and several other ailments **(Ojha and Mishra, 1985)**. But due to increasing anthropogenic activities and rapidly eroding natural ecosystem, the natural habitat of this species is decreasing. Rauvolfia is threatened in India due to its indiscriminate collection and over exploitation of natural resources for commercial purposes to meet the requirements of pharmaceutical industry, coupled with limited cultivation **(Singh *et al.,* 2009)**. IUCN has kept this plant under endangered status **(Anonymous, 2009; Jain *et al.,* 2003)**. In the present study, an attempt has been made to preserve the plant and to maintain its essence for future generation, as we know the plant is an endangered species. Due to its medicinal values, the root of this plant has been popular both in India and Malaya-peninsula, from ancient times as an antidote to the stings of insects and poisonous reptile. It has also been used as febrifuge and stimulant to uterine contraction for insomnia and most of all for insanity **(Vakil, 1949)**. The plant is vegetatively propogated by root cutting because of poor seed viability and low germination percentage that may be largely ascribed to the presence of cinnamic acid and derivatives in the seed **(Mitra,1976; Sahu, 1979)**.

Further Rauwolfia is threatened with extinction due to its limited cultivation and over exploitation by local people, government agencies and various pharmaceutical houses **(Mamgain *et al.,* 1998).**

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.

But, the conservation of callus for months through indirect regeneration and mass replication of secondary metabolite in hassled free cultivation method is a new invention and there is also a need to apply in vitro methods for the regeneration and conservation of this valuable endangered plant. Hence, an effort has been made to develop an efficient protocol for the recovery of plants through organogenesis of *Rauvolfia serpentina*. It was hypothesized that, use of suitable plant growth regulators (PGR) as supplement of basic MS media could improve the in vitro plantlet regeneration and conservation potentiality of Rauvolfia. Different growth regulators as supplement of MS medium were optimized for rapid in vitro multiplication and conservation of *Rauvolfia serpentina* callus using leaf explant as an initial plant material.

Rauwolfia serpentina belongs to the family Apocynaceae. It is a small, erect, evergreen perennating undershrub, 15-45 cm high. taproot tuberous, soft, leaves thin, in whorls of three, large, flowers white or pinkish, irregular corymbose cymes.

Figure.-1 A field growing a medicinal Plant of *Rauwolfia serpentina.*

2. Materials and Methods

2.1 Glassware's Equipments: The glassware's i.e. beakers, glass bottles with polypropylene lids, conical flasks, pipettes, test tubes, Petri plates, etc. will be of borosilicate Glassware.

2. 2 **Nutrient Medium:Murashige and Skoog, (1962).**

2.2.1 Component of Nutrient Media: Plant cell and tissue culture media generally contain some or all of the following components:

1. Inorganic macronutrients.
2. Inorganic micronutrients.
3. Carbon source.
4. Vitamins,
5. Amino acids.
6. Phytohormones
7. Gelling and solidifying agents.

2.2.1. **Inorganic Macronutrients**: These include Nitrogen (N), Phosphorus (P), Calcium (Ca), Magnesium (Mg). These elements are needed in large quantities and are added to nutrient media in the form of their like Ammonium nitrate (NH_4NO_2), Potassium nitrate (KNO_3), Magnesium sulphate ($MgSO_4.7H_20$), Potassium dihydrogen orthophosphate (KH_2PO_4), Sodium dihydrogenphosphate (NaH_2PO_4), Ammonium sulphate $(NH_4)_2SO_4$, and Calcium chloride(CaCl2.2H20),

2.2.2**. Inorganic Micronutrients**: These include Iron (Fe), Manganese (Mn), Zinc (Zn), Boron (B), Copper (Cu), Molybdenum (Mo) and Cobalt (Co). Iron is the most critical of these and is generally supplied in chelated forms like Fe-EDTA (Ethylene Diamine Tetra Acetic Acid).

2.2.3. **Carbon Source**: Sucrose (30 gm/1) is the most commonly used for all cultured plant materials including even green shoots, Autoclaving hydrolyses the sucrose, which enhances its availability to plant cells.

2.2.4 **Vitamins**: For optimum tissue growth, the following vitamins are required: ionositol, thiamine, pyridoxine & nicotinic acid of which thiamine is essential.

2.2.5 **Amino acids**: Amino acids show beneficial effect during culturing of cells & are capable of synthesizing all required amino acids, but apparently in sub- optimal quantities.

2.2.6 **Phytohormones**: Phytohormones are the organic compounds other than nutrients, which influences growth & development of plants. These are also used for inducing growth and differentiation in the explants. The critical hormones used in the nutrient media are auxins, cytokiniens and gibberellins.

2.2.6.1. **Auxin**: Auxins are used to induce cell division, cell elongation, root formation and to suppress the growth of auxiliary shoots. Both naturally occurring and synthetic auxins are used in medium. The naturally occurring auxin is IAA. Commonly used auxins include IAA, IBA, NAA and 2, 4-D,

2.2.6.2. **Cytokinins**: Cytokinins are generally associated with cell division and promotion of auxiliary shoot formation. The commonly used cytokinins in the media include BAP, Kinetin.

2.2.6.3. **Gibberellins**: Gibberellins generally promote callus growth. GA3 is the most commonly used gibberellins.

2.2.7. **Gelling or solidifying agents**: Agar, a polysaccharide, is most widely used solidifying agent, and it renders the nutrient media as a moist gel. Agar is generally used at a concentration of 0.8% and it melts at 60-100 °C and solidifies at 40°C.

2.3 Preparation of Stock Solution of Plant Growth Regulator: The stock solutions of various chemicals will be prepared in distilled water and stored in sterile reagent bottles in a refrigerator after filtering the contents, Benzyl amino purine (BAP), IAA (Indole acetic acid) will be dissolved first in 1N NaOH solution and then, the required amount of distilled water will be added to make the required concentration of stock solution. Fresh solutions of organic salts and vitamins will be used in the culture media.

2.4 Preparation of culture media: Distilled water was used for the preparation of medium. The amounts of macro and micro nutrients, organic salts, vitamins, growth regulators and sucrose were added to the double distilled water kept in distilled water. The final volume was made in a graduated cylinder/breaker by adding double distilled water. The **pH** of the solution was adjusted to **5.7-5.8** using either **0.1N HCl** or **0.1N NaOH.** For solidification of the medium, agar powder (Tissue culture grade, agar-agar type) @ 0.8% w/v was added to warm solution and then, boiled for proper dissolving and melting of agar powder. Then the medium was poured in glass vessel (i.e. culture tube or culture bottle). After that the vessel was covered by lid or with aluminium foil. Basal nutrient media will be used during this investigation are given in table 1. i.e. Induction of callus from leaf segments in MS medium with different concentrations of growth regulators. It will be used in present project.

2.5.1 Procedure

The medium was prepared in sterile flasks. Required quantities of stock solutions 50 ml of stock 1, 5ml of stock 2, 10ml of stock 3, 10ml of stock 4, 10ml of stock 5 will be added. The pH of the solution was adjusted 5.8 by adding NaOH and HCl drop by drop. Final volume of medium will be made to 1 litter by adding distilled water. Adding of 8% agar, which was dissolved in warm water followed by constant stirring. The medium thus prepared was dispensed into different culture tubes or culture bottles each having 20 to 30 ml of the media. The medium was autoclaved at 15 lbs. pressure and 121°C temperature for 20 minutes. The medium was finally allowed to cool and solidify.

The tissue culture technique involves the isolation, sterilization, inoculation and cultivation of plant cells, tissues and organs under aseptic conditions in culture vials, containing nutrient medium. In tissue culture, the organized structures like shoot tips, root tip etc. are culture in-vitro to obtain their development as organized structure under controlled environmental conditions (temperature, light, humidity, aeration) which effectively controls the expansion of any genotype or phenotypic potential in explants.

2.6 Isolation of explant

2.6.1 Source of explants: The explant parts such as nodal segments shoot tip, and *In vivo* germinated seeds were taken from plants of *Rauwolfia serpentina* plant material was collected from the Botanical garden of Rajasthan University. It's Plant Herbarium is kept in RUBL Herbarium, Department of Botany, University of Rajasthan, Jaipur, India. After collecting Plant, It was grown in .S. S. Jain Subodh P.G. Autonomous College, Jaipur, Rajasthan, India.

The specific differences in the regeneration potential of different organs and explants have various explanations. The significant factors include differences in the stage of the cells in the cell cycle, the availability of or ability to transport endogenous growth regulators, and the metabolic capabilities of the cells. The most commonly used tissue explants are the meristematic ends of the plants like the stem tip, auxiliary bud tip and root tip. These tissues have high rates of cell division and either concentrate or produce required growth regulating substances including auxins and cytokinins.

2.7. Sterilization of explants

Young stem segments, young shoot tip and seeds were surface sterilized by first washing them in Extran detergent in running tap water for 10-15 min. to remove soil particles and debris, after that were wash with distilled water, dipping in absolute alcohol foe 2 min. and also dipping in 20 % (v/v) commercial sodium hypochlorite solution for 5-6 min. wash with sterilized distilled water for one times, followed immediately, immersing them in 0.1% (w/v) $Hgcl_2$ solution for 5 min treatment under laminar Air Flow Unit, They were thoroughly washed 5-6 times in sterile distilled water. This entire treatment was carried out at low temperature to prevent damage to the shoots.

2.8. Inoculation

Before inoculation, inside the laminar air flow chamber the ultraviolet lamp was put on carefully to avoid contamination for at least 20 minutes, the working table 1 of laminarair flow chamber was wiped thoroughly with 70% ethanol before use. The material required for inoculation was steam sterilized. The hands were cleaned with 70% ethanol. Then, the individual explants were inoculated in individual culture tubes or culture bottles having solidified culture medium. Forceps and scalpels were flame sterilized before each inoculation. The explants were then, cultured on MS medium supplemented with different concentration of BAP, Kn, AdS and NAA.

2.9. Incubation of culture

After inoculation cultures were kept in the incubation room where the temperature and light period was maintained at 25^0 ± 2C and 16 hr. photoperiod with approximate 1500 lux intensity of light respectively. Source of the light was from the four florescent tube lights each of four feet and 40 W (Philips make) a total of 22 culture bottles for each treatment has been kept on the culture racks. After three week period callus developed in the medium or shoots/roots develop from explants.

3. Results

Callus induction

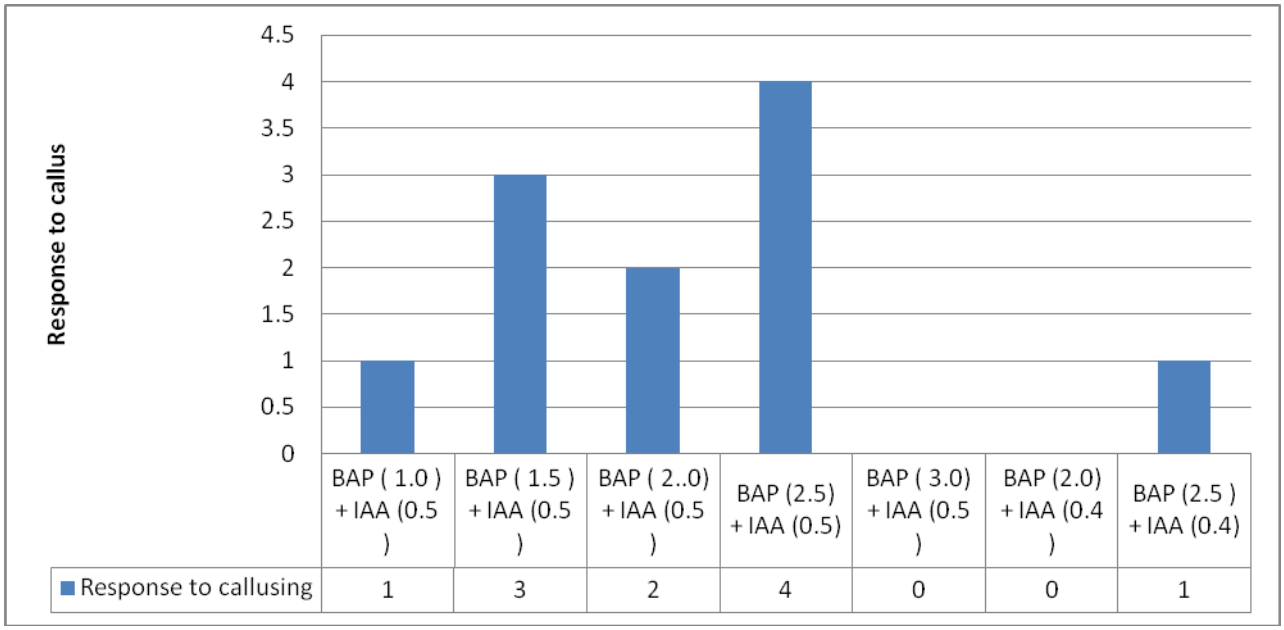
In an effort to propagate the plants from the callus and also to understand the morphogenic response from the leaf explants were used in the experiment. The MS medium contained different doses of growth regulators as shown in table 1.

Table 1-Induction of callus from leaf segments in MS medium with different concentrations of growth regulators.

Sr. No.	Medium composition	Response to callusing
1.	MS + BAP (1.0 mg/l) + IAA (0.5 mg/l)	+
2.	MS + BAP (1.5 mg/l) + IAA (0.5 mg/l)	+++
3.	MS + BAP (2.0mg/l) + IAA (0.5 mg/l)	++
4.	MS + BAP (2.5 mg/l) + IAA (0.5 mg/l)	++++
5.	MS + BAP (3.0mg/l) + IAA (0.5 mg/l)	_
6.	MS + BAP (2.0 mg/l) + IAA (0.4 mg/l)	_
7.	MS + BAP (2.5 mg/l) + IAA (0.4mg/l)	+

Mark indicate (-) no callusing, (+) least callusing, (++) fair callusing, (+++) good callusing, (++++) very good callusing

Above table explains the two different combinations of hormones, namely BAP + IAA with different concentrations were applied for callus induction. No callus induction was observed when BAP (3.0mg/l) + IAA (0.5 mg/l) and BAP (2.0 mg/l) + IAA (0.4 mg/l) was applied in MS medium. Least callusing was observed when BAP(1.0 mg/l) + IAA (0.5 mg/l) and BAP (2.5mg/l) + IAA (0.4mg/l) was applied in MS medium. Fair callusing was observed when BAP (2.0mg/l) + IAA (0.5 mg/l) was applied in MS medium. Good callusing was observed when BAP (1.5 mg/l) + IAA (0.5 mg/l) was applied in MS medium. . Very good callusing was observed when BAP (2.5 mg/l) + IAA (0.5 mg/l) was applied in MS medium.



Young excised leaf segments were inoculated on MS medium supplemented with 2,4 –D (2mg/l) and Kn (0.5 mg/l). The swelling around mid –vein was first sign of response to callus induction as normally known. In medium with low concentration of 2,4-D and Kn, swelling appears in about one week after the inoculation. Similar results have also been reported by **Chaturvedi (1965).** Callus was frequently induced only when the leaf segments were placed when their abaxial face thouch the medium. Callus was evident when the in vitro leaf segments were inoculated on MS medium augmented with 2, 4-D (2mg/l) and Kn (1mg/l) after two week of inoculation. Callus induction was well marked with 2,4-D (2mg/l to 3mg/l), in the presence of Kn, as reported by **Gupta *et al.*(1966)** in *Nicotiana tabacum*. Callus formation on the MS medium containing CH (100 mg/l), Ads (5mg/l) and IAA (0.5 mg/l) showed development of chlorophyll and growth of cells around veins after three week of culture period. This is in accordance with **Chaturvedi *et al.* (1965)**.

Figure -2:- Callus induction in *R. serpentina*

Above figure illustrating that when leaf explants in MS medium was inoculated and incubated in culture room that MS containing BAP (2.0 mg/l) and IAA (0.5 mg/l). Frequency of callus induction (70%).

Figure -3:- Shoot induction from nodal explants of *Rauwolfia serpentina*

Above figure was illustrated that When MS Medium supplemented with BAP (2.5 mg/l) + IAA (0.5 mg/l) added then after 3 weeks frequency of shoot induction was 45%.

4. Summary

The Present study on in vitro organogenesis of *Rauwolfia serpentina* is one of the most important medicinal shrubs, very useful for various types of ailements e.g. (antidote for snake bites ,fever, insomnia, dysentery etc.) The aim of the study is to develop and efficient plantlet regeneration protocol. In vitro multiplication is an alternative method to conventional propagation that deals with rapid and mass multiplication of a rare species hybrid elite genotype etc.

Murashige and Skoog (1962) medium were used throughout the experiments, with suitable growth regulators. The explants consisted of shoot apices, nodal bud, young leaves and primordial from *in-vivo*. The explants disinfected with 0.1% $HgCl_2$ and 0.1%SLS followed by washing with sterile distilled water were cultured on the MS medium containing different growth regulators.

Two different combinations of hormones, namely BAP + IAA with different concentrations were applied for callus induction. . Least callusing was observed when BAP(1.0 mg/l) + IAA (0.5 mg/l) and BAP (2.5mg/l) + IAA (0.4mg/l) was applied in MS medium. Fair callusing was observed when BAP (2.0mg/l) + IAA (0.5 mg/l) was applied in MS medium. Good callusing was observed when BAP (1.5 mg/l) + IAA (0.5 mg/l) was applied in MS medium. Very good callusing was observed when BAP (2.5 mg/l) + IAA (0.5 mg/l) was applied in MS medium. When IAA Concentration was kept constant 0.5 mg/l and BAP concentration was increased from 1.0mg/l to 2.5mg/l, Callus induction was better each step.

The other experimental findings are as followed: callus induction, shoot regeneration.

The leaf segments from the axenic plants as well as from in vivo grown plants were subjected to callus induction on MS modified medium having different concentration of 2,4-D, BAP, NAA and IAA. The medium containing 2,4-D and BAP was most effective for callus induction in presence of NAA. The two combinations of the growth substances as BAP (3mg/l) + NAA (1 mg/l) and 2,4-D (3mg/l) + NAA (0.5 mg /l) gave the satisfactory performance with regard to callus induction from the leaf explants. However, callus induction was appreciably high using the MS medium supplemented with CH (200mg/l) + IAA (1 mg/l). The callus when transferred on to the shoot regeneration medium containing CH (200mg/l) +Ads (5 mg/l) + IAA (1mg/l) exhibited primarily some nodule like structures which later on gave rise to shoot development after three week of culture. The phenomenon was observed in the 75% cultures.

5. Conclusion

From the above investigations it is concluded that *Rauwolfia serpentina* showed a potential for callus formation The callus formation obtained when explants inoculated on MS medium supplemented with different concentrations of BAP and IAA in different combinations. The best callus formation was achieved when BAP (2.5 mg/l) + IAA (0.5 mg/l) was applied in MS medium. When can be exploited for large scale regeneration and to supply planting material in bulk for use in pharmaceutical industry. These data and necessary inputs towards the designing the manuscript. All authors discussed the methodology and Result,

6. References

1. **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. 2006: 5: 659-661.
2. **Bhatara V S, J N Sharma, S Gupta and Y K Gupta.** "*Rauwolfia serpentina*: The first antipsychotic." American Journal of Psychiatry.1997:154: 894-896.
3. **Chaturvedi, H. C., Mitra, G. C. and Prabha, C..**Histogenesis of callus tissue from different organs of *Rauwolfia serpentina.* Indian. J. Exp. Biol. 1965: **4**:216-222.
4. **Gupta, G. R. P., Guha, S and Maheshwari, S.C..**Differentiation of buds from leaves of *Nicotina tabaccum Linn.*In sterile culture Phytomorphology. 1966: **16**:175-182.
5. **Haberlandt, G..**Culturever such emit isoliertenphlanzenzellerstizungrber. Ciasse 111 Abster. 1902: **1**: 69-92.
6. **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. 2005: 68: 848-852.
7. **Jain, V., Singh, D., Saraf, S., Saraf, S.**.In vitro micropropagation of *Rauvolfia serpentina* through multiple shoot generation. Anc. Sci. Life. 2003: 23: 1–5.
8. **Kirillova N V, M G Smirnova and V P Komov.**"Sequential isolation of superoxide dismutase and ajmaline from tissue culture of *Rauwolfia serpentina* Benth." PrikladnaiaBiokhimiiaMicrobiologiia. 2001: 32:181-185.
9. **Kirtikar K R and B D Basu.** "Indian medicinal plants" Bishen Singh Mahendra Pal

Singh, Publishers, Dehradun, India.1993: 2, pp.289.

10. **Mamgain SK, Goel AK, Sharma SC.** Conservation of assessment of some important threatened medicinal plants of India. J. Non-Timber Fore. Prod.1998: 5: 1-9.
11. **Mitra GC.**Studies on the formation of viable and non-viable seeds in *Rauwolfia serpentina* benth. Indian J. Exp. Biol. 1976: 14(1): 54-56.
12. **Murashighe,T. and Skoog, F..**A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol Plant. 1962:15: 483-497**.**
13. **Ojha J, Mishra U.** Dhanvantari Nighantuh, with hindi translation and commentary, Ist ed. Deptt. Of Dravyaguna, Institute of Medical Sciences, BHU, Varanasi. 1985 p. 204.
14. **Pathania S, V Randhawa and G Bagler.**"Prospecting for novel plant derived molecules of *Rauvolfia serpentina* as inhibitors of aldose reductase, a potent drug target for diabetes and its complications."PLoS ONE. 2013: 8: 613-627.
15. **Singh, R. K., Singh, A., Rath S. and Ramamurthy, A..**A review of sarpagandha - whole herb v/s reserpine alkaloid in the management of hypertension. Int. Ayurvedic Med. J. 2015: 3: 555-569.
16. **Singh, P., Singh, A., Shukla, A. K., Singh, L., Pande, V. and Nailwal, T. K..**Somatic Embryogenesis and In Vitro Regeneration of an Endangered Medicinal Plant Sarpgandha (*Rauvolfia serpentina*. L). Life Sci J. 2009: 6: 57-62.
17. **White, P. R..** Potentially unlimited growth of exised plant callus in an artificial nutrientAmer J. Bot. 1939: 26: 59-64.
18. **Vakil RJ.**A clinical trial of *Rauwolfia serpentina* in essential hypertention. Br. Heart J. 1949: xi(4): 350
19. **Von Poser G, H H Andrade, K V Da Silva and J A Henriques**. "Genotoxic, mutagenic and recombinogenic effects of Rauwolfia alkaloids."Mutation Research. 1990: 232: 37-43.

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	Website: www.ijarbs.com
Quick Response Code	Subject: Biotechnology
DOI:10.22192/ijarbs.2025.12.01.004	

How to cite this article:
Anurag Jain, Geeta Chaudhary, Pawan Kumar Katariya. (2025). Study of phytohormones effect on micropropagation and callus induction in *Rauwolfia serpentina*. Int. J. Adv. Res. Biol. Sci. 12(1): 35-43.
DOI: http://dx.doi.org/10.22192/ijarbs.2025.12.01.004