NDC E-BIOS (Online) Volume 2, pp 63-70 (2022)
ISSN: 2583-6447

ORIGINAL ARTICLE

COMPARATIVE STUDY OF RATE OF CALLUS INDUCTION OF *RAUWOLFIA SERPENTINA*

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Abstract: *Rauwolfia serpentina [Rauwolfia serpentina (L.) Benth. Ex Kurz.] has been the subject of research for its pharmacologically active natural constituents namely monoterpenoid indole alkaloids especially reserpine. The plant is vegetatively propagated because of poor seed viability and low germination rate. Therefore in vitro propagation of Rauwolfia serpentina is essential for the production of life supporting alkaloids, to satisfy the growing commercial demand of the plant and decrease the load on the wild population by conservation of this endangered plant. In the present study, we are reporting indirect morphogenesis of Rauwolfia serpentina in different combinations of plant growth regulators and indirect organogenesis mediated shoot regeneration of Nicotiana plumbaginifolia for comparison of its callus induction and proliferation rates with Rauwolfia serpentina.*

Key words: *in vitro propagation,* morphogenesis, organogenesis, callus, growth regulators, *Rauwolfia serpentina*, *Nicotiana plumbaginifolia*

Communicated: 12.12.2022 Revised: 27.12.2022 Accepted: 28.12.2022

1. INTRODUCTION

Formulation and study of plant-based drugs have become an area of rising interest in the world of modern medicine and have been relevant since ancient times because of their biosafety attributes. *Rauwolfia serpentina* (Family: Apocynaceae) is a well-known medicinal plant, even in ancient literature of Indian subcontinent [1]. In the Charaka Sanhita (approximately 1000-800 B.C.), it was mentioned in its Sanskrit name 'Sarpagandha', which was a useful antidote against snake venom and insect stings [2]. Around 50 alkaloids are reported to be present in this plant. Among these alkaloids, reserpine is the most important, for the actions like lowering blood pressure and sedation [3-5]. The influence of reserpine was proved with successful trials in the cases of gynaecology, hypertension, neuropsychiatry and geriatrics [6, 7]. The application of herbal products from *Rauwolfia, including reserpine* was reduced during late 60s of the twentieth century, due to some reports of its side effects [8]. Later, it was proved that the use of *Rauwolfia in* low dose is very much useful for patients, if selected on the basis appropriate case history of hypertension [9]. The plant provides a safe and effective natural source of pharmaceutically effective active constituents, useful for management of high blood pressure [4]. For its high level of demand as a source of herbal medicine, micropropagation of *Rauwolfia serpentina* is very much essential for its bulk production at

industrial level. The hairy roots of this plant show a remarkable capacity of regeneration into complete plants, with high rate of survival with intact active constituent of its secondary metabolites, in laboratory. The plant propagates vegetatively and the presence of cinnamic acid and derivatives in the seed leads to poor level of seed viability and germination percentage [10, 11]. *Rauwolfia serpentina* is threatened with extinction due to limited cultivation and overexploitation [12] that lead to the necessity of its *in vitro* propagation to meet its increasing commercial demand, in pharmaceutical field. Improvements in plant tissue culture techniques for the mass propagation of *R. serpentina* are highly desirable to share the load on the existing natural population as the conventional propagation method is not sufficient for steady supply of raw materials.

2. MATERIALS AND METHODS

Collection and Sterilization of Explant

Disease free plants were collected from the garden of Scottish Church College and Garia Krishna nursery, Kolkata. The plants were maintained in the polyhouse, regularly watered and sprayed with 0.001% Bavistin solution at 7-day intervals prior to explant inoculation. Juvenile leaves, portions of stems i.e., nodes and internodes were collected from healthy saplings. The explants were washed using tap water in running condition, for 15 minutes, then dipped in 0.1% Triton solution for 5 minutes and washed thoroughly in distilled water. Then they were treated in 0.01% $HgCl_2$ solution for 2 minutes and washed in sterile distilled water under laminar air flow thrice till no trace of surface sterilant was left. After blotting the explants dry, they were cut and aseptically transferred into culture tubes containing culture medium.

Transfer of Explants in Media and Incubation

Explants were aseptically implanted on MS medium (Murashige and Skoog medium) supplemented with different combinations of auxins and cytokinins under controlled conditions of temperature (25±2°C), light i.e., 2000 lux for 16 h/day provided by fluorescent tubes and 60-70% humidity following standard technique described in previous reports [13, 14].

Abbreviations used: BAP – Benzoyl aminopurine (synthetic cytokinin), IBA – Indole butyric acid (synthetic auxin), IAA – Indole Acetic acid (auxin).

3. RESULTS AND DISCUSSION

Apical and marginal portions of leaves and sections of stems i.e., nodes and internodes were chosen as explants [15-17].

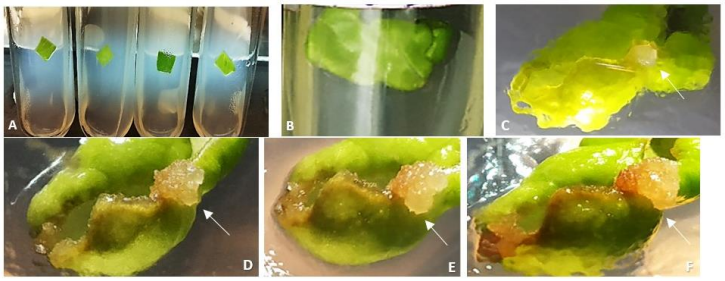

Figure 1. A-G. Pictures of *in vitro* response of leaf explants of *R. serpentina* cultured on MS media supplemented with BAP (1mg/l) and IBA (0.125mg/l). (A) Explant inoculation on day 0 (B) Swelling and enlargement of explants after 7 to 14 days (C) First callus induction after 21 days,

In both *Rauwolfia serpentina* and *Nicotiana plumbaginifolia*, juvenile leaves from the apical meristematic portion of the stem of approximately 1cm^2 length was reported to have better and quicker response than mature leaves and larger/smaller sized explants [14, 17]. The callus induction and development with time were observed *in vitro*, with different nutrient combinations (Figure 1-3).

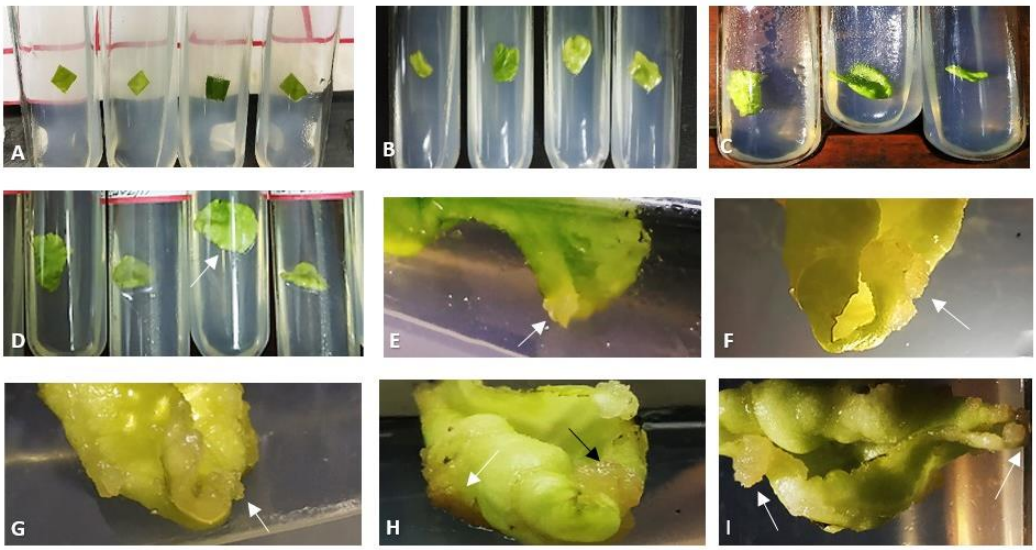

Figure 2 A-I. Pictures of *in vitro* response of leaf explants of *R. serpentina* cultured on MS media supplemented with BAP (1mg/l) and Kinetin (0.1mg/l). (A) Explant inoculation on day 0 (B)-(C) Swelling and enlargement of explants between 7 to 14 days (D) First callus induction after 14 days (E)-(G) Further callus induction between 28 to 35 days, friable appearance (H) Initiation of callus proliferation after 42 days (I) Further callus proliferation after 49 days

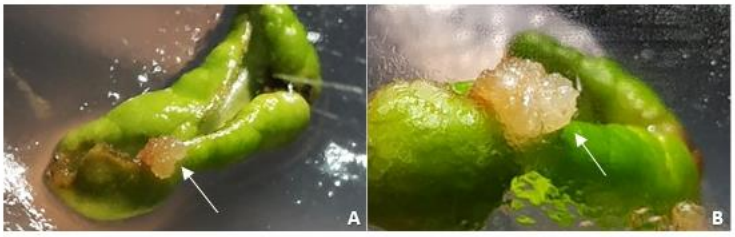

Figure 3. Minute (very slowly proliferating) Callus [A] in response to MS + BAP (1mg/l) + Kinetin (0.1mg/l); Moderate (slowly proliferating) Callus [B] in response to MS + BAP (1mg/l) + IBA (0.125mg/l)

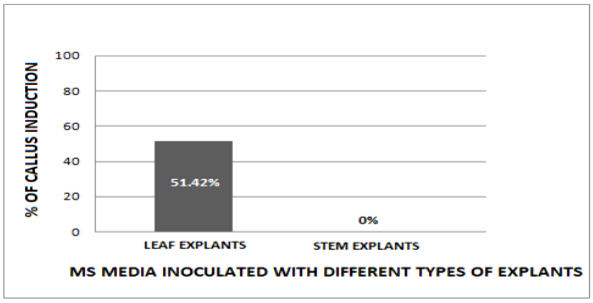

Figure 4. Comparison between percentage of callus induction on MS media supplemented with different types of explants i.e., leaf (apical, left margin, right margin) and stem (nodes, internodes)

Stem explants of *Rauwolfia* irrespective of their dimensions and position did not respond to callus induction media (Figure 4) and showed a much greater percentage of contamination and browning instead when the same sterilisation protocol was followed (Figure 5) Hence usage of stem portions as explants was discontinued.

Figure 5. Inoculated stem explants of *Rauwolfia serpentina* showing contamination and browning

Initial cultures with leaf explants showed a high percentage of contamination which was reduced to a great extent when the plants were sprayed with 0.001% Bavistin. The Murashige and Skoog medium is widely used as effective plant tissue culture medium for all sort of tissue culture work [18, 19]. The culture tubes containing culture media were put on slant for availability of higher surface area for callusing. 16h/day photoperiod was provided for potential adventitious budding.

In *Rauwolfia serpentina* , among MS media supplemented with 4 different combinations of plant growth regulators, MS + BAP (1mg/l) + IBA (0.125mg/l) and MS + BAP (1mg/l) + Kinetin (0.1mg/l) showed best results for callus induction i.e. 65% and 60% respectively followed by MS + BAP (0.1mg/l) + IAA (1mg/l) and MS + BAP (1mg/l) + IAA (0.1mg/l) showing low rates of callus induction i.e., 30% and 20% respectively with no callus proliferation (Figure 6).

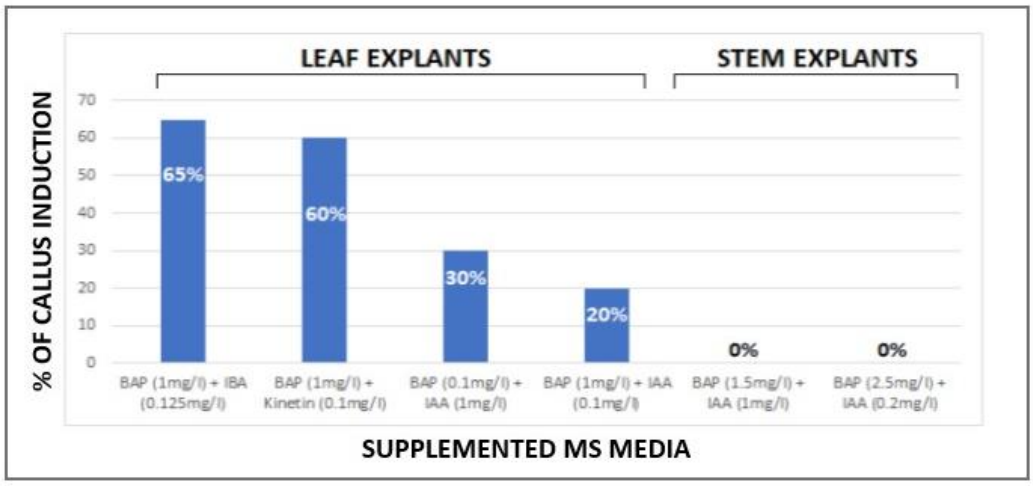

Figure 6. Graph showing comparison between percentage of callus induction based on the choice of explant in response to different combinations of plant growth regulators

The rate of callus proliferation is higher and faster on MS media supplemented with BAP (1mg/l) and IBA (0.125mg/l) followed by MS media supplemented with BAP (1mg/l) and Kinetin (0.1mg/l) [Figure 7].

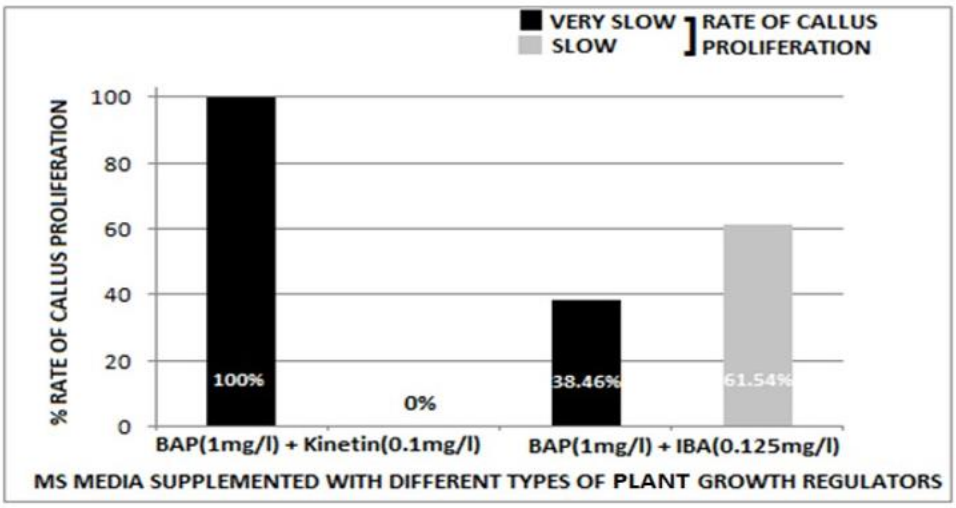

Figure 7. Comparison between percentage of callus proliferation of leaf explants in response to two best supplemented callus induction media (MS)

On MS media supplemented with BAP (1mg/l) and IBA (0.125mg/l), the explants swelled and enlarged between 7 to 14 days, dedifferentiated into callus after 21 days and callus proliferation occurred after 42 days from the browning edges of the explant [Figure 1]. On MS media supplemented with BAP (1mg/l) and Kinetin (0.1mg/l, swelling and enlargement of explants occurred between 7 to 14 days, first callus induction occurred after 14 days and callus proliferation was observed after 42 days [Figure 2].

Nicotiana plants, used as model species for molecular, genetic, plant physiology studies and in experimental botany such as plant transformation experiments due to its easy availability and high growth rate, requires *in vitro* regeneration and has been taken as a standard in this experiment. Cultures were put on slant with leaf explants after following the same sterilisation protocol and incubation was done under similar conditions.

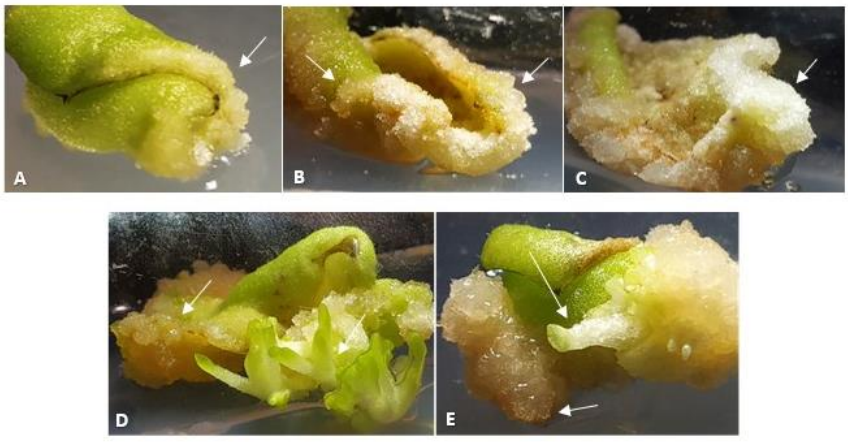

Figure 8 A-E. Pictures of *in vitro* response of leaf explants of *N. plumbaginifolia* cultured on MS media supplemented with BAP (1mg/l) and Kinetin (0.1mg/l). (A) Callus initiation after 14 days (B) Callus proliferation after 21 days, dry and friable callus, (C) Dry, semi solid callus after 28 days (D)-(E) Shoot regeneration and further callus proliferation between 28 to 35 days

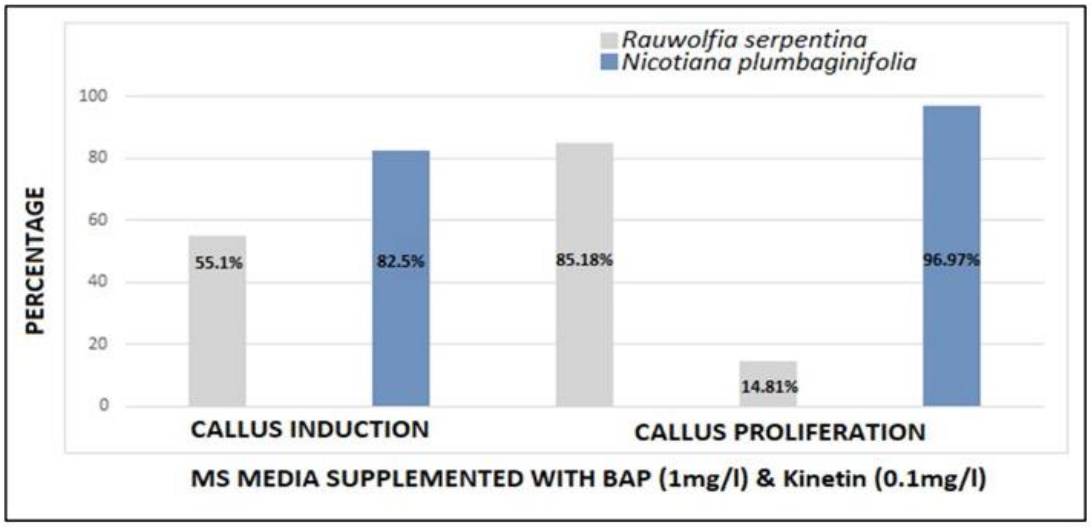

Figure 9. Comparative percentage of callus induction and callus proliferation of leaf explants of *Rauwolfia serpentina* with that of *Nicotiana plumbaginifolia*.

In *Rauwolfia serpentina*, the initiation of callus induction is faster on MS media supplemented with BAP (1mg/l) and Kinetin (0.1mg/l) i.e., after 14 days followed by MS media supplemented with BAP (1mg/l) + IBA (0.125mg/l) i.e., after 21 days. Among MS media supplemented with 4 different combinations of plant growth regulators, MS + BAP (1mg/l) + IBA (0.125mg/l) showed the highest rate of callus induction i.e., 65% [Figure 6]. Callus proliferation is higher and faster on MS media supplemented with BAP (1mg/l) and IBA (0.125mg/l) [Figure 7].

In *Nicotiana plumbaginifolia,* the initiation of callus induction on MS media supplemented with BAP (1mg/l) and Kinetin (0.1mg/l) occurred after 14 days always [Figure 8]. Percentage of callus induction is 82.5% and callus proliferation is 96.97% [Figure 9].

4. CONCLUSION

Taking the results obtained from callus culture of *Nicotiana plumbaginifolia* as a standard, it can be concluded that *Rauwolfia serpentina* responds very slowly in callus induction medium according to the experiments done as compared to the experimentally recorded response of *Nicotiana plumbaginifolia* [Figure 8].

Juvenile leaves of approximately $1cm^2$ were found to be the best choice of explant for callusing in *Rauwolfia seprentina* in these experiments. Usage of 0.001% Bavistin prior to explant inoculation eliminated contamination in culture media to a great extent. 0.1% Triton (detergent) and 0.01% $HgCl_2$ were found to be the most potent surface sterilant for leaf explants.

Stem portions taken as explants showed no response in callus induction media [Figure 4-6]. Further callus, cell culture, induction of organogenesis, morphogenesis and genetic engineering experiments can be initiated. Standardized propagation system can be used for propagating elite plant that are well adaptive with disease tolerance and high reserpine content. Such *In vitro* techniques as tissue culture offer new

openings for clone propagation, genetic manipulation and production of inbred lines supplementing the vegetative production of plant species.

5. CONFLICTS OF INTEREST

Authors of the article do not have any conflict of interest.

6. ACKNOWLEDGEMENT

The authors would like to thank Dr. Madhumanjari Mandal, Principal, Scottish Church College, Kolkata for providing all the essential facilities to carry out the project work in the Botany department.

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