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In vitro regeneration of plantlets from nodal culture of *Rauwolfia serpentina* L.

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Abstract

Rauwolfia serpentina L. holds an important position in the pharmaceutical world because of its immense anti-hypertensive propesties. *Rauwolfia* is threatened with extinction due to its limited cultivation, over-exploitation by people and various pharmaceutical houses. To cope up with this alarming situation, the tissues culture have come as a boon. The present study is an attempt for *in vitro* regeneration of *Rauwolfia serpentina* plantlets from axillary node and to node and to study the effect of plant growth hormones like BAP and kinetin in regeneration of plantlets.

Keywords: *Rauwolfia serpentina*, plant tissue culture, BAP, kinetin

1. Introduction

In vitro culture or plant tissue culture is the rearing of plant cell, tissue, organ in artificial medium containing carbon source micronutrient, macronutrients vitamin and hormone, under suitable physical condition of light, temperature and humidity for regeneration of whole plant. The technique of plant culture occupies a key role in the second green revolution in which gene modifications and biotechnology are being used to improve crop yield and quality. This technology has been employed to a wide range of crop plants and tree species to solve the biological problems. This state of art technology has become possible by continuous efforts of many scientists who carried out the basic work.

With the better understanding of the technique of plant tissue culture and nutritional requirements of plant cells it was possible to develop newer technologies by culturing plant organs (anther, ovary, ovule, petal, leaf and meristem) leading to establishment of new research lines viz, haploids, virus free plants, *in vitro* fertilization, embryo rescue and direct regeneration from leaf discs for genetic engineering. Subsequently, it has

become possible to grow isolated epidermal cells, gland cells or even protoplasts and to regenerate from such specialized cells or individual protoplasts. Regeneration of plants and production of useful, metabolites through plant biotechnology has become an industrial application. The techniques of cell line selection, anther and pollen culture, procedure of protoplast isolation, culture, fusion and regeneration of plant are proving invaluable in the field of applied botany. The use of cell culture for the production of high value compounds for the food and pharmaceutical industries has provided new perspectives and sharpened the focus of the ways in which plant tissue culture can aid man. The application of genetic engineering in plant tissue culture for genetic modification in plant is still in progress.

Rauwolfia serpentina **L***: R. serpentina* commonly known as sarpagandha is a small, woody, perennial medicinal shrub which belongs to the family Apocynaceae. In Sanskrit, sarpagandha means one which smells like serpent. The root of *R. serpentina* is a rich source of indole alkaloids viz. reserpine, recinnanine, serpentine, ajamaline, ajmalicine etc. According to ayurveda, its root and whole plant is used for the treatment of cardiovascular disorder, snake bite, rheumatism, hypertension, insanity, epilepsy, eczema and leaves are used in the removal of opacities of the cornea. After reports of therapeutic properties, natural reserves of *R. serpentina* are declining due to over exploitation by the local and tribal people. The IUCN has assigned an endangered status to *R. serpentina*.

In vitro propagation offers not only a means for mass multiplication of existing germplasm stocks, but also for the conservation of important elite and RET (rare, endangered and threatened) species which are facing the danger of extinction.

2. Materials and methods :

The explants i.e. axillary node of *Ranwolfia serpentina* was collected from the botanical garden of Department of Life Sciences, Dibrugarh University. For the *in vitro* culture of axillary node of *R. serpentina*, Murashige and skoog media, 1962 (commonly known as MS medium) was prepared supplemented with 0.5 mg/l BAP (6 – Benzyl amino purine) and Kinetin (6 – furfuryl amino purine), both exclusively and in combination.

After incubation, the culture tubes were checked regularly and periodically for contamination with microorganisms, any changes in the explants and initiation of growth. The periodic studies were done after 5 days, 7 days and 15 days.

3. Results :

The results were drawn from the periodic observations which were made after 5 days, 7 days and 15 days from inoculation. The results of present study are elaborated below:

Table 3.1: Proliferation of *R. serpentina* using nodal explants –

RESPONSE AFTER 5 DAYS

Sl. No.	HORMONAL COMBINATION (0.5 mg/L)	No. OF EXPLANTS INOCULATED	CHANGES OBSERVED	No. OF SHOOTS PER EXPLANT
1	MS basal	03	No growth	No growth
2	MS + BAP	03	Shoot emergence	01
3	MS + Kinetin	03	No growth	No growth
4	MS + BAP + Kinetin	03	No growth	No growth

Table 3.2: Proliferation of *R. serpentina* using nodal explants

RESPONSE AFTER 7 DAYS

Sl No.	Hormonal combination (0.5 mg/l)	No. of explants inoculated	Changes observed	No. of shoots per explant
1	MS basal	03	No growth	No growth
2	MS + BAP	03	One leaf (2 cm long) is seen	01
3	MS + Kinetin	03	No growth	No growth
4	MS + BAP + Kinetin	03	Multiple shoot induction	05

Table 3.3: Proliferation of *R. serpentina* using nodal explants

RESPONSE AFTER 15 DAYS

Sl No.	Hormonal combination (0.5 mg/l)	No. of explants inoculated	Changes observed	No. of shoots per explant
1	MS basal	03	No growth, browning of media	No growth
2	MS + BAP	03	Shoot elongates, number of leaves increase to 5	03
3	MS + Kinetin	03	No growth, browning of media	No growth
4	MS + BAP + Kinetin	03	Shoot elongates	05

Results were drawn from the above periodic observations which are as given below:

3.1: Growth of nodal explants of *Rauwolfia serpentina* in MS basal medium :

The present results on the growth of nodal explants in basal medium showed very poor growth in all the experimental samples. No growth and proliferation was observed after 5 and 7 days of culture. The explants and media became brown in color after 15 days of culture (Table 3.1-3.3).

3.2: Growth of nodal explants of *Rauwolfia serpentina* in MS + 0.5 mg/l BAP medium:

Regeneration was observed from the nodal explants in MS + 0.5 mg/l BAP medium which showed good response. Growth started after 5 days from inoculation. After 15 days, further proliferation of shoots and leaves was noticed (Table 3.1 – 3.3).

3.3: Growth of nodal explants of *Rauwolfia serpentina* in MS + o.5 mg/l Kinetin medium:

Table 3.1-3.3 shows the effect of Kinetin (0.5 mg/l) along with MS basal medium on nodal explants which showed no response to this combination. No regeneration or growth and proliferation were observed after 5 and 7 days of culture. The explants and the media became brown in color after 15 days of culture.

3.4: Growth of nodal explants of *Rauwolfia serpentina* in MS + 0.5 mg/L BAP + 0.5 mg/L kinetin medium.

The combination of MS + 0.5 mg/L BAP + 0.5 mg/L kinetin showed positive response on the growth of nodal explants. There was multiple shoot induction after 7 days of culture. After 15 days, there was further elongation and proliferation of shoots with 54 shoots per explants (Table 3.1 – 3.3)

Two different hormones viz. BAP and kinetin, exclusively and in combination were applied to induce direct regeneration was found when only BAP was applied. Multiple shoot regeneration (5 shoots per explants) was observed in combination of BAP and kinetin hormones. The regeneration was totally absent when only kinetin was applied.

The fig 3.1 represents the overall result obtained as a result of *in vitro* culture of explants in different PGR combination.

Fig. 4.1

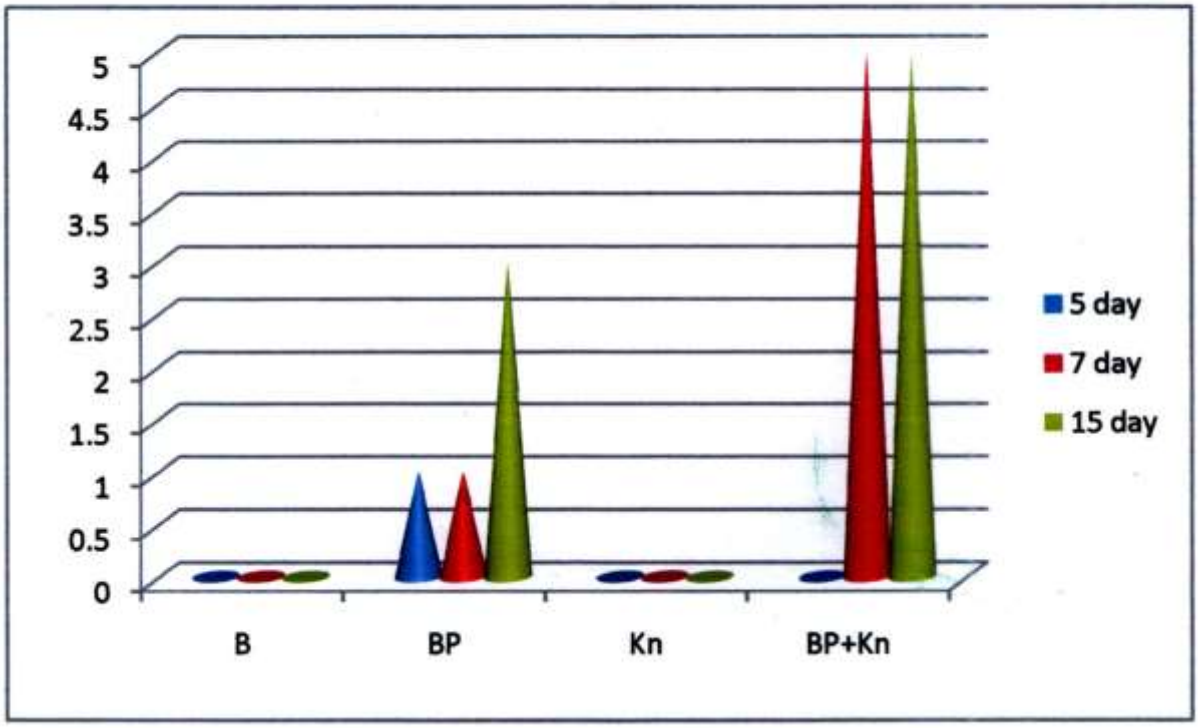


Photo plates:

Photo Plate I showing response in different cultural tubes after 7 days

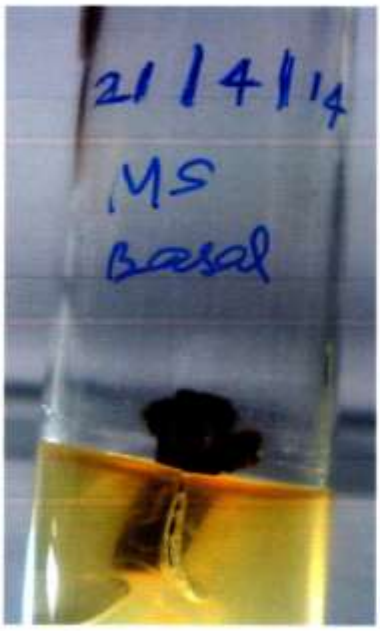
a. No growth in MS Basal Medium

b. Shoot regeneration in MS+0.5mg/L BAP

c. No growth in MS+0.5mg/L Kinetin

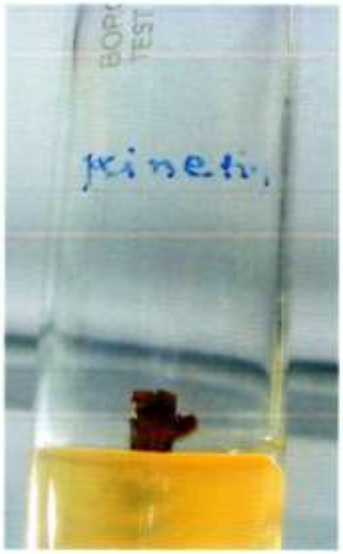
d. Multiple shoot induction in MS+0.5mg/L BAP+0.5mg/L Kinetin

Photo Plate II showing response in different cultural tubes after 15 days



a. Browning of explant and medium in MS Basal Medium

b. Shoot elongation and proliferation in MS+0.5mg/L BAP



c. Browning of explant and medium in MS+0.5mg/L Kinetin

d. Shoot elongation MS+0.5mg/L BAP+0.5mg/L Kinetin

4. Discussion:

The present study makes an attempt to study direct regeneration of *Rauwolfia serpentina* plantlets via axillary node by tissue culture method and to study the affect of the hormones BAP and kinetin on micropropagated plantlets. The results were drawn from the periodic observations which were made after 5 days, 7 days and 15 days from inoculation. It was found that among the various hormonal combinations used for shoot regeneration MS + 0.5 mg/l BAP was found to be most effective. However, MS + 0.5 mg/L BAP + 0.5 mg/L kinetin was best suitable for multiple shoot regeneration. Moreover after 15 days, it was observed that in the culture tubes containing MS basal and MS + 0.5mg/L kinetin, there was browning of media. It is due to secretion of phenolic compounds. Therefore pretreatment of explants is required to eliminate such problems.

Through this present study, we learnt a very genuine method of *in vitro* culture of *Rauwolfia serpentina*. It was found that regeneration of plants from axillary nodes is a very reliable method of *in vitro* propagation as its regeneration is very difficult from seeds and other sources. The seeds are mostly non viable due to abortive embryos. Further we could learn about the effect of the cytokinins, BAP and kinetin on the explants used here.

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