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Research Article

Tissue Culture Studies In *Raulfai tetraphylla* L.

Shilpa Chavan, Afreen Attar, Ganesh Kolekar and Narayan Pandhure

Tissue Culture Laboratory, Department of Botany,
Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431001, India.
*Email: drnarayan14872@gmail.com

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Abstract

Rauvolfia tetraphylla L. is an endangered plant, known for its medicinal properties. It contains various indole group alkaloids with reserpine most prominent among them. Reserpine is a hypotensive agent that is in great demand for the modern pharmaceutical industries. In the present work, leaf explants were induced to produce calli by using the phytohormones 2,4-D, NAA, IBA and IAA. Among these, 9 µM 2,4-D was found suitable for maximum callus induction (95%). The calli produced in this hormone concentration were subjected to NaCl salt treatment (0, 25, 50, 75 and 100 mM) and the effect of salinity on callus growth and reserpine accumulation was observed. The callus growth was normal up to 50 mM concentration of NaCl and there was a reduction in growth of the calli at 75 mM salt treatment whereas at 100 mM concentration complete cessation of callus growth was noticed. An increase in reserpine accumulation was noticed with increases measured up to 75 mM NaCl concentration.

INTRODUCTION

The genus *Rauvolfia* belongs to the family Apocynaceae that consists of around one thousand species, five of which are native to India1. *R. tetraphylla* is economically important because of the presence of alkaloids, which are localized in the roots2. The roots are useful in the treatment of hypertension, cardiovascular diseases and as a tranquilizing agent.

The extract of the root is valuable for intestinal problems. Roots are believed to stimulate uterine contraction in case of difficult delivery3. About 30 indole alkaloids are reported in *Rauvolfia* and reserpine holds the first place among them. Other frequently reported alkaloids are ajmalicine, reserpitine, deserpitine, sarpagine, rescinnamine and yohimbine4. Tryptophan is the starting material in the biosynthetic pathway of reserpine that is converted to tryptamine by tryptophan decarboxylase enzyme. Tryptamine is combined with secologanin in the presence of strictosidine synthetase enzyme and yields strictosidine. Various enzymatic conversion reactions lead to the synthesis of reserpine from strictosidine5. *R. tetraphylla* roots are often used as a substitute to the *R. serpentina* roots which gained export value in recent years. *R. tetraphylla* L. is a woody shrub that grows up to 1½ m in height. Tender parts of this plant are puberulous. Leaves are four at each node, elliptic and ovate. Inflorescence develops in axillary or terminal, 5-7 flowered corymb. Flowers are white or yellowish white. Fruit is a drupe and seeds are ovoid6.

The indiscriminate collection and limited cultivation of both *R. tetraphylla* and *R. serpentina* made these plants unavailable normally and they are listed as endangered7, 8. When plant material is rare and difficult to acquire, or when chemical synthesis is not possible and cost and demands are high, plant tissue culture technology provides a valuable alternative to obtain the desired product9. Plant tissue culture and hairy root

cultures are promising potential alternative sources for the production of high value secondary metabolites of industrial importance10, 11. By controlling the composition of the culture medium and the environment, secondary metabolite synthesis may be enhanced *in vitro*. Cheng and Cheng12 successfully produced more reserpine in calli of some Chinese herbs, in a quantities approaching *in situ* level, by modifying the medium components.

Culture media and Growth Conditions:

Full strength M.S medium (Murashige and Skoog, 1962) supplement with 3 %sucrose (Hi media Mumbai, India) ,0.2% Clerigel (Hi-media, Mumbai India)and different combination of Auxin (2,4-D ,NAA,IBA) and Cytokinins (BAP,KIN) at the concentration 0.5,1.0…….3.0 mg/l was used as the callus induction medium. The pH of the medium was adjusted to 5.8 before the addition of Clerigel .Culture bottles were filled with 50 ml of the media .The media was autoclaved at 15 lbs(121 c) for 15 min. Cotyledon explant were used to induce the embryonic callus from *Raulfia tetraphylla* L. (corr).The surface sterilized seeds were incubated to callus induction medium.

The cultures were incubated at 25+2 with 16 h photoperiod with the light intensity of 3000 Lux under cool white florescent lamps .All the experiment were conducted in 5 replicates & repeated for 3 times. The No of days, frequency of callus formation ,color of callus were determined after 4 weeks of culture the morphology of embryonic callus was also observed every 2 weeks after the callus formation .

Table 1: Effect of different concentration of PGR's on direct somatic embryonic callus from cotyledon explant of *Raulfia tetraphylla* (L.) corr.

Concentrations of Plant Growth Regulator (mg/l)				**Frequency of Callus formation**	**Frequency of Direct Somatic embryonic callus**	**Color of callus/ somatic embryo**
NAA	2,4-D	BAP	KIN			
0.1	-	2.0	-	+	+	Yellowish green
0.2	-	2.0	-	+++	+++	Yellowish light green
0.3	-	2.0	-	++++	++++	Yellowish green
0.4	-	2.0	-	+++++	+++++	Whitish
-	1.0	-	-	+	-	Creamish
-	1.5	-	-	+++	-	Creamish
-	2.0	-	-	++++	-	Creamish
-	2.5	-	-	+	-	Creamish
1.0	0.5	-	0.5	+	+	Yellowish
1.0	0.5	-	1.0	+++	+++	Yellowish
1.0	0.5	-	1.5	+++	++++	Yellowish
1.0	0.5	-	2.0	+++++	+++	Creamish

+ : very weak ; +++: Moderate; ++++: Profuse; +++++: Very profuse

Development and plant regeneration from Somatic Embryogenesis:

In order to develop and further regeneration the somatic embryos from the embryonic callus, which were represented by the compact, yellowish green with nodular structure and slow growing .The callus was transferred to the medium similar to the initation medium.

Somatic embryos were indicated by the formation of yellowish green colour smooth and zygotic embryo shape, which has different developmental stages. Mean while the plantlet regeneration was indicated by the development of morphologically normal plantlets which shoots and roots.

RESULT AND DISCUSSION:

Effects of different Auxins & Cytokinins on the cotyledon explants for callus induction:

Effects of different Auxins & Cytokinins were studied by using cotyledon as explants. The basic culture medium utilized in present piece of work was Murashige and Skoog medium (MS) supplemented with different concentration of Auxins and cytokinins. Result reveled that when 2,4-D and NAA tried alone and in combination of BAP and KIN shows induction of callus. Maximum proliferation was achieved on (2 mg/ L) 2, 4 D when it was tried alone and in combination 2 mg/L of BAP and from 0.2 mg/L to 0.4 mg/L of NAA increased the proliferation in cotyledons tissue. Highest rate of callus indication was found on MS medium supplemented with NAA 1mg/L + 0.5mg/L of 2, 4 D + 2.0 mg/L of KIN and 0.4 mg/L of NAA with 2.0 mg/L of BAP. Callus formed in present study were showing variability in terms of color and texture, Yellowish, Green, Light Green, White, creamish color callus were frequently observed with different type of texture viz. smooth, rough, crystalline. Callus develop in present piece of work were show direct somatic embryogenesis with different type of shapes.

Table 2 : Effect of different concentration of PGR's on Shoot induction From Embryonic Callus Of *Raulfia tetraphylla* (L.) corr.

Concentrations of Plant Growth Regulator (mg/l)				**No of shoot induction**	**Mean ± SE**
NAA	2,4-D	BAP	KIN		
0.1	-	2.0	-	10	8 ± 0.577
0.2	-	2.0	-	22	18 ± 1.632
0.3	-	2.0	-	36	28 ± 1.527
0.4	-	2.0	-	52	45 ± 2.848
-	1.0	-	-	-	-
-	1.5	-	-	-	-
-	2.0	-	-	-	-
-	2.5	-	-	-	-
1.0	0.5	-	0.5	07	5 ± 0.577
1.0	0.5	-	1.0	12	9 ± 0.881
1.0	0.5	-	1.5	32	28 ± 1.201
1.0	0.5	-	2.0	25	19 ±1.452

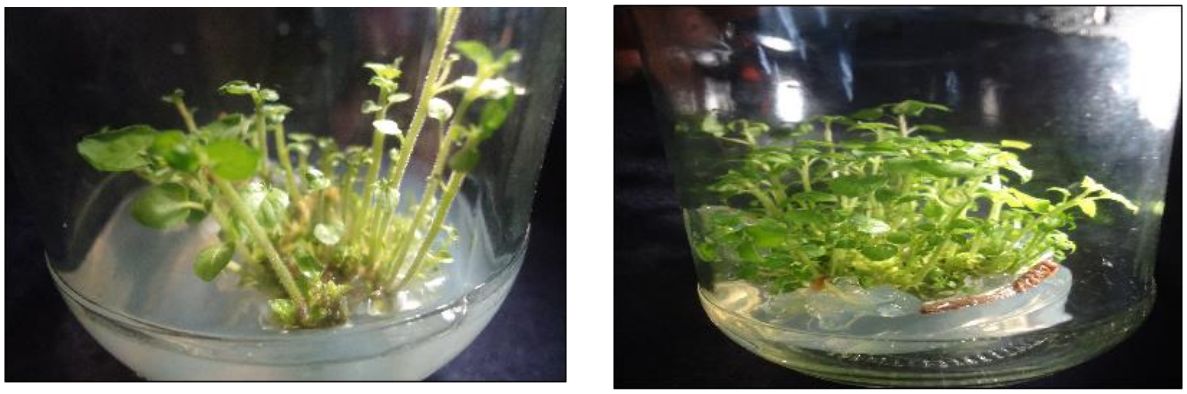

Fig. 1 : Effect of different concentration of PGR's on Shoot induction From Embryonic Callus Of *Raulfia tetraphylla* (L.) corr.

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