

## TISSUE CULTURE TECHNIQUES IN THE PROLIFERATION OF *RUTA GRAVEOLENS L*

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### ABSTRACT

There is a high demand for plant material from *Ruta graveolens* in the production of herbal medicines. Proliferation of shoots from nodal explants was achieved on MS medium supplemented with various concentration cytokinins and auxins. The result showed shooting and rooting from nodal culture of *Ruta graveolens*. Shoot Proliferation was dependent on cytokinins (6 Benzyl amino purine). The highest rate for rooting (85%) was obtained using IBA 2.5 mg/L<sup>-1</sup>. the efficient protocol in vitro regeneration of the whole plant can be used as a fast and reliable method for *Ruta graveolens*.

**Keywords:** Herbal Medicines, Cytokinines, Auxins, Shoot Proliferation

### INTRODUCTION

*Ruta graveolens* L., commonly known as rue, belongs to the Rutaceae family. It is exploited for its ornamental, aromatic and culinary uses, and is thought also to have potential medicinal and pharmaceutical value. *In vitro* cultures of *R. graveolens* are promising methods for the production of secondary metabolites, notably flavonoids, furanocoumarins, acridine alkaloids, furanoquinolins, essential oils and coumarins (Hornok, 1992; Junghanns, 1998; Diwan & Malpathak, 2007).

Production of furanocoumarins has garnered significant interest in the medical community, and there are available information which revealed that micropropagated shoots are richer in furanocoumarins than any other plant material (Massot *et al.*, 2000).

Furanocoumarins have wide applications in the pharmaceutical industry, being used to treat various diseases such as vitiligo, psoriasis, multiple sclerosis, cutaneous lymphomas. It is an anti-HIV agent (Bisaccia, 1993) and shows anti-inflammatory, anti-viral and anti-bacterial properties (Aljaiyash *et al.*, 2014). *R. graveolens* also helps to protect against cell damage by free radicals and is used to treat varicose veins and as a traditional supplement to improve eyesight (Hoffmann, 2010).

To exploit *Ruta* tissue culture successfully as a means for pharmaceutical production and other applications would require its mass propagation to be improved so as to reduce the cost of its production. Research has been undertaken previously to develop protocols for the micropropagation of *R. graveolens* (Faisal *et al.*, 2005; Bohidhar *et al.*, 2008; Diwan & Malpathak, 2008; Ahmad *et al.*, 2010).

Its medicinal values are because of the many secondary metabolites it contains, such as furocoumarins, furoquinolines, and acridone alkaloids. Amongst furocoumarins, bergapten has been used for the treatment of various skin diseases such as vitilago and psoriasis (Song and Tapley, 1979).

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All the parts of the plant material have active principles, although they are mostly encountered in leaves (especially before blooming). A pale yellow or greenish volatile oil, rue oil, often with a fluorescence, is obtained (0.06%) on steam distillation of the fresh plant materials. We describe an efficient protocol for rapid clonal multiplication of *R. graveolens* through high frequency shoot induction from nodal explants followed by successful outdoor establishment of regenerated plants.

## **MATERIALS AND METHODS**

### ***Plant Materials and Sterilization***

Nodal of *Ruta graveolens* were collected from a healthy true to type mother plants. After removing the leaves were thoroughly washed with tap water. The nodal explants were sequentially treated with 70% alcohol for 30 seconds, then treated with 100% (v/v) hypochlorite (the active ingredient was 3.85% sodium hypochlorite) mixed with few drops of Tween 20 for 20 minutes in order to sterilize the surface.

This was then followed with treatment of 50% (v/v) hypochlorite of the same active ingredient for 10 minutes. The explants were further trimmed to remove the remaining leaves and rinsed with sterile distilled water before initiation.

### ***Culture Conditions and Media for Nodals Proliferation***

The explants were placed in culture vessels containing 20 mls of culture media containing MS basal salts supplemented with 30 g/l sucrose, vitamins; glycine 2 g/l, pyridoxine 0.5 g/l, Nicotinic acid 0.5 g/l, Thiamine 0.1 g/l and Myo inositol at 0.1 g/l. The media was also supplemented with different concentrations of BAP and NAA and solidified with 8 g/l of agar (Table 1). The pH was adjusted to 5.8 prior to autoclaving at 121°C for 15 min.

### ***Roots Initiation Media***

Good established shoots were transferred to root initiation media. This media consisted of MS basal salts with 30 g/l sucrose, vitamins glycine 2 g/l, pyridoxine 0.5 g/l, Nicotinic acid 0.5 g/l, Thiamine 0.1 g/l and Myo inositol at 0.1 g/l. The media also contained 0.8 g/l of activated charcoal (AC) to mimic the soil environment. It was supplemented with different concentrations of auxin as treatments for rooting; IBA with or without BAP as shown in (Table 2) and solidified with 8 g/l of agar.

Each treatment was replicated five times and one explant was cultured in each culture bottle. The pH of the media was adjusted to 5.8 before addition of agar. The media were autoclaved at 121°C and 1.05kg/cm<sup>3</sup> for 15 minutes. The cultures were incubated at 25°C ± 1°C and 16 and 8 hrs light and darkness respectively.

### ***Data Collection and Analysis***

After four weeks the following parameters were measured; number of shoots and number of roots. The data collected were analyzed for statistical significance using analysis of variance (ANOVA). Fisher least significance was used to compare means at p = 0.05 level of significance.

## **RESULTS AND DISCUSSION**

### ***The Effect of BAP Concentration on Nodal Proliferation Rate***

The findings of this study demonstrated the effects of cytokinins on shoots formation and multiplication. In this experiment, the use of BAP alone or in combination with NAA had significant ( $p \leq 0.05$ ) effect on shoots proliferation (Table 3; Figure A & B). However, different concentrations regimes of BAP with and without NAA had significant effect on the number of shoots produced

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It was observed that the number of shoots produced increased with increase in concentration. A significantly ( $p \leq 0.001$ ) highest number of branches were observed when 3 mg/l was used (9.5 shoots per explants, Table 3).

Slightly increase in number of shoots was also observed in other treatments with low concentrations compared with the control. The initial response of explants to shoot formation due to addition of cytokinin is mediated by an increase in the cytosolic calcium concentration which is promoted by its high uptake from the media. This affects cytoskeleton and regulates exocytosis.

Generally, this study indicates that increasing concentration of BAP for this particular variety enhanced the branches formations.

Addition of 3 mg/l BAP with 0.25mg/l NAA to the growth media showed best results compared with all other treatments (Figure 1).

***The Effect of IBA and BAP Concentration on the Number of Roots per Explant Produced in Vitro***

In this study, there was significant difference between MS media supplemented with IBA alone and media supplemented with IBA and BAP in terms of number of roots produced per explant (5.1 roots per explants, Table 4, Figure C). However, significant ( $p \leq 0.001$ ) increase in number of roots produced was observed with increased concentration. The concentration of 2.5 mg/l exhibited superiority over all other treatments in terms of number of roots produced (Figure 3). However, auxins are known to induce quick and further roots initiation. In terms of rooting, our experiment indicated that better response to roots initiation of this variety was 3 mg/l.

**Table 1: Different Concentrations of BAP and NAA Used for Shoots Proliferation**

Treatments	Concentration (mg/l)		
MS + BAP		1.0	
MS + BAP		2.0	
MS + BAP		3.0	
MS + BAP		4.0	
MS + (BAP + NAA)	1 + 0.00	1 + 0.25	1 + 0.50
MS + (BAP + NAA)	2 + 0.00	2 + 0.25	2 + 0.50
MS + (BAP + NAA)	3 + 0.00	3 + 0.25	3 + 0.50
MS + (BAP + NAA)	4 + 0.00	4 + 0.25	4 + 0.50

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**Table 2: Different Concentrations of IBA and BAP Used for Rooting**

Treatments	Concentration (mg/l)		
MS + IBA		1.0	
MS + IBA		1.5	
MS + IBA		2.0	
MS + IBA		2.5	
MS + (IBA + BAP)	1.0 + 0.00	1.0 + 0.25	1.0 + 0.50
MS + (IBA + BAP)	1.5 + 0.00	1.5 + 0.25	1.5 + 0.50
MS + (IBA + BAP)	2.0 + 0.00	2.0 + 0.25	2.0 + 0.50
MS + (IBA + BAP)	2.5 + 0.00	2.5 + 0.25	2.5 + 0.50

**Table 3: The Effect of Different Concentration of BAP and NAA on Nodal Proliferation Rate (Mean ± SE)**

NAA mg/l	BAP mg/l			
	1.0	2.0	3.0	4.0
0.0	1.2	3	5.7	7.3
0.25	2.2	6.6	<b>9.5</b>	7.2
0.50	2.5	5.5	7.2	6.1

**Table 4: The Effect of Different Concentration and Combination IBA and BAP on Root Development (Mean ± SE)**

BAP mg/l	IBA mg/l			
	1.0	1.5	2.0	2.5
0.0	1.9	3.4	4.3	<b>5.1</b>
0.25	1.1	1.6	2.5	3.2
0.50	1.0	1.4	2.1	2.4

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**Figure (A): Initiation Stage of Shoot Formation from Nodal of *Ruta Graveolens***      **Figure (B): Proliferation & Elongation of Multiple Shoots from Nodal**



**Figure (C): Fully Rooted Plantlets Developed**

**Conclusion**

The optimum concentration of BAP for shoots proliferation of *Ruta graveolens* was 3 mg/l as reflected by increased number of branches. The use of BAP in combination with NAA was found to enhance number of branches at concentration of 3 mg/l BAP with 0.25 mg/l NAA respectively.

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As for rooting 2.5 mg/l of IBA increased the number of roots formed and reduce the time required to wait for roots to be formed in basal media.

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