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RESEARCH ARTICLE

Effect of Plant Growth Regulators on Indirect Organogenesis in *Ruta graveolens.L*

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Abstract

Ruta graveolens is important medicinal and aromatic plant of Rutaceae. The present study was undertaken to evaluate the most suitable concentration of growth regulators for indirect organogenesis in *Ruta graveolens.*Callus was initiated from stem,and petiole on MS basal media supplemented with various combinations of auxins and cytokinines,the callus obtained from different explants was cultured on MS medium supplemented with various combinations of PGR's.Studies were carried out to evaluate the shoot regeneration capacity of callus using plant growth regulators on growth of *Ruta graveolens L.* Among the different concentration of plant growth regulators used in the study;it was found that MS fortified with 4.44µM BA+2.69μM NAA resulted in highest shoot regeneration frequency 86.66% and highest mean number of shoots (28.00±1.15) with maximum shoot length (3.96 ± 0.3).

INTRODUCTION

Ruta graveolens L.is an aromatic and medicinal herb native to the Mediterranean and temperate zone (Bradly 1998; Gonzalez-Trujano, *et al.;* 2006) the plant is mainly distributed Mediterranean and south east Europe countries and cultivated quiet widely in different countries including India.The plant is having wide spectrum of different phytoconstituents.More than 120 natural compounds belonging to four different classes have been identified **(**Kuzovkina *et al.;* 2004).The active principles of clinical importance are Psoralens and Methyl-nonyl-ketone, which are responsible for hepatoxicity, photosensitization and effects on the uterus respectively. Because of its medicinal properties; Furocoumarins (FCS) have gained wide applications in Pharmaceutical industry (Diwan and Malpathak, 2007). Among the various members who have investigated the FCS content in different plants; *R. graveolens* was found to contain highest concentration of such furocoumarins (Poutarud *et al.*; 2000).

The plant is prescribed in Indian systems of medicine for the treatment of dropsy, neuralgia, rheumatism, menstrual and other bleeding disorders. The plant is also reported to treat internal infections, inflammations, eczema and external ulcers (Wink, 1998).The plant is demonstrated to be potent anti HIV agent (Bissacia,*et al* 1993). The whole herb is abortifecent, anthelmentic, antispasmodic and carminative, expectorant, ophthalmic and rubefacient (Ivanova *et al.*; 2005). Furocoumains e.g. Xanthotoxin, Burgaptin, Isopimpinelin have been applied in the treatment of skin diseases e.g. Psoriasis, Mycosis fungoids or in pigmentation disorder e.g. Vitilago (Ekierts *et al.*; 2005). The volatile oil from *R. graveolens* possesses phototoxical, bacteriostatistical and anthelmentic activities (Petit-Paly *et al.*; 1988).

A number of workers have attempted to multiply *Ruta graveolens* using indirect organogenesis. Bohidar *et al* (2008), Ahmad *et al* (2010), Tejavathi *et al* (2010) obtained complete plants of *Ruta graveolens* from cultured explants.

The present communication demonstrates an indirect regeneration method for producing a large number of plants from explants of *Ruta graveolens.*The present studies have been carried out to establish in vitro cultivation of *Ruta graveolens*, through indirect organogenesis from stem and petiole explants

Materials and Methods:

Plant material- Plant material was collected from aromatic and medicinal plant garden of S.H. Kelkar and company Pvt. Ltd. Mulund, Mumbai. Plants of *R.graveolens* L. were further maintained in the aromatic and medicinal plant garden of V. G.Vaze College campus.

Surface sterilization of explants: The different explants viz. stem internode, petiole and leaf discs were washed with running tap water for 10 min. to remove dust and other contaminants; followed by washing with 5% Teepol® for 10 min, then thoroughly washed with running tap water. The cleaned explants were then treated with 3% Dettol solution. The washed explants were further treated with 1% Bavistin (systematic fungicide). Sterilized explants were then rinsed with distilled water and transferred to laminar air flow. These explants were then exposed to 70% ethanol for 30 sec. followed by three washes with sterilized distilled water. 0.1% $HgCl_2$ (w/v) for 4 min.and repeatedly washed with sterilized distilled water.The explants were then trimmed and the trimmed explants were soaked into Ciplox solution for 1 min. surface sterilized explants were blot dried with sterilized tissue paper.Such explants were aseptically inoculated onto Murashige and Skoog (MS 1962) medium supplemented with different combinations of auxins and cytokinins.

***In vitro* Callus induction-** Callus initiation experiments were carried out using, petiole, and internodal segments of stem. MS basal medium supplemented with different combinations of growth hormones such as NAA, IAA, 2, 4-D, either singly or in combinations. 3% Sucrose was used as a carbon source.The gelling agent was 0.8% (w/v) Agar (Bacteriological Grade, Qualigens, Mumbai). The *pH* of the medium was adjusted to 5.7 with 0.1N NaOH or 0.1N HCl as per requirement and autoclaved.20 tubes each of above mentioned combinations were inoculated with desired explants for callus induction.

Culture conditions:

The cultures were incubated at 25 ± 2^0C under 16 hrs photoperiod with cool, white fluorescent tube light (3000 Lux) with 80% relative humidity.

After callus induction; the calli were transferred to the fresh medium of same combination after every 15 days of incubation for further proliferation and maintenance.

Indirect organogenesis

The callus obtained from different explants was sub cultured on MS medium supplemented with various combinations of PGR's. Following combinations of medium were used for organogenesis experiments. MS + BA (2.22 µM, 4.44 µM, 6.66 µM, 8.87 µM, 11.09 µM, 13.31 µM), MS + Kinetin (2.32 µM, 4.65 µM, 6.96 µM, 9.29 µM, 11.61 µM), MS + BA (4.44 µM) + IAA (2.85 µM), MS + BA (4.44 µM) + IAA (5.14 µM), MS + BA (4.44 µM) + IAA (7.99 µM), MS + BA (4.44 µM) + IAA (11.42 µM), MS + BA (4.44 µM) + NAA (2.69 µM), MS + BA (4.44 µM) + NAA (5.37 µM), MS + BA (4.44 µM) + NAA (8.06 µM), MS + BA (4.44 µM) + NAA (10.74 µM). 20 tubes each of above mentioned combinations were inoculated with callus for indirect organogenesis. Newly developed shoots were sub cultured on same medium after every 3 weeks for further proliferation and maintenance.

Results and Discussion: Callus induction

The manipulation of plant growth regulators is essential to optimize the induction of callus. The role of auxin alone or in combination with cytokinin for callus induction and proliferation is well documented (Roy and De, 1990). The effects of different growth regulators on callusing were one of the objectives of this study. Though the extensive studies have been done on callus culture of *R.graveolens* for biochemical and phytochemical studies but very few efforts have been taken to study potent ability of callus for organogenesis. The present investigation was therefore carried out to check the potent ability of callus of *R. graveolens* for organogenesis using different PGRs.

Preliminary studies showed stem and petiole were best for callusing where as leaf discs did not respond to callusing. Similar observations were recorded by Saeeda and Benjamin (2007). Callus induction was observed at the cut surfaces of stem and petiole within 2-3 weeks. Explants placed horizontally on agar medium produced callus more

rapidly than the explants which were placed vertically in the medium. No callus was induced when explants were cultured on MS basal medium.

Among the different concentration of 2, 4-D used in the study; 4.52 µM gave the best result. It gave early initiation (within 15 days) with 100% response and degree of callusing was much higher than the other concentrations of 2, 4-D. The callus produced by the explants was compact and yellow green colour.Srivastava .P, and Pandey .A (2011) obtained the maximum induction and proliferation of callus on MS+6.0 µM 2,4-D, with white green friable callus from leaf explants of *Vigna mungo* var. *silvestris*

Effect of auxin on petiole as explants

The different concentrations of 2, 4-D evaluated for callusing. This study showed that the time taken for callus initiation from petiole explants was shorter (12 to 19 days) than the internodal explants. 4.52 µM 2, 4-D showed early initiation (12 days) with the higher degree of callusing than other concentrations.

Indirect Organogenesis

Studies were also carried out to evaluate the shoot regeneration capacity of callus. The callus obtained from petiole, and stem internode was used for shoot induction.

Effect of BA on shoot induction

The callus was transferred to shoot induction media containing MS+ BA (2.22 µM, 4.44 µM, 6.66 µM, 8.87 µM, 11.09 µM, 13.31 µM). The calli become more yellow greenish and appeared highly competent for bud induction. BA (4.44 µM) showed 75% shoot induction with 20 ± 1.15 of mean number of shoots/ unit callus and mean length of shoot was 2.53 ± 0.88 cm .With concentrations of BA more than 4.44 µM and less than 4.44 µM the no of shoots were decreased (Table 1.1). The shoot premordia developed in this medium were green nodular, which later developed into shoots. The results obtained here are in agreement with Saeeda *et al* (2007). They observed the formation of shoot premordia from callus produced by internodal segment of *R. chalepensis* when inoculated on the same medium combination. Further increasing BA concentration from 6.66µM to 11.09µM resulted in decrease in shoot regeneration frequency. However Ahmad *et al* (2010) obtained 70% shoot bud induction with BA (7.50µM). The length of shoots recorded in 4.44µM is lesser than other concentrations of BA e.g. 6.66µM BA and 8.88µM BA.

Effect of Kinetin on shoot induction

Effect of Kinetin at different concentrations (2.32 µM, 4.65 µM, 6.96 µM, 9.29 µM, 11.61 µM) was also evaluated for shoot bud induction from callus. At the lower concentration of kinetin induction of shoot was much lower than higher concentration of kinetin It was observed that at the 2.32 µM kinetin resulted in lowest regeneration frequency (45.00%) and lowest number of shoots /unit callus (12.00 ± 0.58) with maximum mean length of shoot length was 1.17 ±0.33 cm (Table 1.2). It was also observed that the Kinetin at 11.61 µM showed 78.33% shoot with regeneration with 19.00 ± 0.58 of mean number of shoots/ unit callus and mean length of shoot was 3.20 ± 0.06 cm

BA was therefore found to be more effective than the Kinetin in induction of shoot buds from the callus.

Effect of BA and IAA on shoot induction

The combined effect of BA (4.44 µM) and IAA at different concentrations (2.85 µM – 11.42 µM) was also evaluated. It was observed that BA (4.44 µM) + IAA (7.99 µM) resulted in highest regeneration frequency (66.66%) and highest number of shoots (21.00 ± 2.08) with maximum mean length of shoot length was 3.3 ± 0.17 cm. The concentration of IAA (7.99 µM) showed synergistic effect with BA and the increased shoot morphogenetic response was recorded (Table.1.3). Similar observations with auxin and cytokinin concentrations have been recorded in *Hypericum performata* (Wojeik and Podostolski, 2007).

Effect of BA ands NAA on shoot induction

The combined effect of BA (4.44 µM) and NAA at different concentration were studied. It was observed that NAA at 2.69µM in combination with BA 4.44 µM resulted in highest shoot regeneration frequency 86.66% and highest mean number of shoots (28.00 ± 1.15) with maximum shoot length 3.96 ± 0.3 (Table 1.4). The lower concentration of NAA with BA showed maximum regeneration frequency. Similar observations were made by Janarthanam *et al* (2009). They used leaf derived callus of *Steveia rebaudiana* Bertoni to study the shoot regeneration. Also Shivanna

et al (2009) studied shoot formation in *Biophytum sensitivum* (Linn.) DC. Ghosh *et a*l (2003) reported in *Rauwolfia tetraphylla* L. Kannan *et al* (2005) showed the similar results while propagating the shoots at large scale from *Withania somnifera.*

Conclusion:

Effect of different concentrations and combinations of plant growth regulators on indirect shoot morphogenesis in stem callus studies on *R. graveolens* showed, MS+ 4.44 µM BA+2.69 µM NAA is better for indirect organogenesis than other plant growth regulators in terms of percent callus response, mean number of shoots/ unit callus and mean length of shoot.

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Table.1.1 Effect of different concentration of BA on indirect shoot morphogenesis in stem callus of *R. graveolens*

Sr. No.	Concentration of BA (µM)	% Callus response	*Mean No. of shoots/ unit callus	*Mean length of shoot (cm)
1	0.0	-	-	-
2	2.22	58.3	15.66 ± 0.88	1.46 ± 0.12
3	**4.44**	**75**	**20 ± 1.15**	**2.53 ± 0.88**
4	6.66	76.6	17 ± 1.53	2.5 ± 0.11
5	8.88	73.33	14.67 ± 0.33	2.4 ± 0.92
6	11.09	75	12.33 ± 0.42	2.63 ± 0.12

Table. 1.2. Effect of kinetin on indirect shoot morphogenesis in stem callus of *R. graveolens*

Sr. No.	Concentration of Kinetin (µM)	% Callus response	*Mean No. of shoots/ unit callus	*Mean length of shoot (cm)
1	0.0	-	-	-
2	2.32	45.00	12.00 ± 0.58	1.17 ±0.33
3	4.65	83.33	15.00 ± 0.58	1.23 ± 0.03
4	6.96	88.33	15.67 ±0.33	2.27 ±0.09
5	9.29	86.33	17.67 ± 0.33	2.27 ± 0.19
6	11**.61**	**78.33**	**19.00 ± 0.58**	**3.20 ± 0.06**

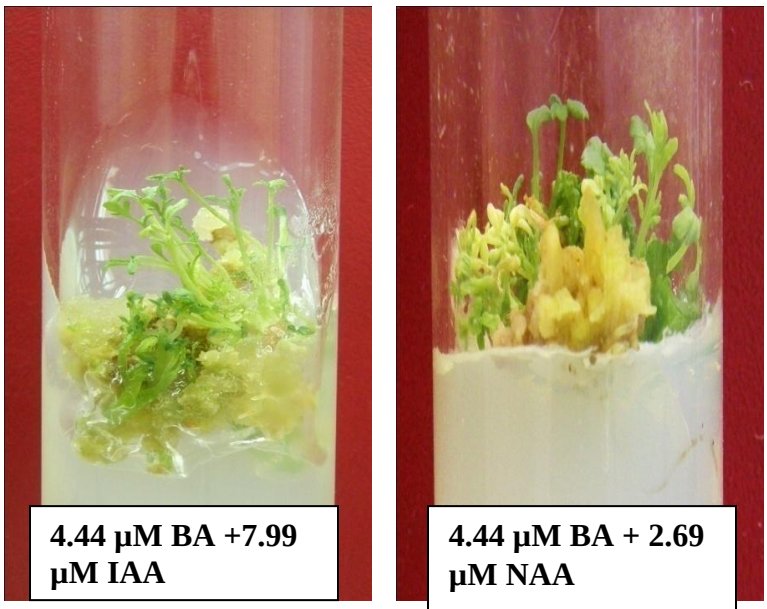
Table.1.3. Effect of different concentration of BA and IAA on indirect shoot morphogenesis in stem callus of *R. graveolens*

Sr. No.	Concentration (µM)		% Callus response	*Mean No. of shoots/ unit callus	*Mean length of shoot (cm)
	BA	IAA			
1	4.44	0.0	75	20 ± 1.15	2.53 ± 0.88
2	4.44	2.85	44.00	13.67 ± 0.33	1.33 ± 0.33
3	4.44	5..14	58.33	17.67 ± 1.20	2.1 ± 0.15
4	**4.44**	**7.99**	**66.67**	**21.00 ± 2.08**	**3.3 ± 0.17**
5	4.44	11.42	51.67	11.33 ± 0.33	1.43 ± 0.09

Table 1.4.Effect of different concentration of BA and NAA on indirect shoot morphogenesis in stem callus of *R. graveolens*

Sr. No.	Concentration (µM)		% Callus response	*Mean No. of shoots/ unit callus	*Mean length of shoot (cm)
	BA	NAA			
1	4.44	0.0	75	20 ± 1.15	2.53 ± 0.88
2	**4.44**	**2.69**	**86.67**	**28.00 ± 1.15**	**3.96 ± 0.30**
3	4.44	5.37	71.67	24.33 ± 0.33	3.50 ± 0.25
4	4.44	8.06	68.33	22.66 ± 0.33	3.37 ± 0.09
5	4.44	10.74	50.00	19.33 ± 0.67	3.10 ± 0.10

*Values represent mean ± standard error of 20 replicates per treatment in three repeated experiments



Effect of BA and BA in combination with IAA & NAA on indirect organogenesis

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