

Regeneration of the Medicinal Plant *Ruta Graveolens* L. from Hypocotyl

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Abstract:

*In this study, culture conditions were adapted to produce intact plants of *Ruta graveolens* L. via in vitro propagation from hypocotyl explants cultures. The results show that the direct formation of shoots from hypocotyl in agar-solidified MS medium containing 6-benzyladenine (BA) 0.25 mg L⁻¹ and 2,4-Dichlorophenoxy acetic acid (2,4-D) 0.25 mg L⁻¹. They were kept in light or dark conditions, and did not enhance callus induction. While hypocotyl explants were grown on MS medium containing 0.25 mg L⁻¹ of BA and 0.50 mg L⁻¹ of 2,4-D induced callus, at ends segments, and shoots induction occurred from intermediate the tissue kept the induction medium. In light and dark, region, hypocotyl explant lost and converted to shape callus on MS containing 0.25 mg L⁻¹ BA and 0.75 mg L⁻¹ 2,4-D. In vitro regenerated shoots were rooted easily on half- strength free growth-regulators MS medium. Plantlets with well-developed root and shoot systems were successfully acclimatized and transferred in pots containing garden soil.*

Key words: *Ruta Graveolens* L., Hypocotyl, in vitro propagation

1. Introduction

Ruta graveolens L. (Rutaceae) which is commonly known as rue is a strongly scented medicinal and aromatic plant of Europe,

mostly grown in the Mediterranean region (Anonymous, 2003). The roots and aerial parts of Rue plant contain more than 120 compounds that belong to different classes of natural products such as alkaloids (skimianine, arborine and graveolene), coumarins (herinarin and umbelliferon, essential oil, flavonoids (rutin glycoside I and its aglycone quercetin II) and furoquinoline (Duke *et al.*, 2002). It has been traditionally used as a sedative to relieve menstrual and gastrointestinal disorders (Gatierre-pajares *et al.* 2003). Also it shows hypotensive (El-Sherbeny *et al.*, 2007), antifertility (Ratheesh *et al.*, 2009), analgesic and anti-inflammatory effects (Trease and Evans, 2009). Micropropagation has become an important part of the commercial propagation of many plants because of its advantages as a multiplication system. Several techniques for *in vitro* plant propagation have been devised, including the induction of axillary and adventitious shoots, the culture of isolated meristems and plant regeneration by organogenesis and/or somatic embryogenesis (Davey and Anthony, 2010; Al-Mahdawe *et al.*, 2013). Direct shoot bud induction in nodal segments of *Ruta graveolens* through axillary shoot multiplication has been reported earlier (Faisal *et al.*, 2005). Indirect multiple shoot bud induction and plant regeneration from leaf segment-derived callus of *Ruta graveolens* has been developed (Ahmad *et al.*, 2010). The target of the present investigation is to find a rapid plant regeneration protocol from hypocotyl as a first step for new studies focused on the isolation of its natural products.

2. Materials and Methods

Ruta graveolens L. seeds were collected from 2 year- old plants grown at the botanical garden (Agriculture and Forestry College, Mosul University). Seeds were washed in running tap water, treated for 5 min with 3% sodium hypochlorite NaOCl solution and then rinsed three times with sterile distilled

water. Sterilized seeds were germinated on agar- solidified MSO medium (Murashige and Skoog, 1962) and kept in dark for three days at 25°C. The produced plantlets were maintained at 25±2°C for 16 h photoperiod with a photon flux density of 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ provided by cool white fluorescent lamps (Ahmad *et al.*, 2010). Hypocotyl explants were excised from 21 days old plantlets, and sliced into lengths of 1.0 cm. Then they were placed horizontally on the surface of callus-induction MS basal medium supplemented with 0.25 mg L⁻¹ of BA in combination with 0.25 , 0.5 and 0.75 mg L⁻¹ of 2,4-D. The MS medium lacking plant growth regulators (MSO) was used as control. Ten replicates of hypocotyl segments were maintained in lighting conditions (for 16 h photoperiod with a photon flux density of 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$), and the other in the dark. Data of explants forming callus, average fresh weight, number of shoots and the total number of regenerated shoots were recorded after 8 weeks. Regenerated shoots (1.5-2 inches length) were excised and transferred to basal MS medium either half- and full-strength for rooting. Plantlets with well developed shoot and root were removed, washed carefully with tap water and transplanted into plastic pots (80 mm diameter) containing sterile peat and covered with transparent polyethylene bags to ensure high humidity. The bags were removed after 2 weeks to acclimatize plantlets to field condition. The surviving plants were transplanted into the garden condition after 3 months.

3. Results and discussion

Table 1 indicates that the process of callus induction from hypocotyl was affected clearly by concentration of 2,4-D supplementation, as shown below.

Table 1 Callus formation from hypocotyl explant of *Ruta graveolens* L. in light and dark condition.

Media (mg.L ⁻¹)	Light		Dark	
	Callus formation (%)	Fresh weight* (mg/explant)	Callus formation (%)	Fresh weight* (mg/explant)
MSO(control)	0.0	-----	0.0	-----
MS+0.25 2,4-D+0.25 BA	0.0	-----	0.0	-----
MS+0.50 2,4-D+0.25 BA	40.0	0.02	20.0	0.04
MS+0.75 2,4-D+0.25 BA	50.0	0.26	50.0	0.27

*Each value represents 10 replicates.

It was noted that hypocotyl explants grown on agar-solidified MS medium supplemented with the mixture containing 0.25 mg L⁻¹ of each 2,4-D and BA and incubated in light or dark conditions failed to produce callus, whereas those grown on induction medium containing either 0.50 or 0.75 mg L⁻¹ of 2,4-D in the presence of 0.25 mg L⁻¹ of BA accelerated calli production.

Many authors found that 2,4-D was one of the auxins commonly used in plant tissue culture (George *et al.*, 2008). It enhanced elongation, division and callus proliferation of cells when interacted with cytokinins. The finding that cytokinins in the presence of auxin behaved as a key to start cell division (Goodwin, 1985). Other investigators (Taiz and Zeiger, 2006) reported that 2,4-D is the popular auxin used in callus formation to inhibit organs differentiation depending on its concentration. It was reported that high conc. of auxin and low conc. of cytokinins sustained the production of shiny callus (Slater *et al.*, 2003). Again the priority of callus produced and its fresh weight that was incubated in dark condition rather than light. This may explain that dark inhibits the oxidation of phenolic compounds and affects the cell wall thickness. The latter promotes the permeability of growth regulators and

nutrients into culture which finally led to callus stimulation (George and Sherrington, 1993).

The nine day old calli produced at the edges of explants was yellow in color and friable as shown in **Fig. 1.A**. Explant cultured on agar-solidified MS medium provided with 0.25 mg L^{-1} BA and 0.75 mg L^{-1} 2,4-D completely converted to a mass of callus five weeks of culture as illustrated in **Fig. 1.B**. The production of callus may be due to the accumulation of hormones at wound sites (Ahmad *et al.*, 2010), and according to the role of explant type, it seems likely that hypocotyls were suitable for callus formation, this may be attributed to their meristematic cell that is capable of the division and to the high level of endogenous auxins (Grout, 1990). Hypocotyl explants cultured on agar-solidified MS medium provided with 0.25 mg L^{-1} of BA and 0.25 or 0.50 mg L^{-1} of 2,4-D produced shoots along the explant as elucidated in **Fig. 1.C**. They appeared firstly as green spots gradually differentiated to shoots. Whereas callus induced at the terminal ends of explant, shoots were developed from the dark green tissues located at mid region of explant in the presence of 0.50 mg L^{-1} 2,4-D.

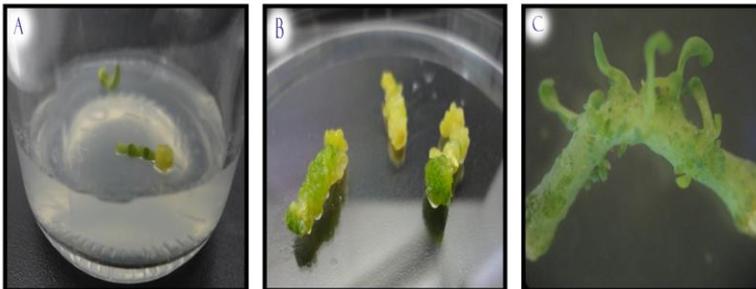


Fig.1. (A): callus stimulation and shoot bud emergence from hypocotyl explant in MS+ 0.25 mg L^{-1} BA + 0.50 mg L^{-1} , after 5 weeks. **(B):** callus stimulation from hypocotyl explant in MS+ 0.25 mg L^{-1} BA + 0.75 mg L^{-1} , after 5 weeks. **(C):** Shoot bud emergence from hypocotyl explant in MS+ 0.25 mg L^{-1} BA + 0.25 mg L^{-1} , after 5 weeks.

In this investigation, the results indicated that hypocotyl explant grown in light conditions were better than those grown

in dark condition in aspect of the total number of regenerated plants as shown in **Table 2**.

Table 2 Regeneration of *Ruta graveolens* L. plants from callus grown in light or dark conditions.

Media (mg.L ⁻¹)	Light		Dark	
	Reg. Explant (%)	Total No. of explant	Reg. Explant (%)	Total No. of explant
MSO(control)	0.0	0.0	0.0	0.0
MS+0.25 2,4-D+0.25 BA	5.0	22.0	4.0	13.0
MS+0.50 2,4-D+0.25 BA	5.0	15.0	3.0	7.0
MS+0.75 2,4-D+0.25 BA	0.0	0.0	0.0	0.0

The regenerated shoots were excised and rooted easily as shown in **Fig. 2. A** in agar solidified half strength MSO medium. They successfully adapted to soil environment producing intact plant as explained in **Fig. 2.B**.

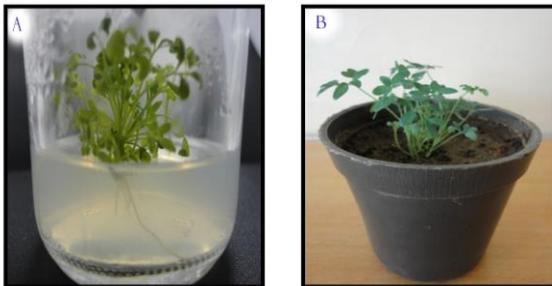


Fig. 2. (A): Rooting of regenerated shoot in agar-solidified 1/2MSO, after 4 weeks. **(B):** regenerated plantlets grown in soil pot.

The obtained results were difficult to be explained due to the effects of various factors such as light intensity, wavelength and photoperiod; this is coupled with the effect of explant type and plant specie. All these factors affected tissue orientation either to callus or shoot formation. Also they reported that the adsorbed light in the present pigment of phytochromes act as a key controlling differentiation process in plant tissue (George *et al.*, 2008).

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