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In vitro Culture of *Ruta graveolens* L.

Hamdy K. Atta-Alla[1*], Islam H. Abo El Soud[1], and Manal H. Eid[2]
[1]Horticulture Department, Faculty of Agriculture, Suez Canal University 41522 Ismailia, Egypt
[2]Agricultural Botany Department, Faculty of Agriculture, Suez Canal University 41522 Ismailia, Egypt

ABSTRACT

Shoot multiplication for *Ruta graveolens* L. was achieved when shoot tips excised from mature plants and cultured on Murashige and Skoog (MS) medium containing BA at 0.5, 1.0 and 2.0 mg l^{-1} combined with NAA at 0.1, 0.2 and 0.3 mg l^{-1}. Highest rate of shoot proliferation was obtained after 8 weeks with the medium supplemented with BA at 0.5 mg l^{-1} and 0.3 mg l^{-1} NAA. Complete plants with strong, fibrous roots were obtained after transferring the separated shoots into rooting medium (1/2 MS or MS media with or without NAA). The survival rate of transplants during acclimatization reached 100% and the plants grew normally under the greenhouse conditions.

Key words: Micro-propagation, *Ruta graveolens*.

INTRODUCTION

Ruta graveolens L. (commonly known as Rue), belongs to family Rutaceae is a perennial medicinal plant synthesizing several types of metabolites, notably flavonoids, alkaloids, essential oils and furanocoumarins (Brickell and Zuk, 1996).

The plant is commonly used in the treatment of various diseases such as skin diseases (psoriasis), to relieve symptom of hangover, applied externally as a poultice against rheumatic pain (Atta and Alkofahi, 1998). Rue's active ingredients may have antifungal property, which could be beneficial to agriculture and medicine (Retheesh and Helen, 2007).

Ruta is a cross-pollinated plant, thus shoots obtained would not be genetically identical to parent plant and the genetic makeup may vary with individual shoot. This may lead to variations in secondary metabolites production. Besides, the seed-set is low and seeds exhibit dormancy (Faisal *et al*., 2005). Therefore, there is an urgent need to look for alternate means of propagation which could ensure large-scale production of plants to fulfill the growing demands.

Tissue culture provides a means of rapid propagation of a large number of uniform plants while maintaining their genotype (Arikat *et al.*, 2004).

The efficient of *in vitro* regeneration can be used as a fast and reliable method to transform *Ruta graveolens* genetically for its active principles (Faisal *et al*., 2005). Micropropagation procedures and evaluation of active constituents were reported for *Ruta graveolens* (Diwan and Malpathak, 2008).

This study reports an efficient protocol for regeneration of plants of *Ruta graveolens* using shoot tips derived from mature plants.

MATERIALS AND METHODS

Shoot tips were excised from Rue plants grown in the field, surface sterilized by immersion in 70% (v/v) ethanol for one minute, followed by soaking in 20% Clorox for 20 min. The explants were then rinsed three times with sterile distilled water.

Under aseptic condition, shoot tip explants (0.5 cm) were inoculated on basal MS (Murashige and Skoog, 1962) medium containing 3% sucrose and supplemented with BA (0.5, 1.0, 2.0 mgl^{-1}) and NAA (0.1, 0.2, 0.3 mgl^{-1}). The pH of medium was adjusted to 5.8 prior to the addition of 0.8% agar and autoclaved at 121^0 C for 15 min. Cultures were then incubated at 24±1°C with a 16 hr photoperiod at a fluorescent light intensity of approximately 25 µmol $m^{-2}s^{-1}$. After 8 weeks, the number of proliferated shoots and shoot length were recorded.

For root induction, the proliferated shoots were individually separated and cultured on 1/2 MS or MS medium supplemented with NAA at 0.0, 0.1 or 0.2 mgl^{-1}. After 4 weeks, the number of roots and their lengths were recorded.

The rooted shoots were carefully removed from the culture jars, then planted in trays containing sand: peat moss: vermiculite (1:1:1 v/v/v/). The trays were placed under plastic cover where they were irrigated with a fine mist of water for 3 weeks and transferred to grow under the greenhouse conditions. The percentage survival was determined after 4 week.

In both multiplication and rooting experiments, three explants were cultured per vessel. All experiments were repeated twice. Completely randomized design was used for statistical analysis in factorial arrangement with 12 replications (vessels) in each treatment and mean comparisons were made using Duncan's Multiple Range test at 5% significant level according to Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The greatest number of multiple shoots (8.68 shoots/explants) was obtained from explants cultured on medium containing 0.5 mgl^{-1} BA. Generally, increasing BA concentrations led to a significant decrease in the

* *Corresponding author: hamattaeg@yahoo.com*

number of formed shoots. Meanwhile, it was observered that BA at 0.5 mgl^{-1} gave the highest significant average of shoot length as 4.61 cm. In this concern, Baskaran and Jayabalan (2005) reported that higher concentration of cytokinin reduced shoot number as well as shoot length. The lower concentration of BA showed better response resulting in multiple shoot induction and shoot elongation on *Eclipta alba*.
Generally, application of NAA to the culture media has different effects on shoot number. The treatment with NAA at 0.2 mgl−1 significantly produced the highest number of shoots and the shoot length.

Data in Table (1) show that various concentrations of BA and combinations with NAA helped in induction of shoot proliferation from shoot tips of *Ruta graveolens*. However, the best shoot multiplication was observed on medium containing BA at 0.5 mgl^{-1} and 0.3 mgl^{-1} NAA. This treatment produced the highest significant number of shoots per explant (13.13 shoot) (Fig. 2A). On the other hand, the greatest significant length of shoots (4.92 cm) was obtained on medium supplemented with BA at 0.5 mgl^{-1} plus NAA at 0.2 mgl^{-1} as shown in Table (1).

Further increase in the cytokinin/auxin ratio had no effect on the number of proliferated shoots. Similar results were reported by Faisal *et al*. (2005) on *Ruta graveolens*.

These results confirmed that some plant species have enough levels of endogenous hormones and do not require high levels of exogenous growth regulators for plant regeneration (Wala and Jasrai, 2003). It was considerable to mention that in this study the shoot multiplication was direct and without formation of any callus. Thus, a method of *in vitro* shoot propagation involving no callus formation phase is preferred for production of true-to-type plants.

For root induction, multiple shoots were individually separated and transferred to rooting media (1/2 MS or full MS containing different concentrations of NAA).
Root formation occurred on micro shoots in all tested media within 10-15 days. Further incubation for one week led to a very vigorous root growth (Fig. 2B).

However, full strength MS medium supplemented with 0.2 mgl^{-1} NAA significantly produced the highest number of roots (8.06 roots/plantlet) (Fig. 1A) as well as the root length (Fig. 1B). Increasing the NAA concentrations from 0 to 0.2 mgl^{-1} increased the number of roots.

The rooted shoots were carefully removed from the culture vessels and placed into plastic trays (Fig. 2C) for hardening off. Successful acclimatization took 3 weeks. All plants survived and grew normally. Subsequently,

Table (1): Effect of BA and NAA on the shoot multiplication of *Ruta graveolens*.

BA mg l^{-1}	NAA mg l^{-1}	Number of shoots/ explant	Shoot length (cm)
0.5	0.1	3.2^{b}	4.85^{a}
	0.2	10.92^{a}	4.92^{a}
	0.3	13.33^{a}	3.58^{ab}
1.0	0.1	5.40^{b}	3.65^{ab}
	0.2	5.67^{b}	3.42^{ab}
	0.3	4.88^{b}	2.88^{c}
2.0	0.1	4.00^{b}	4.29^{ab}
	0.2	2.22^{b}	4.17^{ab}
	0.3	3.75^{b}	2.94^{c}

Means with the same letters in the same column are not significantly different according to Duncan's multiple range test 5%.

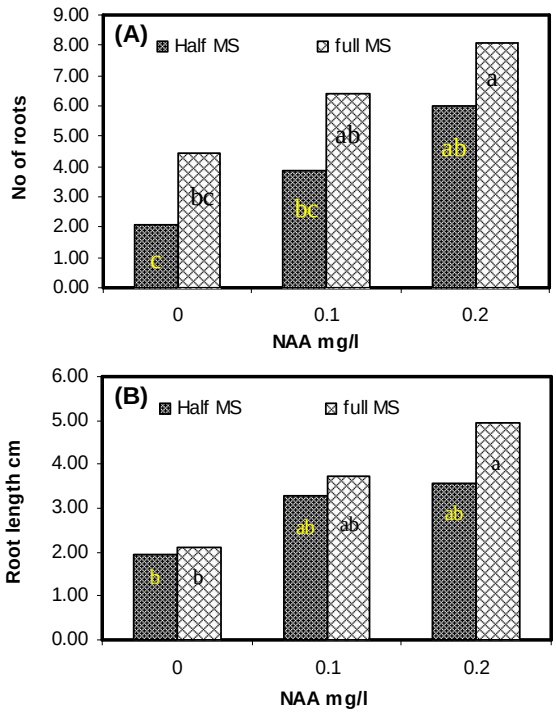

Figure (1): Effect of media and NAA concentrations on *in vitro* rooting of *Ruta graveolens*.

the acclimatized plantlets were transferred to greenhouse conditions (Fig. 2D). Further studies are in progress in this direction and other study to compare the activity of regenerated plants to that of field-grown plants for secondary metabolites components.

In conclusion, our results suggested that tissue culture technique could be successfully used as a rapid method to propagate *Ruta graveolens* plants using MS medium supplemented with 0.5 mgl^{-1} BA plus 0.3 mgl^{-1} NAA. The rooting was optimized using MS medium supplemented with 0.2 mg l^{-1} NAA.

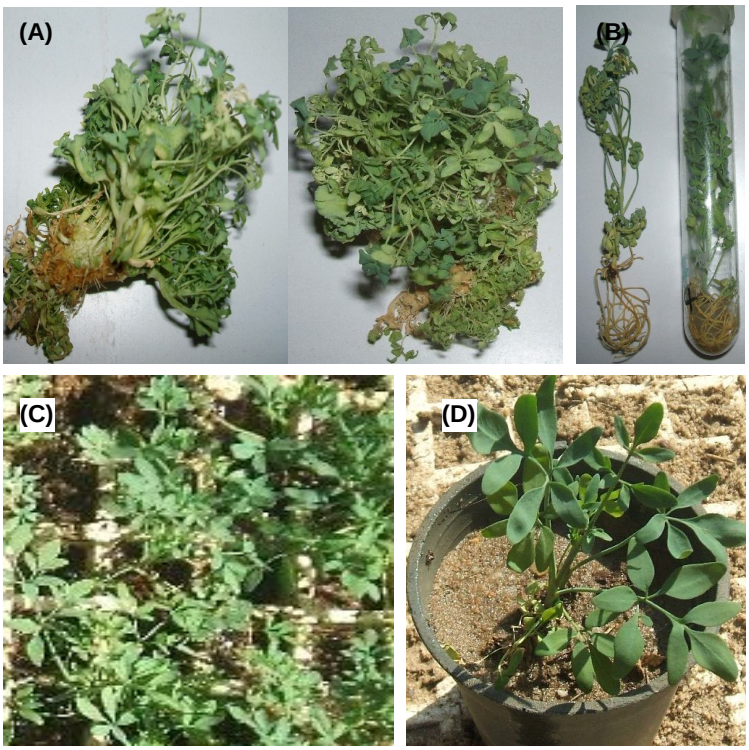

Figure (2): Micropropagation and acclimatization of *Ruta graveolens*: **(A)** Multiplication of cultures with BA and NAA, **(B)** Root development, **(C)** Successful hardening of rooted plantlets after 3 weeks, and **(D)** Plants under plastic green house conditions.

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إكثار السذب بواسطة زراعة الأنسجة

حمدي كمال عطاالله[1] ، إسلام حسن أبو السعود[1] ، منال عيد[2]
[1]قسم البساتين، كلية الزراعة، جامعة قناة السويس
[2]قسم النبات الزراعى، كلية الزراعة، جامعة قناة السويس، الإسماعيلية، مصر

الملخص العربـــى

تم الإكثار بإحداث تضاعف فى نبات السذب عندما فصلت القمم النامية وزرعت على بيئة مورشاج وسكوج مضاف إليها بنزيل ادينين بتركيزات 0.5 و1.0 و 2.0 مليجرام /لتر ونقثالين حمض الخليك بتركيزات 0.1 و0.2 و 0.3 مليجرام/لتر، وقد وُجد أن أعلى معدل تضاعف تم الحصول عليه فى البيئة التى تحتوى على 0.5 مليجرام/لتر بنزيل ادينين و0.3 مليجرام/لتر نقثالين حمض الخليك بعد 8 أسابيع، كونت الأفرع المتضاعفه بعد فصلها ونقلها إلى بيئة التجذير جذوراً جيده فى ببيئة مورشاج وسكوج سواء بكامل أو نصف تركيزاتها مُضافاً إليها نقثالين حمض الخليك أو بدون، وتم أقلمة ونمو النباتات تحت ظروف الصوبة بنسبة نجاح وصلت إلي 100%.