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RESEARCH ARTICLE

EFFECT OF TWO CULTURE MEDIA (MS & B5) ON SHOOT INDUCTION OF RUTA GRAVEOLENS L.

[1,*] Abhilasha Bhargava, [1]Dr. Ranjana Varma, [2]Dr. Alka Varma and [3] Dr. Jaya Sharma

[1,2]Institute for Excellence in Higher Education, Bhopal
[2]Sarojini Naidu Girl's PG College, Bhopal
[3]SAGE University, Bhopal

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ABSTRACT

A Micropropagation protocol is presented for the conservation of critically treated plant species. The present work was carried out in the different types of concentration with two basal media (MS & B5) and undertaken to develop a basic and simple protocol for shoot induction via. micropropagation of Ruta graveolens L., the best result is shown in MS media. The effect of two types of media on the behavior of in vitro connective micropropagation protocol was developed. Two media were tested B5 and Ms with different growth hormones for induction stages. MS medium is to be the best medium for plant tissue culture, especially shoot induction. High frequency of induced shoot was obtained on BAP (2 mg/l) KN(1.5mg/l) and NAA(1mg/l). Very low concentration of growth hormones did not support in vitro shoot induction in Ruta graveolens L. The plant tissue culture methods provide unique protocol and developed in the biodiversity conservation and for the further investigation further work for standardized of efficient in vitro protocol for the best shoot multiplication in and in - vitro Rooting in under progress in our laboratory.

Key words: Ruta graveolens L. in -vitro propagation, medicinal plant conservation, MS, B5, media.

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INTRODUCTION

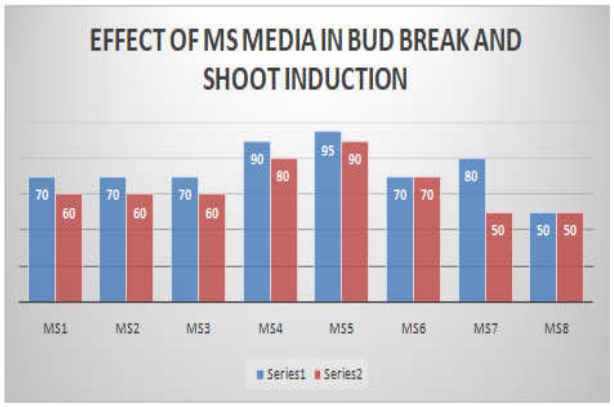
Ruta graveolens L. belongs to family Rutaceae its locally knows as Sitab. Rutaceae is a perennial medicinal plant synthesizing several types of metabolites, notably flavonoids, alkaloids, essential oils and furanocoumarins (Brickell and Zuk, 1996).It is one of the common plants used in Indian system of medicine various parts of the plants are used the treatment of skin diseases, to rheumatic pain). Rue's active ingredients may have antifungal property, which could be beneficial to agriculture and medicine (Atta and Alkofahi, 1998; Retheesh and Helen, 2007). It has been, brought to knowledge that Ruta, which is homothetically potentiated completely destroys the brain cancer cells (Pathak *et al*., 2003African traditional healers are using dry leaves of *Ruta graveolens* for diabetes, headache, urinary ailments disorders, and cardiovascular system (Thring etal., 2006). In homeopathy, for the treatment of muscular strength, injuries, eyestrain, joints, Arthritis, toothache, rheumatism, back pain and headache, *Ruta graveolens* are abundantly used. (Narayanappa M.*et al*., 2016).As the germination rate is often poor. Natural condition seed germination about very low percentage andthe seed-set is low and seeds exhibit dormancy (Faisal *et al*., 2005). The current requirement in out country is to high because this are uses. In very popular garden of South America, Ruta graveolens is being grown not only for medicinal and ornamental uses but also for charm against evil (Mohd Faisal.*et al*., 2005).so in-vitro micropropagation is very necessary so the plant requires conservation to meet its demand in medicine and other uses. The biotechnology approach such as plant tissue culture is an alternate and variable methods for and conservation of economically and medicinally important plants (Jaya at all 2012). The media compositions and plant growth regulator's play in vital role in vitro micro propagation there for optimization of these condition is a prerequisite for *in vitro* related work. There is no reported available in vitro propagation in Bhopal (as pr my deep study). Ruta graveolens that made us interested to develop micropropagation protocol for this endangered medicinal, economically important plants. The present study was based on under taken to effect of 2 basal media with different PGR concentration standardize a protocol for in -vitro shoot induction of *Ruta graveolens plants* by using different explants by tissue culture micropredation to meet its demand in medicine.

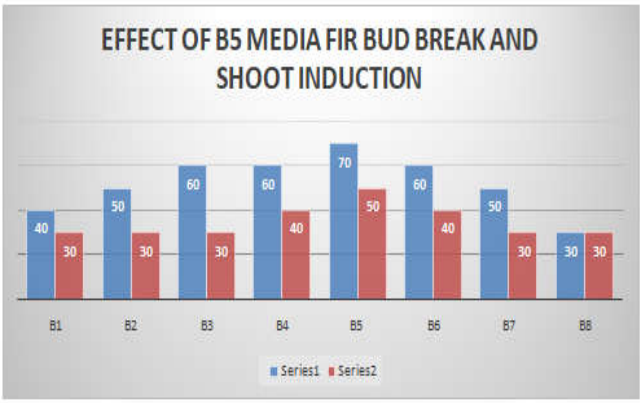
Corresponding author: **Mrs. Abhiladha Bhargava,**
Institute for Excellence in Higher Education, Bhopal.

MATERIAL AND METHODS

The research was conducted at the Department Biotechnology Institute for Excellence in Higher Education, Bhopal Madhya Pradesh (India).

Collection and Authentication of Plant Material- The shoots were collected in the month of Nov.-Dec. From mature plants growing inside the Jawahar chock park, no 2 Bhopal and the plant were identified by collages taxonomists.





Selection of Explants: Meristems were used as explants for this experiment. Explants were cut and reduced to a length of2 cm using surgical blad. Surface Sterilization Procedure -Shoots were thoroughly washed under running tap water for 30 min to remove all the dirt and soil particles adhering to them, then treated with 5% tween-20 is the type ofchemical this is use for for 5 minutes with constant stirring followed by 3-4 rinses in sterile distilled water and further treated with an antifungal agent (Bavistin) for 2 hours and were further with detergent for 20 minutes and rinsed 4-5 times tap water. Thereafter, again explants were kept immersed in distilled water with few drops of wetting agent, labolene for ten minutes, then immediately followed by 2-3-time rinses in distilled water to remove f labolene solutions. Further sterilization procedures were carried out inside a laminar airflow chamber, where shoots were surface sterilization through 1 minute's treatment in 70% (v/v) for half a minute followed by three times rinses in sterile distilled water. Mercuric chloride (0.1%) treatment was given to explants for only 1 minutes followed by 3 times rinsed in sterile distilled water. Thereafter shoots were carefully transferred to be placed over sterile Petri plats to remove excess water and were then and were then inoculated into the culture establishment medium (MS/B5) using sterile forceps under aseptic conditions.

Chemicals and Glass Wear: Experimental explant is selected shoots were cultured on high frequency two basal media(MS/B5)with requard supplemented 3 % (w/v) sucrose (Sd-fine Chemicals, India) for shoot induction. The pH of the medium (supplemented with respective growth regulators) was adjusted carefully to 5.5-5.8with 0.8 % (w/v) agar. All the experiments, the chemicals used were of pure, authentic and analytical grade. The all culture vessels is use to (borosil) andmedium (high quality) into autoclaved at 105 kPa at 125°C for 15 minutes. The explants surface-sterilized is very important were placed vertically on the culture medium. All the cultures were incubated at 25±2°C under 16h light/8h dark photoperiod with an irradiance of 45 - 50 µ mol/ m²/s photosynthetically active radiation (PAR) provided by cool white fluorescent tubes (Philip, India) and with 60 - 65 % relative humidity. All subsequent cultures sub culturing were done at one weeks intervals. Culture media consisted of MS/B5 (Murashige and Skoog 1962) supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar (Himedia, India) was evaluated for their effects on in-vitro growth and development of *Ruta graveolens* .For induction of shoots, explants were cultured on MS/B5 medium supplemented with different concentration of cytokines, including BAP (0.5-2mg/l), NAA (0.1-0-1.0 mg/l) and Kinetin (0.1-2.0 mg/l) either individually or in combination. Tissue culture applications is strongly important to plant conservation in India has been largely restricted to economically important species However, the approach could usefully be extended to conserve all threatened plants so that vital biodiversity and the ecological network is sustained can be preserved (Jiten Chandra *et al*., 2011) *Ruta graveolens* is categorized as a rare and endangered species According to literature survey, this is found out that, in the southeast coast of India, medicinal plant *Ruta graveolens* is being endangered (S.Gurudeepban *et al.,* 2011).

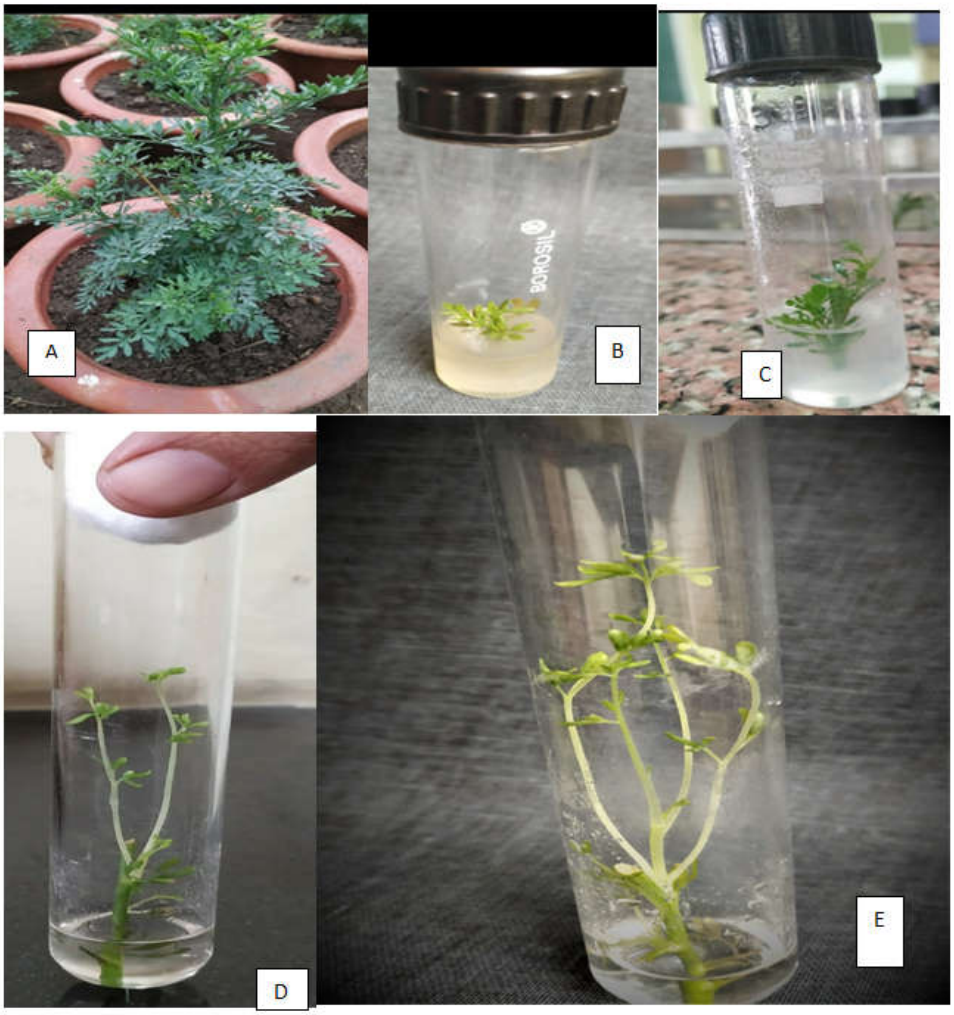
Shoot Induction: Meristems node was excised and inoculated by vertical orientation on the culture medium containing different concentration of BAP (0.5-2mg/l), NAA (0.1-0-1.0 mg/l) and Kinetin (0.1-2.0 mg/l). Ten single explants were assigned randomly to each treatment and the culture was kept under 16 h light/day photoperiod at 25±2°C shoot induction the effects of different media treatments were results to presented.

RESULTS AND DISCUSSION

MS medium supplemented with different levels of BAP, KN, or NAA were tried to induce shoots from meristems explants of Ruta graveolens nodal explants showed initiated on higher concentrations ofBAP (2mg/l), KN (1.5mg/l), or lower levels of NAA (.5mg/l) however these levels failed to induce shoot formation. The combined effect of cytokinin's, KN in combination with BAP or NAA, was tested on in vitro nodal in. Ruta graveolens interestingly, the above cytokinin's when combined resulted in axillaries and meristem as well as bud break. A combination of KN (1.5mg/l) plus BAP (2mg/l), NAA (0.5mg/l) showed maximum (95MS,70B5, %) And shoot induction (90MS, 50B5). The purpose of this study was to develop an in vitro propagation method from mature nodes of Ruta graveolens a medicinally important plant. In the present work we have, for the first time in Bhopal District established a rapid and reproducible method for high-frequency from mature node segments of Ruta graveolens. Similar observations were previously reported with other persons (Yogeshwari Mishra *et al*., 2000 and Charan Singh *et al.*,2011) Earlier studies have compared the effectiveness of different type's PGR concentrations. In the present investigation, the interaction ofKN with NAA or BAP with MS basal media was established that resulted in efficient shoot regeneration. Further, the in vitro regenerated shoots are using high quality experiments. However, nodal segments incubated on medium supplemented with KN + NAA+BAP produced healthy shoots and overall shoot. Quality did not differ much when these media formulations were used.

Table 1. Effects of MS & B5 media and concentration of plant growth regulators on *in-vitro shoot* initiation from meristems shoot of *Ruta graveolens* L. after approx. -2 weeks of culture.

S.no	Plant Growth Regulators mg/l	Media	Percentage Explants Bud Break	Percentage Explants Shoot Proliferation	Mean Length of Shoots in (cm.)Approx.
1.	0.5BAP+0.5KN	MS	70	60	2-3
2.	1.0BAP+0.5 KN+0.1NAA	MS	70	60	2-3
3.	1.5 BAP+1.0KN+0.5 NAA	MS	70	60	3-4
4.	1.5 BAP+1.5.0KN+0.5 NAA	MS	90	80	4-5
5.	2.0 BAP+1.5 KN+0.5NAA	MS	95	90	4-5
6.	3.0 BAP+1.5 KN+1.0NAA	MS	90	70	4-5
7.	5.0 BAP+2.0KN+2.0NAA	MS	80	50	3-.0
8.	5.0 BAP+3.0KN+2.0NAA	MS	50	50	2-.0
9.	0.5BAP+0.5KN	B5	40	30	2.0
10.	1.0BAP+0.5 KN+0.1NAA	B5	50	30	2.0
11.	1.5 BAP+1.0KN+0.5 NAA	B5	60	30	2.0
12.	1.5 BAP+1.5.0KN+0.5 NAA	B5	60	40	2.0
13.	2.0 BAP+1.5 KN+0.5NAA	B5	70	50	3.0
14.	3.0 BAP+1.5 KN+1.0NAA	B5	60	40	3.0
15.	5.0 BAP+2.0KN+2.0NAA	B5	50	30	2.0
16.	5.0 BAP+3.0KN+2.0NAA	B5	30	30	2.0



Photos- (A) Mature Plant (B) Shoot induction (C) Shoot induction (D- E) Shoot length

Very effective results of a combination of two or more cytokinin's in to shoot induction from various explants has also been reported for Gymnocladus dioicus L. (Geneve, 2005); Eclipta Alba (Baskaran and Jayabalan, 2005); and Momordica tuberose Roxb (Aileni *et al*., 2008).The role of cytokinin's in shoot differentiation from nodal segments was reported in several plants species but only a few reports were successful in inducing organogenesis from mature node explants (Ramesh *et al*., 2002).

The greatest number of multiple shoots (8.68 shoots/explants) was obtained from explants cultured on medium containing 0.5 mg/l−1 BA. Generally, increasing BA concentrations led to a significant decrease in the number of formed shoots. Meanwhile, it was the observer that BA at 0.5 mg/l−1 gave the highest significant average of shoot length as 4.61 cm. In this concern, (Baskaran and Jayabalan (2005)) Increase PGR concentration 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.

Here we report on shoot induction from mature node explants of Ruta graveolens . In conclusion, in vitro growth and development from nodal explants of Ruta graveolens was highly influenced by the type of BAP+NAA+KN+MS basal medium combination used for propagation. This results is presented also demonstrate that mature nodal segment as a explants of Ruta graveolens offer great potential as a source tissue for shoot induction(Mohammad Faisa *et al*. (2005). The procedure reported in this study may facilitate improvement, conservation, and mass propagation of this medicinally important plant species.

Conclusion

It can be concluded that the MS showed a high% of shoot induction as compared to B5 media. The protocol defined in this study as outlined below and is demonstrated in fingers. The findings have several implications for managing the diversity of this species as well as the restoration of its degradation. In conclusion, our results suggested that the tissue culture technique could be successfully used as a rapid method to propagate Ruta graveolens plants using MS medium supplemented with 0.5 mg/l-2 BA plus 0.1 mgl-1 NAA. The present experiment has shown that it is possible to in vitro shoot induction and use for further experiments' and done by plantlets.

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Abbreviations

BAP-6 benzyl amino purine, NAA – Naphthalene Acetic acid, KN-kinetin, Mg-mile gram, PGR-plant growth regulator.

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