STUDIES ON ORGANOGENESIS FROM NODAL EXPLANT OF RUTA GRAVEOLENS L.

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INTRODUCTION
Ruta graveolens L., a member of Rutaceae, is well known for its wide utilities such as ornamental, aromatic and culinary in addition to medicinal properties. Medicinal value of this taxon is attributed to the accumulation of flavonoids, furanocoumarins, acridine alkaloids, furanoquinolins and also essential oils which led to its recognition as one of the sought after traditional medicinal plants by pharmaceuticals. Further, Ruta graveolens has been identified as one of the potential sources of allelochemicals (Feo et al., 2002). Large scale destruction of natural habitats due to population pressure and over exploitation has become a major threat to important bioresources of medicinal plants. Hence there is a need to conserve this taxon. Conventional propagation through seeds is not sufficient enough to produce required number of plants as there is a poor viability of seeds. Establishment of in-vitro gene banks is one of the promising ex-situ approaches for conservation of elite germplasm.

Attempts have been made previously to develop protocols for the micropropagation of Ruta graveolens (Castro and Barros, 1997; Faisal et al., 2005, 2006; Bohidhar et al., 2008). The present studies have been carried out to establish in vitro gene bank of Ruta graveolens through direct and indirect organogenesis from nodal explants.

MATERIALS AND METHODS
Potted healthy plants were procured from University of Agricultural Sciences, Bangalore. They were maintained in the Botanical garden, Department of Botany, Bangalore University, Bangalore. Nodes of about 1cm were excised from healthy plants and used as explants.

Surface sterilization: The explants were washed with Tween-20 for 5-10min. After thorough washing under running water for 30 min they were treated with Bevastin, a fungicide (0.1%) for 5 min. Further surface sterilization was carried out by treating the explants with saturated chlorine water followed by mercuric chloride (0.1%) for 2 min each. After each treatment the explants were washed thoroughly with double distilled water.

Culture medium: The surface sterilized explants were inoculated to Murashige and Skoog’s medium supplemented with various growth regulators. Direct and indirect regeneration from the cultures were observed depending on the media composition. MS + 2, 4-D (2.26μM) + Kin(9.29μM) + GA₃ (1.44μM) was found to be best for direct regeneration, while MS + NAA(10.74μM) + BAP(13.20μM) + L-glutamine (1.36mM) + GA₃ (2.88μM) for indirect regeneration from the callus raised on NAA supplemented medium. Rooting of the regenerated shoots was obtained on ½ MS + NAA (0.01μM) + L-glutamine (1.36mM). Acclimatized plants are maintained in Botanical garden of the department. About 95% survivals were recorded.

ABSTRACT
Ruta graveolens L., commonly known as Rue is widely exploited for its active principles which are of high pharmaceutical value. In vitro techniques were employed to multiply and conserve this traditional medicinal plant. Nodal explants were cultured on Murashige and Skoog’s medium supplemented with various growth regulators. Direct and indirect regeneration from the cultures were observed depending on the media composition. MS + 2, 4-D (2.26μM) + Kin(9.29μM) + GA₃ (1.44μM) was found to be best for direct regeneration, while MS + NAA(10.74μM) + BAP(13.20μM) + L-glutamine (1.36mM) + GA₃ (2.88μM) for indirect regeneration from the callus raised on NAA supplemented medium. Rooting of the regenerated shoots was obtained on ½ MS + NAA (0.01μM) + L-glutamine (1.36mM). Acclimatized plants are maintained in Botanical garden of the department. About 95% survivals were recorded.
of the explants largely depends on the critical balance between factors that influence the gene expression include tissue or organ selected for explant, season or growing conditions and the ontogeny of the source plants (Edvin et al., 2008). In the present study, the explants collected from June to December responded better to the culture conditions than other seasons. Similar season dependent morphogenetic response in in vitro conditions was also reported in other medicinal plants (Sahoo and Chand, 1998; Ahuja et al., 1992; Patnaik and Chand, 1996; Ramaswamy et al., 2004). Apart from these facts, the morphogenetic potential of the explants largely depends on the critical balance between the endogenous and exogenously supplied growth regulators.

When the explants were grown on basal MS medium, there was no response even after several subcultures to the fresh media. However, direct and indirect organogenesis from the explants was observed when MS medium was supplemented with various growth regulators.

### Table 1: Effect of growth regulators on multiple shoot regeneration from the cultures of R. graveolens L.

<table>
<thead>
<tr>
<th>MS + Growth regualtors + Amino acids</th>
<th>Mean ± SD</th>
<th>95% CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS + NAA (0.27 μM) + Kin(9.29 μM) + GA₃ (1.44 μM) + 12.13± 11.80</td>
<td>6.93± 6.7</td>
<td>9.16-14.0</td>
</tr>
<tr>
<td>MS + NAA(5.37 μM) + Kin(9.29 μM) + GA₃ (2.88 μM)</td>
<td>0.57</td>
<td>8.08</td>
</tr>
<tr>
<td>MS + NAA (0.27 μM) + Kin(9.29 μM) + GA₃ (2.88 μM)</td>
<td>7.87± 7.7</td>
<td>15.64-23.31</td>
</tr>
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<tr>
<td>MS + NAA (0.27 μM) + Kin(9.29 μM) + GA₃ (2.88 μM)</td>
<td>9.00± 8.7</td>
<td>17.88-26.66</td>
</tr>
<tr>
<td>MS + NAA(5.37 μM) + Kin(9.29 μM) + GA₃ (2.88 μM)</td>
<td>0.69</td>
<td>9.26</td>
</tr>
<tr>
<td>MS + NAA (0.27 μM) + Kin(9.29 μM) + GA₃ (2.88 μM)</td>
<td>12.00± 11.6</td>
<td>23.66-34.32</td>
</tr>
<tr>
<td>MS + NAA(5.37 μM) + Kin(9.29 μM) + GA₃ (2.88 μM)</td>
<td>1.11(**)</td>
<td>12.42</td>
</tr>
<tr>
<td>MS + NAA (0.27 μM) + Kin(9.29 μM) + GA₃ (2.88 μM)</td>
<td>9.00± 8.8</td>
<td>17.88-26.66</td>
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<td>MS + NAA(5.37 μM) + Kin(9.29 μM) + GA₃ (2.88 μM)</td>
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**NOTE:** Results are represented as Mean ± SD. Data within a column followed by the symbol (***) represent 5% level of significance compared to all treatments by Tukey post-hoc test.
principle sites of cytokinin biosynthesis. Hence, exogenous supply of cytokinin is needed to bring out the morphogenetic potential of the nodal explants. Multiple shoot induction was also recorded when BAP (13.20μM)/ kin (9.29μM) was combined with 2, 4-D (2.26μM) + GA₃ (1.44μM). The best combination for the proliferation of multiple shoots was found to be MS + 2, 4-D (2.26μM) + Kin (9.29μM) + GA₃ (1.44μM).
with 18 ± 1.05 shoots per culture (Fig. 2). When these new shoots grew into 5 to 6 cm in length having 4 to 5 nodes were cut into segments containing a single node and subcultured to the same combinations, resulted in the formation of 18 ± 1.05 shoots per culture. Thus from a single explant it was possible to obtain nearly 1000 shoots within a period of 10 to 12 weeks. Thus obtained shoots were healthy with elongated internodes. BAP is considered as a potential hormone to induce multiple shoots in several taxa (George and Sherrington, 1984). Tejavathi et al., (2009) have observed the induction of maximum number of multiple shoots/explant from the nodal explants of mulberry when cultured on BAP supplemented medium compared to other cytokinins such as Kin, 2-ip and Zea. However in the present studies, kinetin either alone or with 2, 4-D and GA₃ was found to be better than BAP combinations. This is in conformity with the observations made by Ramaswamy et al., (2004) in Solanum surattense. While Benneth and Davies (1986) and Bhat et al., (1995) have reported that kin is less effective than BAP in shoot multiplication was reduced in the present studies. Similar combinations. This is in conformity with the observations made by Ramaswamy et al., (2004) in Solanum surattense. Cytokinin concentration has been several times reported to show the synergistic effect with BAP and enhanced the induction of shoot buds from callus and increased the morphogenetic potential than other cytokinin and auxin combinations. Callus mediated shoot morphogenesis has been accomplished in several medicinal plants (Agarwal and Sardar, 2006). High frequency callus mediated shoot regeneration can be further exploited by biotechnological approaches to develop the elite clones with high content of active principles.

Rooting and acclimatization

Plantlets with well developed roots in in vitro is essential for the successful establishment of regenerated plants in field. Well developed shoots thus obtained from both direct and indirect regeneration were transferred to various strengths of media containing different auxins. Presence of IAA, NAA and 2, 4-D in the medium promoted the formation of basal callus without any root induction as was observed by Bohidhar et al., (2008) in the same taxon. Faisal et al., (2005, 2006) had recorded rooting of microshoots of Ruta graveolens on ½ MS + IBA (0.5μM). Bohidhar et al., (2008) are also of the opinion that IBA is a potential hormone for root induction than other auxins in the same taxon. IBA, as root inducing hormone is well established in several taxa (Santos et al., 2003; Cheepala et al., 2004). However the ½ MS with NAA (0.0054μM) and L-glutamine (1.36 mM) proved to be the best for induction of roots from the basal parts of the regenerated shoots in the present investigation (Fig. 5). Presence of L-glutamine in the medium promoted root induction of microshoots in Helianthus annus and inter specific hybrids of Helianthus (Witzens et al., 1988).

The plantlets were then first transferred to the plastic pots containing soilrite and watered regularly. After four weeks under lab conditions (25 ± 2°C), they were transferred to earthen pots containing pot mixture of sand: soil: farmyard manure in the ratio of 1:1:2 and maintained in polyhouse (Fig. 6). Thus hardened plants showed 95% survival.

REFERENCES


Faisal, M., Ahmad, N. and Anis, M. 2005. In vitro regeneration and


