Micropropagation Studies in *Lavandula angustifolia*

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**Abstract**

*Lavandula angustifolia* (Family Labiates) is a medicinal herb mainly found in Mediterranean area. It is a well known herb in ayurvedic system of medicines and has traditionally been used to treat disorder of liver, fever and several conditions including infertility infection, anxiety. It is also used in aromatherapy as antidepressants. There are few reports on tissue culture of *Lavandula angustifolia* that too mainly on micropropagation. Present investigation on micropropagation studies in *Lavandula angustifolia* was undertaken to identify suitable explants and media for micropropagation. The explants were collected for medicinal and aromatic plant project from herbal garden of Shoolini University. Among the different media concentration tried for the establishment of culture, the media MS+ BAP 0.002mg/l gave maximum percent establishment *i.e.* 55% for nodal segment. MS+ BAP 0.002mg/l proved superior for establishment and require least days for establishment. The media MS+ 0.5mg/l IAA + 2.00mg/l of BAP gave maximum no. of multiple shoots per culture *i.e.* 16 after establishment of explants. The percent of shoot multiplication from nodal segment shown a trend as M2>M1>M3>M4 (56>49>42>36) and that of shoot form on nodal explants are T4>T3>T2>T1 (16>10>5>3).

**Keywords**: Micropropagation, Growth regulators, Culture media, *Lavandula angustifolia*.

1. **Introduction**

*Lavandula* is a genus of the Labiatae family. *Lavandula* consists of about 20 species of small evergreen shrubs, having aromatic foliage and flowers. *Lavandula* spp. is some of the most popular medicinal herbs with great economic interest (Nobre, 1996). *Lavandula* is one of the most important aromatic crops that are geographically grown in Mediterranean countries (Baytop, 1984). *L. dentata*, as is an important essential oil producing plant characterized by high contents of 1, 8-Cineole, Fenchol, Borneol and Camphor (Sudriá *et al.*, 1999). Commercially, the lavender oil is widely used in fragrance industry including soaps, colognes, perfumes, skin lotions, and other cosmetics (Paul *et al.*, 2004). In food manufacturing lavender oil is employed in flavouring beverages, ice creams, candy, baked goods and chewing gum (Kim and lee, 2002). Lavender is also used in aromatherapy as a relaxant (Gheladini *et al.*, 1999). Lavender oil is also used as an antibacterial agent (Lawless, 1992). Lavandula species can be vegetatively propagated by woody stem cutting, but their rooting and risks of modification induced by repeated vegetative propagation (Moutet, 1980) can be overcome by Micropropagation.

Micropropagation is a tissue culture technique used for obtaining a large number of genetically identical plantlets (Nguyen and Kozai, 1998). Tissue culture technique could also be applied for cultivar improvement both in in-vitro micropropagation and shoot regeneration (Penniza *et al.*, 1990). Research on micropropagation has been focused mainly on the biochemical effects of medium composition (sugar, mineral salts, phytohormones, vitamins, amino acids etc.) on the different rooting and the growth of explants under aseptic conditions.

Micropropagation can become a feasible technique to obtain good quality plantlets with low production cost (Nguyen and kozai, 1998). Limited tissue culture work has been done on *Lavendula* species (Calvo, 1980). Only one study has been reported on micropropagation of *L. Latifolia* through axillary buds (Quazi, 1980). However, the multiplication rate achieved by this method was very...
low. Several species of Lavender has been used, like L. dentata (Jorden et al., 1998), L. angustifolia (Quazi, 1980) L. latifolia (Panniza and Tognoni, 1992), and L. stoechas (Nober, 1996). In the present study we evaluated the effect of different culture media and plant growth regulators on shoot proliferation and rooting.

The culture media used for micropropagation consist of the salt and vitamin in the Murashige and Skoog media. In micropropagation shoot regeneration has been obtained from shoot tips, nodal segment, and flower tissue axillary bud but never from leaves (Panniza and Tognoni, 1988). For the micropropagation of plant nodal segments measuring 2 to 3 cm in length were excised from 5 year old plant of Lavandula and implanted on the culture media. Cultures were maintained at 25± 2 °C with 16 h photoperiod with irradiance of 10-20μmol-2s-1. (Echeverrigaray, 2005).

Hence, it is possible that by use of optimum concentration of growth regulators and suitable shooting medium a rapid multiplication system can be developed for propagating of English lavender cuttings. Shooting efficiency would be better when done in controlled conditions such as optimal concentrations of growth regulators. Keeping these points in view, the research work was conducted to study the micropropagation of Lavandula angustifolia by using nodal segments and to study the effect of growth regulators.

2. Materials and Methods

Murashige and Skoog medium was used for the present study. The stoke solutions for the growth regulators were prepared as per procedure given by. The specific medium pH 5.6-5.8 poured hot at the rate of 25-30 ml per sterilized bottle. The bottles were plugged with caps or non sterile cotton and autoclaved at 15 lbs (1.06kg/m2) pressure and 121°C for 15-20 minutes (Bhojwani and Razdani, 1983). The inoculated culture was incubated at 25±2°C in an air condition culture room. Photoperiod was maintained 16 hrs (3000-3500 lux) supplied by cool white florescent tube light daily followed by 8 hrs of darkness as suggested by (Conger, 1981)

The explants of optimum size were collected from the field grown one year old plant of Lavandula angustifolia from the Herbal Garden of Shoolini University and washed thoroughly under running tap water for 25 minutes followed by distilled water containing detergent (Tween-20) solution for 10 minutes with constant shaking. The nodal segment of 0.5 to 1cm was taken for the incubation sterilized with HgCl2. The culture bottle after incubation were kept in culture room at 25± 2°C temperature of photoperiod of 16 hrs light and 8 hrs dark in culture room.

Treatment combinations shoot multiplication and percentage shoot multiplication per explants is used as, T1,MS + 0.5mg/l IAA and 0.5mg/l BAP, T2, 0.5mg/l IAA and 1.0mg/l BAP, T3,MS + 0.5mg/l IAA and 2.0mg/l BAP, T4,MS +1.0mg/l IAA and 1.0mg/l BAP.

3. Results and Discussion

3.1 Effect of BAP on Explants Establishment from Nodal Segment

The effect of different concentration of BAP for explants establishment from nodal segments is furnished in Table 1. Significant results were found when concentration of BAP was increase up to certain level (viz. 0.002mg/l) after that it reduces with further increase.

Thus the treatment group M2 (MS + 0.002mg/l BAP) was found to give better response for explants establishment among various treatment conditions (Plate 1). The number of days for explants establishment ranged from 23-30 (Table 1). The treatment group M1, M3, M4 maximum days for explants establishment. The percentage establishment of explants was ranged from 40-55. The treatment M2 (MS + 0.002mg/l BAP) recorded the significantly maximum percent response for establishment (55%) and the minimum percent response for establishment (40%) was recorded (36%) by treatment M4 (MS + 0.02mg/l BAP).

Plate 1: Establishment of nodal segment in Lavandula angustifolia

3.2 Effect of Plant Growth Regulator Combination on Multiple Shoot Formation and Percent Shoot Multiplication from Nodal Segments

Effect of different concentration of IAA and BAP on multiple shoot furnished in Table 2.
Table 1: Effect of different media on explants establishment and explants establishment percentage

<table>
<thead>
<tr>
<th>Treatment code</th>
<th>Treatment detail</th>
<th>Days required for establishment of nodal segments</th>
<th>Explants establishment percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>M₁</td>
<td>MS+BAP 0.001mg/l</td>
<td>25</td>
<td>49</td>
</tr>
<tr>
<td>M₂</td>
<td>MS+BAP 0.002mg/l</td>
<td>23</td>
<td>56</td>
</tr>
<tr>
<td>M₃</td>
<td>MS+BAP 0.01mg/l</td>
<td>28</td>
<td>42</td>
</tr>
<tr>
<td>M₄</td>
<td>MS+BAP 0.02mg/l</td>
<td>30</td>
<td>36</td>
</tr>
</tbody>
</table>

Table 2: Effect of media composition on shoot multiplication and shoot multiplication percentage

<table>
<thead>
<tr>
<th>Treatment code</th>
<th>Treatment detail</th>
<th>No. of multiple shoot developed per nodal segment</th>
<th>Percentage of shoot multiplication</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>MS+IAA 0.5mg/l+IAA 0.5mg/l+BAP 0.5mg/l</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>T₂</td>
<td>MS+IAA 0.5mg/l+IAA 1.0mg/l+BAP 1.0mg/l</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>T₃</td>
<td>MS+IAA 0.5mg/l+IAA 2.0mg/l+BAP 2.0mg/l</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>T₄</td>
<td>MS+IAA 1.0mg/l+BAP 1.0mg/l</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

3.2.1 Number of Multiple Shoots per Explants: The number of shoots formed per explants ranged from 2-16. The treatment T₃ (MS + 0.5mg/l IAA + 2.00mg/l of BAP) recorded the maximum number of shoots per explants (16). Minimum number of shoots per explants (2) was recorded by T₁ treatment (MS + 0.5mg/l IAA + 0.5mg/l BAP) in Plate 2.

Plate 2: Multiple shoot formation from nodal segment of Lavandula angustifolia

3.2.2 Influence of Plant Growth Regulators Combination: The effect of different combination of BAP for explants establishment from nodal segment are furnished in Table 1. Optimum explants establishment was obtained when concentration of BAP was increasing up to a certain level (0.002mg/l) after that it reduce with further increase.

3.2.3 Days Required for Establishment: The number of days required for explants establishment ranges from (23-30 days) for explants with treatment M₂ (MS + 0.002mg/l BAP). The treatment M₂, M₃, M₄ were recorded maximum number of days (30) for explants establishment.

3.2.4 Percent of Shoot Multiplication: The percent of shoot multiplication from nodal segment was ranged BAP) recorded the maximum number of shoots per explants (16). from 40-55 percent Table 2. Treatment M₂ (MS + BAP 0.002mg/l) recorded highest number of percent establishment explants (56%). treatment M₄ (MS + BAP 0.02) recorded minimum number of percent establishment explants (36%).

3.3 Effect of Plant Growth Regulators Combination on Multiple Shoot Formation and Shoot Multiplication

3.3.1 Number of Multiple Shoots per Explants: The number of shoots formed per nodal explants was ranged from 2-16 (Table 2). The treatment T₃ (MS + 0.5mg/l IAA + 2.00mg/l of BAP) recorded the maximum number of shoots per explant (16). Minimum number was recorded by T₁ and T₂.

4. Conclusion

The results from present investigation clearly indicated that mass multiplication is reliable and promising for Lavandula angustifolia. Tissue culture techniques can be used to attain rapid multiplication of the Lavandula angustifolia. From the present investigation in vitro response of Lavandula angustifolia clearly indicated that the suitability of nodal segments for micropropagation large scale production. The percent of shoot multiplication from nodal segment shown a trend as M₂>M₁>M₃>M₄ (56>49>42>36) and that of shoot form on nodal explants are T₄>T₃>T₂>T₁ (16>10>5>3).
References


