In vitro Propagation of Two Lavandula Species: Lavandula angustifolia and Lavandula latifolia L. Medica

Areej A. M. Al-Bakhit, Jamal S. Sawwan* and Mohsen S. Al-Mahmoud**

ABSTRACT

A *In vitro* propagation of *Lavandula angustifolia* and *Lavandula latifolia* was studied. *In vitro* Cultures of *Lavandula angustifolia* and *Lavandula latifolia* were established by germinating seeds of each species on Murashique and Skoog (MS) medium supplemented with 0.5 mg/L Naphthaline Acetic Acid (NAA) incubated for three weeks in the dark at 28° C. The best shoot multiplication rates (2.0) and highest proliferation of *Lavandula angustifolia* were obtained when internodal segments were subcultured on MS medium supplemented with 1.5 mg/L Kinetin and 0.05 mg/L NAA. Shoots were rooted on MS medium supplemented with 0.4 mg/L of NAA or Indole Butyric Acid (IBA). Plants were successfully transferred to soil after two weeks of acclimatization in the greenhouse. Shoot multiplication rates and proliferation of *Lavandula latifolia* were best when MS medium was supplemented with 0.5 mg/L Benzyl Amino Purine (BAP), 0.05 mg/L NAA or with 1.0, 1.5 or 2.0 mg/L Kinetin and 0.05 mg/L NAA Shoots were rooted on MS supplemented with 0.3 mg/L NAA.

KEYWORDS: *Lavandula*, Micropropagation, (NAA), (BAP), (IBA), TDZ, Kinetin.

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. are some of the most popular medicinal herbs with great economic interest (Nobre, 1996).

The scent of most, but not all, *Lavandulas* is somewhat similar, namely: refreshing, herbaceous and sweet, imparting a sense of ’clean’.

The medicinal plant preparations play one of the key roles in the modern pharmaceutical industry; but mass, and uncontrollable collection of medicinal plants, may lead to the reduction of their populations.

The objective of this study was to develop a protocol for starting *in vitro* propagation of *Lavandula* spp.

2. MATERIALS AND METHODS

Seeds of *Lavandula angustifolia* were purchased from Park Seeds; whereas, *Lavandula latifolia* seeds were purchased from Tisflor. (Park Seed Company 1 Parkton Ave. Greenwood, SC 29647, USA).

Seeds of the two species of *Lavandula* were surface

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sterilized by washing under running tap water for 15 minutes, and then seeds were rinsed with 95% (v/v) ethanol for 30 sec. The seeds were then soaked in dish washing detergent solution for 20 min., then soaked in a 20% commercial sodium hypochlorite (6.0% active ingredient) for three times (45 min. each). Finally, seeds were rinsed with autoclaved distilled water for three times.

The seeds were inoculated onto two MS media in 100 x 15mm petriplates. The first was hormone free and the other was supplemented with 0.5 mg/L NAA. Seeds were incubated in the dark at 28º C for 14 days; the petriplates were transferred from the dark to 16 hr light/8 hr dark with 40-45 µmol m\(^{-2}\)s\(^{-1}\) photosynthetic photon flux density and 28º C for another week for further growth.

After 21 days, germination percentage and percentage of contaminated dishes were recorded and discarded for each medium.

Seedlings of the two *Lavandula* spp. were transferred to hormone free medium for one week to minimize the carry over effect of the hormone and to study the regeneration capacity of each species under investigation.

### 2.1 Shoot Proliferation:

In order to test different protocols of micropropagation of the two *Lavandula* spp., the experiment was designed to fit split-split-split-plot design in which the two species were the main classes, 2 media were tested (Murshigue and Skoog, 1962; Gamborg *et al.*, 1968) as sub-classes and three synthetic cytokinins were sub-subclasses whereas the concentrations were subclasses of sub-subclasses.

Internodal segments of 1-1.5 cm from seedlings that were grown on hormone free media were sub-cultured in test tubes containing 15 ml of either MS or B5 media. Media were supplemented with one of the following synthetic cytokinins; Kinetin, BA or TDZ at concentrations of 0.0, 0.5, 1.0, 1.5 or 2.0 mg/L and 0.05 mg/L NAA. Each growth regulator concentration was represented by 10 test tubes as replicates.

Test tubes were kept in a growth room at 28ºC±1ºC with light regime of 16 hrs light (photosynthetic photon flux density = 40-45 µmol m\(^{-2}\)s\(^{-1}\)), and 8 hrs dark for 10 weeks. After that, the following data were recorded: number of shoots per explant, shoot length, shoot fresh weight, callus (+/-) judged by vision and callus fresh weight. Contaminated test tubes were discarded.

### 2.2 Rooting of Shoots:

The objective of the rooting experiment was to test the effect of two different synthetic auxins on rooting of the two *Lavandula* spp. in order to complete the micropropagation protocol. The experiment was designed to fit split-split-split-plot design in which the two species were the main classes, two synthetic auxins were tested as sub-classes; whereas, the concentrations were sub-subclasses.

Internodal segments of 1-1.5 cm from the shoot multiplication experiment were sub-cultured in 15 x 150 mm test tubes, containing 15 ml of MS media supplemented with one of two different synthetic auxins: NAA or IBA at concentrations of 0.0, 0.1, 0.2, 0.3, 0.4 or 0.5 mg/L. Each treatment was represented by 5 test tubes.

After 6 weeks the number of roots, root length and shoot length were recorded.

### 2.3 Acclimatization of Plantlets:

The plastic covers of the test tubes that contained the rooted shoots from the rooting experiment were removed for two days in the growth room before moving them to
the greenhouse. In the greenhouse, rooted plantlets were removed from the medium; roots were washed thoroughly with water to rinse away any media to avoid contamination. The plantlets were moved to pots filled with 1:1 moss peat and perlite mixture. The plantlets were irrigated immediately with a solution of inorganic macronutrients. Light regime of 14 hrs light and 10 hrs dark was used for 2 weeks. To maintain high relative humidity, pots were placed in plastic bags; a small opening was left on the top of the plastic bag to improve air circulation. Relative humidity was reduced by gradual enlarging the hole. Complete removal of the plastic bag took place after two weeks of placement.

2.4 Statistical Analysis:

The micropropagation experiment and the rooting experiment were set to be split-split-split-plot design. Since B5 medium did not show any growth, the media as sub-classes were ignored and the experiment was set as follows: the two Lavandula spp. were the main classes, the three synthetic cytokinins and a control were the sub-classes and the different concentrations were the sub-sub-classes. Data were transformed according to square root for analysis (Steel and Torrie, 1980).

The rooting experiment was set to be split-split-split-plot design. The two Lavandula spp. were the main classes, the two synthetic auxins and a control were the sub-classes and the different concentrations were the sub-sub-classes. Data were transformed according to least squared mean (Steel and Torrie, 1980).

The transformed data in the experiments were statistically analyzed by SAS system software, and significance was tested according to probability test at 5% using the Least Significant Difference (LSD).

3. RESULTS AND DISCUSSION

3.1 Seed Germination and Plant Establishment

Seeds of Lavandula angustifolia, and Lavandula latifolia were germinated successfully in vitro on MS medium containing 0.05 mg/L NAA, with germination percentages of 72% and 78% for Lavandula angustifolia and Lavandula latifolia seeds respectively Table (1). Contamination percentage ranged from 7 to 13% as compared to 100% for non-sterilized seeds Table (1).

Seed sterilization not only reduced the contamination percentage, but also enhanced the germination percentage for the two species. Comparing the in vitro germination percentage to that stated on the label (50%), it is noted that in vitro seed germination percentage was much higher than on the label. Thus, seeds have proved to be good starting material for in vitro plant establishment for both L. angustifolia and L. latifolia.

3.2 Shoot Proliferation

Results indicate that MS was superior to B5 media when supplemented with the same plant growth regulators, Fig. (1). Several reports indicated that MS media was superior; Jordan et al., 1998 for Lavendula dentate, Sanchez-Gras and Calvo, 1994 for Lavendula latifolia and Panizza, and Tognoni, 1988 for Lavandin cv Grosso. Jordan et al., (1998) suggested a protocol for Lavandula dentate micropropagation by using MS medium as the basal medium for inter-nodal segment explants. Also micropropagation of Lavandula latifolia, Lavandula stoechas cultured on Margara medium gave necrotic growth and the plants died later, while MS medium did not give those symptoms (Nobre, 1996). Contrary to others, Quazi (1980) reported the successful use of B5 medium as a basal medium for multiplication.
of Lavandula spp., no other authors reported similar result in any Lavandula spp. The differences in plant responses and performance when cultured in different culture media, may be due to the differences in osmotic potential of the media, or to presence and/or absence of specific compounds in the media (Conger, 1982).

All tested synthetic cytokinins, along with 0.05 mg/L NAA, showed significant effect over the control in all shoot multiplication and growth parameters for the two Lavandula species (Table 2; Figures 2 and 3). In L. angustifolia, the control gave significantly the least number of shoots, shoot length, number of internodes per shoot and shoot weight. On the other hand, in L. latifolia, BAP showed higher number of shoots and shoot weight while in L. angustifolia TDZ gave significantly heavier vegetation. Kinetin was best in the shoot length and number of internodes for both species. However, BAP, Kinetin and TDZ were significantly better in number of shoots, shoot length, number of internodes and shoot weight as compared to the control Table (2).

In addition, Kinetin was better in the number of internodes than TDZ and the control, and was not different from BAP. Calvo and Segura, (1988) reported that a combination of BA and IAA, or NAA gave better organogenesis compared to IAA or NAA alone; media containing 2,4-D were less effective in bud induction than those containing IAA or NAA for Lavandula latifolia. Shoots of Lavandula latifolia were proliferated from inter-nodal segments, grown on MS medium supplemented with IAA and BA, and those cultures of IAA, or BA alone, gave no, or low shoot proliferation (Sanchez-Gras and Calvo, 1996). Adult plants of Lavandula dentata were micropropagated from nodal segments that were cultured on MS medium supplemented with either, BA or Kinetin with NAA (Jordan et al., 1998).

Number of shoots in both spp. was not affected by cytokinin type and conc. Table (3). The longest shoots were obtained when Kinetin was used at concentrations of 1.0 or 1.5 mg/L (1.89 and 2.55 cm respectively) while, the greatest number of internodes resulted from the use of Kinetin at 1.5 mg/L (4.79 internodes). For shoot weight, all tested synthetic cytokinins gave the same shoot weight except for Kinetin at 0.5 and 2.0 mg/L, which gave the lowest weight Table (3).

For Lavandula latifolia, there was no significant difference in number of shoots, among different concentrations of different tested synthetic cytokinins, but for other growth parameters the best attributes were obtained at 0.5 mg/L BAP, combined with 0.05 mg/L NAA, and at 1.0, 1.5 or 2.0 mg/L Kinetin combined with 0.05 mg/l NAA Table (3).

The results of this experiment indicated that the two Lavandula species responded differently to treatments, and that Lavandula angustifolia was more sensitive to cytokinin’s concentrations than Lavandula latifolia. This difference between the two species may be due to their genetic make up.

Many authors reported similar differences within species of the same genera to different treatments (Pierik, 1987). Moreover, Calvo and Segura, (1989) reported that explants of Lavandula latifolia, but not Lavandula stoechas were induced to form buds, and shoots on MS media supplemented with different hormonal combinations.

Sanchez-Gras and Calvo (1996) reported that 0.05 mg/L IAA and 0.8 mg BA was the best combination for shoot proliferation.

Hypocotyl explants of Lavandula latifolia were cultured on MS Medium Supplemented with 0.1 mg/L
NAA, and 2.0 mg/L IBA for bud induction (Calvo and Segura, 1989); maximal response of *Lavandula angustifolia* was achieved when BA exogenous levels were 1.0 or 2.0 mg/L (Segura and Calvo, 1991); the best shoot multiplication rate of *Lavandula dentata* was obtained from 0.1 mg/L BA or 4.0 mg/L Kinetin (Jordan et al., 1998).

### 3.3 In vitro Rooting:

Results of the rooting experiment indicated that for *Lavandula angustifolia* Table (4), NAA and IBA at 0.4 mg/L gave the greatest number of roots (2.6 roots), longest roots (1.4 and 2.26 cm) and shoots (6.32 and 6.64). For the callus diameter, 0.4 and 0.5 mg/L IBA gave larger diameter. As 0.5 mg/L IBA did not bring any root emergence, the large callus does not indicate a superior parameter. For the purpose of protocol establishment, the best growth regulator and concentration may be weighted to be NAA or IBA at the level of 0.4 mg/L Table (4).

*Lavandula latifolia* gave different results than those obtained in *L. angustifolia*. For example: the greatest number of roots were those from IBA at 0.4 mg/L (3.8 roots), the longest roots were those from 0.3 mg/L NAA and 0.4 and 0.5 mg/L IBA, the longest shoots were obtained in 0.2 to 0.4 mg/L NAA, and finally, the largest callus diameters were when 0.1-0.4 mg/L NAA or 0.3-0.5 mg/L IBA were used.

In vitro rooting of *Lavandula stoechas* shoots resulted in 100% rooting, when basal medium contained 1.0 mg/L NAA (Nobre, 1996). On the other hand, the number of roots and root length of *Lavandula vera* was increased by increasing the concentration of NAA in MS media; shoots of *Lavandula latifolia* and *Lavandula dentata* were rooted on MS half strength and hormone free media (Sanchez-Gras and Calvo, 1996 and Jordan et al., 1998).

For *Lavandula latifolia* 0.3 mg/L NAA was the best. Although this concentration of this synthetic auxin did not give the largest number of roots but it did give better shoot length and root length as well as good callus diameter, the reason for this choice is that plantlets of higher shoots showed better survival in the acclimatization process.
Figure 1: Performance of *Lavandula* cultures on MS and B5 media.

Figure 2: Effect of different synthetic cytokinins (BAP, Kinetin, TDZ) over the control on shoot multiplication of *Lavandula angustifolia*.
Table (1): Effect of seed sterilization on seed germination and culture contamination of *Lavandula angustifolia* and *Lavandula latifolia*.

<table>
<thead>
<tr>
<th>Spp.</th>
<th>Germination %</th>
<th>Contamination %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sterilized</td>
<td>Nonsterilized</td>
</tr>
<tr>
<td></td>
<td>seeds</td>
<td>seeds</td>
</tr>
<tr>
<td>L. angustifolia</td>
<td>72%</td>
<td>32%</td>
</tr>
<tr>
<td>L. latifolia</td>
<td>78%</td>
<td>3%</td>
</tr>
</tbody>
</table>

Table (2): Influence of BAP, Kinetin or TDZ on number of shoots per explant, shoot length, number of internodes and callusing (+/-) in explants of *in vitro* grown *Lavandula angustifolia* (I) and *Lavandula latifolia* (II) cultured on MS medium.

<table>
<thead>
<tr>
<th>Growth regulator</th>
<th>No. of shoots per explant</th>
<th>Shoot length (cm)</th>
<th>No. Of internodes per shoot</th>
<th>Shoot weight (g)</th>
<th>Callus (+/-)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td>I</td>
<td>II</td>
<td>I</td>
</tr>
<tr>
<td>BAP</td>
<td>3.05 a’</td>
<td>8.5 a</td>
<td>1.40 a</td>
<td>1.42 b</td>
<td>2.78 ab</td>
</tr>
<tr>
<td>Kinetin</td>
<td>1.55 a</td>
<td>4.18 b</td>
<td>1.67 a</td>
<td>1.71 a</td>
<td>3.05 a</td>
</tr>
<tr>
<td>TDZ</td>
<td>4.60 a</td>
<td>4.25 b</td>
<td>1.19 b</td>
<td>1.34 b</td>
<td>2.41 b</td>
</tr>
<tr>
<td>Control</td>
<td>0.50 b</td>
<td>0.8 c</td>
<td>0.35 c</td>
<td>0.53 c</td>
<td>0.60 c</td>
</tr>
</tbody>
</table>

* Means followed by different letters differed significantly (p<0.05) according to LSD-test.
Table (3): Influence of different concentrations of synthetic cytokinins on number of shoots per explant, shoot length, number of internodes per shoot, shoot weight and callusing (+/-) in explants of in vitro grown *Lavandula angustifolia* (I) and *Lavandula latifolia* (II).

<table>
<thead>
<tr>
<th>Growth regulator</th>
<th>No. of shoots per explant</th>
<th>Shoot length (cm)</th>
<th>No. of internodes per shoot</th>
<th>Shoot weight (g)</th>
<th>Callus (+/-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP</td>
<td>I: 1.9a*</td>
<td>4.50a</td>
<td>1.15cde</td>
<td>2.18a</td>
<td>2.80b</td>
</tr>
<tr>
<td></td>
<td>II: 5.0a</td>
<td>6.10a</td>
<td>1.80b</td>
<td>1.09c</td>
<td>2.91b</td>
</tr>
<tr>
<td></td>
<td>I: 2.5a</td>
<td>13.40a</td>
<td>1.17cde</td>
<td>1.30bc</td>
<td>2.39b</td>
</tr>
<tr>
<td></td>
<td>II: 2.8a</td>
<td>10.00a</td>
<td>1.15cde</td>
<td>1.44bc</td>
<td>2.71b</td>
</tr>
<tr>
<td>Kinetin</td>
<td>I: 1.0</td>
<td>1.9a</td>
<td>5.0a</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II: 4.50a</td>
<td>6.10a</td>
<td>1.89ab</td>
<td>2.20a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I: 2.0</td>
<td>1.15cde</td>
<td>1.17cde</td>
<td>1.30bc</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II: 2.8</td>
<td>10.00a</td>
<td>1.15cde</td>
<td>1.44bc</td>
<td></td>
</tr>
<tr>
<td>TDZ</td>
<td>I: 5.8a</td>
<td>5.20a</td>
<td>1.62bc</td>
<td>1.76ab</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II: 3.4a</td>
<td>3.20a</td>
<td>1.42bcd</td>
<td>1.63b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I: 6.2a</td>
<td>5.90a</td>
<td>1.26bcde</td>
<td>1.28bc</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II: 3.0a</td>
<td>2.70a</td>
<td>0.38de</td>
<td>0.85d</td>
<td></td>
</tr>
</tbody>
</table>

* Means followed by different letters differed significantly (p<0.05) according to LSD-test.

Table (4): Effect of different concentrations of synthetic auxins on rooting of *in vitro* grown *Lavandula angustifolia* (I) and *Lavandula latifolia* (II) on MS medium.

<table>
<thead>
<tr>
<th>Growth regulator</th>
<th>No. of roots per shoot</th>
<th>Root length (mm)</th>
<th>Shoot length (cm)</th>
<th>Callus diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA</td>
<td>I: 0.0c*</td>
<td>0.00 d</td>
<td>0.00 d</td>
<td>2.74 d</td>
</tr>
<tr>
<td></td>
<td>II: 0.0c</td>
<td>0.00 d</td>
<td>0.10 cd</td>
<td>3.98 c</td>
</tr>
<tr>
<td></td>
<td>I: 0.4bc</td>
<td>1.0 c</td>
<td>0.14 d</td>
<td>4.74 bc</td>
</tr>
<tr>
<td></td>
<td>II: 0.5</td>
<td>0.00 d</td>
<td>0.86 bc</td>
<td>4.56 bc</td>
</tr>
<tr>
<td>IBA</td>
<td>0.0 c</td>
<td>0.00 d</td>
<td>0.00 d</td>
<td>2.74 d</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.00 d</td>
<td>0.00 d</td>
<td>3.96 c</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>0.16 d</td>
<td>0.38 cd</td>
<td>6.40 a</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>0.38 cd</td>
<td>0.54 cd</td>
<td>6.02 bc</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.00 d</td>
<td>0.17 a</td>
<td>4.64 bc</td>
</tr>
</tbody>
</table>

* Means followed by different letters differed significantly (p<0.05) according to LSD-test.
REFERENCES


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