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Research article

Micropropagation of lavender (*Lavandula angustifolia*)

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Abstract

Lavender (*Lavandula angustifolia*) is widely used in medicine. Lavender essential oil has a sedative effect, antispasmodic, prevent respiratory failure. Lavender should be preserved and studied deeply about biotechnology in the production of oil. Lavender seeds are disinfected best at a javel concentration of 75% in 10 minutes. Medium appropriate for plant growth is WPM. WPM supplemented with BA (0.1 mg/L), sucrose (30 g/L) is suitable for the process of creating the bud. IAA concentration (0.5 mg/L) is suitable for rooting process in *in vitro* culture. Lavender stem is strong and compact, dark green, thick blade, healthy roots when activated carbon was added into the media at concentrations 1.0 g/L.

Introduction

Lavender (*Lavandula angustifolia*) belongs to Lamiaceae originated from the Mediterranean. It is an annual shrub with specific purple and fragrance, high drought tolerance, dislike of humidity. At the middle ages, this plant has been used for condiment and medicinal herb [1]. Lavender is widely used in medicine. Lavender oil was used for reducing the pain, controlling the convulsion, treating rheumatism, healing wounds, protecting the stomach and enhancing the health for human beings [2]. The main components of lavender oil includes linalool, 4-ol-terpinen, α-terpineol, linalyl anthranilate, geranyl acetate, coumarin, herniarinborneol, lavandulol acetate and mineral components such as Mn, Cu, Ca, Mg, Zn, Fe, Na [3,4] effective against fungal infections popular on human skin [5,6]. Especially the lavender oil has also a sedative effect, antispasm and useful for patients of respiratory distress syndrome [7].

Lavender is widely grown in many countries such as: Banstead (UK), Furano, Hokkaido (Japan), Dungeness Sequim (United States), Y Le (China). In Vietnam, it has only been grown in Da Latcity [8] and many people know this plant through perfumes, scented bags and medicinal products. The demand of Lavender flowers in medicine, beauty, cuisine, etc. in our country more and more increased. However, the domestic supply has not been guaranteed because of the difficulties in the planting and caring process.

The plant cell and tissue culture technique has been launched and constantly developed with many considerable achievements. This technique plays an important position in the field of plant propagation [9]. With many advantages, it has been used for the proliferation of plant with large quantities in a short time, keeping the genetic characteristics of the mother plant [9]. Lavender asexually propagated by plant tissue culture methods will be grow and develop well and higher in productivity. Because the seedlings selected with the best quality, high-yielding, and disease-free is used as material for this technique [10]. There are some tissue culture works on Lavendula species [11-13]. Several species of Lavender has been used, like *L. dentata* [11], *L. stoechas* [12], *L. angustifolia* [13]. However, to build a complete protocol for micropropagation of *Lavandula angustifolia*, some steps in the procedure need to be improved for increasing survival rate of plantlets. In this study, we investigated propagation of Lavender by plant tissue culture aimed to produce high quality plantlets to meet the domestic demand.

Experimental

Materials

Lavender seeds are provided from the seed collection of plant biotechnology Lab, Faculty of Agricultural Sciences and Biotechnology, Nguyen Tat Thanh University. Media used for this study was MS (Murashige-Skoog, 1962), WPM (Lloyd &McCown 1980), LV (Litvay, 1985). The substances added to the culture medium includes sucrose, BA (benzyladenine), NAA (α-naphthaleneacetic acid), IBA (indole-3-butyric acid), IAA (indole-3-acetic acid), activated charcoal.

Culture conditions: the experiments were carried out at 26 ± 2°C in temperature, 70% - 80% in humidity, 33.3 µmol/m^2/s in light intensity (8 hours/day).The culture media were sterilized at 1 at min 20 minutes.

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Methods

The experiments were arranged in a completely randomized design (CRD) with 3 replications. Each treatment was cultured 15 explants. Data was recorded and subjected to analysis of variance with the SAS 9.1 statistical software package.

Experimental design

Experiment 1: The examination of the concentrations of javel solution (NaOCl 5%) and treatment time on the rate of sterile explants: 20 seeds were washed with soap, rinsed with tap water, and then moved into the laminar flow bench. Subsequently, those were disinfected with ethanol 70% in 1 minute, followed by the different concentrations of javel solution (50%, 70% and 100%) added with several drops of Tween-80 in different periods (10 and 15 minutes) and rinsed with sterile distilled water. After sterilization, the seeds were cultured on MS medium supplemented with sucrose 30 g/L, agar 8 g/L.

Experiment 2: The effects of mineral media on the growth of Lavender shoots: The *in vitro* 2cm-long shoots with 2 leaves were cultured on different mineral media: ½ MS (half of macro and micro minerals), MS, WPM and LV added with sucrose 30 g/L, agar 8 g/L.

Experiment 3: The effects of BA concentrations on the shoot induction of Lavender: The *in vitro* 2cm-long shoots with 2 leaves were cultured on WPM medium added with sucrose 30 g/L, agar 8 g/L, and different concentrations of BA (0.1 – 0.3 – 0.5 – 1.0 mg/L).

Experiment 4: The effects of auxin concentrations on the root formation of Lavender shoots: The *in vitro* 2cm-long shoots with 2 leaves were cultured on WPM medium added with sucrose 30 g/L, agar 8 g/L, and different concentrations of auxinIAA (0.1 – 0.3 – 0.5 - 1 mg/L), IBA (0.1 – 0.3 – 0.5 - 1 mg/L), NAA (0.1 – 0.3 – 0.5 - 1 mg/L).

Experiment 5: The effects of active charcoal on the growth of *in vitro* shoots of Lavender: The *in vitro* 2cm-long shoots with 2 leaves were cultured on WPM medium added with sucrose 30 g/L, agar 8 g/L, IAA (0.5 mg / l), and activated charcoal (0.5 - 1 - 1.5 – 2.0 - 2.5 g/L)

Data collection

The percentage of germinatedseeds (%) = (number of germinated seeds/total of seeds) x 100.

The rate of sterile seed (%) = (number of sterile seeds/total of original seeds) x 100.

The leaf number of shoot = the total of leaf number after 4 weeks - the initial number of leaves.

The number of generated shoots= the number of shoots after 4 weeks - the initial number of shoots.

The height of the shoot is calculated from the junction of the roots to the top of the shoot tip.

The average height of shoots (cm) = the total height of shoots/ the total number of shoots.

The length of the roots is calculated from the junction of roots to the root tip of the longest root.

The average length of roots (cm) = the total length of roots/the total number of roots.

The number of roots is calculated by counting the total number of roots after 4 weeks.

Time of root formation is recognized from the incurred date.

Results and Discussion

The examination of the concentrations of Javel solution (NaOCl 5%) and treatment time on the rate of sterile explants

According to the results in Table 1, the concentration of javel solution and sterilization time is proper at a concentration of 75% during 10 minutes (75.45% of sterile seeds and 30.71 % of germinated seeds). When sterilization of Lavender seeds was at a concentration of 50% javel in 10 and 15 minutes, the rate of sterile sample was low from 50% to 73.33%. While increasing javel concentration (100%), the rate of sterile seeds increased markedly (85.71 - 100%) but the germination rate decreased significantly (17.65 - 22.86%).

The effects of mineral media on the growth of Lavender shoots

The shoots cultured on the different mineral media after 4 weeks showed that MS and ½ MS media was not suitable for growth of Lavender shoots; Lavender shoots developed on LV medium (0.66 shoots, 3.36 cm in height, and 6.06 leaves) but still less than on the WPM medium.

Table 1. Results of sterilization of Lavender seeds

Conc. of javel (%)	Treatment time (minutes)	Rate of aseptic explants (%)	Rate of germinated seeds (%)
50	10	50.00^{c}	27.33^{ab}
50	15	73.33^{b}	25.41^{ab}
75	10	75.45^{b}	30.71^{a}
75	15	83.53^{ab}	20.17^{b}
100	10	85.71^{ab}	17.65^{c}
100	15	100.00^{a}	22.86^{b}



Figure 1. Sterilization of Lavender seeds. (A) Germinated seeds of Lavender (B) Lavender shoots grown after 4 weeks

Table 2. Effects of mineral media on the growth of Lavender shoots

Mineral media	Shoot number (shoots/explants)	Height of shoots (cm)	Leaf number (no)
½ MS	0.00	2.00[c]	2.00[b]
MS	0.00	2.00[c]	3.13[b]
WPM	2.46[a]	5.28[a]	15.20[a]
LV	0.66[b]	3.36[b]	6.06[b]

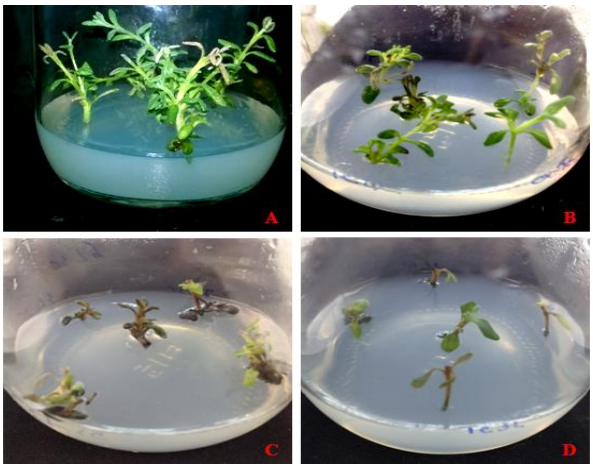


Figure 2. The growth of Lavender shoots on the mineral media (A) WPM (B) LV (C) MS (D) 1/2 MS.

WPM medium was best for growth and development of Lavender shoots with number of generated shoots (2.46 shoots), leaf number (15.20 leaves), and height of shoot (5.28 cm).

The demand of mineral compositions is different for each different species of plant. For micropropagation of *Lavandula dentata* from axillary buds of field-grown adult plants, Murashige and Skoog (MS) medium was used [11]. For micropropagation of *Lavandula Stoechas* from single node explants, a basal medium containing Margara N30K macro-salts was used [12]. In this study, WPM medium is suitable for growth and development of Lavender shoots (*Lavandula angustifolia*).

The effects of BA concentrations on the shoot induction of Lavender

The results of the effect of BA on Lavender shoot induction *in vitro* showed the statistically significant differences among treatments. At the concentration of BA (0.1 mg/L), the number of generated shoots was the highest with 3.06 shoots/explant, 6.35 cm in height and 18.86 leaves. When the concentration of BA increased (0.3 to 1 mg/L), the possibility of shoot induction decreased markedly (2.73 - 2.40 in number of induced shoots, 5.28 - 2.71 cm in height, and 13.26 - 8.26 leaves). This showed that the concentration of BA (0.3-1 mg/L) inhibited the growth of Lavender buds. Thus, the concentration of BA at 0.1 mg/L is suitable for induction of Lavender buds. The shoots are stout, upright, green, and uniform.

According to Echeverrigaray et al. (2005), MS medium supplemented with a combination of 2.2 µM BA and 2.5 µM IBA gave highest multiplication rate from axillary buds of field-grown adult plants of *Lavandula dentata* [11]. In another study, the highest shoot number and length of *Lavandula angustifolia* on MS + 1 mg/L BAP + 0.05 mg/L NAA reached 3.9 and 47.2 mm, respectively [13]. In this study, the concentration of BA at 0.1 mg/L is suitable for induction of buds. The shoots are stout, upright, green, and uniform.

The effects of auxin concentrationson the root formation of Lavender shoots

Based on root number, root length, root germinated time, treatments with IBA and NAA showed that they were not suitable for culture of root induction. In the treatments with IAA, the growth of lavender shoot was best at the concentration of 0.1 mg/L with 2.26 roots and 1.06 cm in root length. When the concentration of IAA increased to 0.3 mg/L, the number of roots was 1.80 roots/ shoot and root length was 0.94 cm. At a concentration of IAA (0.5 mg/L), the root induction of shoot was best with 2.60 roots/shoot, 1.65 cm in root length, and the growth of shoots was slow down at higher concentration of IAA (1.0 mg/L).

Table 3. Effect of BA concentrations on induction of Lavender buds

BA (mg/L)	Shoot number (shoots/explants)	Height of shoots (cm)	Leaf number (no)
0.0	2.46[b]	2.76[c]	9.47[bc]
0.1	3.06[a]	6.35[a]	18.86[a]
0.3	2.40[b]	2.71[c]	8.26[c]
0.5	2.73[ab]	5.28[b]	13.26[b]
1.0	2.66[ab]	2.88[c]	9.53[bc]

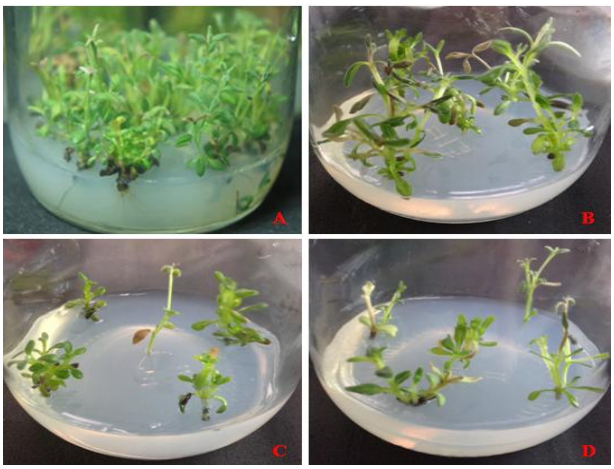


Figure 3. Induction of Lavender shoot on media added with. (A) 0.1 mg/L BA (B) 0.3 mg/L BA (C) 0.5 mg/LBA (D) 1.0 mg/L BA.

According to Echeverrigaray et al. (2005), the best condition for rooting of *Lavandula dentata*s hoots was MS medium

plus 2.5 µM NAA [11]. In another study, *In vitro* rooting of the *Lavandula Stoechas* shoots was highest on basal medium containing 5.4 µM NAA [12]. In this study, the root induction of *Lavandula angustifolia in vitro* shoot was best at concentration of IAA (0.5 mg/L) with 2.60 roots/shoot, 1.65 cm in root length.

The effects of active charcoal on the growth of *in vitro* shoots of Lavender

The results of influence of active charcoal on the growth of Lavender buds showed a statistically significant difference. After 4 weeks of culture, the number of generated buds on the medium with active charcoal was 2.33 shoots, the height of shoot reached 2.80 cm, the number of roots reached 1.53 roots per shoot, and root length reached 2.66 cm. When the concentration of active charcoal increased from 0.5 to 1 g/L, the growth of Lavender buds enhanced significantly with 3.80 shoots, 3.24 cm in shoot height, 3.33 roots and 2.93 cm in root length, the growth of shoots was the best. However, the concentration of active charcoal increased from 1.5 to 2.5 g/L, the growth of shoots diminished. Therefore, WPM medium supplemented with active charcoal (1.0 g/L) was chosen as the culture medium for *in vitro* Lavender buds. After 60 days of culture, the plantlets were transferred nursery garden reached high survival rate and best growth and development (Figure 6).

Table 4. Effect of concentration of IBA, IAA, and NAA on root induction of Lavender buds

Auxin (mg/L)	Root Number	Root length (cm)	Time of root induction (day)
Control (0.0)	2.20^{a}	1.22^{ba}	12.66^{fg}
IBA (0.1)	0.86^{a}	0.43^{b}	20.00^{cbd}
IBA (0.3)	1.33^{a}	0.99^{ba}	9.00^{hg}
IBA (0.5)	1.26^{a}	0.78^{ba}	13.00^{feg}
IBA (1.0)	1.06^{a}	0.58^{ba}	17.33^{ed}
IAA (0.1)	2.26^{a}	1.06^{ba}	19.00^{cd}
IAA (0.3)	1.80^{a}	0.94^{ba}	22.33^{cb}
IAA (0.5)	2.60^{a}	1.65^{a}	7.66^{h}
IAA (1.0)	2.53^{a}	1.15^{ba}	11.33^{fgh}
NAA (0.1)	1.33^{a}	0.61^{ba}	24.00^{b}
NAA (0.3)	1.53^{a}	0.84^{ba}	13.66^{fe}
NAA (0.5)	1.46^{a}	0.68^{b}	20.00^{cbd}
NAA (1.0)	0.86^{a}	0.44^{b}	29.00^{a}

Conclusion

Lavender seeds sterilized at the javel concentration of 75% during 10 minutes were best. The induction of buds was on WPM medium supplemented with BA (0.1 mg/L), sucrose 30 (g/L) and agar 8 (g/L). WPM medium supplemented with IAA (0.5 mg/L), active charcoal (1.0 mg/L), sucrose 30 (g/L) and agar 8 (g/L) was suitable for root formation and the growth of Lavender plantlets.

Table 5. Effect of the concentration of active charcoal on the growth of Lavender buds

Active charcoal (g/L)	Shoot number (shoots /explants)	Height of shoots (cm)	Root number (no)	Root length (cm)
0.0	2.33^{ba}	2.81^{a}	1.53^{ba}	2.66^{a}
0.5	3.20^{ba}	3.00^{a}	2.53^{ba}	2.89^{a}
1.0	3.80^{a}	3.24^{a}	3.33^{a}	2.93^{a}
1.5	2.86^{ba}	2.83^{a}	1.66^{ba}	2.74^{a}
2.0	2.13^{ba}	2.76^{a}	1.40^{b}	2.52^{a}
2.5	1.53^{b}	2.57^{a}	0.80^{b}	1.50^{a}

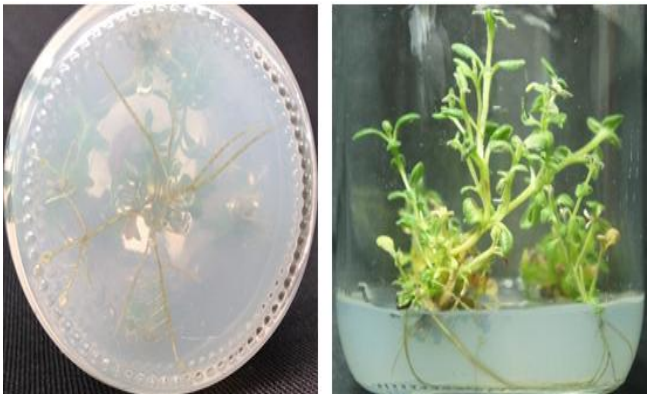


Figure 4. The roots of Lavender buds cultured on WPM medium supplemented with IAA (0.5 mg/L)

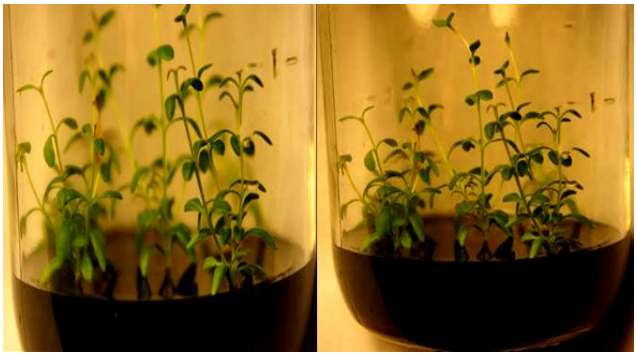


Figure 5. Lavender plantlets on WPM medium supplemented with active charcoal (1 g/L)



Figure 6. Lavender plantlets were transferred the garden

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