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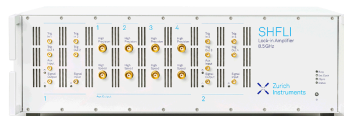

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Tissue Culture of *Lavandula* angustifolia L.

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Abstract. Objective: The study is to establish the key techniques of tissue culture of *Lavandula* angustifolia L. Method: Using stem with buds of *Lavandula* angustifolia L. as explant, the effect of disinfection time on contamination rate, types and ratio of plant regulator on propagation index and rooting, types of transplanting substance on plantlet survival rate were studied. Results and conclusion: The proper disinfection time of 0.1 % $HgCl_2$ was 15min, by which the survival and contamination rate of plantlets respectively were 83.3 % and 25.0 %; MS +1.0mg/L BA + 0.3 mg/L IBA was the proper medium for propagation, which propagation index was 4.92; White + 0.4 mg/L NAA was the proper medium for rooting, which rooting rate and the average number of roots per plantlet respectively were 66.7% and 9.4; the survival rate of plantlets on transplanting substance which were peat and perlite mixed with an equal volume was 66.7 %.

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INTRODUCTION

Lavender (*Lavandula* angustifolia L.) It is a perennial dicotyledonous plant of lavender genus in Labiatae.which has been widely used in medicine since ancient times. Its stems and leaves can be used in medicine. It has the functions of invigorating stomach, sweating and relieving pain, and is now widely used in medicine. Ornamental and flavor raw materials [1], has a very high prospects for development and utilization. At present, the propagation of lavender is mainly using seed and cutting propagation, the speed of propagation is limited. The use of plant tissue culture has the advantages of fast propagation, uniform progeny and no seasonal restriction. However, there are only a few reports on in vitro culture and plant regeneration of lavender [2-3]. In order to establish a more perfect culture and regeneration system of lavender in vitro, this study studied the propagation, rooting and transplanting of lavender with bud stem segment, and laid a technical foundation for its wide application in production.

MATERIALS AND METHODS

Material

The experimental material was Lavender with narrow leaf, which was purchased from Yili, Xinjiang, and the stem with buds of current year old branches was used as explant.

Sterilization of Explants

Remove lavender from narrow leaf and cut into long stem of 1~2 cm. Wash with a small amount of washing powder and rinse with water for 2 h. After that, the water was soaked in 75% ethanol for 30 s, washed with aseptic water for 2 times or 3 times, then soaked with 0.1% $HgCl_2$ for 5 min, 10 min, 15 min and 20min, respectively (Table 1). Finally, the dry water was washed with aseptic water for 3 times, then the water was absorbed by filter paper, and then fed into MS +0.5 mg/L BA+ 30g/L sucrose+ 6.0g/L Agar medium. the explant contamination rate and survival rate were counted after 8 days.

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TABLE 1. Effect of $HgCl_2$ treatment time on the contamination rate of Lavender explants

Treatment	Disinfection time/min	Number of plants to be tested /plant	Number of polluted plants /plant	Number of dead plants /plant	fraction surviving /%	Pollution rate /%
1	5	72	72	0	100.0%[c]	100.0%[d]
2	10	72	60	0	100.0%[c]	83.3%[c]
3	15	72	18	12	83.3%[a]	25.0%[a]
4	20	72	14	48	33.3%[b]	19.44%[b]

Note: different letters represent significant differences at the level of $P < 0.05$.

Proliferation Culture

The stem segments with buds about 2cm were cut from the established sterile materials and inoculated in MS medium with different levels of BA (0.5 mg /L, 1.0 mg /L, 2.0 mg /L) and IBA (0.1 mg /L, 0.3 mg /L, 0.5 mg /L) (Table 2), in which sucrose 3% and Agar 0.6% were obtained. The proliferation and growth of adventitious buds in explants were observed 38 days later.

TABLE 2. Effects of different concentrations of BA and IBA on adventitious bud proliferation of lavende

Treatment	BA concentration mg/L	IBA concentration mg/L	Inoculation number	Total number of differentiated buds	Proliferation multiple	Bud growth
5	0.5	0.1	72	195	2.70[b]	The Plexus bud is not obvious, the bud is strong.
6	0.5	0.3	72	211	2.93[b]	The Plexus bud is not obvious; the bud is strong.
7	0.5	0.5	72	186	2.58[b]	The Plexus bud is not obvious; the bud is strong.
8	1.0	0.1	72	312	4.33[a]	The Plexus bud is obvious and the bud is stronger.
9	1.0	0.3	72	354	4.92[a]	The Plexus bud is obvious and the bud is strong.
10	1.0	0.5	72	303	4.21[a]	The Plexus bud is obvious and the bud is strong.
11	2.0	0.1	72	274	3.80[ab]	The Plexus bud is obvious and the bud growth is weak.
12	2.0	0.3	72	282	3.92[ab]	The Plexus bud is obvious and the bud is stronger.
13	2.0	0.5	72	274	3.81[ab]	The Plexus bud is obvious and the bud growth is weak.

Rooting Culture

The material about 2 cm was inoculated into rooting medium (Table 3). The basic medium for rooting was White, supplemented with different levels of NAA (0.2 mg /L, 0.4mg / L, 0.8 mg /L) or IBA (0.2 mg /L, 0.4 mg /L, 0.8mg / L), where sucrose 1.5% and Agar 0.6%. After 25 days, the number of rooting and root growth were counted.

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TABLE 3. Effects of different hormone types and levels on adventitious root formation in lavende

Treatment	culture medium	Inoculation number	Number of rooting plants	Average rooting numbe	Rooting rate (%)	Root growth
14	White + IBA 0.2 mg/L	72	18	2.0^{d}	$25.0\%^{c}$	Root fineness
15	White + IBA 0.4 mg/L	72	24	3.5^{d}	$33.3\%^{c}$	Root fineness
16	White + IBA 0.8 mg/L	72	39	3.0^{d}	$54.2\%^{b}$	Root fineness
17	White + NAA 0.2 mg/L	72	36	6.0^{c}	$50.0\%^{b}$	good
18	White + NAA 0.4 mg/L	72	48	9.4^{a}	$66.7\%^{a}$	good
19	White + NAA 0.8 mg/L	72	50	8.0^{b}	$69.4\%^{a}$	good

Transplantation of Test-Tube Seedlings

When the adventitious root length of the test-tube seedling was 0.8 ~ 1.0 cm and the height of the seedling was 3~4 cm, the test-tube seedling was processed with strong light for 3 days, then the tube seedling was removed from the bottle cap for 3 days, and the root culture medium was washed and planted on different substrates (Peat, vermiculite, peat and perlite of equal volume mixing, perlite). Peat and perlite were mixed with perlite in volume (Table 4) and the ambient temperature was controlled at 20~ 30 °C, the relative humidity of air was between 60 ~ 80%, and the survival rate of rooting seedlings was calculated after 25 days.

The culture temperature is 25 °C, the light intensity is 4800 lx, and the light intensity is 16 h/d.

Statistical Analysis

The test data were analyzed by ANVOA with SPSS13.0 software, and the experiment was repeated 3 times.

RESULTS AND ANALYSIS

Effect of $HgCl_2$ Treatment Time on the Rate of Contamination in Explants of *Lavandula angustifolia*

$HgCl_2$ treatment time has a great effect on the establishment of explants (Table 1). When 5~10min was treated with 0.1% $HgCl_2$, the sterilization effect was poor, and the contamination rate of explants was between 83.3% and 100.0%. When 15min was treated, the sterilization effect was better, the contamination rate of explants was 25.0% and the survival rate was 83.3%. When the sterilization time exceeded 10min, the explants began to die, and the mortality increased with the increase of sterilization time. According to the survival rate and contamination rate of explants, 0.1% $HgCl_2$ sterilization was more suitable for 15min treatment.

Effects of Different Concentrations of BA and IBA on Adventitious Bud Proliferation of Lavende

After 5 days of explant inoculation, the single bud began to germinate, and about 28 days after inoculation, the single bud began to germinate. There were significant differences in bud proliferation and growth between different levels of BA and IBA combinations (Table 4). When the concentration of BA was 0.5 mg/L, the multiplication effect of each treatment was the worst, and the multiplication multiple was between 2.58 and 2.93; When the concentration of BA was 1.0 ~ 2.0 mg/L, there was no significant difference in the multiplication ratio of each treatment bud, and the multiplication multiple was between 4.21 and 4.92. However, when the concentration of BA was 1.0 mg/L, the growth of bud was strong, the leaves were flat, and the average height of axillary bud was higher. However, when the concentration of BA was 2.0 mg/L, the growth potential of cluster buds in 11 ~ 13 treatments was weaker than that of 1.0 mg/L BA treatment, and some buds were vitrification. Combined with multiplication

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times and bud growth, it was considered that MS + 1.0 mg/L BA + 0.3 mg/L IBA was a suitable medium for the multiplication of adventitious buds of *Lavandula angustifolia*.

Effects of Different Hormone Types and Levels on Adventitious Root Formation in Lavende

The high 2 cm plantlets were inoculated into the rooting medium (Table 3), and about 9 ~ 12 days after inoculation, the white root tips began to grow at the base of some tissue culture plantlets, and the root length was about 0.7cm after about 3 weeks. The rooting effect of NAA was better than that of IBA, and the rooting rate increased with the increase of auxin concentration. When the concentration of NAA was 0.4 mg/L and 0.8 mg/L, there was no significant difference in rooting rate of test-tube seedlings, which were 66.7% and 69.4%, respectively, and the root growth was good. However, the average rooting number of 0.4 mg/L NAA treatment was higher than that of 0. 8 mg/L NAA treatment, which was 9.4, significantly higher than that of 0.8 mg/L NAA treatment. According to the rooting rate and the average number of rooting, White 0.4 mg/L NAA was considered to be a suitable rooting medium for adventitious buds of lavender.

Effects of different transplanting substrates on the survival rate of lavender plantlets in vitro

The rooting plantlets were transplanted into different substrates, and the temperature was controlled at 20 ~ 30 °C, and the relative humidity of air was between 60% and 80%. The transplanting experiment showed that the plantlets with equal volume mixed peat and perlite could grow well, and the survival rate of transplanting was 66.7%, and the survival rate of peat and perlite was 60% and 50%, respectively. Using vermiculite as substrate, the growth of test-tube seedlings was slower, and the survival rate of transplanting was the lowest, which was 40.0% (Table 4).

TABLE 4. Effects of different transplanting substrates on survival rate of lavender plantlets in vitro

Transplanting substrate	Transplanting seedling numbe	Survival seedling numbe	rate of survival (%)
Turf	72	42	60.0%[b]
Peat: perlite (1:1)	72	48	66.7%[a]
roseite	72	29	40.0%[d]
pearlite	72	26	50.0%[c]

DISCUSSION

The disinfection of explants needs to take into account survival rate and disinfection rate. The longer the disinfection time, the lower the contamination rate, but the survival rate was related to genotype, explant type and young degree. The results showed that the explants had relatively high survival rate and low contamination rate when 15min was treated with 0.1% $HgCl_2$. The results of Shao Mingyue *et al.* [3] showed that 0.1% $HgCl_2$ was more suitable for 6 ~ 8min treatment of lavender, which might be due to genotypic differences [3].

Lavender multiplication culture can select leaves, stem nodes and buds as explants, but buds are more suitable for rapid propagation. The species and proportion of cytokinins and auxins are important factors affecting organ formation in proliferative culture. The multiplication of stem was related to the concentration of BA. The multiplication ratio of stem increased with the increase of BA concentration. However, when the concentration of BA was too high (2.0 mg/L), the proliferation of stem was inhibited and vitrification appeared. Adding a certain concentration of IBA can promote the proliferation of stem. According to stem quality and multiplication quantity, MS + 1.0 mg/L BA + 0.3 mg/L IBA culture medium is suitable for the proliferation of lavender.

The rooting of test-tube seedlings was affected by genotypes, culture medium and culture conditions [3, 4]. In general, low salt medium was used for rooting of test-tube plantlets, and low salt environment reduced nitrogen concentration to a favorable level suitable for rooting[]. In this study, White was used as the basic medium for rooting and NAA and IBA were used as auxin for rooting induction. The results showed that the rooting effect of NAA was better than that of IBA, and the rooting rate increased with the increase of NAA concentration. However, in the

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course of culture, 0.8 mg/L NAA rooting could easily form callus at the base of the test-tube seedling, thus affecting the domestication and transplanting of the test-tube seedling in the next stage. The average root number of 0.8 mg/L NAA was less than 0.4 mg/L NAA, so White + 0.4 mg/L NAA was considered as a suitable rooting medium for lavender.

When domesticated and transplanted, the composition of substrate also significantly affected the survival of test-tube seedlings. In this study, the same volume of peat and perlite was used as substrate, the growth of test-tube seedlings was fast and the survival rate of seedlings was high (66.7%). However, the seedling survival rate of Suchen was 96.7% after transplanting and acclimating lavender with the same proportion of substrate [5]. The survival rate of plantlets transplanted in tissue culture was affected by light, temperature and humidity, but the survival rate of rooting seedlings of lavender was not high in this experiment.

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REFERENCES

1. M. Tsuro, M. Inoue and H. Kameoka, Scientia Horticulturae **4**, 309-317 (2001).
2. S. Gonçalves and A. Romano, Biotechnology Advances **31**, 166-174 (2013).
3. M.Y. Shao, Z.Y. Ye, M.F. Chen, *et al.*, Modern Agricultural Science and Technology **22**, 50-51 (2011).
4. S. Predieri, G. BrizziI and E. Gatti, Advances in Horticultural Science **13**, 68-70 (1999).
5. J. Qi, S. Zhang, H.W. Li, *et al.*, Jilin Forestry Science and Technology **41**, 41-42 (2012).

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