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The Effect of Phytohormones on Lavender (*Lavandula Angustiflia* Mill.) Organogenesis

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Abstract: Lavender (*L. angustifolia*) is a genus of flowering plants in the mint family, Lamiaceae. It is an aromatic shrub with multiple stems growing 30 to 60 cm tall. It is native to Asia, Europe and Mediterranean regions and grows on hilly areas and coastlines. The evergreen leaves are narrow and long and covered in fine hairs, which normally contain the essential oils. The leaves are also pinnately toothed, or pinnate. The plant blooms between April and June, and flowers are purple produced on violet bracts. All parts of the plant have a strong and scented odor and a bitter taste. The flowers and leaves are used as an herbal medicine. Lavender essential oil is extracted by distilling flowers and leaves. Essential oil has higher levels of linalyl acetate (40%), butyric acid, propionic acid, valeric acid, free linalool. Considering the lack of information about lavender tissue culture, the current experiment was aimed to study the effect of phytohormones on lavender callus induction and organogenesis. The lavender explants (leaf and apical meristem) were cultured onto MS media supplemented with different phytohormones. According to the observations, 5 mg/L NAA and 5 mg/L KIN treatment caused leaf and root formation, which were originally derived from dense and green calli. The MS medium containing 5 mg/L IBA and 5 mg/L Kin was effective just on leafy explants so that explants formed into leaves after callus induction. Application of 5 mg/L NAA and 5 mg/L BAP on apical meristem explants produced shoot and root after callus induction. However, complete plants were produced after sub-culturing the samples. In conclusion, MS medium supplemented with 5 mg/L NAA and 5 mg/L BAP was selected as the best medium for lavender tissue culture.

Key words: Lavender, tissue culture, organogenesis, phytohormone.

1. Introduction

Lavandula Angustifolia L. is an important aromatic, ornamental and essential oil producing plant. It is commonly known as Lavender that belongs to the family Lamiaceae cultivated in the Mediterranean areas. The oil is used in perfumery, cosmetics, flavoring, detergents, insecticides and pharmaceutical industries. It has medicinal properties such as sedative, diuretic, diaphoretic, antiseptic carminative, spasmolytic, antidepressent and anti-rheumatic properties.

The propagation by seeds is slow and also exhibits variation in growth rate, growth by stem is not only slow but its rooting ability is also poor and due to repeated vegetative propagation it leads to modification of the morphological and chemical characteristics [1]. Also results in clonal degeneration and reduced life span, deterioration in vigor, low diseases resistance and tolerance to environmental stress conditions. Thus micropropagation through axillary buds, leaves and apical buds has been proposed as a useful technique and alternative method to cope up with this problem. All living cells of a plant are capable of differentiating and dedifferentiating into whole plants. This inherent property of the cells called “cellular totipotency” has led to the concept of tissue culture studies. Plant tissue culture was originally developed as a research tool in order to study the biochemistry and physiology of

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plants. Plant tissue culture has advanced the knowledge of fundamental botany, especially in the field of agriculture, horticulture, plant breeding, forestry, somatic cell hybridization, phytopathology and industrial production of plant secondary metabolites etc. Plant tissue culture has turned into a standard procedure for modern biotechnology and has become one of the cornerstones of present day agriculture [2]. Micropropagation involves the production of plants from very small plant part tissue or cell grown aseptically. The ability to grow plant organs and tissue such as stem, root, flower and callus has been used in scientific laboratories for many decades as a research tool for genetics, botanists and plant pathologists. The oil in lavender's small, blue-violet flowers gives the herb its fragrant scent [3]. The flowers are arranged in spirals of 6-10 blossoms, forming interrupted spikes above the foliage. Plant tissue culture is a practice used to propagate plants under sterile conditions, often to produce clones of a plant. Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation.

Chambdon et al., (1992) Studied on the micro propagation of Lavenders and Lavandins, a tissue culture protocol was established for the mass propagation of disease free plantlets from commercial hybrid stock plants. When regenerates were planted in fields, quality characteristics, including essential oil concentrations, were similar to parent plants. Tissue cultured plantlets could therefore be a good source of disease-free plantlets for regeneration purposes. Raev et al., (1996) [4] induced polyploidy in lavender.

Bhojwani and Razdan, (1996) [5] practice that shoots emerge from a culture, they may be sliced off and rooted with auxin to produce plantlets which, when mature, can be transferred to potting soil for further growth in the greenhouse as normal plants. The aim of this study was to optimize the protocol for lavender species for their micro propagation and conservation.

2. Material and Methods

In vitro axillary bud explants of *Lavandula angustifolia* L., were used as explants and were inoculated on Murashige and Skoog's basal medium (MS) supplemented with phytohormones for initiation and multiplication of shoots. Preparation of MS medium (murashig and skoog medium) is the most suitable and most commonly used basic tissue culture medium for plant regeneration from tissues and callus and tissues. Generally the MS medium consists of different components. Generally the MS medium consists of different components (Table 1).

Then this medium is subjected to autoclave for 15-20 min at 121 ℃. Then the medium is poured into test tubes. These tubes are allowed to solidify in laminar airflow cabinet.

Selection of the plant:

(1) Apparently good looking healthy and of good commercial value as well as of expected medicinal important plants were selected.

(2) The plants were sprayed with the fungicide and insecticide.

(3) Meristem tips.

Sterilization and inoculation of explants:

(1) Collect the nodal explants from the plants in garden.

(2) Wash the explants with detergents like labolin and then with distilled water for 3 to 5 times.

(3) Later the explants are treated with fungicides like bavostine.

(4) Now the explants again wash with distilled water for 3 times.

(5) The sterilized explants were sent to laminar air flow cabinet.

(6) In laminar air flow cabinet explants washed with autoclaved distilled water for three times.

(7) Later the explants are treated with 0.1% mercuric chloride for 1-2 min.

(8) Remove the ends of the nodal segments by using scalpels and inoculate the explants into the MS medium containing tubes.

Table 1 Composition of culture medium, MS (Murashige and Skoog) medium.

Chemical	Formula	Concentration
Macronutrients (10 X)		100 mL/L
Ammonium nitrate	NH_4NO_3	16.5
Potassium nitrate	KNO_3	19.0
Calcium chloride	$CaCl_2.2H_2O$	4.4
Magnesium sulfate	$MgSO_4.7H_2O$	3.7
Potassium dihydrogen orthophosphate	KH_2PO_4	1.7
Micronutrients (100 X)		10 mL/L
Manganese sulphate	$MnSO_4.4H_2O$	2.23
Zinc sulphate	$ZnSO_4.7H_20$	0.86
Potassium iodide	KI	0.086
Cupric sulphate	$CuSO_4.5H_2O$	0.0026
Sodium molybdate	$Na_2MoO_4.2H_2O$	0.025
Cobalt (ous) chloride	$CoCl_2.6H_2O$	0.0026
Boric acid	H_3BO_3	0.62
Vitamin source (100 X)		10 mL/L
Nicotinic acid	$C_6H_5NO_2$	0.05
Thiamine hydrochloride	$C_{12}H_{17}CIN_4OS.HCl$	0.01
Pyridoxine hydrochloride	$C_8H_{12}N_2O_2.2HCl$	0.05
Glycine	$C_6H_{12}O_6$	0.2
Iron source (100 X)		10 mL/L
Sodium EDTA	$C_{10}H_{14}N_2O_8Na_2H_2O$	2.78
Ferrous sulphate	$FeSO_4.7H_2O$	3.72
Myo-inositol		0.1 g (freshly add)
Sucrose	$C_2H_5NO_3$	30 g
Phytagel		2 g

(9) The inoculated tubes are incubated at cold temperature and suggested photoperiod.

(10) After 7-10 days, we observe the rhizogenesis and shoot development.

2.1 Culture Medium and Culture Conditions

Leaf and apical meristem were placed on semisolid modified MS medium supplemented with different combinations of Kin (0.0, 1.0, 1.5, 2.5, 3.0, 3.5, 4.5, 5.0 mg/L) and BAP

(0.01 with 1.5, 2.0, 2.5, 3.0, 3.5, 4.5, 5.0 mg/L) or BAP or Kin with IAA, IBA and NAA for shoot proliferation. Multiplication was observed in modified MS medium supplemented with different combination of hormones.

2.2 Induction of Rooting and Acclimatization

Rooting was initiated in the half strength modified MS basal medium containing 30 gm/L sucrose supplemented with different concentrations of IAA or IBA and/or NAA. All the cultures were incubated for 16 hours at 24 ± 2 ^oC under fluorescent light (3000-4000) Lux. Number of roots per shoot were assessed after four weeks.

3. Results and Discussion

The rapid production of pathogen free plants is one of the fundamental goals of the plant tissue culture. In the present study high concentration of cytokinine reduce the number of shoots as well as shoot length. The shoot crown bud were cultured on modified MS medium and supplemented with various concentrations of Kin, BAP, individually and in different combination of plant regulatory hormone for shoot regeneration [6]. MS medium was found to be more effective than other medium for medicinal plants [7]. Various morphogenic responses were obtained with different combination and concentration of either Kin, BAP or with IAA, IBA, NAA (Table 2).

Experimental observations revealed that higher concentration of cytokinine reduced the number of micropropagated shoots [8] and some research worker

Table 2 Influence of Cytokenin with Auxin added to the modified MS medium on shoot bud induction from shoot crown explant.

BAP (mg/L)	Kin (mg/L)	IAA (mg/L)	IBA (mg/L)	NAA (mg/L)	Percentage of explants producing shoots	Mean No. of shoots per explant
0	0	0	0	0	0	0
1.0	0	0	0	0	30	0.4
1.5	0	0	0	0	50	1.1
2.0	0	0	0	0	65	2.2
2.5	0	0	0	0	87	1.2
3.0	0	0	0	0	75	1.1
3.5	0	0	0	0	71	1.1
4.0	0	0	0	0	65	0.34
5.0	0	0	0	0	45	0.15
5.5	0	0	0	0	42	0.12
6.0	0	0	0	0	35	0.09
0	1.0	0	0	0	55	1.12
0	1.5	0	0	0	67	3.22
0	2.0	0	0	0	92	4.75
0	2.5	0	0	0	85	4.20
0	3.0	0	0	0	81	3.50
0	3.5	0	0	0	73	3.30
0	4.0	0	0	0	71	3.0
0	4.5	0	0	0	63	2.20
0	5.0	0	0	0	56	2.0
0	5.0	0	0	0	50	1.2
0	6	0	0	0	45	1.0
2.0	0	0.5	0	0	75	3.5
0	2.0	1.0	0	0	80	3.8
0	2.0	0.5	0	0	80	4.7
2.0	0	1.0	0	0	92	4.9
2.0	0	0	0.5	0	76	3.52
0	2	0	1.0	0.5	82	3.92
0	1	0	0.5	1.0	82	4.9
2.0	0	0	1.0	0.5	75	5.1
2.0	0	0	0	1	73	3.92
0	0	0	0	1.0	25	1.0

reported cytokinine was very critical for shoot elongation of many other plant species. It was also revealed that BAP alone or in combination with other cytokinine induced shoot organogenesis from shoot tips. In our plant species greater numbers of morphologically distinct multiple shoots were developed in MS medium containing BAP combined with Kn, but some research workers reported that BAP and kinetin combination developed fewer multiple shoots in some species [9]. The minimum number of roots was produced at the base of shoot at low concentration of Kin or BAP or combination of Kin and BAP. Rooting is completely arrested at kinetin concentration more than 5 mg/L. The number of maximum shoots was obtained in MS medium supplemented with Kin (3 mg/L) and BAP (2 mg/L), but shoots were small when the concentration of kinetin was raised (Table 3). The shoot length was strongly affected by high concentration of cytokinine [10].

Experimental observation support that MS medium containing low concentration of auxin along with

Table 3 Influence of different concentrations of Cytokinines and NAA, IAA, IBA, added to the MS medium on shoot multiplication from shoot crown explant after 4 weeks of culture.

BAP (mg/L)	Kin (mg/L)	IBA (mg/L)	IAA (mg/L)	NAA (mg/L)	Mean number of shoots/culture
0	0	0	0	0	0
2	0	0	1	0	7.45
2	0	1	0	0	15.25
2	0	0	0	1	10.0
0	2	0	1	0	12.60
0	2	1	0	0	28.25
0	2	0	0	1	20.75
1.5	0	0	0	0	7.25
2	0	0	0	0	9.20
2	3	0	0	0	73.2
2	4	0	0	0	18.6

Table 4 Influence of different concentration of Auxin (IAA, IBA, NAA) added to the strength modified MS medium on rooting of in vitro formed shoots.

IBA (mg/L)	IAA (mg/L)	NAA (mg/L)	Mean % of shoots rooted	Mean no of roots / shoot
0	0	0	0	0
0.1	0	0	65.45	2.2
0.25	0	0	72.25	3.25
0.5	0	0	80.2	3.45
1	0	0	92.2	23.14
2	0	0	55.8	12.8
0	0.1	0	48.2	9.25
0	0.25	0	45.2	2.1
0	0.5	0	65.4	2.3
0	1.0	0	72.0	3.2
0	1.5	0	48.2	9.5
0	2	0	42.8	6.2
0	0	0.1	57	2.1
0	0	0.25	60	2.2
0	0	0.5	65.0	3.2
0	0	1	74	13.2
0		0.5	57.2	11.24

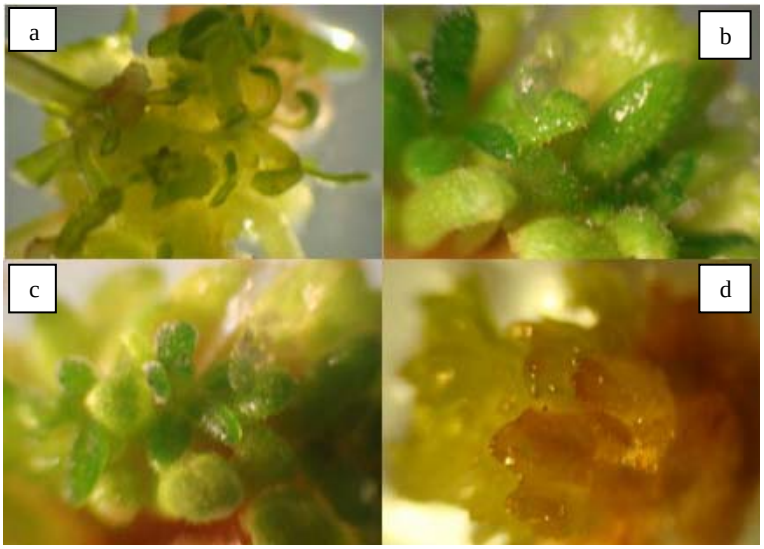

Fig. 1 Shoot bud differentiation and regeneration from shoot crown explants.
(a) Medium with BAP (0.5 mg/L); (b) Medium with BAP (1 mg/L); (c) Medium with NAA (0.5 mg/L); (d) Medium with NAA (1 mg/L).

cytokinine increases the rate of shoot multiplication [11].

Seventy-three shoots/culture was produced at a combination of Kin (3 mg/L) and BAP (2mg/L) and 8 shoots/culture was produced at a combination of Kin (6 mg/L) and BAP (2 mg/L) (Table 4). Shoots were small when the concentration of kinetin was raised. The shoot length was strongly affected by concentration of cytokinines. Excised shoots were rooted on 1/2 strength modified MS medium [12] with different type of auxin. Four week old healthy excised shoots on transfer to 1/2 strength MS medium containing 3% sucrose supplemented with 1 mg/L IBA formed vigorous fibrous and thick strong roots during the culture of 4 week (Fig. 1). IBA was most effective in inducing rooting of several other plants [13]. Higher concentration of IBA or IAA or combination of IAA and NAA was not favourable.

4. Conclusion

Efficient plant regeneration is the primary objective of many studies in plant tissue culture. Micropropagation system provides a method for rapid regeneration of various medicinal crops of high economic value. The improved *in vitro* plant culture system has the potential for commercial production of medicinal crops on large scale. During the past decade remarkable progress resulted in plant biotechnology has been witnessed with a constant flow of improved transformation regeneration protocols for many medicinal crops. A good regeneration protocol is always needed for genetic transformation studies for up-regulation of secondary compounds. In such instances usage of natural organic extracts in culture medium rejuvenates the *in vitro* plant system resulted with good regeneration frequencies and enhanced shoot multiplication, however these findings have been reported and well discussed in this review. Supplementation of natural organic extracts as additives for standardizing regeneration protocols of commercially important medicinal crops has been increased significantly. These promissory organic extracts described in this review would certainly be of increasing importance in near future in the field of medicinal plants research, such as genetic transformation studies and scale-up of secondary compounds through cell suspension cultures in bioreactors.

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