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Research article

STUDIES ON INFLUENCE OF GROWTH REGULATORS IN MICROPROPAGATION OF *LAVANDULA ANGUSTIFOLIA*

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ABSTRACT: *Lavandula angustifolia (*Family Labiates) is a medicinal herb 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 and anxiety. There are few reports on tissue culture of *Lavandula angustifolia* that too mainly on micropropagation. Present study explored an *in vitro* micropropagation of *Lavandula angustifolia*. *In vitro* callus formation was established by using nodal segments on Murashique and Skoog, (1962) medium (MS) supplemented with IAA at 0.1mg/l, l BAP at 0.002mg/l and 2-4D at 0.2mg/l, significantly recorded complete callus formation after 6 weeks of incubation at 25±1ºC. The callus was allowed for organogenesis and then shoot multiplication was carried out at 4 concentrations of BAP (0.5, 1, 2 and 1mg/L) and IAA (0.5, 0.5, 0.5 and 1mg/L) on MS medium. The shoot regeneration medium for shoot multiplication and proliferation with higher number of shoots was recorded at 0.5mg/l of IAA and 2.0mg/l BAP. However, the growth was very steady and originating from the base. MS medium without any growth regulators tabulated the tallest shoot length of 35 mm, but the shoots were clustered not properly differentiated.

Key words: *Lavandula angustifolia***,** callus, shoot multiplication, Murashige and Skoog (MS)

INTRODUCTION

Lavandula aungustifolia is a genus of Labiateae family. It is a hardy perennial shrub rich in aromatic essential oils and is valuable for its pharmaceutical, aromatic and culinary properties. A total of 20 species of *Lavandula* have been described in the literature. There is confusion with the naming of lavenders round the world, owing to differences in their appearance under different climatic and/or husbandry conditions (Lis-Balchin, M. 2002). *Lavandula aungustifolia* is the one of the most important aromatic crops that is geographically grown in Mediterranean countries (Baytop, T. 1999). This genus is relatively rich in phenolic constituents, with 19 flavones and 8 anthocyanins (Harborne, J.B., Williams C.A. 2002).

Lavandula angustifolia is a carminative, spasmolytic, tonic, antidepressant, nervous headache, neuralgia, rheumatism, depression, insomnia, windy colic, fainting, toothache, sprains, sinusitis, stress and migraine (Bertram, T. 1995). Lavandula oil is used in aromatherapy which involves massage using a much diluted essential oil's (Lis-Balchin, M. 2002). Besides, it is also a very beautiful ornamental pot plant. *Lavandula angustifolia* plants are commercially propagating by stem cuttings, but have a poor rooting ability (Andrade, L.B. et al, 1999). Also, the seeds have a poor germination percentage (Takano, T. et al, 1990), therefore these methods are not efficient enough to produce mass production needed. Micropropagation offers a potential to deliver large quantities of disease-free and large number of genetically identical plants within a short span of time. Limited tissue work has been done in lavandula species (Calvo, M.C., Segura J. 1989). Several species of lavender have micropropagated used, like *L. dentata* (Echeverrigaray, S et al, 2005), L. *vera* (Andrade, L.B. et al, 1999) *L. latifolia* (Panizza, M., Tognoni F. (1992). So the aim of the current study is a trial to develop a commercial protocol for direct micropropagation of this aromatic and medicinal plant.

MATERIALS AND METHODS

Plant Material

The plants of *Lavandula aungustifolia* was collected from herbal garden of Shoolini University, Solan. In vivo grown nodal segments harvested from six month old plants were used as an explant to study regeneration potentiality.

Sterilization of explants

The explants were washed under running tap water for 30 minutes and were surface sterilized under aseptic conditions inside the laminar air flow hood by using 70% ethyl alcohol for 30 minutes and 0.1% of mercuric chloride for 2 minutes with one drop of Tween-20. All the traces of used disinfectants were removed by rinsing the explants 3-4 times in sterilized distilled water inside the laminar air flow hood. The nodal segments were then incubated in sterile bottles containing Murashige and Skoog (1962) medium with 30g/L of sucrose and 8g/L of agar and incubated at 25±1ºC. The medium has been sterilized at 121°C for 15-20 minutes.

Callus Induction

Explants were taken from the six month old *Lavandula aungustifolia*. The each explant was cultured on (MS) Murashige and Skoog (1962) medium comprising 30g/L sucrose and 8g/L of agar with two different hormonal combinations of BAP (0.002mg/l) + IAA (0.1mg/l) + 2-4, D (0.2mg/l) and BAP (0.1mg/l) + IAA (0.001mg/l) + 2-4, D (0.2mg/l) . The pH of the medium was adjusted at 5.8 by using 1 N HCl and 1N NaOH. The medium was cooked and distributed into 250ml jars containing 25ml of nutrient medium. Jars were covered and autoclaved at 121°C for 20 minutes. Explants were inoculated on the MS medium and incubated at 25°C in growth chamber. Data were recorded after a month of inoculation for complete callus induction.

Shoot Proliferation

For shoot proliferation, explants were taken from the *in vitro* initiated callus of *Lavandula aungustifolia*. The callus was excised from length of 5mm approximately and was inoculated on MS medium with two different hormonal combinations BAP (0.002mg/l) + IAA (0.1mg/l) and BAP (0.001mg/l) + IAA (0.001mg/l) inside the culture cabinet. Data were recorded after four weeks of inoculation for shoots proliferation.

Studying on influence of growth regulators concentration on proliferation of shoots and shoots number.

For studying the influence of growth regulators concentration on proliferation of shoots, the explants with length of 4mm approximately were cultured on MS medium with different concentration and combinations of BAP and IAA.

1. MS medium without Auxins and Cytokinins (Control)
2. MS medium with hormones combination of 0.5mg/l IAA and 0.5mg/l BAP.
3. MS medium with hormones combination of 0.5mg/l IAA and 1.0mg/l BAP.
4. MS medium with hormones combination of 0.5mg/l IAA and 2.0mg/l BAP.
5. MS medium with hormones combination of 1.0mg/l IAA and 1.0mg/l BAP.

And the explants were inoculated onto the medium inside the culture cabinet. Data were recorded after 4 weeks for:

1. Shoot number per explants*
2. Shoot length (mm)*

* The experiment was conducted in three triplets.

RESULTS

Callus Induction

Nodal segments of *Lavandula aungustifolia* were cultivated in different hormonal combinations in semi-solid medium. The friable calli and increase in callus size were observed in MS medium containing the hormonal combination of BAP (0.002mg/l) + IAA (0.1mg/l) + 2-4, D (0.2mg/l). This might be because of more auxin concentration led to rapid growth (Table 1 & Fig. 1).

Table 1: Effect of different concentration of 2,4-D with NAA and BAP for the initiation of callus culture.

S.No	Treatment	Response
1	MS +BAP (0.001)+ 2,4-D (0.1 mg/l) + IAA (0.1 mg/l)	No Response
2	MS +BAP (0.001)+ 2,4-D (0.2 mg/l) + IAA (0.1 mg/l)	No Response
3	MS +BAP (0.001)+ 2,4-D (0.2 mg/l) + IAA (0.2 mg/l)	No Response
4	MS +BAP (0.001)+ 2,4-D (0.1 mg/l) + IAA (0.2 mg/l)	No Response
5	MS +BAP (0.002)+ 2,4-D (0.1 mg/l) + IAA (0.1 mg/l)	No Response
6	MS +BAP (0.002)+ 2,4-D (0.2 mg/l) + IAA (0.1 mg/l)	Callus formation
7	MS +BAP (0.002)+ 2,4-D (0.2 mg/l) + IAA (0.2 mg/l)	No Response
8	MS +BAP (0.002)+ 2,4-D (0.1 mg/l) + IAA (0.2 mg/l)	No Response
9	Control	No Response

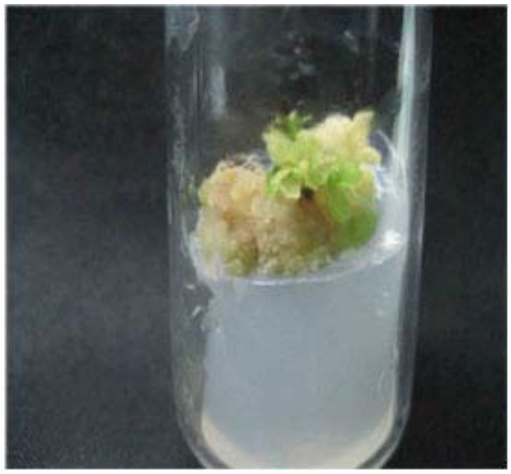

Figure 1: Callus induction of *Lavandula aungustifolia*

Shoot multiplication

The explants were taken from the *in vitro* initiated callus of *Lavandula aungustifolia* were cultured on MS medium with two different hormonal combinations BAP (0.002mg/l) + IAA (0.1mg/l) and BAP (0.001mg/l) + IAA (0.001mg/l) according to the literature . Rapid shoot multiplication was observed on MS medium supplemented with combinations BAP (0.002mg/l) + IAA (0.1mg/l). Data was recorded after different days of incubation (Fig. 2).

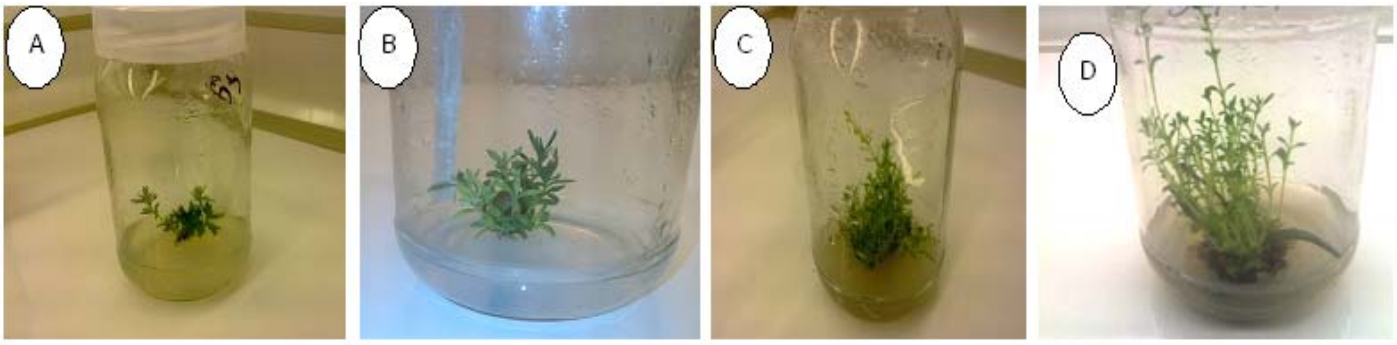

Figure 2: Shoot multiplication of *Lavandula aungustifolia* after different incubation days (A) 12 days (B) 18 days (C) 25 days and (D) 34 days.

Influence of growth hormones on proliferation rate and shoot number

Nodal segments of *in vitro* micropropagated *Lavandula aungustifolia* plants with length of 4mm approximately were cultured on MS medium with different concentration and combinations of BAP and IAA with respect to control (Table 2& Fig. 3). The shoot growth was found to be the found best at IAA (0.5mg/l) +BAP (2.0 mg/l).

So, it can be cleared from present study that auxin concentration is an important factor affecting the callus growth and less concentration of auxin itself leads to less growth (Fig. 3).

Table 2: Effect of different concentrations and combinations of growth hormones on in vitro shoot multiplication of *Lavandula aungustifolia*.

S.No	Medium	Auxin (mg/l)	Cytokinin (mg/l)	Proliferation Rate(Shoot/Explant)		SHOOT LENGTH(mm)	
				Initial	Final	Initial	Final
1	MS(Control)	0	0	1	2	4	35
2	MS	IAA(0.5)	BAP(0.5)	1	3	4	20
3	MS	IAA(0.5)	BAP(1.0)	1	5	4	27
4	MS	IAA(0.5)	BAP(2)	1	16	4	30
5	MS	IAA(1)	BAP(1)	1	10	4	25

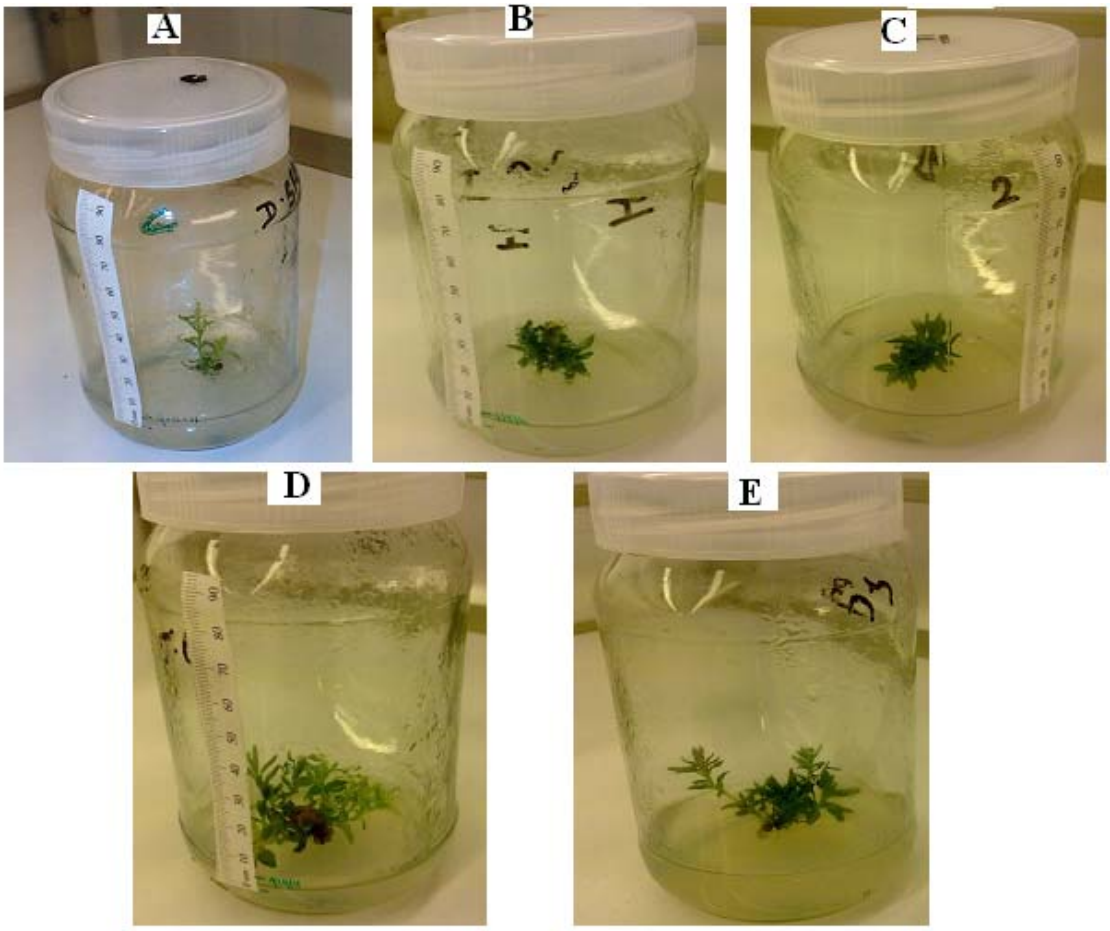

Figure 3: Shoot proliferation of *Lavandula aungustifolia* when MS medium supplemented with (A) without phytohormones (Control), **(B)** IAA (0.5mg/l) and BAP (0.5 mg/l), **(C)** IAA (0.5 mg/l) and BAP (1.0 mg/l), **(D)** IAA(0.5 mg/l) and BAP (2.0 mg/l), and **(E)** IAA (1.0 mg/l) and BAP (1.0 mg/l).

A) The first concentration was control with no auxin or cytokinin showing proliferation rate of 2 shoot /explant from 1 and shoot length of 35 mm from 4mm.
B) The second concentration took was MS+ IAA(0.5 mg/l) + BAP(0.5mg/l) showing proliferation rate of 3 shoot/explant from 1 and shoot length of 20mm from 4 mm
C) The third concentration took was MS+ IAA(0.5 mg/l) + BAP(1.0mg/l) showing proliferation rate of 5 shoot/explant from 1 and shoot length of 27mm from 4 mm.
D) The fourth concentration took was MS+ IAA(0.5 mg/l) + BAP(2.0mg/l) showing proliferation rate of 3 shoot/explant from 1 and shoot length of 30mm from 4 mm.
E) The fifth concentration took was MS+ IAA(1.0 mg/l) + BAP(1.0mg/l) showing proliferation rate of 3 shoot/explant from 1 and shoot length of 25mm from 4 mm.

DISCUSSION

Gros and Calvo, (1996) studied the micropropagation of *Lavandula angustifolia* through nodal segments of mature plant which were initially cultured on MS medium supplemented with two different macronutrient combination of BAP, KN and 20% of coconut milk. After 15 days of culture in rooting media plantlets developed into mature plants which were later on transferred to green house for initial acclimatization. In contrast to their study, MS+ (0.002mg/l) + IAA (0.1mg/l) + 2-4, D (0.2mg/l) was found to be the best medium for callus induction in the present investigation.
Similarly in 1999 Andrade evaluated the effect of growth regulators on in vitro shoot proliferation and rooting of micropropagated Lavendula versa. Highest multiplication was obtained using Murashige and Skoog (1962) medium supplemented with varying concentration of TDZ and BA. In the present study MS medium supplemented with IAA (0.5mg/l) +BAP (2.0 mg/l) was found to be the best for the proliferation of shoots.

CONCLUSION

Callus were obtained after two month of incubation period and then transfer to shooting medium supplemented with IAA at 0.1g/L and BAP at 0.002g/L. For studying the influence of growth regulators on micropropagated *Lavandula aungustifolia* different concentration and combinations of IAA at 0.5 and 1.0mg/L and BAP at 0.5, 1.0 and 2.0mg/L. after four weeks of incubation on different combinations of growth regulators the result came out to be as follows. MS medium supplemented with BAP at 2.0mg/L and IAA at 0.5 g/L recorded highest shoot number of 16 with shoot length of 30mm and was found to be best shoot proliferation medium. It was found surprisingly that MS Murashige and Skoog, (1962) medium without growth regulators resulted in best shoots length of 35mm.

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Conflict of Interest

No potential conflict of interest to disclose.

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