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Research Article

In-vitro Propagation of *Lavendula officinalis* through Shoot Tips and Seeds

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ABSTRACT

In the present study a successful procedure for rapid proliferation of shoots and seed germination of Lavendula officinalis Chaix was formulated. Shoot tip cultures were preferred as the pre-existing meristem easily develops into shoots while maintaining clonal fidelity. The superiority of BAP and IAA over Kn and IBA combination has been Investigated during the present study. The BAP + IAA combination showed more shoot proliferation then KN + IBA combination. Among the various: auxin cytokinin combinations, the best in terms of shoot multiplication were recorded in BAP (0.8mg/1) + IAA (0.4mg/1) after sixth days of inoculation.

In order to break the dormancy of the lavender seeds an efficient protocol was carried out under in-vitro laboratory conditions. Seeds of Lavendula officinalis subjected to scarification showed 6.25% germination in light and same percentage of seed germination was recorded in complete darkness. Seeds of Lavendula officinalis subjected to acid wash showed 12.5% germination in light. At dark treatment the seeds treated with sulphuric acid showed 6.25 % of germination. Seeds subjected to 48 hours chilling treatment showed 25% of germination in light. The dark chilling treatment showed 18.75% of germination. Best results (31.25%) were recorded in seeds treated with GA_3 (200ppm) in ordinary light and 12.5% of seed germination was recorded in GA_3 seeds kept in complete darkness.

Key words: *Lavendula officinalis, Invitro propagation, Seed dormancy, Shoot tip.*

INTRODUCTION

Lavendula officinalis Chaix is among the most important aromatic plants of now-a-days. *Lavendula officinalis Chaix syn Lavendula angustifolia Mill*. (Family lamiaceae) is one of the most important aromatic plants in France, Spain, Bulgaria and Russia.

The name *lavender* comes from the Latin root *lavare*, which means, "to wash." *Lavender* most likely earned this name because it was frequently used in baths to help purify the body and spirit. The genus lavender consists of 28 – 30 species. True lavender is commonly known as *English lavender*. Lavenders are mainly classified in four categories on the bases of origin, plant type and varieties[4].

Lavendula officinalis is propagated through layering, cuttings and seeds. But the poor rooting ability of stem cuttings, as well as the lack of selected clones, restrains its industrial exploitation[8].

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Multiplication through seeds also involves segregation which results drop in quality and quantity of essential oil[11]. Accordingly, an alternative procedure is required for propagating Lavendula plants efficiently. Multiplication through invitro seeds and shoot tip explants are among the useful methods which can be employed for clonal propagation. Clonal propagation through tissue culture can be achieved in a short time and space. The increasing demand of plant based drugs has led to the decline in the supply of medicinal plants from their natural habitats and as such there is a great need for cultivation of medicinal and aromatic plants in vitro cultures for meeting the growing demands. Plant tissue culture is considered as a noble method to select, multiply and conserve the critical genotypes of plants by using techniques such as micro propagation. However tissue culture studies on *lavendula officinalis* have largely been restricted to monitor monoterpene synthesis in shoots regenerated from callus culture[13].

MATERIAL AND METHODS

I. *In vitro* propagation through shoot tip

During the present investigation *Lavendula officinalis Chaix* was subjected to *in vitro* studies, so as to develop efficient protocol for the shoot proliferation and seed germination of the plant. Carlos *et al*.[5], developed an efficient protocol for the micro propagation of *Lavendula latifolia* through nodal bud culture. They succeeded to produce multiple shoots from these explants on MS medium supplemented with BA.

For the *in vitro* proliferation of shoots tender shoot tips (2cm) in length were excised as explants from the mature plant. These explants when inoculated on MS Basal medium showed no response.

In order to induce the production of multiple shoots in these explants, MS Basal medium was fortified with a range of cytokinins (BAP, Kn) and auxins (IAA, IBA) which were added in a wide range of concentrations

Preparation and sterilization of the nutrient media:

During the present investigation modified murashige and skoog's basal medium[9] was used. The preparations of MS stock and vitamin stock solutions are given in table 1 and 2 respectively. All hormones, vitamins and constituents were dissolved separately in double distilled water to form a stock solution and were stored in a refrigerator. Different combinations and concentrations of phyto hormones viz; auxins (like IBA, IAA), cytokines (BAP, Kinetin) were added to the media. Andrade *et al*.[1], evaluated the effect of growth regulators on shoot propagation and rooting of common lavender (*Lavendula Vera* DC).They evaluated that the highest multiplication rate from nodal segments is on MS medium fortified with 1.0 mg-1 TDZ (2.25 M) or BA (2.0 M).

Table 1For preparation of 20x MS stock of 500 ml, the composition includes:-

Table 1: Preparation of MS stock

S. No.	Components	Concentration
01	KNO_3	19 gm
02	NH_4NO_3	16.5 gm
03	$MgSO_4.7H_2O$	3.7 gm
04	$MNSO_4.H2O$	223.0 mg
05	$ZnSO_4.7H_2O$	86.0 mg
06	$CuSO_4$	0.25 mg
07	$CaCl_2.2H_2O$	4.4 gm
08	KI	8.3 mg
09	H_3BO_3	62.0 mg
10	KH_2PO_4	1.7 mg
11	$CaCl_2.6H_2O$	0.25 mg
12	Distilled Water	500 ml

Table 2: Preparation of MS stock vitamins:

		Ix	**100x**
S. No.	**Ms Vitamin**	**Mg/ltr**	**Mg/100ml**
01	Nicotinic Acid	0.5mg	50mg
02	Pyridoxine HCL	0.5mg	50mg
03	Thiamine HCL	0.1mg	10mg
04	Glycine	2mg	200mg
05	Inositol is added separately at a concentration of 100mg/ltr		
06	Folic acid	2mg	200mg
07	Biotin	2mg	200mg

3.6 Sub culturing:

The cultures showing better response were maintained by subculturing on a fresh medium at regular interval of 3-4 weeks.

ii. In vitro propagation through seeds

Seeds were collected from the field gene bank of Indian Institute of Integrative medicine (CSIR) Srinagar and were separately subjected to various treatments.

Sand paper Scarification

Sand paper of "size 100" was used .The seeds were kept between two sheets of sand paper and rubbed six times. After scarification seeds were kept at light and dark conditions.

Sulphuric Acid (H2SO4) treatment

Seeds were kept in a muslin cloth and then dipped in concentrated H2SO4 treatment for 3 minutes followed by a wash in distilled water. After washing in distilled water seeds were kept at light and dark conditions. Sulphuric acid used was 99.9% pure, Merck's Sulphuric acid.

Chilling treatment

Seeds were subjected to 48 hours chilling treatment in -20. After chilling seeds were kept at light and dark conditions

Gibberlic Acid Treatment

Gibberlic Acid treatment of 200 ppm prepared by dissolving 200 mg of gibberlic acid in 1000 ml of distilled water. The seeds were kept in the solution for about 24 hours followed by a wash in autoclaved double distilled water.

RESULTS AND DISCUSSION

For the *in vitro* proliferation of shoots tender shoot tips (Explants 2cm in length) when These explants were inoculated on MS Basal medium they did not showed any response. In order to induce the production of multiple shoots in these explants, MS Basal medium was fortified with a range of cytokinins (BAP, Kn) and auxins (IAA, IBA) which were added in a wide range of concentrations of which the most effective ones are given in (Table 3). Shoot tip explants inoculated on MS Basal medium augmented with various concentrations of cytokinin (Kn) and auxin (IBA). MS Basal medium augmented with various concentrations of Kn and IBA responded at concentrations of Kn (0.3mg/1) + IBA (0.1mg/1), Kn (0.6mg/1) + IBA (0.2mg/1) and Kn (1.0mg/1) + IBA (0.5mg/1). Of these the best result was observed on Kn (0.6mg/1) + IBA (0.2mg/1) in which multiple shoots were produced in 40% of cultures after two weeks of inoculation (Fig. 3). MS Basal medium augmented with Kn (0.3mg/1) + IBA (0.1mg/1) showed the regeneration of multiple shoots in 30% of cultures after 20 days of inoculation and the MS Basal medium augmented with Kn (1.0mg/q) + IBA (0.5mg/1) showed the production of multiple shoots in 20% of cultures after 25 days of inoculation. . In the inoculated culture medium an exudation of some blue pigments was also released into agar medium. Similar, exudations were also reported by[10,3]. in the immobilized cultured cells of *lavendula vera* in the presence of L-cysteine and also in callus cultures of *Lavendula angustifolia*. Although micro propagation in *lavendula vera DC* and *lavender* has been achieved earlier[12,6]. but the plants raised were from the leaf derived callus.

Table 3: Effect of cytokinin and auxins on shoot regeneration from shoot tip explants of lavendula officinalis Chaix in Ms Basal medium after four weeks of culture

S. No	Treatment	%Regeneration
01	MS + BAP (0.4mg/1) + IAA (0.2mg/1)	25
02	MS + BAP (0.5mg/1) + IAA (0.3mg/1)	50
03	MS + BAP (0.8mg/1) + IAA (0.4mg/1)	70
04	MS + Kn (0.3mg/1) + IBA (0.1mg/1)	30
05	MS + Kn (0.6mg/1) + IBA (0.2mg/1)	40
06	MS + Kn (1.0mg/1) + IBA (0.5mg/1)	20
07	MS Basal	-

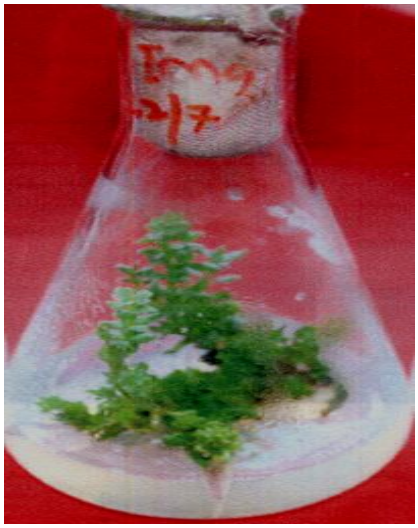


Fig. 1: Lavendula officinalis producing multiple shoots from shoot tip explants cultured on MS + BAP (0.8mg/l) + IAA (0.4mg/l)

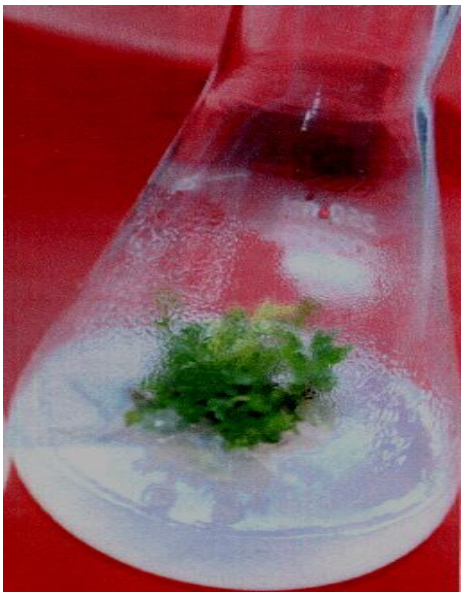


Fig. 2: Lavendula officinalis producing multiple shoots from shoot tip explants cultured on MS + Kn (0.6mg/l) + IBA (0.2mg/l)

Fig. 3: *Lavendula officinalis* producing multiple shoots from shoot tip explants cultured on MS + BAP (0.5mg/l) + IAA (0.3mg/l)

Fig. 4: *Lavendula officinalis* producing multiple shoots from shoot tip explants cultured on MS + KN (0.3mg/l) + IBA (0.1mg/l)

***In vitro* Seed germination of Lavendula officinalis Chaix:**

During the present study it was observed that

●Seeds whose seed code was scarified with sand paper showed 6.25% of germination in light Fig. 6 (Table 4, Graph 1). At dark treatments these scarified seeds show same %age of germination 6.25 %.(Table 4, Graph1).

●Seeds washed with sulphuric acid showed 12.5% of seed germination in light Fig 7 (Table 4, Graph 1). At dark treatment the seeds treated with sulphuric acid showed 6.25 % of germination. (Table 4, Graph 1).

●Seeds subjected to 48 hours chilling treatment showed 25% of germination in light Fig 8 (Table 4, Graph 1). The dark treatment showed 18.75% of germination. (Table 4, Graph1).

●Maximum germination in light i.e. 31.25% was observed in seeds treated with GA_3 Fig. 9 (Table 4, Graph 1). GA_3 treated seeds at darkness showed 12.5% of germination. (Table 4, Graph 1).

Table 4: *In vitro* seed germination studies of *Lavendula officinalis* subjected to various treatments

Treatment	**No. of seeds germinated in light & darkness**		**% germination in light & darkness**	
Control	0	0	0	0
Sand paper Scarification	1	1	6.25	6.25
Acid Wash (3 min)	2	1	12.5	6.25
48 hours chilling	4	3	25	18.75
GA_3 200ppm	5	2	31.25	12.5

various treatments

Seeds of lavendula officinalis subjected to acid wash showed 12.5% germination in light. Similar studies have been observed in Atropa belladonna seeds treated with sulphuric acid (75%) followed by sodium hydroxide (30%) recorded seed germination. Best results (31.25%) were recorded in seeds treated with GA3 (200ppm) in ordinary light and 12.5% of seed germination was recorded in GA3 seeds kept in complete darkness. During the present work the percentage of seed germination was more in the seeds kept in light as compared the seeds subjected to dark treatments. The relationship of seed size / seed germination with light requirements has also been observed in germination of hyptis suaveolens seeds by Felippe *et al.*[7],. During the study of seed germination of this plant it was also observed that the gibberlic acid treatment without pre-freezing significantly increased the percentage of seed germination as was observed by Aoyama *et al.*[2], on lavender seeds.

Fig. 6: Seeds scarified with sand paper

Fig. 7: Seeds washed with sulphuric acid

Fig. 8: Seeds sub. 48 hours chilling treat.

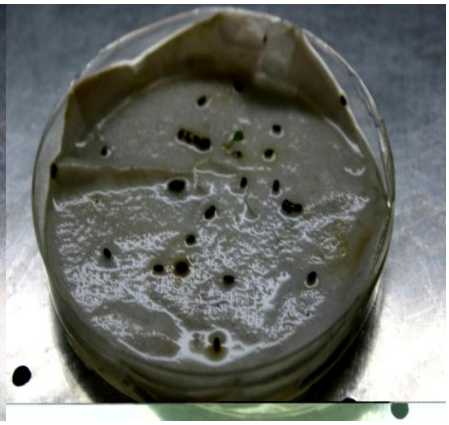
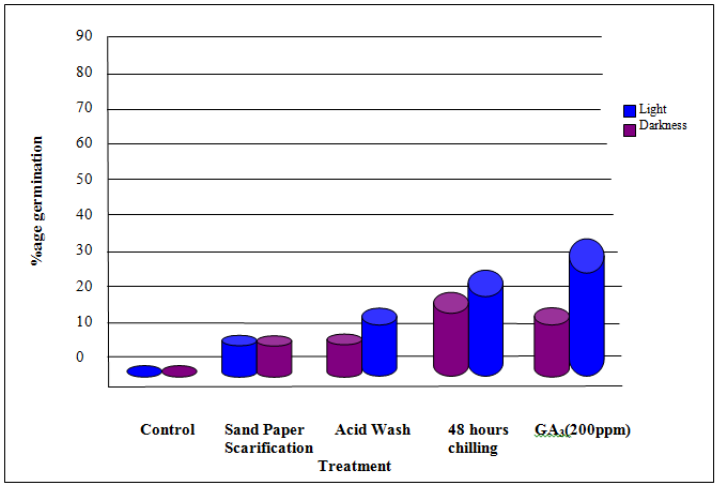


Fig. 9: Seeds treated with



Graph 1: Percentage of germination in seeds of *Lavendula officinalis* Chaix

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