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Effect of BAP on Shoot Regeneration in Shoot Tip Cultures of *Lavandula officinalis*

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ABSTRACT

The present study involves *invitro* propagation through multiple shoot regeneration in cultured shoot tips of field grown plants of *Lavandula officinalis*, an economically important shrub. MS medium (1962) fortified with various phytohormonal regimes was used to get the culture response. Indirect multiple shoots were obtained on MS (x1/2) medium enriched with BAP (5µM), whereas direct shoot multiplication was observed on same medium with slightly higher concentration of BAP (5.5µM & 6.0µM). Elongation of microshoots was favoured by MS (x1/2) basal medium which simultaneously induced rooting of shoots too. Rooting in isolated shoots was also recorded with root inducing hormone NAA.

***Key words*:** *Lavandula officinalis*, Shoot tip culture, Multiple shoots, Plantlets

Abbreviations: MS (x1/2) – Murashige and Skoog (Half-salt strength); NAA-Naptheleneacetic acid; IBA-Indole-3-butyric acid; IAA- Indole-3-acetic acid; BAP-6 Benzyl-aminopurine; TDZ-Thidiazuron

INTRODUCTION

Lavandula is a genus of family Lamiaceae, a family of perennial herbs, shrubs and subshrubs mainly native to warm temperate regions from the Canary Island to India. Several species of this genus are cultivated for ornament, for their pleasant aromatic scent, as honey plants and for the extraction of oil from the flowers (Anonymous, 1982).

Lavandula officinalis Chaix (=*Lavandula vera* DC) is commonly known as true lavender. It is native of southern Europe occurring on dry, barren soil. It is a low shrub with terminal spikes of very fragrant bluish flowers. Many horticultural forms and hybrids of this species occur (Hill, 1986). Lavander has a clean odour and dried flowers are used in sachets and for scenting purposes. Its flowers and flowering tops yield an essential oil, Lavander oil. It is used in perfumery, soap industry and in production of Lavander water which is mixture of the oil in water and alcohol. It is also used medicinally as a carminative and mild stimulant and for flavouring pharmaceutical preparations (Hill, 1986). It has a great therapeutic value as antifungal, anti-inflammatory, carminative, antispasmodic and in skin care. Nowadays it is highly prized

in aromatherapy and is emerging as one of the cash crops of Kashmir valley (Chisti *et al*., 2003).

Lavander is propagated by seeds, which is slow and the plants exhibit too much variation in growth rate and oil composition to be commercially used. Propagation by stem cutting is also slow with poor rooting capacity. Thus, micropropagation is suitable as alternative method for propagation of this economically important shrub (Andrade *et al*., 1999).

MATERIAL AND METHODS

Young shoot tips were collected from mature shrubs of *Lavandula officinalis* growing in University campus. Explants were thoroughly washed with tap water using lab wash cedpol (0.5% v/v) with 2 – 4 drops of Tween-20 (surfactant). Then these explants were surface sterilized with 7% sodium hypochlorite for 10 minutes followed by repeated rinsing with autoclaved double distilled water. Medium used for culturing of explants was Murashige & Skoog's (MS) (1962) basal medium which was supplemented with 3% sucrose. Various concentrations of BAP were separately added to the basal medium. pH of the medium was adjusted between 5.5-5.8 and 0.8% agar was used as jelling agent. The medium after dispensing in suitable culture vials was autoclaved for 20 – 25 minutes at a pressure of 15 lb and at a temperature of 121°C temperature. The cultures were maintained at 23± 5°C with 55-65% RH and exposed to 16 hr light period using cool fluorescent (3000 lux) tubes.

RESULTS

Effect of various BAP concentrations on shoot tip explants of *Lavandula officinalis* is summarized in Table 1. Shoot apices when cultured on MS basal medium, both full and half strength nutrient concentration, showed only elongation of explant beyond which no response was recorded. When MS (x1/2) basal medium was fortified with lower BAP concentrations like 0.5µM, 0.7µM and 1µM no response was registered. On fortifying the medium with 5µM BAP moderate callus formation of explant at basal end took place which was followed by multiple shoot regeneration through callus (Fig. 1). Average number of shoots per explant was 15. On slightly raising the concentration of BAP to 5.5µM and 6µM there was found a total shift in regeneration pathway and direct multiple shoot formation was recorded instead through callus formation. The average number of shoots with BAP 5.5µM was as high as 35 (Fig. 2) while as with BAP 6µM it was comparatively low, i.e., 12 (Fig. 3). On further increasing the BAP concentration (7µM and 8µM) there was again registered a shift in response and only mild non-regenerative compact callus was noticed. Hence BAP 5.5 µM proved to be optimal for multiple shoot regeneration through shoot tip culture.

Fig. 1 – 5. Shoot regeneration in *Lavandula officinalis*: **1. Multiple shoot regeneration through callus on MS (x1/2) + BAP (5µM); 2. Direct multiple shoot formation on MS (x1/2) + BAP (5.5µM); 3. Direct multiple shoot formation on MS (x1/2) + BAP (6µM); 4. Elongation of microshoots on MS (x1/2) basal medium; 5. Rooting of isolated shoots on MS (x1/2) + NAA (2. 5µM)**

Elongation of the raised microshoots took place on MS (x1/2) basal medium (Fig. 4). During elongation phase rooting of microshoots was also registered on the same medium. Isolated shoots also showed rooting on MS (x1/2) medium supplemented with NAA 2.5µM (Fig. 5) but roots formed on this medium were thicker than roots produced on basal medium.

DISCUSSION

The present study carried out on shoot tip explants of *Lavandula officinalis* has shown that the plant possesses a great potential for *invitro* multiplication. The regenerants

were recovered both directly, i.e., without any intervening callus formation and indirectly through callus. Present study registered that lower concentrations of BAP did not favour any callus formation or shoot regeneration which is in contrast to Dias *et al*. (2002) who achieved shoot multiplication of *Lavandula officinalis* on low concentration of BAP. On increasing BAP concentration to 5µM callus formation was recorded at basal end of the explant which was followed by multiple shoot regeneration. Such an observation is in accordance with Sanchez- Gras and Calvo (1996) who also obtained maximum number of shoots on 5µM BAP. However, in the present attempt slight increase of BAP to 5.5µM and 6µM resulted in direct regeneration and multiplication of shoots surpassing any callus formation. But further increase of BAP concentration (7µM and 8µM) showed no shoot multiplication potential at all which is in contrast to Dronne *et al*. (1999) who observed multiple shoots from leaves of lavandin (*Lavandula* X *intermedia* Emeric ex Loiseleur) on 9µM concentration of BAP. On the other hand Panizza and Tognani (1991) and Nobre (1996) reported no effect of BAP concentrations on shoot multiplication in axillary bud proliferation of nodal cuttings of lavandin and *Lavandula stoechas* respectively which is in contrast to present study.

A report published by Andrade *et al*. (1999) wherein they have used combination of TDZ and BAP for achieving multiple shoots in *Lavandula vera,* is running nearly parallel to our results as TDZ has also cytokinin like activity. Significant increase in shoot number was reported in *Lavandula officinalis* (Quazi, 1980) and in *Lavandula latifolia* (Quazi, 1980; Calvo and Segura, 1989) on media supplemented with a combination of BAP and NAA or IAA. Best response for maximum shoot regeneration for shoot tip explants of *Lavandula officinalis* and subsequent rooting was observed on BAP (2mg/l) + IAA (1mg/l) followed by 1/2 MS+ IBA (1mg/l) (Chisti *et al.,* 2003). However, in present study NAA 2.5µM was used alone which was found to be responsible for inducing rooting of isolated shoots.

Different results observed by many workers may be due to difference in age of the plants used for the culture, physiological conditions of plant, response of different genotypes to *invitro* conditions and varied types of interactions between genotypes and growth medium which has been reported in many plant species (Bajaj, 1986). In present study the optimum concentration of BAP for quick clonal multiplication was found to be 5.5µM and the total time period taken for the plantlet regeneration was 12 weeks after using single subculture at every phase.

Table 1: Effect of different concentrations of cytokinin on shoot apices of *Lavandula officinalis* (The results shown are the mean of three repeated experiments)

Growth media & phytohormones	Response*	%age response	Average No. of shoots per	Length of shoot

			explant ±SD	
MS Basal	Elongation of shoot apex	100	-	
MS(x1/2) Basal	Elongation of shoot apex	100	-	
MS (x1/2) + BAP (0.5µM)	No response	-	-	
MS (x1/2) + BAP (0.7µM)	No response	-	-	
MS (x1/2) + BAP (1µM)	No response	-	-	
MS (x1/2) + BAP (5µM)	Moderate callus at basal end followed by multiple shoot formation through callus.	70	15 ± 1.1	5-10 cm
MS (x1/2) + BAP (5.5µM)	Direct multiple shoot formation, no callus formation	70	35 ± 0.8	4-8 cm
MS (x1/2) + BAP (6µM)	Direct multiple shoot formation, no callus formation	50	12 ±1.2	4-6 cm
MS (x1/2)+ BAP (7µM)	Mild compact callus formation at the basal end, no regeneration.	50	-	
MS (x1/2) + BAP (8µM)	Mild compact callus formation at basal end, no regeneration.	50	-	

* Data scored after 8 weeks of culture period; 10 replicates per treatment

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