Production and Composition of Lavender Plants Through Tissue Culture as Affected with Gamma Irradiation Treatments

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Abstract

The effect of BA and NAA concentrations, as well as gamma irradiation, has been investigated on shoot and root formation of *Lavandula officinalis* callus tissues. The effects of gamma irradiation on essential oil production and the composition of volatile oil have been studied as well.

INTRODUCTION

Lavender (*Lavandula officinalis,* Medicus) is one of the important aromatic plants of the Lamiaceae family. Vegetatively it is propagated with poor rooting capability of stem cuttings. Using tissue culture techniques is one of the most successful methods for producing selected clones with rapid multiplication and improvement of this species (Calvo and Segura, 1988 and 1989; Sodi et al 1989; Panizza et al. 1988 and 1990; Mensuali et al, 1993 and Calvo and Sanchaz, 1993).

Concerning the effect of gamma irradiation on Lavender, Chemarin et al. (1972), obtained 87% rooting when gamma irradiation was applied at 0.25 – 0.5 Krd, while it was decreased by raising irradiation rates to 1, 2, 5 and 10 Krd with an increase in the essential oil yield by 5-20% as compared with the control. Watanabe et al. (1982) obtained the greatest amount of free biotin in green cells of *Lavandula vera*, grown under light or from cells which were irradiated with gamma irradiation.

Concerning the composition of lavender's essential oil, it was found that linalool and linalyl acetate were the major components of 20 identified from oils of *Lavandula officinalis* (Tajuddin et al. 1983); linalool 32%, 1.8 cineol 23.5% and camphor 20% (Proenca et al. 1987).

The activity of the volatile compounds of *Lavandula angustifolia* showed that the essential oil and linalool exhibited coricidal properties, which might be attributed to the linalool content.

MATERIALS AND METHODS

The study was carried out at the Department of Ornamental Horticulture, Faculty of Agriculture, Cairo University during the period from 1995-1997. The objective of this study was to investigate:

A – The multiplication of *Lavandula officinalis* plants, through tissue culture techniques, and its essential oil production andcomposition in callus and intact plants.

B – The effect of gamma irradiation on callus performance and its differentiation.

C – Chemical composition of the essential oil of propagated Lavender plants.

The excised explants (shoot tips, 0.5 cm) were cultured after aseptic sterilization, on previously autoclaved MS medium (Murashige and Skoog 1962), supplemented with different hormones, 3% sucrose and 0.8% agar. The pH was adjusted to 5.7 before autoclaving at 120 °C for 15 min. at 1.5atu.

The following treatments were carried out to study the formation of callus.

I. MS (basic) + BA (0, 0.25, 0.05, 0.1 and 0.5 mg/L).

II. MS (basic) + NAA (0, 0.01, 0.05, 0.1 and 0.5 mg/L).

III. Irradiation treatments were carried out on the obtained callus, Different doses of gamma irradiation (0, 20, 40 and 60 gry) were applied using a Co^{60} source from a unit

Gamma chamber 4000 at the dose rate of 4.3 rad/second.

For callus regeneration, different rates of BA (0, 2, 4, 6, 8 and 10 mg/L) were supplemented to the MS medium. Incubation of the treated shoots occurred at 26 ± 2°C for 16 h photoperiod and for 60 days.

Layout of the Experiment and Statistical Analysis

The experiments were devized with a complete randomized design according to Steel and Torrie (1980). The data were subjected to analysis of variance.

The essential oil of lavender was obtained by distillation, and the composition was investigated using GLC (Pye Unican GCV), with FID. The column was 1.5 m long x 4 mm with 10% pega. Separation was carried out according to temperature program: 70 – 190 °C. with a linear increase rate of 4°C/min. Detector temperature was 300 °C and injection block was 250°C. Chart speed was 0.5 cm/min. The carrier gas was nitrogen at a flow rate of 30 mL/min, while hydrogen was 33 mL/min.

RESULTS AND DISCUSSION

Effect of BA and NAA

Using MS medium containing different combinations of BA and NAA in 25 treatments, affected significantly the shoot numbers produced from callus of *Lavandula officinalis* shoot tips. The greatest number of shoots 4.48/explant followed by 4.12 shoots was obtained from the addition of 1.0 mg/L BA, combined with 0 or 0.01 mg/l NAA. Raising the rate of NAA decreased sharply the number of formed shoots/explant. The different combination affected significantly the shoot number/explant. It is clear that higher levels of NAA inhibited shoot formation.

A higher rate of BA (2.0 mg/L) increased the number of shoots/explant; this means that BA was favourable for the differentiation of shoots/explant, which increased gradually as the concentration was raised to reach its maximum value at 1.0 mg/L and then decreased.

Shoot length was affected similarly to that of shoot number; this means that the longest shoots (3.187 cm) were obtained from the treatment containing 1.0 mg/L BA combined with 0.01 mg/L NAA. These results show that the balance between the used growth regulators is necessary to obtain good results.

Concerning callus size, it was clear that the total percentage of callus was the highest when the rates of BA and NAA were increased, i.e. 2.0 mg/L BA and NAA at 0.01 and 0.05 mg/L.

Effect of BA on Multiplication of Lavender

Using different rates of BA (2, 4, 6, 8 and 10 mg/L) had a significant effect on shoot length. The highest value (1.18 cm) was recorded by using 10 mg/L BA.

The differentiation of callus to shoots was not greatly affected by raising BA rates. The values varied between 60 and 70% of callus, which had been differentiated to shoots.

Effect of Gamma Irradiation

Using different rates of gamma rays, (0, 20, 40 and 60 Gy) affected callus differentiated to shoots (Table E). The high rates of differentiation occurred during the first 4 to 5 weeks of the culture period.

As the rate of gamma rays was raised the percentage of callus + shoots decreased to 10% for 60 Gry, as compared with 40 or 60% for the control at the 7 th or 8 th week of culture.

The highest mean value for shoot formation was 86% followed by 72% and 64% for application treatments of 40, 20, 60 and 0 Gry. The percentages of callus + shoots were 36, 28, 14 and 8% for the rates of 0, 20, 40 and 60 Gry, respectively.

Concerning the growth of shoots, gamma rays had been applied at 0, 10, 20 and 30 Gry. The culture period researched was 8 weeks. The data revealed that the highest mean

value for the number of shoots (4.8) was obtained for the control treatment (0 Gry), which was followed by 2.16, 2.08 and 1.68 for 10, 30 and 20 Gry, respectively. This means that application of gamma rays inhibited the formation of shoots. The different doses affected significantly this character. In the case of shoot length, a similar trend was observed as that reported for the number of shoots.

Root Formation

Half strength MS medium supplemented with different rates of NAA was used to study root formation on callus of lavender (tables 4a, b). The highest mean value for roots (0.91) was recorded for the untreated explants, followed by 0.72 and 0.69 for and 20 Gry.

The values decreased as the concentration of NAA was raised, giving the lowest value at the level of 1.0 mg/L. A similar trend had been obtained for root length.

In relation to root formation of the propagated plantlets, it was found that using 0, 10, 20 and 30 Gry showed an inhibiting effect on root formation of lavender plantlets cultured on half MS supplemented with NAA at 0.0, 0.5 and 1.0 mg/L. The different doses and combinations produced significant differences.

The acclimatization had been carried out under green house conditions using culture media consisting of peatmoss, vermiculite and peatmoss + vermiculite. The control treatment resulted in the highest values of survival (58.3%) and formed 4.0 and 4.4 leaves for culture after 8 and 12 weeks. The application of 20 Gry resulted in 46.7 and 43.3% for 8 and 12 weeks, respectively. The number of leaves was 2.7 and 2.4 at both intervals, while the highest dose (40 Gry) produced the lowest values.

In all cases, the highest values were obtained for plants cultivated in a mixture of peatmoss + vermiculite. The stem length was the greatest for this mixture for all doses.

Essential Oil Production

Using different doses of gamma rays (0, 2 and 4 Krd) for callus showed that application of 4 Krd resulted in the highest percentage of essential oil; 0.5 and 0.6 % after 2 and 3 months of acclimatization, respectively. The low rates resulted in a lower content for older plants. The oil yield was the greatest (2.28%) for the untreated callus, while it was lower for higher doses.

In the case of differentiated plantlets, they were subjected to 0, 1, 2 and 3 Krd. It was found that application of 1 Krd was the most effective in increasing the percentage of essential oil giving 0.7 and 0.8 % for 2 and 3 month old plants. Raising the dose reduced the oil content. The oil yield of the produced plants was highest for the untreated 2 or 3 month old plantlets.

Essential Oil Composition

The linalool content was highest for the control callus, with a mean value of 30.6 %. Within the gamma ray treatments, the obtained values were lower than the control, but the values increased as the doses were raised. While linalool was the major component, 1,8-cineol (eucalyptol) was the second one. The mean values varied from 22.5 % to 33.1 %. The other components, i.e. linalyl acetate, caryophylline and limonene were found in lower amounts.

Concerning the propagation, it was found that 2 months of old plants contained increased linalool from 4.7 to 11.7, 15.3 and 18.0 % for 0, 1, 2 and 3 Krd, whereas, for older plants (3 month), the values decreased as the dose was reduced, resulting in 24.3, 24.1, 17.0 and 13.6% for 0, 1, 2 and 3 Krd, respectively.

Concerning 1,8-cineol, it showed a relatively similar trend to that obtained with linalool.

It might be concluded that gamma irradiation affected markedly the composition of lavender oil.

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