# MICROPROPAGATION OF LAVANDULA SPECIES

JENNIFER L. OLIPHANT

*Cyclone Flora*
*14 Clifton Road*
*Takapuna, Auckland 9*

**Abstract.** A micropropagation method for *Lavandula angustifolia* 'Rosea' is described. Shoot tips and nodes were sterilized and placed on a basic Murashige and Skoog medium with Linsmaier and Skoog vitamins, supplemented with 0.3 mg/l benzylaminopurine (BAP). Under culture conditions of light intensity, 2000 lux; photoperiod, 16 hours; and temperature, 25°C, the shoots elongated and axillary growth formed. After 4 weeks the resulting axillary shoots and nodal sections could be used for further multiplication or as cuttings. The cuttings were rooted on a half strength Murashige and Skoog medium supplemented with 1 mg/l indolebutyric acid (IBA). The rooted plantlets were then gradually acclimatized to the greenhouse environment with a 98% success rate.

## REVIEW OF LITERATURE

A method has been devised to propagate the pink flowering lavender, *Lavandula angustifolia* 'Rosea', to provide adequate stock plants for a specialist herb nursery. The method was also successful with the common lavender, *L. angustifolia*, grown at the Department of Scientific and Industrial Research (D.S.I.R.) Lincoln, New Zealand, for assessment in viability trials for lavender oil production.

The technique involves the multiplication of axillary buds, which extends the more restricted work of Quazi in 1980 (1), and avoids the use of callus for plantlet formation which is not always suitable for clonal propagation.

The new techniques in plant molecular biology use programmed bacteria to pass messages into plants. At the moment these messages are passed mostly by bacterial genes which confer resistance to pesticides or disease in the host plant. It is hoped that in the near future plant genes for quality, or ripening, or oil production, or increased fragrancy will be introduced.

To increase the number of such transformed plants a method of propagation is required which ensures the production of true clonal propagules. The propagation of plants from axillary buds, rather than from adventitious buds or callus, is one such method. It lessens the chance of genotypic changes—or somaclonal variation—thought to be stimulated on proliferating tissue by the culture conditions. Stock plants built up in this way will be more true-to-type and this will be retained in the field plants which will be increased by conventional cutting methods.

The technique described below on the micropropagation of *L. angustifolia* 'Rosea' shows that this is feasible.

## MATERIALS AND METHODS

The *L. angustifolia* 'Rosea' plants were held in an acclimatization area, at a temperature of 25°C under low light conditions of 1000 lux and sprayed with a Benlate-thiram fungicide mixture. The new shoot growth consisting of nodal sections with a shoot tip 1 to 2 cm in length, were used as explant material. Disinfestation was achieved with a wash in 0.6% sodium hypochlorite for 20 mins, followed by three rinses in sterile distilled water, and a final dip in 0.2% sodium hypochlorite before plating.

The basic medium trialed for shoot multiplication, contained full and half-strength Murashige and Skoog minerals (2), with Linsmaier and Skoog vitamins, 30g/l of sucrose, 7g/l of Davis agar, with the pH adjusted to 5.7. The strengths of hormones tested were 0 to 1 mg/l BAP, and 0 to 2 mg/l kinetin combined with 0 to 2 mg/l napthaleneacetic acid (NAA). The culture conditions were temperature, 25°C; photoperiod, 16 hours, and light intensity, 2000 lux.

The medium used for root formation was half strength Murashige and Skoog minerals with Linsmaier and Skoog vitamins, 30g/l sucrose, 7g/l Davis agar, with the pH adjusted to 5.7. The hormones tested were indoleacetic acid (IAA) at 1 to 10 mg/l, NAA at 1 to 10 mg/l and IBA at 1 to 10 mg/l.

## RESULTS

The explants rapidly grew new axillary shoots. These shoots were cut and subcultured onto a number of media and, although there was initially some vitrification and etiolation, this was overcome by the use of a higher light intensity.

The media containing BAP levels of 0.5 to 1.0 mg/l, and kinetin at 0.5 to 2.0 mg/l, or in combination with the auxin, NAA at 0 to 2.0 mg/l, all showed poor shoot growth, and produced callus. Successful axillary shoot production with a 4 to 5 fold multiplication rate was obtained using full strength Murashige and Skoog minerals with 0.3 mg/l BAP, or 0.3 mg/l kinetin. (Table l) The reduction of hormone levels to 0.1 mg/l BAP or 0.1 mg/l kinetin one month prior to rooting enhanced the rooting percentages slightly.

Cuttings from the axillary shoots rooted most successfully on half-strength Murashige and Skoog minerals with 1.0 mg/l IBA. (Table l) The roots were established after 4 weeks and the plantlets transferred to soil.

The rooted shoots were rinsed in tepid water containing the fungicide "Euparen" (dichlofluanid, diluted to 1%). They were planted in pumice-sand without fertilizer, and hardened-off in a greenhouse under mist-spray, with further use of a weak fungicide, e.g. "Ridomil". There was a 98% survival rate. After two months they were potted into 7cm propagation tubes and placed in the shade house.

**Table 1.** Media for the micropropagation of *Lavandula angustifolia* 'Rosea'.

| Murashige and Skoog mineral medium at full strength, supplemented with: | |
|---|---|
| myoinositol | 100 mg/l |
| thiamine HCL | 0.4 mg/l |
| sucrose | 30 g/l |
| Davis agar | 7 g/l |
| pH | 5.7 |
| Shoot multiplication: | |
| BAP | 0.3 mg/l |
| Pre-rooting: | |
| BAP | 0.1 mg/l |
| Root elongation: | |
| Murashige and Skoog mineral medium half-strength, supplemented as above, and IBA 1.0 mg/l | |

## DISCUSSION

The micropropagation technique for *L. angustifolia* 'Rosea' was devised for an initial order of 1000 plants. The successful shoot multiplication rate, root development, and transfer to soil, show this to be a feasible commercial production programme.

The *L. angustifolia* from D.S.I.R., Lincoln, has followed a similar pattern, and plantlets have been transferred to soil. Since the propagation of the lavender plants is from the production of axillary shoots, it would be a practical method for use in a molecular biology programme for the improvement of lavender crop production.

**Acknowledgments** The author wishes to thank Rhiannon Edwards and John Braithwaite of Lifetech Laboratories nursery staff for advice on transfer to soil. Also to Dr. J. D. Ferguson, for assistance with plants, and constructive discussion on the paper.

## LITERATURE CITED

1. Quazi, M. H. 1979. In vitro multiplication of *Lavandula* spp. *Ann. Bot.* 45: 361–362, 1980.

2. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* 15: 473–97.