# Lavender Breeding for Commercial Yield©

**Nigel A.R. Urwin**
School of Animal and Veterinary Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga. NSW 2678
Email: nurwin@csu.edu.au

**The mitotic spindle inhibitor colchicine was used to convert diploid *Lavandula* x *intermedia* 'Grosso' and 'Seal' (lavandins), grown for essential oil production, to tetraploids. Lavandins are infertile hybrids of *L. angustifolia* (lavender) and *L. latifolia* (spike lavender) and colchicine treatment of both in vitro grown and conventionally propagated material restored fertility. Seeds obtained from fertile lavandins produced larger plants with larger floral spikes and enhanced yield of essential oil, relative to the parental cultivars. The plants demonstrated hybrid vigour and measurements of nuclear DNA content, by flow cytometry, suggest that these are likely to be triploid hybrids themselves, resulting from cross-fertilisation of the initial tetraploids by diploid *L. angustifolia*.**

## INTRODUCTION

*Lavandula angustifolia* subsp. *angustifolia* Mill. (lavender), *L. latifolia* Medik. (spike lavender), and hybrids between these two species *L.* ×*intermedia* Emeric ex Loisel. (lavandin) are woody perennials of the family Lamiaceae, which for centuries have been grown for their essential oils. The oils are extracted by steam distillation from flowers. The genus *Lavandula* is comprised of 39 species, the taxonomy of which was recently reviewed by Upson and Andrews (2004). *Lavandula angustifolia* cultivars produce high-value, high-quality oils used in perfumes, soaps, and for aromatherapy. *Lavandula* ×*intermedia* cultivars are generally larger plants with larger flowers yielding up to five times more oil than *L. angustifolia,* however the oils are of lower quality and mainly used in soaps, air fresheners, and toiletries (McGimpsey and Porter, 1999). The majority of oils produced from this genus are extracted from *L.* ×*intermedia* cultivars and an estimated 80% of lavandin oil comes from one 'Grosso'. Both lavenders and lavandins are propagated from cuttings and lavandins exclusively so since they are sterile hybrids and do not produce seed.

Chromosome doubling using colchicine has long been used in plant breeding programs. Resulting polyploid plants often have larger leaves, flowers, fruits, and seed (Hancock, 1997). In an attempt to produce higher oil-yielding lavenders and lavandins we used colchicine to induce polyploidy. We previously produced and characterised autotetraploid *L. angustifolia* (Urwin et al., 2007) and here we report the production and preliminary characterisation of polyploid *L.* ×*intermedia* cultivars and discuss their potential benefits for commercial production of essential oils.

## MATERIALS AND METHODS

For conventional propagation of taxa shoots with at least three nodes were cut and the lower node and stem was dipped in Clonex® rooting hormone gel (Growth Technology, Australia) which contained 3 $mg \cdot ml^{-1}$ indole-3-butyric acid (IBA).Cuttings were placed in seed raising mix on heated misting beds and transferred to standard potting mix when roots were established.

Colchicine at various concentrations in 50% glycerol was applied to the terminal nodes of some whole plants grown in pots in the glasshouse after removal of flowers. For cuttings, at least three nodes were removed from plants. The lower pair of leaves was removed and cuttings were submerged in colchicine solutions of various concentrations for different times. Cuttings were rinsed with distilled water and the end of the stem including the lower node stripped of leaves were dipped in Clonex® rooting hormone gel. Cuttings were planted in seed-raising mix and placed on heated misting beds until roots formed.

All lavandin cultivars were cultured in vitro on Murashige and Skoog (MS) medium containing minimal organics and vitamins, 2% sucrose, and 0.9% agar. The hormone benzylamino purine (BAP) was added to the medium after autoclave sterilisation. The stock hormones were filter sterilised and added to sterile media prior to pouring plates. Tissue was taken from glasshouse-grown plants and surface sterilised by a brief rinse with 70% ethanol followed by 1.25% w/v sodium hypochlorite, 0.005% Tween 20 for 20 min with vigorous agitation. Tissue was rinsed in four changes of sterile distilled water. Nodes were excised with 5 mm of stem tissue either side and most leaf tissue was removed. Nodes were semi-submerged in the agar and cultures were incubated at 25 °C under cool white fluorescent lights on a 16-h light, 8-h dark cycle. Every 6 to 8 weeks nodes were excised from new shoots and subcultured on the same medium.

Nodes were placed on fresh medium 3 days prior to treatment and then placed on fresh medium after treatment. Treatments consisted of submerging nodes in filter sterilised 0.1% colchicine, and 2% dimethylsulphoxide (DMSO) solution for various times (DMSO alone in control treatments), followed by rinsing three times in sterile distilled water.

Cultured shoots and shoots surviving colchicine treatments were excised and placed in half-strength MS medium, 1% sucrose, and 0.9% agar without hormones. After root formation plantlets were acclimatised to glasshouse conditions in their Petri-dishes, firstly in shade and then reducing humidity by slowly removing the lids. Plantlets were washed and placed in moist seed-raising mix. Pots were covered with plastic film and subirrigated for several weeks prior to finally removing the film. Plants were then watered and fertilised as required. Plants were propagated and grown under field conditions in the lavender collection at Charles Sturt University. Each taxon in the collection is designated by a "CSU" prefix and a number, e.g., CSU20 and CSU3 are two different accessions of *L.* ×*intermedia* 'Grosso'.

For relative genome size determination by flow cytometry, nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI). Nuclei were released from 0.5 $cm^2$ of leaf tissue by chopping with a razor blade for 30 sec in 2 ml of modified Galbraith's nuclei isolation buffer (Galbraith et al., 1983) containing 45 mM magnesium chloride, 30 mM trisodium citrate, 20 mM 3-morpholinopropanesulfonic acid (pH 7.3), 0.1% triton X-100, and 4 $\mu g \cdot mL^{-1}$ DAPI. Nuclei were passed through a 30-µm nylon filter and were kept on ice for 10 min prior to analysis using a Quanta 488 flow cytometer (Beckman Coulter, Sydney, Australia).

Essential oils were steam distilled from *L.* ×*intermedia* cultivars using a 50-L-capacity stainless steel still and condenser. Water (4 L) was placed in the bottom of the still, the still and condenser were assembled and the still was heated using a gas burner until distillate emerged. The lid and condenser were removed and up to 1 kg of flower heads was placed in a mesh basket above the level of the water.

The still was assembled again and distillate collected until little or no further oil emerged. The time taken for distillation was approximately 20 min and 600–900 ml of distillate was collected in this time. Oil was collected in separating funnel. Oils were immediately stored in dark glass vials with minimal head space to prevent oxidation. Oils were stored at 8 °C prior to analysis. Gas chromatography (GC) was performed by Dr. Robert Lowe at NSW Department of Primary Industry, Wollongbar Agricultural Institute, Wollongbar.

## RESULTS

To generate polyploid *L.* ×*intermedia* we selected two commonly grown cultivars which are used for oil production. These were 'Grosso' and 'Seal'. Shoot cultures of *L.* ×*intermedia* 'Grosso' and 'Seal' were initiated and maintained using standard MS medium containing the cytokinin BAP at a concentration of 0.5 $mg \cdot L^{-1}$. Brief treatments of nodes, whole plants, or cuttings with colchicine at 0.1% were used to induce polyploidy. Previously, we identified *L. angustifolia* tetraploid plants from seeds treated with colchicine by the presence of larger leaves and flowers (Urwin et al., 2007) however on examination of surviving plants no obvious physical differences were observed between the colchicine treatments and controls so we therefore examined these for production of viable seed. Production of polyploids in other hybrids often results in restoration of fertility and production of seed. We found 'Grosso' and 'Seal' plants which had been treated with colchicine produced seed whereas none of the untreated control plants from any experiment produced seed suggesting that some plants carried tetraploid sports.

In one experiment in which shoot cultures were treated with 0.1% colchicine for 16 h, 5 of 21 'Grosso'-derived plants produced seed with one plant producing 80 seeds. In the same experiment 29 of 72 'Seal' derived plants produced seed with one plant producing 90 seeds. Seeds were also recovered from whole 'Seal' plants which had 0.1% colchicine applied to terminal nodes. Seeds from 'Grosso' and 'Seal' plants were visibly larger than those obtained from tetraploid *L. angustifolia* (Urwin et al., 2007). Ten seeds from colchicines-treated *L.* ×*intermedia* 'Grosso' and ten from 'Seal' plants were germinated in Petri dishes and plants were grown in pots until large enough to be transferred to the field. These were then planted in the lavender collection at Charles Sturt University.

The relative genome size of putative polyploids was determined by flow cytometry using DAPI to stain DNA within leaf nuclei. Leaf material was taken from plants grown in the field. All of the 'Grosso'- and 'Seal'-derived seedlings had genome sizes larger than 'Grosso' and 'Seal' (diploids) but smaller than tetraploid suggesting they were likely to be triploid (Fig. 1). None of these plants produced a single seed and therefore the plants were infertile. The plants were extremely vigorous and produced very large flowering spikes with long thick peduncles, much larger than those of either 'Grosso' or 'Seal' or the parent colchicine-treated plants. Flowering spikes were weighed from four selections, two polyploids derived from 'Seal' and two from 'Grosso', and these were compared to spikes taken from various common *L.* ×*intermedia* and *L. angustifolia* cultivars. The spike weights from the polyploid *L.* ×*intermedia* selections were approximately twice that of most control *L.* ×*intermedia* cultivars (Table 1). The polyploid CSU144 derived from 'Grosso' had a mean spike weight nearly three times that of the parent 'Grosso' and flowers were visibly larger (Fig. 2).

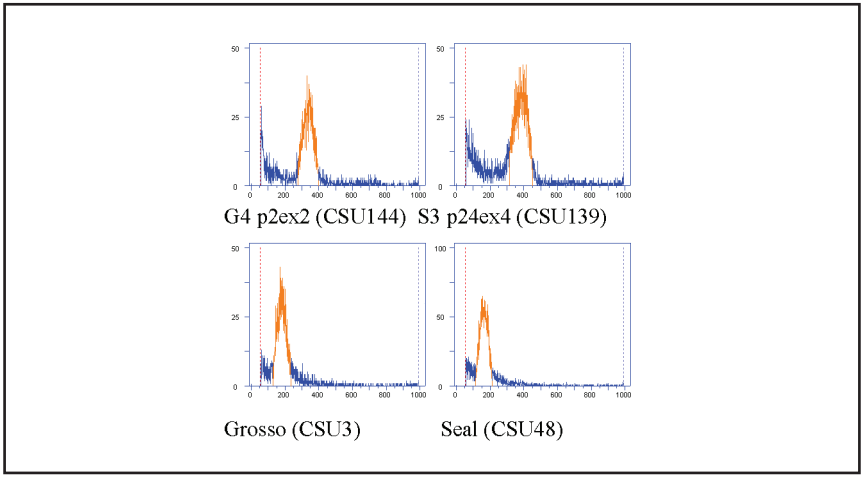


**Figure 1.** Relative genome sizing by flow cytometry of polyploid and diploid *L.* ×*intermedia* taxa. DAPI fluorescence of nuclei (x axis) vs. number of nuclei counted (y axis) in the taxa shown. CSU144 and CSU139 are *L.* ×*intermedia* plants grown from seed obtained from colchicine treated *L.* ×*intermedia* 'Grosso' and 'Seal', respectively

**Table 1.** Average floral spike weight for some diploid and polyploid varieties of *L.* ×*intermedia.* Data shown are the means with +\- SE in brackets and n was between 10 and 15 for all selections. CSU144 and CSU148 are *L.* ×*intermedia* plants grown from seed obtained from colchicine-treated *L.* ×*intermedia* 'Grosso'. CSU139 and CSU153 are *L.*×*intermedia* plants grown from seed obtained from colchicine-treated *L.* ×*intermedia* 'Seal'.

| Taxa | Spike weight (g) |
|---|---|
| G4p2ex2 (CSU144) | 1.66 (1.20–2.12) |
| G7p2ex2 (CSU 148) | 1.56 (1.11–2.01) |
| S3p24ex4 (CSU139) | 1.00 (0.70–1.30) |
| S3p14ex4 (CSU153) | 1.76 (1.27–2.24) |
| 'Abrialii' | 0.93 (0.66–1.19) |
| 'Seal' | 0.91 (0.63–1.18) |
| 'Hidcote Giant' | 0.89 (0.64–1.15) |
| 'Impress Purple' | 0.83 (0.60–1.06) |
| 'Grosso' (CSU 20) | 0.62 (0.44–0.80) |

Two plants from the polyploids were selected on the basis of vigour, plant habit, and flower size for commercial use. These were CSU150 which was a polyploid derived from 'Grosso' and CSU138 a polyploid derived from 'Seal'. These were named *L.* ×*intermedia* 'Riverina Thomas' and 'Riverina Alan', respectively. Preliminary oil yield and quality analysis of oils distilled from these polyploids and others along with three common cultivars of *L.* ×*intermedia,* 'Grosso', 'Abrialii', and 'Impress Purple' suggested oil yield was similar regardless of ploidy level being 14–21 ml·kg$^{-1}$

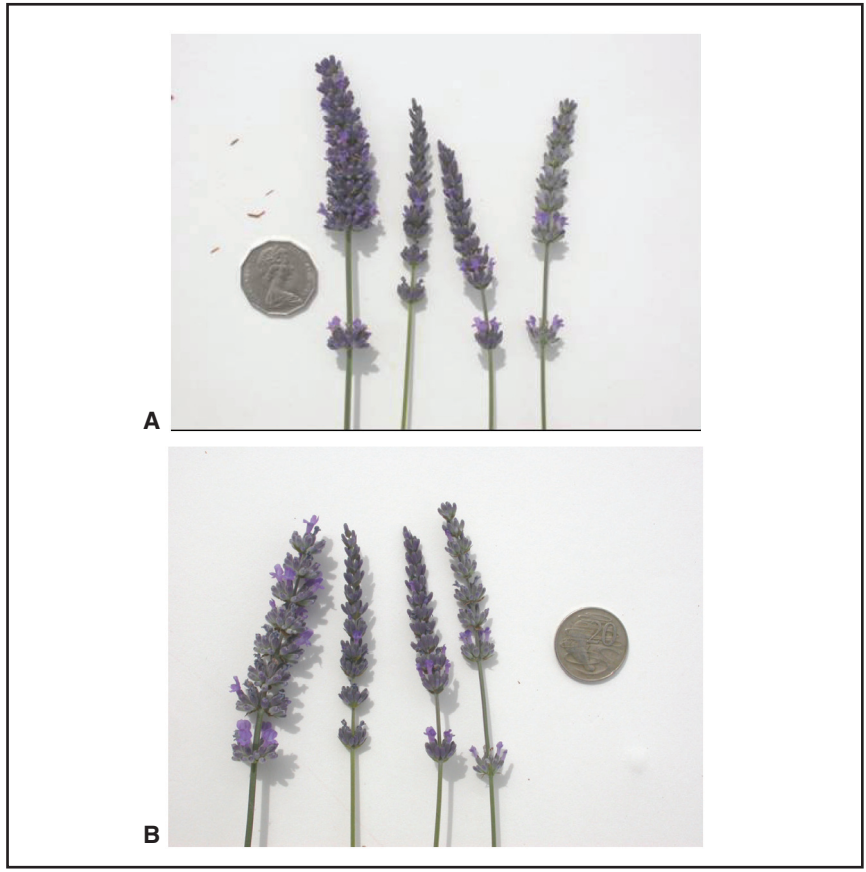


**Figure 2.** Floral spikes from polyploid and diploid *L.* ×*intermedia* taxa. A. Spikes are left to right CSU 144 (polyploid), 'Grosso', 'Abrialii', and 'Seal' (diploids). B. Spikes are left to right CSU153 (polyploid), 'Grosso', 'Abrialii', and 'Seal' (diploids).

(weight of floral tissue). In contrast the yield per plant was approximately 2 to 3-fold greater for polyploids relative to the control cultivars above. In the case of CSU150 compared to 'Grosso' the difference was actually >6-fold although this is based on a single distillation in a single season. The oil yield from that single CSU150 plant was 30 ml compared to 4.8 ml from a single 'Grosso' plant.

Gas chromatographic analysis of the oils distilled from the polyploid selections showed that their composition was similar to other *L.* ×*intermedia* cultivars such as 'Grosso' with minor differences such as a reduction in camphor in CSU138 to levels similar to that of *L. angustifolia* (Table 2).

## DISCUSSION

*Lavandula* ×*intermedia* cultivars are sterile however a single report in the literature by Vinot and Bouscary (1971) reported a fertile lavandin that spontaneously occurred in the field. Chromosome estimates in the sterile lavandin and in

**Table 2.** Composition of essential oils distilled from *L.* ×*intermedia* 'Grosso' and two polyploid selections. Compositions of international standards are labelled ISO and *L. angustifolia* oil standard is included for comparison.

| Component | ISO | ISO | CSU20 | CSU150 | CSU138 |
|---|---|---|---|---|---|
| | *L. angustifolia* French | *L.* ×*intermedia* 'Grosso' | *L.* ×*intermedia* 'Grosso' | *L.* ×*intermedia* 'Riverina Thomas' | *L.* ×*intermedia* 'Riverina Alan' |
| limonene | 0.0–0.5 | 0.0–1.5 | 1.2 | 1.4 | 0.5 |
| β-phellandrene | 0.0–0.5 | | 0.1 | 0.2 | 0.4 |
| cis-β-ocimene | 0.0–10.0 | 0.5–1.5 | 1.0 | 1.4 | 2.9 |
| 3-octanone | 0.0–0.2 | | 0.1 | 0.4 | 0.4 |
| 1,8 cineole | 0.0–1.0 | 4.0–7.0 | 11.1 | 8.8 | 13.2 |
| trans-β-ocimene | 1.5–6.0 | 0.0–1.0 | 0.3 | 0.2 | 3.8 |
| linalool | 25–38 | 24–35 | 23.3 | 34.5 | 40.1 |
| camphor | 0.0–0.5 | 6.0–8.0 | 8.6 | 8.0 | 0.5 |
| terpinen-4-ol | 2.0–6.0 | 1.5–5.0 | 1.9 | 4.8 | 4.1 |
| lavandulol | >0.3 | 0.2–0.8 | 0.7 | 0.7 | 1.3 |
| α-terpineol | 0.0–1.0 | | 0.7 | 0.5 | 0.6 |
| linalyl acetate | 25–45 | 28–38 | 25.7 | 21.9 | 14.4 |
| lavandulyl acetate | >2.0 | 1.5–3.0 | 3.9 | 1.8 | 2.1 |

the fertile derivative were 50 and 100 respectively suggesting polyploidy can occur naturally at low frequency in the wild within this genus. The fertile lavandin is no longer in cultivation or in any collection.

In our study treatment of the lavandins 'Grosso' and 'Seal' with colchicine likely resulted in plants with fertile, tetraploid sports from which we obtained seed. These initial putative tetraploids are being propagated and investigated by flow cytometry to determine actual ploidy level. Preliminary data suggest that they are chimeric with respect to ploidy level between sports but also between cell types within tissues (data not shown) and this might explain the absence of phenotypic differences between these plants and the parental taxa. In contrast plants resulting from seeds collected from the putative tetraploids were much more vigorous and have larger flowers than the parents 'Grosso' and 'Seal'. The increase in yield of oil observed from these new cultivars appears to be due to the increased size of these plants rather than increased yield of oil per flower. Measurements of DNA content of nuclei by flow cytometry revealed that these plants are likely to be triploid, which was also suggested by their sterility since triploid cells cannot easily undergo meiosis. Consequently, it is suggested that these plants resulted from fertilisation of tetraploid 'Grosso' and 'Seal' plants by pollen from *L. angustifolia* cultivars grown close by in the collection. This observation also suggests the fertile lavandins were largely self incompatible. If this is the case then the improved hybrid vigour observed would be expected, since these new *L.* ×*intermedia* selections would have a genome ratio of 2 : 1 for *L. angustifolia* and *L. latifolia* whereas other lavandins have a genome ratio of 1 : 1 for the two species. It is hoped that these new higher yielding selections will be adopted by the Australian lavender industry.

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