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BRIEF COMMUNICATION

Micropropagation of *Lavandula dentata* from axillary buds of field-grown adult plants

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Abstract

Axillary buds from adult field-grown plants of *Lavandula dentata* L. were used to evaluate the effect of growth regulators and culture media on the *in vitro* shoot proliferation and growth. The highest multiplication rate was obtained using Murashige and Skoog (MS) medium supplemented with a combination of 2.2 μM of benzyladenine and 2.5 μM indole-3-butyric acid. The best condition for rooting was MS medium plus 2.5 μM naphthaleneacetic acid. Rooted plantlets were successfully transferred to soil. Short-term culture derived plants (6 month) exhibited a normal development, but a low frequency of not heritable morphological changes were detected in long term culture derived plants (more than 1 year).

Additional key words: lavender, *in vitro* propagation, bud breaking.

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Lavandula species are important ornamental, melliferous and essential oil producing plants. *Lavandula dentata* L. is a Mediterranean highly aromatic shrub species with peculiar dentate leaves and violet spikes. *L. dentata*, as most species of the genera, is an important essential oil producing plant characterized by high contents of 1,8-cineole, fenchol, borneol and camphor (Sudriá *et al.* 1999).

Lavandula species can be vegetatively propagated by woody stem cuttings, but their poor rooting and the risks of modifications induced by repeated vegetative propagation, restrains its application (Moutet 1980, Panizza and Tognoni 1992). Micropropagation may overcome these limitations, allowing the rapid multiplication of selected genotypes. Several species of lavender have been used, like *L. dentata* (Jordan *et al.* 1998, Sudriá 1999, 2001), *L. angustifolia* (Quazi 1980, Segura and Calvo 1991, Andrade *et al.*1999), *L. latifolia* and *L. officinalis* × *L. latifolia* (Panizza and Tognoni 1992, Sánchez-Gras and Calvo 1996), and *L. stoechas* (Nobre 1996). In the present study we evaluate the effect of different culture media and plant growth regulators on shoot proliferation and rooting of *L. dentata* and propose a system for the efficient micropropagation of this species.

Nodal segments measuring 2 to 3 cm in length were excised from 5 year-old plants of *Lavandula dentata* L. cv. Goodwin Creek (*Floricultura Úrsula*, Nova Petrópolis, RS, Brazil) between September and December, 2001, disinfected first in 70 % ethanol for 30 s and then in a 1 g dm^{-3} sodium hypochloride solution containing 0.01 % (v/v) *Tween-20* for 20 min. After rinsing with sterile distilled water, the axillary buds were dissected and implanted vertically onto the culture media. Cultures were maintained at 25 ± 2 °C with 16-h photoperiod with irradiance of 10 - 20 μmol m^{-2} s^{-1}.

The culture media evaluated in this work consist of the salt and vitamin solutions of Murashige and Skoog

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Abbreviations: BA - 6-benzyladenine, 2iP - isopentenyladenine, AS - adenine sulfate, KIN - kinetin, TDZ - thidiazuron, IBA - indole-3-butyric acid, NAA - α-naphtaleneacetic acid, MS - Murashige and Skoog (1962) medium, B5 - Gamborg *et al.* (1968) medium, NN - Nitch and Nitch (1969) medium, LS - Linsmaier and Skoog (1965) medium, QL - Quoirin and Lepoivre (1977) medium.
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(1961; MS), Linsmaier and Skoog (1965; LS), Gamborg *et al.* (1968; B5), Nitch and Nitch (1969; NN), and Quoirin and Lepoivre (1977; QL), with 0.4 mg dm^{-3} thiamine, 100 mg dm^{-3} inositol, 3 % sucrose, and 0.6 % agar (*Merck*, Darmstadt, Germany).

The effect of growth regulators on micropropagation was evaluated on MS basal medium supplemented with 2.2, 4.4 and 8.8 μM of adenine sulfate (AS), benzyladenine (BA), kinetin (KIN), 2-isopentenyladenine (2iP), and thidiazuron (TDZ). Different concentrations of BA (0 to 8.8 μM) alone or in combination with indolebutyric acid (IBA) and naphthaleneacetic acid (NAA) were also tested. The effect of auxin (IBA and NAA) concentration on rooting was evaluated.

The experimental designs were fully randomized with three replicates of 20 explants per treatment. Data were analyzed statistically by analysis of variance (*ANOVA*) followed by the Tukey test, with the level of significance set at 5 %.

Rooted plantlets were washed in tap-water and transferred to plastic chambers containing a sterilized mixture of sand and soil (1:1), covered with a plastic cap that was gradually opened during the acclimatization period of 20 d. Acclimatized plants were transferred to the greenhouse and then to outdoor conditions during spring.

Initially we evaluated the effect of five culture media supplemented with 2.2 μM BA on the multiplication and growth of *L. dentata*. The results showed that MS, B5, LS and NN media support a similar multiplication rate (10.2 to 11.8 shoots/explant), whereas the number of shoots per explant on QL medium was significantly lower (4.6 shoots/explant). Shoot length was significantly higher on MS and LS media. Based on these results MS medium was used in subsequent experiments.

In order to test whether different growth regulators influence the multiplication of *Lavandula dentata*, axillary buds were inoculated on MS medium supplemented with different concentrations of AS, BA, KIN, 2-iP, and TDZ. The media supplemented with 2.2 to 4.4 μM BA and 2.2 μM TDZ were the most effective in promoting shoot development. However, TDZ grown plantlets were stunted with strong hyperhydricity. BA has previously proved to be efficient for the multiplication of *L. dentata* (Jordan *et al.* 1998), and other *Lavandula* species (Calvo and Segura 1989, Panizza and Tognoni 1992, Nobre 1996, Andrade *et al.* 1999). Further tests showed that shoot multiplication was dependent on BA concentration (Table 1). The highest multiplication rates were achieved on MS media supplemented with 2.2 to 8.8 μM BA. However, under high concentrations of BA (8.8 μM) shoots were stunted due to hyperhydricity. Hyperhydricity was also a limiting factor in the propagation of *L. stoekas* (Nobre 1996) and *L. vera* (Andrade *et al.* 1999).

The combinations of BA and auxins (NAA and IBA) were also tested (Table 1). In general, the addition of NAA into the multiplication media resulted in a reduction of the shoot number per explant with no significant changes in shoot length. Conversely, the addition of IBA (1.0 and 2.5 μM) increased shoot multiplication and elongation. However, as was previously observed in *L. vera* (Andrade *et al.* 1999), hyperhydricity was not overcome by the addition of auxins. The best results were obtained in a combination of 2.2 μM BA and 2.5 μM IBA. The present results corroborate those reported for *L. vera* (Quazi 1980) and *L. latifolia* (Quazi 1980, Calvo and Segura 1989).

Table 1. Effect of different BA concentration, alone or in combination with NAA or IBA on *L. dentata* shoot proliferation (Mean ± SE, n = 60). Within each column, values followed by the same letter are not significantly different at the 5 % level according to Tukey's test. n - no callus production, p - callus only being formed at the base of stem explant.

Cytokinin + auxin [μM]	Shoot length [cm]	Number of shoots [$explant^{-1}$]	Callus
0.0 BA	2.08 ± 0.10^{bc}	1.10 ± 0.32^{e}	n
0.4 BA	2.22 ± 0.15^{bc}	2.46 ± 0.37^{e}	n
0.9 BA	2.31 ± 0.12^{bc}	2.58 ± 0.65^{e}	n
2.2 BA	2.45 ± 0.18^{bc}	12.84 ± 1.58^{bc}	n
4.4 BA	2.11 ± 0.12^{bc}	12.63 ± 1.02^{bc}	n
8.8 BA	1.86 ± 0.07^{c}	8.92 ± 0.45^{d}	p
2.2 BA + 1.1 NAA	2.05 ± 0.15^{bc}	10.72 ± 1.40^{cd}	n
2.2 BA + 2.7 NAA	1.79 ± 0.16^{c}	7.80 ± 0.88^{d}	p
4.4 BA + 1.1 NAA	2.16 ± 0.18^{bc}	9.20 ± 0.82^{cd}	p
4.4 BA + 2.7 NAA	2.16 ± 0.18^{bc}	8.81 ± 0.97^{d}	p
2.2 BA + 1.0 IBA	2.79 ± 0.26^{b}	14.02 ± 0.97^{b}	n
2.2 BA + 2.5 IBA	3.53 ± 0.17^{a}	18.60 ± 1.13^{a}	n
2.2 BA + 5.0 IBA	2.65 ± 0.15^{b}	9.62 ± 1.20^{cd}	p
4.4 BA + 1.0 IBA	2.52 ± 0.15^{bc}	13.60 ± 1.11^{bc}	n
4.4 BA + 2.5 IBA	2.62 ± 0.16^{b}	14.83 ± 1.05^{b}	n
4.4 BA + 5.0 IBA	2.51 ± 0.15^{bc}	12.20 ± 1.21^{bc}	n

Rooting of 2.2 μM BA grown plantlets was easily achieved after 15 d on MS hormone-free medium. In this condition rooting percentage was 100 %. Similar results were reported in several *Lavandula* species, including *L. dentata* (Jordan *et al.* 1998). However, the addition of NAA significantly increased the number of roots per explant, as well as root and shoot length (Table 2). The best results were obtained on MS medium supplemented with 2.5 μM NAA. The efficiency of NAA in root induction was previously reported for lavandins (Panizza and Tognoni 1992), *L. stoechas* (Nobre 1996), and *L. vera* (Andrade *et al.* 1999). Rooted plantlets were acclimatized well first to the greenhouse and then to outdoor conditions. The establishment of micro-propagated plants occurred at a high rate (87 %). All the plants transferred to the field (565 plants) were

Table 2. Effect of auxin concentration on plant growth and rooting rates of micropropagated plantlets of *L. dentata* (Mean ± SE, $n = 60$). Within each column, values followed by the same letter are not significantly different according to Tukey's test. $P = 0.05$.

Auxin [μM]	Shoot length [cm]	No. of shoots [plantlet^{-1}]	No. of roots [plantlet^{-1}]	Root length [cm]
0.0	3.12 ± 0.19^{c}	1.20 ± 0.15^{b}	9.85 ± 0.92^{c}	3.64 ± 0.33^{b}
0.5 IBA	3.91 ± 0.27^{bc}	1.10 ± 0.10^{b}	10.45 ± 1.27^{c}	4.02 ± 0.41^{ab}
1.0 IBA	3.72 ± 0.20^{c}	1.00 ± 0.00^{b}	10.05 ± 0.94^{c}	3.58 ± 0.21^{b}
2.5 IBA	3.94 ± 0.34^{bc}	1.00 ± 0.00^{b}	14.33 ± 1.79^{c}	4.07 ± 0.50^{ab}
5.0 IBA	3.41 ± 0.25^{c}	1.90 ± 0.17^{a}	14.85 ± 1.52^{c}	3.97 ± 0.63^{ab}
0.5 NAA	4.19 ± 0.24^{bc}	1.00 ± 0.00^{b}	13.55 ± 1.12^{c}	4.47 ± 0.27^{ab}
1.0 NAA	4.27 ± 0.38^{abc}	1.15 ± 0.15^{b}	12.25 ± 1.06^{c}	4.33 ± 0.41^{ab}
2.5 NAA	5.35 ± 0.31^{a}	1.05 ± 0.05^{b}	25.42 ± 1.54^{b}	5.09 ± 0.19^{a}
5.0 NAA	5.09 ± 0.35^{ab}	1.00 ± 0.00^{b}	32.15 ± 3.02^{a}	4.78 ± 0.42^{ab}

grown until maturity. No morphological changes were detected in plants derived from short term *in vitro* cultures (6 month). However, several plants (>1 %) derived form long term cultures (more than one year) exhibited some non-heritable morphological changes. The most notorious changes were shoot fasciation, and an increase in the number of axillary buds per node and flowers per spike. These morphological anomalies can be attributed to a residual effect of benzyladenine, a growth regulator that has been associated with shoot, leaf, stem and flower changes during the micropropagation of several species (George 1996), and that affects the formation of secretory glands and the essential oil production of micropropagated *L. dentata* plantlets (Sudriá *et al.* 1999, 2001). As these alterations were not longer observed in new shoots formed during greenhouse and field culture, they did not compromise plant development, flowering and production.

The present results show that micropropagation though axillary budding is a reliable method for the rapid multiplication of *L. dentata*, allowing for the selection and propagation of elite clones of this important ornamental and aromatic plant. In fact, this protocol has been used to transfer more than 12 000 plants to agriculturists of the northeast region of Rio Grande do Sul State, Brazil.

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