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Trichomes Morphology and Essential Oils Characterization of Field-Growing and *In Vitro* Propagated Plants of *Lavandula pedunculata*

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The selection of native *Lavandula* species and their economic exploitation have increased in the last few years. Micropropagation techniques have been used as an alternative for vegetative propagation allowing the multiplication of selected genotypes and chemotypes. Our previous studies showed that the essential oils of *Lavandula pedunculata* have an important antifungal activity against dermatophyte strains [1]. Therefore, a new line of investigation concerning the *in vitro* culture of this species is justified. In the present study we compare the morphology of the leaf trichomes and the chemical composition of their essential oils in both field-growing and *in vitro* propagated plants.

In vitro cultures of axillary buds of *L. pedunculata* were established in Murashige and Skoog medium (MS) supplemented with 0.25mg/L BA (benzyladenine) and 10mg/L ascorbic acid. Leaf trichomes were examined using scanning electron microscopy (SEM). The essential oils from both the aerial parts of field-growing plants and the rooted plantlets kept *in vitro* during 1 month were isolated by hydrodistillation and analysed by gas chromatography and gas chromatography/mass spectroscopy.

In vitro cultures (Fig. 1) were established with a success of 84.5%. After 18 days in culture, an average of 4.07 ± 0.35 shoots per explant with more than 0.5 cm in length were obtained. Shoots were then separated and transferred to MS medium. After a month in culture an increase of 1.91 ± 0.20 cm was observed, with an average number of 5.04 ± 0.52 nodes per plantlet. In this medium, 62.8% of the shoots rooted spontaneously avoiding the use of another medium and allowing a considerable gain in time of the regeneration process. SEM examinations showed that both the *in vivo* and *in vitro* samples presented three types of glandular trichomes: peltate (Fig. 2A), capitate type I (Fig. 2B), and capitate type II(Fig.2C) and a "mixed" type of trichomes (Fig.2D) with characteristics of both glandular and non-glandular ones. Nevertheless, the *in vitro* samples showed fewer glandular trichomes and the non-glandular ones were smaller and in lower number (cf. Fig 2F and 2G). The essential oils of the *in vitro* plantlets consisted of the same main components as the original field-growing parent (fenchone, 1,8-cineole, and camphor) and presented no remarkable compositional variations (Table 1).

Our study shows that the essential oils of *in vitro* propagated plantlets of *L. pedunculata* have a similar composition to those isolated from field-growing plants. Therefore, micropropagation through axillary shoot proliferation is a reliable method for the rapid multiplication of this species allowing essential oil production in any time of the year. This will be of value in plant conservation without damage of the natural resources.

Reference

[1] M. Zuzarte, A.M. Dinis, C. Cavaleiro, J. Canhoto, M.J. Gonçalves, L. Salgueiro. 2007, II Encontro Nacional de Pós-graduação em Ciências Biológicas, Porto, pp 37.

Fig. 1. *In vitro* plantlet of *L. pedunculata* after 18 days in culture.

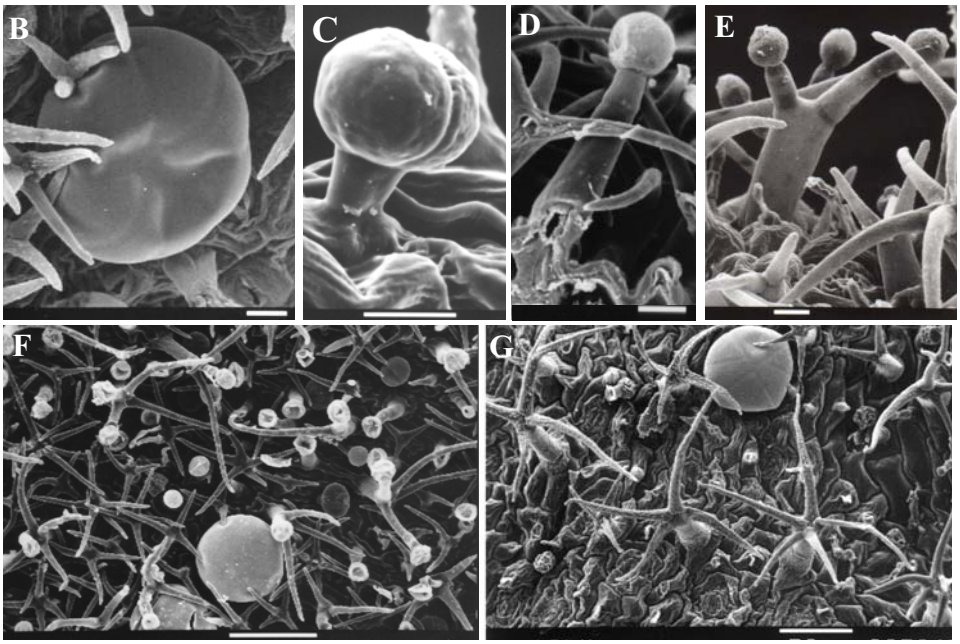

Fig. 2. SEM micrographs of *L. pedunculata* leaf trichomes. A. Peltate trichome. B. Capitate type I trichome C. Capitate type II trichome. D.“Mixed” type trichome. Scale bars = 10 µm F. Leaf adaxial surface of a field-growing plant. G. Leaf adaxial surface of an *in vitro* plantlet. Scale bars = 50 µm

TABLE 1. Essential oil composition (%) of *L. pedunculata* isolated from a field-growing plant and the respective *in vitro* shoot cultures.

Compounds	**Field-growing**	***In vitro***
α-pinene	8.6	7.4
camphene	3	1.9
ß-pinene	1.8	7.1
limonene	3	5
1,8-cineole	**9.8**	**7.1**
fenchone	**48.6**	**34**
linalol	1.8	3.5
α-fenchol	1.2	3
camphor	**14.1**	**7.2**
cis-verbenol	0.7	2.1
trans-verbenol	0.4	1.1
borneol	1.3	t
terpineol-4	0.7	t
myrtenal	0.7	t
bornyl acetate	1.7	t
α-cadinol	0.5	t
Monoterpene hydrocarbons	16.4	21.4
Oxygen-containing monoterpenes	81	58
Oxygen-containing sesquiterpenes	0.5	t

t – traces (<0.05%)

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