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Etherio, a new variety of *Lavandula angustifolia* with improved essential oil production and composition from natural selected genotypes *growing* in Greece

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ABSTRACT

Lavender oil is a popular essential oil which unfortunately is not produced in any Greek region due to problems reported before from insufficient lavender cultivars or varieties used. The aim of the present study was to create a synthetic *Lavandula angustifolia* variety from native Greek plants which however present very well adaptation in local fields. Thirty native plants of *L. angustifolia* from 10 different habitats were quantified and qualified for essential oil. The best two plants of each population were cross pollinated and the seeds came out from this pollination cultivated until new plants test again for oil quantity and quality. This cycle process was being repeated for 6 years until it resulted in a final plant genotype with high amount of essential oil. The essential oil yield reached under laboratory extraction 2.6% (w/fw) while under field steam distillation was 2.3%. The name of this synthetic variety is *L. angustifolia* var. *etherio* and the major essential oil compounds are linalool 26.9% and linalyl acetate with 22.8%. *L. angustifolia* var. *etherio* was reproduced by tissue culture and 2 ha of this variety are cultivated for 6 years now. The variety showed high transplantation degree, high adaptation, plant viability, rich flower production and high essential oil yield.

1. Introduction

The genus *Lavandula* contains many different species which belong to the 'Labiatae' family that geographically grown in Mediterranean countries (Baytop, 1984). Labiatae are generally known for their multiple pharmacological effects such as anticonvulsant, sedative, antispasmodic, analgesic, antioxidant, local anaesthetic activity and it has been used for medicinal purposes (Lis-Balchin and Hart, 1997; Ghelardini et al., 1999; Hosseinzadeh et al., 2000; Kovatcheva et al., 2001).

Essential oils obtained from aromatic plants, are complex mixtures of several chemical compounds including terpenes, alcohols, aldehydes and phenols. Lavender oil, obtained from the flowers of *Lavandula angustifolia*, is composed mainly of linalyl acetate, linalool, lavandulol, 1,8-cineole, lavandulyl acetate and camphor (Lis-Balchin and Hart, 1999). Because of its delightful odour, lavender is one of the most useful medicinal plants and has found wide application in perfumes and cosmetics, colognes, skin lotions and other cosmetics (Paul et al., 2004). In food manufacturing, lavender essential oil is employed in flavouring beverages, ice-cream, candy, baked goods, and chewing gums (Kim and Lee, 2002). Recently, aromatherapy is becoming increasingly popular, and lavender is used in aromatherapy as a relaxant (Valnet, 1986; Hohmann et al., 1999; Lis-Balchin and Hart, 1999). Several therapeutic effects of lavender, such as sedative, relaxant, carminative spasmolytic, antioxidant, antiviral and antibacterial activities as well as several gastrointestinal nervous and rheumatic disorders have been reported (Duke, 1989; Gamez et al., 1990; Leung and Foster, 1996; Cavanagh and Wilkinson, 2002). An *in vitro* cytotoxic activity of lavender oil and its main components linalyl acetate and linalool on human skin cells has been reported by Prashar et al. (2004).

The world demand for lavender essential oil is still increasing. It is estimated that over 200,000 ha are being cultivated in Europe and the quality of produced essential oil is important especially for medicinal, pharmaceutical and aromatherapy uses. Greece is a Mediterranean country with favourable climatic and edaphic conditions for aromatic and medicinal plants. *L. angustifolia* and *Lavandula stoechas* are grown naturally on calcareous substrates, slight well drainage slopes in altitudes ranged from 100 to 800 m. Despite of growing interest and the commercial importance of lavender, farmers are not interested to imply in this production due to problems raised from unsatisfied adaptation and low productivity presented by commercial varieties of *Lavandula*. The aims of the present study were: first to find native populations of *L. angustifolia* in Greek region; second, to evaluate these populations for essential oil quantity and quality; third, to pollinate the most valuable

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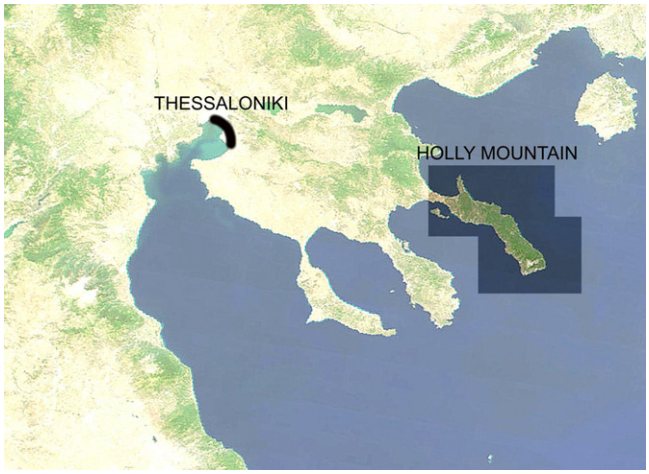

Fig. 1. The Holly Mountain where native plants of *Lavandula angustifolia* were selected.

plants in order to create a synthetic variety and finally to cultivate this plant in order to assess the adaptation of the final genotype, the essential oil productivity and quality and to collect field data useful for farmers, cosmetic and pharmaceutical industry.

2. Materials and methods

2.1. Area of the investigation and plant collection

Native plants of *L. angustifolia* Mill. were selected from the Peninsula of Holly Mountain in Greece which is regarded as one of the less disturbed area in the country (40°16′40 N, 24°11′53 E, Fig. 1). Ten populations of *L. angustifolia* growing in an altitude ranging from 330 up to 710 m above sea level were used. Thirty plants from each population were marked and assessed for their essential oil content. The extraction of the oil in the field was achieved by organic solvent extraction and followed by hydro distillation in the selected plants.

In the solvent extraction the plant material was put into a plastic pipette tip and was kept by non-absorbent cotton from both sides. The cotton on the bottom filters the solvent and regulates the flow through the plant material thus controlling the time of extraction. The cotton on the top prevents accidental losses of the plant material when applying the solvent. The extraction medium was diethyl ether with 10^{-4} molar (M) of n-tetradecane to act as an internal standard for any losses. Approximately 2 g of lavender flowers was put into the capsule after a precise weighting and covered by the top cotton. Five millilitres of solvent (diethyl ether) was added on the top and left to flow through the plant sample, percolating the essential oil. The washing lasts about 15 min and three successive washings were taken. At the end of each washing an elastic tube was fitted on the open top, which was connected with an air pump. The air pressure breaks the structures which are resistant to the solvent treatment. The dilution was transferred and left at room temperature for evaporation (in a fume cupboard) and the essential oil was measured. This technique is fast, reliable, efficient and no further filtering is needed. After essential oil quantification the two plants of each population (in total 20), with the highest amount of essential oil, were further marked and their essential oils subjected to GC–MS analysis for oil qualification.

2.2. Plant isolation and handmade cross-pollination

L. angustifolia is an insect pollinated plant. The selected plants were isolated before the pollination period. This was achieved by

Fig. 2. Similar metallic cylinder with that used for plant isolation and cross-pollination.

using a metal cylinder (60 cm diameter and 80 cm high) constructed by permanent galvanised mess (5 cm × 10 cm). Up to 50 cm the cylinder was covered around by thin colourless plastic and the rest by nylon mess (5 mm × 5 mm) in order to prevent any insect penetration to selected plant (Fig. 2). *L. angustifolia* is a perennial shrub and has about 100–150 branches. In the top 20 plants before the pollination period the most of the branches were removed leaving only forty of those in every plant. During the pollination period, 20 flower branches from each selected plant was cut and put in 20 different trays as shown in Fig. 3. Each tray had one branch from all selected plants in total 20 branches (These 20 trays gave 20 buckets which were used for hand made cross-pollination). Dur-

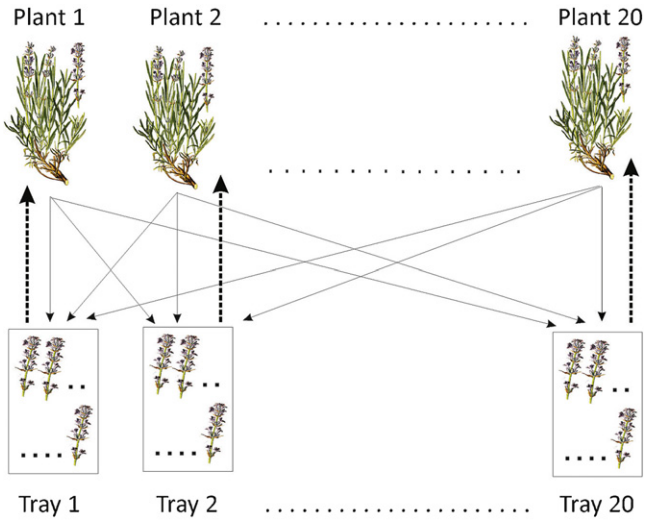

Fig. 3. Strategy which was followed for hand made cross-pollination between the 20 lavender plants with the highest amount of essential oil.