Mycorrhiza (2006) 16:443–446
DOI 10.1007/s00572-006-0062-9

SHORT NOTE

Arbuscular mycorrhiza alter the concentration of essential oils in oregano (*Origanum* sp., Lamiaceae)

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Received: 25 January 2006 / Accepted: 14 June 2006 / Published online: 15 August 2006

Abstract The effect of root colonization by *Glomus mosseae* on the qualitative and quantitative pattern of essential oils (EO) was determined in three oregano genotypes (*Origanum* sp.). To exclude a simple P-mediated effect through mycorrhization the effect of P application to plants on the EO accumulation was also tested. In two genotypes the leaf biomass was increased through mycorrhization. Root colonization by the arbuscular mycorrhizal fungus (AMF) did not have any significant effect on the EO composition in oregano; however, in two genotypes the EO concentration significantly increased. As EO levels in P-treated plants were not enhanced, we conclude that the EO increase observed in mycorrhizal oregano plants is not due to an improved P status in mycorrhizal plants, but depends directly on the AMF–oregano plant association.

Keywords Arbuscular mycorrhiza · Glomeromycota · Essential oil · *Origanum vulgare* · Oregano

Introduction

Essential oils (EO) are volatile, lipophilic mixtures of secondary plant compounds, mostly consisting of monoterpenes, sesquiterpenes, and phenylpropanoids. Besides their many ecological functions in plants (Harborne and Tomas-Barberan 1991; Harrewijn et al. 2001) they are used as flavors and fragrances, as antimicrobials and antioxidants, and as medicines (Deans and Waterman 1993). EO compounds are formed by all plants but only those species able to store them in larger quantities are used as EO crops.

In large-scale production of plants containing EOs, even relatively small increases of their EO content can result in an enhanced product yield of economic interest. Thus, any agricultural practice or treatment increasing EO levels is of practical interest.

During the establishment of the arbuscular mycorrhizal (AM) symbiosis, a range of chemical and biological parameters is affected in plants, including the pattern of secondary plant compounds. The accumulation of flavonoids (Harrison and Dixon 1993; Morandi 1996; Vierheilig et al. 1998a; Larose et al. 2002), cyclohexanone derivatives and apocarotenoids (Fester et al. 2002; Maier et al. 1995; Vierheilig et al. 2000a,b), phytoalexins (Sundaresan et al. 1993; Yao et al. 2003), phenolic compounds (Devi and Reddy 2002; Grandmaison et al. 1993), triterpenoids (Akiyama and Hayashi 2002), and glucosinolates (Vierheilig et al. 2000c) in plants colonized by AM fungi (AMF) has been reported.

Recently, Adams et al. (2004) observed that EO levels in vetiver roots are modulated in the presence of nonidentified bacteria and fungi, and AMF were suggested to be involved in the altered EO accumulation. In studies on *Ocimum basilicum* (Copetta et al. 2006) and *Mentha arvensis* (Freitas et al. 2004), it was shown that AM fungal root colonization increases the EO content and in *O. basilicum* alterations of the EO composition have been reported (Copetta et al. 2006).

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The objective of the present study was to evaluate the effect of mycorrhization by *Glomus mosseae* on the qualitative and quantitative accumulation patterns of EOs in oregano (*Origanum* sp., Lamiaceae), an important Mediterranean aromatic plant with new applications in food and feed based on its antimicrobial and antioxidant properties (Kintzios 2002). To exclude a simple P-mediated effect through mycorrhization, we also tested the effect of P application on the EO accumulation pattern.

Materials and methods

Biological materials and growth conditions

Three genotypes of *Origanum* were grown in the greenhouse: *Origanum vulgare* ssp. *hirtum* var. Kalitera and two breeding strains of *O. vulgare* ("Cona" and "b13/2"). A single plant of each genotype was chosen and cuttings were prepared from this mother plant to reduce genetic variability within the genotypes in the experiment because *Origanum* populations are known to be heterogeneous. The rooted cuttings were transplanted into small pots (7×7 cm) containing a commercial soil substrate [Frux ED63: 75% peat; clay (traces); pH ($CaCl_2$) 5.5–6.5; N 200–350 mg/l; P_2O_5 200–350 mg/l; and K_2O 300–500 mg/l]. After 2 months, plant roots were washed and root samples (about 30 root pieces with a length of 1–2 cm taken randomly from the root system) were taken to verify whether plants were AM-free (see "Determination of root colonization").

Plants were then transferred to 300-ml pots (one plant per pot) into a mixture (autoclaved for 20 min at 121°C; 1:1:1, by vol.) of sand:expanded clay:soil. Plants were planted into a hole in the substrate where the inoculum (5 g per plant) had been previously added. The inoculum consisted of colonized root pieces of beans (*Phaseolus vulgaris* L. cv. Sun Gold), sporocarps, spores, and hyphae of *G. mosseae* (Vierheilig et al. 1993) (BEG 12; International Bank of Glomeromycota; http://www.kent.ac.uk/bio/beg/).

Plants were grown in a random design in the greenhouse (day/night cycle: 16 h; 22°C/8 h; 19°C; relative humidity 50–70%; light intensity 400 µE s^{-1} m^2 by Radium HRI-T4W/DH lamps) for 12 weeks (six plants per treatment) and watered with a nutrient solution with or without P. The nutrient solution without P consisted of $Ca(NO_3)_2$ 0.472 g/l, K_2SO_4 0.256 g/l, $MgSO_4$ 0.136 g/l, MoO_3 0.07 g, NH_4NO_3 8 mg/l, $Fe_6H_5O_7$ ×3 H_2O 50 mg/l, $Na_2Bo_4O_7$ × $4H_2O$ 1.3 mg/l, $MnSO_4$ × 4 H_2O 1.5 mg/l, $ZnSO_4$ × 7 H_2O 0.6 mg/l, $CuSO_4$ × 5 H_2O 0.54 mg/l, $Al_2(So_4)_3$ 0.028 mg/l, $NiSO_4$ × 7 H_2O 0.028 mg/l, $Co(NO_3)_2$ × 6 H_2O 0.028 mg/l, TiO_2 0.028 mg/l, $LiCl_2$ 0.014 mg/l, $SnCl_2$ 0.014 mg/l, KJ 0.014 mg/l, and KBr 0.014 mg/l. To the nutrient solution with P, KH_2PO_4 (0.136 g/l) was added. At the end of the experiment the shoot biomass (dry weight, DW) was determined (dried at 35°C).

The "−P" (no P and no AMF) and the "+M" (no P but with AMF) treatment received the nutrient solution without P, whereas the "+P" (no AMF but with P) treatment received the nutrient solution with P. Six plants per treatment and per genotype were tested.

Qualitative and quantitative EO analysis

Some leaves were harvested at the beginning of flowering and were dried at 35°C. Leaves (0.2 g) were extracted with 2 ml dichloromethane for 30 min in an ultrasonic bath and fenchone was added as an internal standard. Gas chromatography/mass spectrometry analyses were performed on an HP 6890 coupled with an HP 5972 mass selective detector and fitted with HP-5MS 30 m×0.25 mm capillary column coated with a film thickness of 0.25 µm. The analytical conditions were: carrier gas, helium; injector temperature, 250°C; split ratio, 20:1; temperature program, 40 to 240°C with 3°C/min. Components were identified by comparing Kovats retention indices and the spectra with published data (Adams 1995; McLafferty 1989).

P content

The P content in dried leaves was determined with the ammonium-vanadate-molybdate method (Gericke and Kurmies 1952). Values are given as percentage of P of plant dry weight.

Determination of root colonization

To visualize the AM fungal colonization, roots were cleared by boiling for 4 min in 10% KOH, rinsed three times with tap water and stained according to the method of Vierheilig et al. (1998b) by boiling for 4 min in a 5% ink (Shaeffer; black)/household vinegar (equal to 5% acetic acid) solution. After staining, the percentage of root colonization was determined according to the method of Newman (1966).

Statistical analysis

The experiment was analyzed by two-factorial ANOVA with genotypes and AM fungal treatment as the two factors. Bonferroni multiple mean comparison test was used to test for significant differences between treatments. All statistical analyses were performed with SPSS for Windows Release 11.5.2.1 (SPSS, Chicago, IL, USA).

Results and discussion

In the mycorrhizal treatment all oregano plants were well colonized with percentage of root colonization levels as follows: *O. vulgare* var. Cona 53±13%, *O. vulgare* ssp. *hirtum* "Kalitera" 60±8%, and *O. vulgare* b13/2 50±13%. To our knowledge these are the first data showing that oregano is highly mycorrhizal.

The P content in leaves of *O. vulgare* was clearly enhanced when plants were colonized by the AMF (+M treatment) compared to the nonmycorrhizal control treatment ("−M" treatment); however, highest P levels were obtained in all plants with the +P (P added but no AMF) treatment (% P in dry weight: *O. vulgare* var. Cona: control (−P) 0.103a, +M 0.114b, +P 0.129c; *O. vulgare* ssp. *hirtum* Kalitera: control (−P) 0.055a, +M 0.081b, +P 0.139c; *O. vulgare* b13/2: control (−P) 0.067a, +M 0.128b, +P 0.148c; values within each oregano genotypes followed by the same letter were not significantly different).

It is interesting to note that only in *O. vulgare* var. Cona these high P levels in the +P treatment are reflected in an enhanced shoot biomass (Fig. 1). Mycorrhization significantly increased the shoot biomass in *O. vulgare* var. Cona and *O. vulgare* ssp. *hirtum* var. Kalitera, confirming results on the positive effect of mycorrhization on plant growth of EO-containing plants as also reported with *O. basilicum* (Copetta et al. 2006).

In terms of EO composition, the effect of mycorrhization differed in *O. vulgare* and *O. basilicum*. In contrast to *O. basilicum* (Copetta et al. 2006), in *O. vulgare* the EO composition was not altered through mycorrhization (percentage of EO composition in the −P treatment: sabinene 13.1%, myrcene 2.2%, alpha-phellandrene 0.0%, alpha-terpinene 1.3%, *para*-cymene 5.1%, beta-phellandrene 0.6%, gamma-terpinene 24.2%, *trans*-sabinene hydrate 0.0%, *cis*-sabinene hydrate 32.6%, linalool 0.0%, terpinene-4-ol 1.1%, alpha-terpineol 0.3%, beta-caryophyllene 0.0%, carvacrol 0.0%, beta-caryophyllene 3.8%, germacrene D 14.0%, and *trans-trans*-alpha-farnesene 1.6%), although the total concentration of EOs differed depending on the treatment.

Mycorrhization increased the EO concentration in *O. vulgare* var. Cona and *O. vulgare* b13/2 compared to the −M and the +P treatment (Fig. 1), showing that mycorrhization not only positively affects the EO concentration in *O. basilicum* (Copetta et al. 2006) and *M. arvensis* (Freitas et al. 2004), but also in *O. vulgare.*

In the large-scale production of plants containing EOs, even relatively small increases of the EO concentration in combination with an improved plant growth can result in an enhanced EO yield of economic interest. Combining the EO concentration with the data on shoot biomass, we found a similar EO yield per plant in the −P and the +P treatment of *O. vulgare* ssp. *hirtum* Kalitera and *O. vulgare* b13/2. However, in the mycorrhizal treatment of all three *O. vulgare* genotypes, the EO yield per plant was always enhanced (EO yield increase per plant compared to −P treatment: *O. vulgare* var. Cona, more than tenfold; *O. vulgare* ssp. *hirtum* Kalitera, around threefold; and *O. vulgare* b13/2/, around twofold). This increased EO yield per plant was of specific interest in *O. vulgare* ssp. *hirtum* Kalitera where the EO concentration was unaffected by mycorrhization, but the highly enhanced shoot biomass through mycorrhization finally resulted in an increased EO yield per plant.

Out of the three tested oregano genotypes, two showed a clear increase in the EO concentration through mycorrhization. As no similar increases were observed in the +P

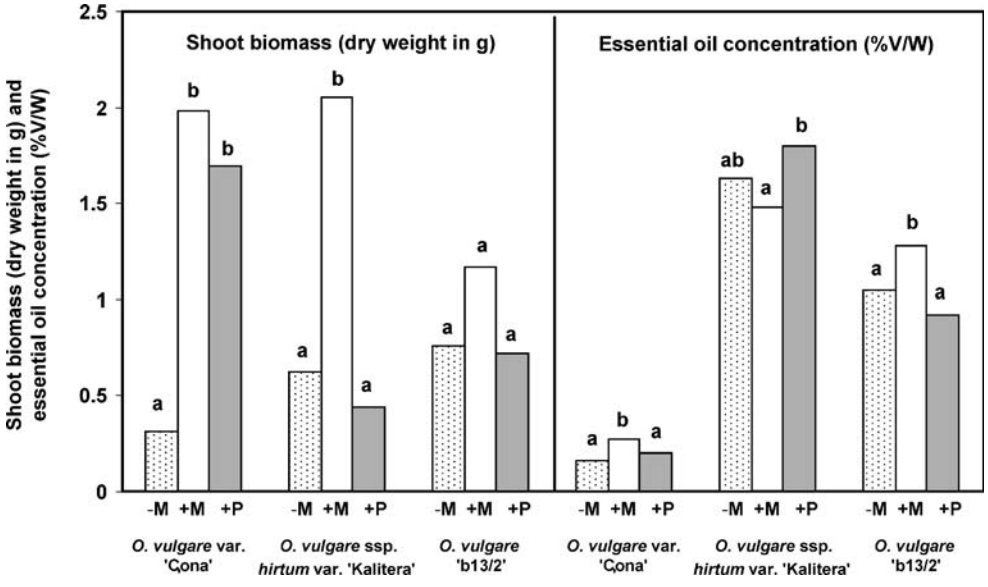

Fig. 1 Shoot biomass and content of EO in leaves of mycorrhizal (*+M*) and nonmycorrhizal (*−M*) oregano genotypes and of nonmycorrhizal oregano genotypes supplied with a P solution (*+P*). Within each genotype *bars* with the same *letter* are not significantly different

treatments of these plants a P effect on the EO concentration can be excluded. Moreover, it shows that the positive effect of mycorrhization is highly dependent on the genotype of the plants and is not a general characteristic of oregano.

Acknowledgements This work was partially funded by a grant of the Thai government to TK and by the Austrian Science Fund (FWF; project L194-B06).

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