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Effects of three AM fungi on growth, distribution of glandular hairs, and essential oil production in *Ocimum basilicum* L. var. *Genovese*

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Abstract The essential oils of basil are widely used in the cosmetic, pharmaceutical, food, and flavoring industries. Little is known about the potential of arbuscular mycorrhizal (AM) fungi to affect their production in this aromatic plant. The effects of colonization by three AM fungi, *Glomus mosseae* BEG 12, *Gigaspora margarita* BEG 34, and *Gigaspora rosea* BEG 9 on shoot and root biomass, abundance of glandular hairs, and essential oil yield of *Ocimum basilicum* L. var. *Genovese* were studied. Plant P content was analyzed in the various treatments and no differences were observed. The AM fungi induced various modifications in the considered parameters, but only *Gi. rosea* significantly affected all of them in comparison to control plants or the other fungal treatments. It significantly increased biomass, root branching and length, and the total amount of essential oil (especially α-terpineol). Increased oil yield was associated to a significantly larger number of peltate glandular trichomes (main sites of essential oil synthesis) in the basal and central leaf zones. Furthermore, *Gi. margarita* and *Gi. rosea* increased the percentage of eugenol and reduced linalool yield. Results showed that different fungi can induce different effects in the same plant and that the essential oil yield can be modulated according to the colonizing AM fungus.

Keywords *Ocimum basilicum* L. · AM fungi · Essential oil · Glandular hairs · Lamiaceae

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Introduction

Many aspects of arbuscular mycorrhizal (AM) interactions were studied (e.g., growth effect, nutritional exchanges, biocontrol toward plant pathogens, tolerance to water stress, and adverse environmental conditions), but little is known about the potential of AM fungi to affect the secondary metabolic pathways of plants. Several papers have investigated secondary compound patterns of mycorrhizal roots: terpenoids (Akiyama and Hayashi 2002), carotenoids (Maier et al. 1995; Walter et al. 2000; Fester et al. 2002), flavonoids (Morandi 1996; Larose et al. 2002), glucosinolates (Vierheilig et al. 2000), phenols (Zhu and Yao 2004), and phenylpropanoids (Weiss et al. 1997). In addition, indirect evidence suggests that AM fungi can also affect the volatile compounds produced in the leaves (Guerrieri et al. 2004). Furthermore, phytohormone levels can be altered in both arbuscule-containing cells and whole tissues of mycorrhizal plants (Allen et al. 1980; Dixon et al. 1988; Kaldorf and Ludwig-Müller 2000; Torelli et al. 2000; Hause et al. 2002).

Ocimum basilicum L. (sweet basil) is an economically important plant (Werker et al. 1993). Its essential oils are synthesized and stored in glandular hairs and are used as flavorings in foods and beverages, as fragrances, as fungicides, or insecticides in pharmaceutical and industrial products (Simon et al. 1990; Grayer et al. 1996; Hasegawa et al. 1997; Miele et al. 2001; Mondello et al. 2002; Pascual-Villalobos and Ballesta-Acosta 2003). The qualitative and quantitative improvement of essential oil production represents an area of high commercial interest. In recent years, genetic techniques were applied to identify and control the genes involved along the process (Lange and Croteau 1999; Mahmoud and Croteau 2002).

The present study consists of a comparative analysis of the effects induced by three AM fungi, *Glomus mosseae* (Nicolson & Gerdemann) Gerd. & Trappe BEG 12, *Gigaspora margarita* Becker & Halle BEG 34, and *Gigaspora rosea* Nicolson & Schenck BEG 9, on plant development and on the qualitative and quantitative synthesis of a number of essential oils of *O. basilicum* var. *Genovese* leaves.

Materials and methods

Experimental design and plant culture

Sweet basil (*O. basilicum* L., var. *Genovese*) seeds (Zorzi, Padova, Italy) were surface sterilized by gently shaking in a 1% NaClO solution for 3 min and rinsed six times for 5 min and four times for 20 min in sterile deionized water (Gamalero et al. 2004). The seeds were pregerminated on moist sterile filter paper at 24°C in the dark for 3 days.

Sterile pregerminated basil seeds were transplanted into plastic pots with 100 ml quartz sand (diameter 2–3 mm) on the bottom and with 600 ml substrate made of 1:1 fine quartz sand (0.6–1.2 mm):vermiculite (Punto Elle, Turin, Italy). Culture substrates were sterilized at 180°C for 2 h. Four treatments were considered: control plants without mycorrhiza (C), plants inoculated with *G. mosseae* (Nicolson & Gerdemann) Gerd. & Trappe BEG 12, *Gi. margarita* Becker & Halle BEG 34, or *Gi. rosea* Nicolson & Schenck BEG 9. Inoculation of AM fungi was obtained by incorporating 30% (v/v) of an inoculum–quartz sand mix (BIORIZE) into the growth substrate. A total of 28 plants per treatment were prepared. Plants were kept in a growth chamber with a 16/18 h light/dark photoperiod, 26/22°C light/dark thermoperiod, 150 μEm^{-2} s^{-2} light irradiance at pot height (Sylvania 58W), and watered to saturation three times per week with a modified Long Ashton nutrient solution containing 32 μM phosphate (Trotta et al. 1996). Plants were harvested 21, 42, and 63 days after sowing and were processed as described below. All experiments were duplicated and the results shown are from one representative experiment.

Morphology

The following parameters were determined: leaf number; root, leaf, and shoot fresh weight; shoot and total root length; number of root tips; root/shoot fresh weight ratio; and root branching (i.e., number of root tips/total root length). Values for total root length and number of root tips were obtained using root systems fixed in 70% ethanol and stored at 4°C. Digital images of the root systems were created and analyzed by means of Mac Rhizo version 3.9 software package and the associated scanner (Regent Instruments, Montreal, Canada).

Mycorrhizal colonization

Mycorrhizal colonization and arbuscule abundance were estimated according to Trouvelot et al. (1986) after staining with 1% methyl blue in lactic acid.

Count of glandular trichomes

Five plants for each treatment were used for the glandular trichome count of the abaxial side of leaves. From each plant, three leaves (of the same age and position in each plants) belonging to three different couples of leaves were taken and examined by stereomicroscopy (Stemi SV6 Zeiss, Jena, Germany). Peltate hairs were counted in three portions (1 cm^2 each) of the basal (close to the petiole), central, and apical zone of the leaf. Samples were gently laid down on plastic transparent sheets to measure projected area (PA) by means of McRhizo version 3.9. Results were normalized with reference to fully developed leaves (with a PA of 25 cm^2). Images of the trichomes were taken connecting the stereomicroscope to a PC using the Axio Vision version 4.1 software.

Scanning electron microscopy

Portion of leaves, sampled from the basal zones, of three samples per treatment were fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, at pH 7.2 for 2 h at 4°C. After washing in the same buffer, the tissue was postfixed by immersion in a solution of 1% osmium tetroxide in cacodylate buffer for 1 h. The material was dehydratated through a series of 15-min incubations at room temperature in 30, 50, 70, and 90% ethanol, followed by two washes in 100% ethanol, and one in 100% acetone. The 100% acetone was replaced by fresh acetone then the samples were capped and incubated overnight at room temperature. Leaf zones were then dried to critical point using CO_2 (K850 Emitech), mounted on stubs, and coated with a thin layer of gold (SEM Coating System, Biorad, Segrate, Italy). Observations were carried out on a Siemens Autoscan scanning electron microscope (Munich, Germany).

Chemical analysis of essential oils

After 42 and 63 days of growth, five plants per treatment were used for the chemical evaluation of essential oil content. All leaves were collected and weighed and oils were extracted in *n*-hexane (Sigma) for 2 days (Tsuro et al. 2001). The extracts were dried over anhydrous sodium sulfate for 5 days and concentrated to 1 ml with a rotovapor

Table 1 Morphological parameters of *O. basilicum* plants at three harvests (21, 42, and 63 days after sowing)

	Parameters	C	*G. mosseae*	*Gi. margarita*	*Gi. rosea*
21 days	Leaf number	4.2±0.2 a	5.2±0.5 b	4.0±0.0 a	4.4±0.4 ab
	Leaf fresh weight (g)	0.214±0.032 a	0.291±0.021 b	0.190±0.020 a	0.200±0.022 a
	Leaf area (cm^2)	11.6±1.6 a	15.8±1.2 b	9.2±1.2 a	11.0±1.0 a
	Shoot length (cm)	4.2±0.3 ab	4.9±0.6 a	3.9±0.4 ab	3.6±0.3 b
	Shoot fresh weight (g)	0.381±0.054 a	0.540±0.047 b	0.385±0.025 a	0.467±0.017 b
	Shoot dry weight (g)	0.025±0.003 ab	0.032±0.003 a	0.020±0.002 b	0.023±0.001 b
	Root fresh weight (g)	0.203±0.028 a	0.333±0.047 b	0.226±0.025 a	0.233±0.017 a
	Total root length (cm)	282.3±43.4 a	313.2±32.7 a	287.7±33.8 a	272.8±11.5 a
	Number of root tips	210.6±50.3 a	224.8±22.5 a	202.4±9.2 a	272.8±20.7 a
	Root branching degree	0.705±0.069 a	0.725±0.045 a	0.724±0.045 a	0.996±0.047 b
	Root/shoot fresh weight	0.535±0.027 ab	0.614±0.017 c	0.588±0.013 bc	0.500±0.021 a
42 days	Leaf number	11.6±0.9 a	13.5±1.2 a	15.2±1.6 a	24.2±2.1 b
	Leaf fresh weight (g)	3.120±0.156 a	3.218±0.183 a	3.507±0.123 a	5.157±0.220 b
	Leaf area (cm^2)	169.9±14.4 a	166.7±10.7 a	180.5±12.9 a	250.1±13.9 b
	Shoot length (cm)	26.0±1.8 ab	27.6±2.2 a	23.0±1.6 b	26.9±1.4 ab
	Shoot fresh weight (g)	4.680±0.143 a	4.920±0.150 a	4.922±0.163 a	7.153±0.101 b
	Shoot dry weight (g)	0.323±0.029 a	0.503±0.058 b	0.405±0.034 ab	0.531±0.054 b
	Root fresh weight (g)	4.787±0.366 a	5.740±0.123 b	5.741±0.255 b	5.683±0.306 b
	Total root length (cm)	2.694.5±76.6 a	2.207.6±140.2 c	2,852.5±205.3 a	3,657.9±310.6 c
	Number of root tips	1,958.7±134.5 a	1,212.9±121.9 b	1,839.1±211.6 a	2,064.8±287.1 a
	Root branching degree	0.726±0.042 a	0.540±0.025 b	0.627±0.044 ab	0.552±0.041 b
	Root/shoot fresh weight	1.042±0.091 a	1.094±0.055 a	1.108±0.052 a	0.920±0.058 a
63 days	Leaf number	28.4±3.4 a	25.6±3.4 a	32.4±2.9 a	55.8±4.3 b
	Leaf fresh weight (g)	4.054±0.356 a	3.981±0.414 a	6.472±0.382 b	8.500±0.581 c
	Leaf area (cm^2)	185.5±26.5 a	206.0±32.5 a	223.1±20.1 a	341.8±24.3 b
	Shoot length (cm)	46.9±1.7 a	48.5±2.2 a	50.5±1.6 ab	54.7±1.4 b
	Shoot fresh weight (g)	8.502±0.351 a	9.075±0.417 a	11.763±0.628 b	15.511±0.636 c
	Shoot dry weight (g)	1.011±0.081 a	1.523±0.096 b	1.157±0.039 a	1.629±0.118 b
	Root fresh weight (g)	9.804±0.617 a	6.606±0.207 c	9.651±0.610 a	12.887±0.407 b
	Total root length (cm)	6,542.3±335.8 a	4,699.6±240.4 c	7,054.3±209.2 a	9,013.3±345.2 b
	Number of root tips	4,234.6±272.8 a	3,832.4±197.5 a	5,905.1±221.2 a	8,818.0±605.0 b
	Root branching degree	0.648±0.028 a	0.822±0.034 b	0.837±0.019 b	0.973±0.038 c
	Root/shoot fresh weight	1.169±0.086 a	0.741±0.039 b	0.826±0.040 b	0.840±0.034 b

Values are the means of five repetitions±standard errors. *G. mosseae* enhanced growth parameters after 21 days of growth, but not at the following harvests. *Gi. rosea* was the most effective fungus in promoting plant growth after 42 and 63 days of growth. All the fungi increased root branching, but the effect due to *Gi. rosea* was the greatest.

Different letters indicate statistically significant differences ($p<0.05$) comparing treatments (across the lines of the table).

C: control, nonmycorrhizal plants; *G. mosseae*: inoculated with *G. mosseae*; *Gi. margarita*: inoculated with *Gi. margarita*; and *Gi. rosea*: inoculated with *Gi. rosea*

Buchi R-114 at 30°C. Gas chromatography/mass spectrometry analyses were performed on a Varian CP 3800, associated to an autosampler (Varian CP 8400) and a mass spectrometer (Varian Saturn 4000). The analytical conditions were: helium was the carrier gas, 1 ml of sample was injected at 250°C with a column flow of 1.2 ml/min in a RTX-200 column (60 m×0.25 mm, 0.25 μm film thickness). Components were identified according to databases and quantified by comparison with certified standards for 12 oils (α-pinene, β-myrcene, limonene, eucalyptol, linalool, camphor, α-terpineol, eugenol, caryophyllene, menthol, 4-allyl anisole, and skatol).

P content

Five plants from the last harvest (63 days) were used for P determination. Approximately 0.5 g (dry weight) of shoots was taken. Samples were weighed and then digested in 10 ml concentrated HNO_3 in a CEM MARS 5 microwave digestor. The digested material was filtered on 45-μm filters, and then deionized water was added to a final volume of 100 ml. Metal concentration was assessed by means of a calibration curve after measurement by Inductively Coupled Plasma Optic Emission Spectrometry using an IRIS Advantage ICAP DUO HR

Table 2 Mycorrhizal colonization (% M) and arbuscule abundance (% A) in the root systems of *O. basilicum* at three harvests

	% M			% A		
	21st day	42nd day	63rd day	21st day	42nd day	63rd day
C	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
G. mosseae	50.2±7.4 b	59.7±3.3 c	77.9±2.3 b	39.2±4.0 b	29.3±5.2 b	25.0±3.4 b
Gi. margarita	21.4±5.0 c	25.8±4.5 b	38.1±4.0 c	20.5±5.2 c	21.6±3.9 b	36.5±4.0 c
Gi. rosea	70.6±8.2 d	60.4±2.7 c	67.9±4.2 d	69.6±8.2 d	49.4±2.9 c	62.7±4.2 d

Gi. rosea produced the highest intensity of colonization with many arbuscules. *G. mosseae* abundantly colonized basil roots, but without many arbuscules. *Gi. margarita* colonization was the lowest of the three, but with intermediate percent A. Different letters indicate statistically significant differences ($p<0.05$) among treatments (along the columns of the table).

C: control, nonmycorrhizal; *G. mosseae*: inoculated with *G. mosseae*; *Gi. margarita*: inoculated with *Gi. margarita*; and *Gi. rosea*: inoculated with *Gi. rosea*

series (Thermo Jarrell Ash, Franklin, MA, USA) spectrometer. A certified standard with known P content was analyzed with the samples to confirm the correctness of the procedure.

Statistical analysis

Data were statistically analyzed by ANOVA followed by Fisher's probable least-squares difference test with cut-off significance at $p \leq 0.05$.

Results

Morphology

Several differences among the four treatments could be detected after 21 days of growth. In comparison with the other treatments, *G. mosseae* induced significant increases in shoot length, number of leaves, leaf area, and total biomass of *O. basilicum* plants (Table 1). On the following sampling dates, *Gi. rosea* plants showed a more branched root system and a significantly larger development of shoots. After 63 days of growth, the ratio between root and shoot weight was significantly higher in control plants

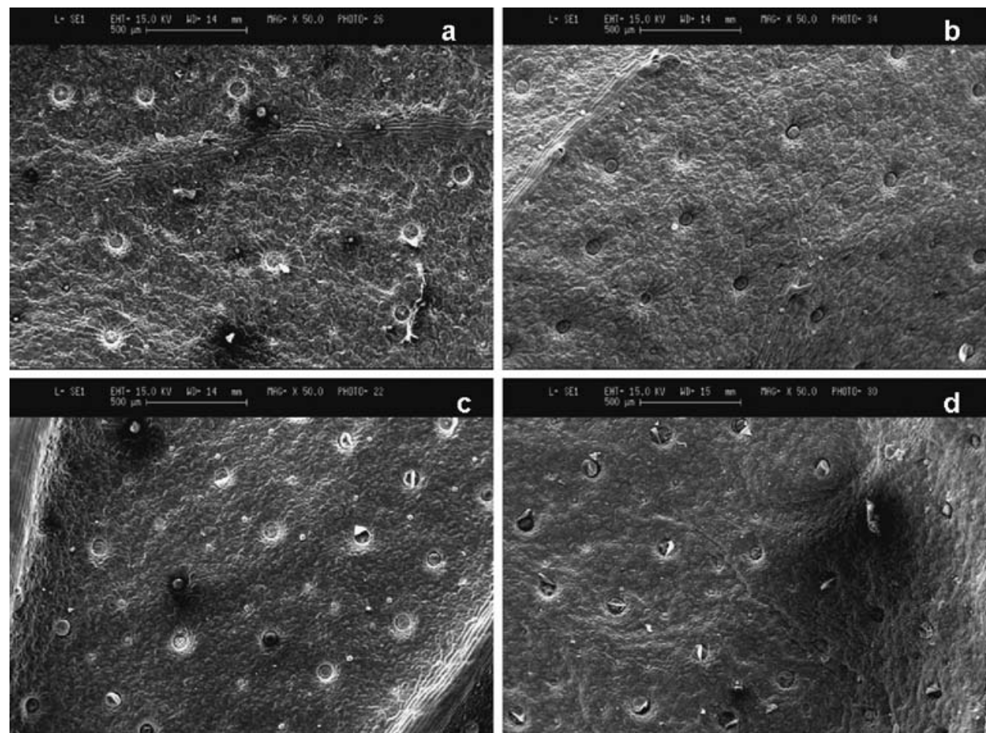

Fig. 1 SEM images of leaf glands of *O. basilicum*. **a** Non-mycorrhizal plants, **b** plants colonized by *G. mosseae*, **c** plants colonized by *Gi. rosea*, **d** plants colonized by *Gi. margarita*. No differences can be observed between the different treatments

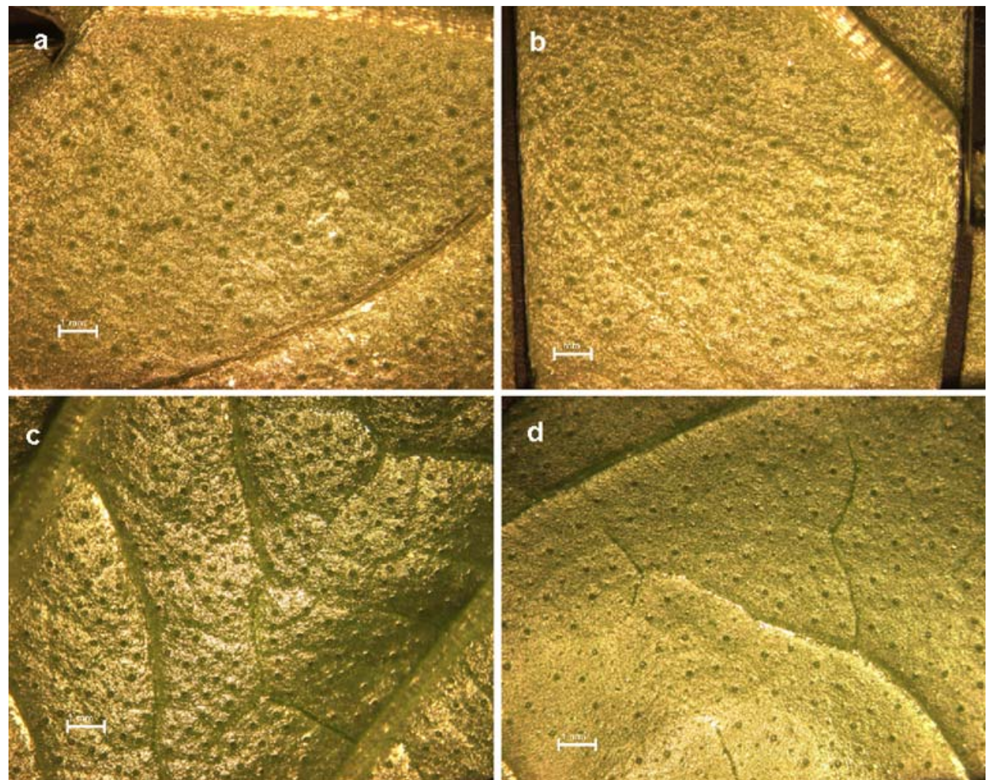

Fig. 2 Light microscope images of the abaxial surface of *O. basilicum* leaves. **a** Basal and **b** distal parts of the leaf of a nonmycorrhizal plant; **c** basal and **d** distal parts of the leaf of a plant colonized by *Gi. rosea*. Glands are more abundant in the basal portion of the leaf (in both treatments) and in AM plants. See also Figs. 3 and 4

compared to mycorrhizal ones. Root systems of mycorrhizal plants were significantly more branched than those of control plants. *Gi. rosea* plants had the highest degree of root branching, significantly higher than *G. mosseae* and *Gi. margarita* plants (Table 1).

Mycorrhizal colonization

No AM colonization was detected in control roots. The three fungal species colonized the root system of basil in different ways. *G. mosseae* quickly produced abundant intercellular hyphae, but relatively few arbuscules that often appeared collapsed. The arbuscule density (% A) decreased with time (Table 2). In roots inoculated with *Gi. rosea*, colonization reached the highest intensity and arbuscules were very abundant. Colonization with *Gi. margarita* was less extensive than with the other two fungi, but arbuscules were abundant in the colonized part of the roots (Table 2).

Scanning electron microscopy

Analyses of the trichomes by scanning electron microscopy (SEM) did not show any alteration or structural modification in the peltate and capitate glands, or of the nonglandular trichomes induced by AM fungi (Fig. 1).

Count of glandular trichomes

A gradient in the abundance of trichomes was observed along the leaf major axis. Trichomes were significantly more abundant in the basal zone (close to the petiole) than in the apical zone (Figs. 2 and 3). Mycorrhizal colonization did not alter such a pattern, but leaves from AM plants had a larger number of peltate glands per square-centimeter than control leaves in all the considered zones. However, significant differences could be detected only when comparing control and *Gi. rosea* plants in the basal zone (Figs. 2 and 3).

More detailed statistical analyses showed that in all treatments the number of peltate glands increased in the upper leaves of plants. Comparing the density of peltate glands in the three zones, it was always higher in mycorrhizal plants, whatever the level of the couples of leaves, with significant differences for the basal zone of the leaf and for intermediate zone of the fifth couple of leaves of *Gi. rosea* samples in comparison with all the others (Fig. 4).

Essential oils

Eugenol was the most abundant component of the essential oils of *O. basilicum* var. *Genovese*, followed by linalool, eucalyptol, β-myrcene, and α-terpineol. Caryophyllene, α-

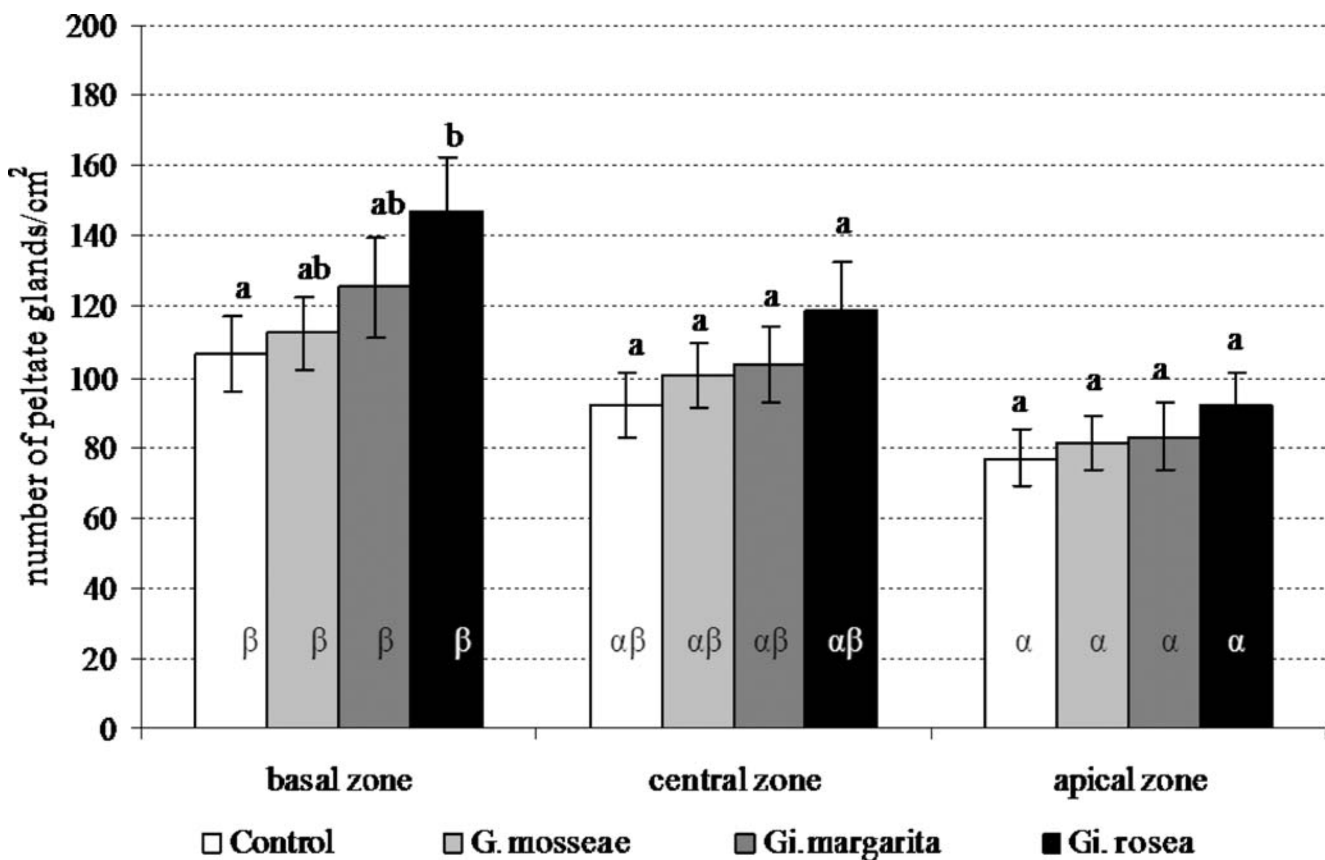

Fig. 3 Number of peltate glands on the abaxial surface of *O. basilicum* leaves. Different *letters* indicate statistically significant ($p<0.05$) differences among treatments. *Normal script* is used for comparison between different fungal treatments, while *Greek letters* are used for comparisons within the same fungal treatment. *Control*: nonmycorrhizal plants, *G. mosseae*: plants inoculated with *G. mosseae*, *Gi. margarita*: plants inoculated with *Gi. margarita*, and *Gi. rosea*: plants inoculated with *Gi. rosea*

pinene, limonene, and camphor were present in smaller amounts. Menthol, estragol, and skatol were not detected. After 42 days of growth, no differences could be observed between control and mycorrhizal plants or between the various fungal treatments. At the end of the experiments, plants colonized by *Gi. margarita* showed a significant decrease in the yield of eucalyptol, linalool, and caryophyllene, in comparison with all the other treatments, while those colonized by *Gi. rosea* increased α-terpineol content and total amount of oils (Table 3). In addition, plants colonized by *Gi. margarita* and *Gi. rosea* showed higher percentages of eugenol and lower linalool (Table 4).

P content

P content in shoots of *O. basilicum* from the four different treatments did not show any significant difference. Total P was 963±65 mg/kg in control plants, 772±30 mg/kg in *G. mosseae*-colonized plants, 951±168 in *Gi. margarita*-colonized plants. and 983±197 mg/kg in *Gi. rosea*-colonized plants.

Discussion

Increased growth and development in AM plants, compared to nonmycorrhizal ones, was reported for many different species (reviewed in Smith and Read 1997). The results of the present work concerning *O. basilicum* are in agreement with such reports. Different effects on plant development were observed, depending on the fungal species. At the end of the experiments, the strongest growth effect was observed with *Gi. rosea*. *G. mosseae* stimulated root and shoot growth in the first weeks of growth but such effects were not lasting and at the last harvest (63 days), shoot weights were not different from those of the nonmycorrhizal controls and root systems were significantly smaller. *Gi. margarita* increased plant growth only from the second harvest onward (42 days), while plants inoculated with *Gi. rosea* showed improved growth at all harvests. In addition, all mycorrhizal plants showed a higher degree of root branching, while root/shoot weight was lower than in the controls, consistent with previous literature (Smith and Read 1997; Berta et al. 2002).

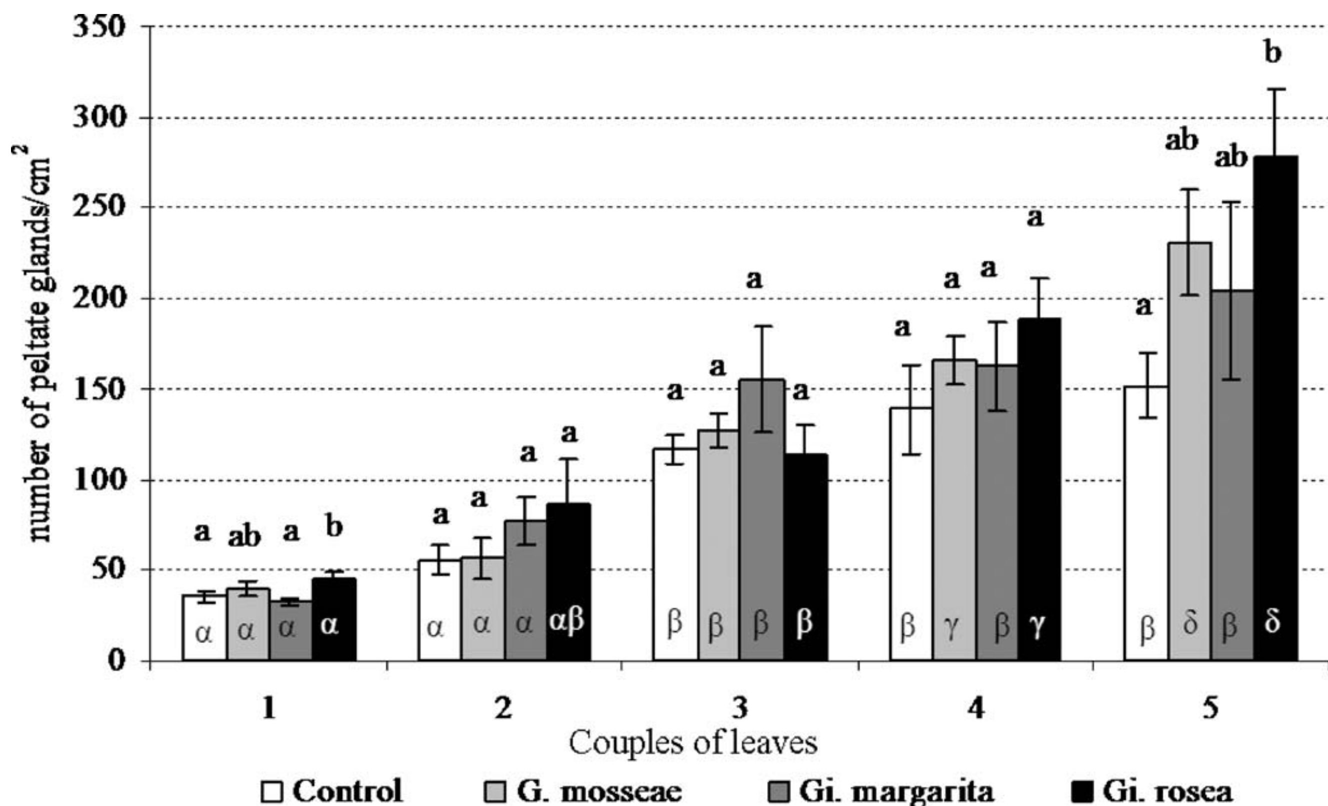

Fig. 4 Number of peltate glands in the basal zone on the abaxial surface of *O. basilicum* leaves, considering leaf *pairs 1* to *5*. *Pair 1* is the oldest and lowest one; *pair 5* is the uppermost and youngest one. *Normal script* is used for comparison between different fungal treatments, while *Greek letters* are used for comparisons within the same fungal treatment. *Control*: nonmycorrhizal plants, *G. mosseae*: plants inoculated with *G. mosseae*, *Gi. margarita*: plants inoculated with *Gi. margarita*, and *Gi. rosea*: plants inoculated with *Gi. rosea*

Differences in the colonization of *O. basilicum* by *G. mosseae*, *Gi. margarita*, and *Gi. rosea* were reported by Dickson (2004) who introduced eight new intermediate morphologies, in addition to the classic *Arum*- and *Paris*-types. Basil is colonized by *G. mosseae* as *Arum*-type (intercellular hyphae and arbuscules), by *Gi. margarita*

Table 3 Essential oil concentrations in *O. basilicum* leaves (μg/g) according to quantitative chemical analyses

	C	*G. mosseae*	*Gi. margarita*	*Gi. rosea*
α-Pinene	1.074±0.159 ab	1.447±0.259 b	1.208±0.188 ab	0.801±0.153 a
β-Myrcene	2.938±0.112 a	3.721±0.428 a	3.056±0.242 a	3.585±0.639 a
Limonene	0.956±0.087 a	1.368±0.257 a	1.426±0.113 a	0.936±0.161 a
Eucalyptol	41.863±4.761 a	50.117±7.890 a	23.715±2.091 b	42.299±5.898 a
Linalool	221.921±25.640 a	239.182±43.432 a	70.677±5.618 b	152.603±21.093 a
Camphor	0.168±0.039 a	0.725±0.363 a	1.080±0.390 a	7.841±3.528 b
α-Terpineol	4.333±0.295 a	5.144±0.762 a	3.906±0.273 a	7.074±0.426 b
Eugenol	296.529±57.123 ab	347.311±93.569ab	185.486±48.443 b	348.628±17.573 a
Caryophyllene	0.681±0.123 a	0.808±0.193 a	2.304±1.156 a	0.725±0.079 a
Menthol	n.d.	n.d.	n.d.	n.d.
4-Allyl anisole	n.d.	n.d.	n.d.	n.d.
Skatol	n.d.	n.d.	n.d.	n.d.
Total (μg)	2,384.606±263.368 a	2,097.123±640.313 a	2,398.607±548.142 a	4,786.386±407.997 b
Total (μg/g)	570.463±75.588 a	649.822±145.704 a	292.923±21.264 b	564.492±36.485 a

Eugenol was the most abundant component, followed by linalool, eucalyptol, β-myrcene, and α-terpineol. Some oils were not detected. *Gi. rosea* increased α-terpineol and total amount of oils. Different letters indicate statistically significant differences ($p<0.05$) among treatments (across the lines of the table).

C: control, nonmycorrhizal; *G. mosseae*: inoculated with *G. mosseae*; *Gi. margarita*: inoculated with *Gi. margarita*; *Gi. rosea*: inoculated with *Gi. rosea*; and *n.d.*: not detected

Table 4 Percentage composition of oil extracts from *O. basilicum* according to quantitative chemical analyses

	C	*G. mosseae*	*Gi. margarita*	*Gi. rosea*
α-Pinene	0.197±0.033 ac	0.230±0.020 c	0.407±0.034 b	0.136±0.020 a
β-Myrcene	0.547±0.081 a	0.618±0.077 a	1.042±0.021 b	0.616±0.080 a
Limonene	0.180±0.037 a	0.217±0.019 a	0.488±0.013 b	0.162±0.021 a
Eucalyptol	7.670±1.041 a	8.058±0.548 a	8.078±0.209 a	7.348±0.631 a
Linalool	39.388±2.494 a	37.960±2.064 a	24.487±2.658 b	26.722±2.508 b
Camphor	0.033±0.009 a	0.165±0.092 a	0.310±0.147 ab	1.390±0.625 b
α-Terpineol	0.795±0.085 a	0.833±0.071 a	1.340±0.073 b	1.260±0.047 b
Eugenol	51.072±3.650 a	51.788±2.789 a	62.997±2.389 b	62.232±2.696 b
Caryophyllene	0.117±0.016 a	0.133±0.025 a	0.755±0.353 b	0.132±0.021 a
Menthol	n.d.	n.d.	n.d.	n.d.
4-Allyl anisole	n.d.	n.d.	n.d.	n.d.
Skatol	n.d.	n.d.	n.d.	n.d.

Gi. margarita and *Gi. rosea* increased the proportion of eugenol and lowered that of linalool. Different letters indicate statistically significant differences ($p<0.05$) among treatments (across the lines of the table).

C: control, nonmycorrhizal; *G. mosseae*: inoculated with *G. mosseae*; *Gi. margarita*: inoculated with *Gi. margarita*; *Gi. rosea*: inoculated with *Gi. rosea*; and *n.d.*: not detected

according to I1 style (intercellular and intracellular hyphae, arbuscules), and by *Gi. rosea* in I2 style (intracellular hyphae noncoil, producing the arbuscules), confirming that these three fungal species interact differently with basil.

Chemical analyses showed that in *O. basilicum* var. *Genovese*, eugenol was the most abundant oil, followed by linalool, eucalyptol, and all the other oils in decreasing order. Quantitative and semiquantitative analyses did detect any difference after 42 days of growth. At the end of the experiments (63 days), it was clear that *Gi. rosea* significantly increased the concentration of camphor, α-terpineol, and the total amount of essential oils (also in relation to the increased number of leaves), while plants treated with *Gi. margarita* had significantly decreased eucalyptol, linalool, eugenol content, and the total concentration of essential oils. In addition, while both *Gigaspora*s affected the proportion of several oils in *O. basilicum*, *G. mosseae* did not alter their proportion relative to control plants, in agreement with data about oregano by Khaosaad et al. (2006). Information about the effects of AM fungi on the production of essential oils is scanty, and only a few papers concerning a limited choice of species have been published up to now. Nemec and Lund (1990) reported that *Glomus intraradices* induces significant variations in the proportion and composition of leaf volatiles in *Citrus Jambhiri*. Three studies carried out on *Mentha arvensis* indicated a relation between the presence of AM fungi, increased growth, essential oil accumulation, and improved mineral uptake (Khaliq and Janardhanan 1997; Gupta et al. 2002; Freitas et al. 2004). Similar results were published about *Coriandrum sativum* (Kapoor et al. 2002b). Kapoor et al. (2002a, 2004) also conducted experiments on three different plant species (*Anethum graveolens* L., *Trachyspermum ammi* L., and *Foeniculum vulgare* Mill.) and two fungal species (*G. macrocarpum* and *G. fasciculatum*) showing that both fungi increased plant growth, phosphate content, and the concentration of essential oils in the fruits. In general, the authors underlined the importance of improved mineral nutrition for essential oil yield. However, we did not observe any improvement in phosphate nutrition after AM colonization, and Khaosaad et al. (2006) showed that *G. mosseae* increases the concentration of essential oils in two genotypes of *O. vulgare* but not in P-fertilized nonmycorrhizal plants. The increased yield in total essential oils in *Gi. rosea*-treated *O. basilicum* plants could be related to the increased number of peltate glands, the structures responsible for oil production (Gang et al. 2001). Our data on the distribution of peltate glands on the leaf surface are in agreement with previous reports concerning other species of the Lamiaceae, showing that the density of glands decreased from the basal to the distal part of the leaves (Werker et al. 1993; Voirin and Bayet 1996; Ioannidis et al. 2002). After 63 days, *O. basilicum* colonized by *Gi. rosea* presented a larger number of glands in comparison with the other mycorrhizal treatments, suggesting that colonization by this fungus can stimulate the production of peltate glands. This greater number of glands may be related to alterations in the hormonal profile of the plants because increased levels of auxins, cytokinins, and gibberellins were recorded in AM plants (Allen et al. 1980; Dixon et al. 1988; Torelli et al. 2000).

The production of essential oils can be modified by other ectomycorrhizal or nonmycorrhizal fungi. For example, ectomycorrhizal colonization increases α-pinene, β-pinene, and δ-carene content in young pine plantlets (Napierale-Filipiak et al. 2002; Werner et al. 2004). Also, Mucciarelli et al. (2003) observed that colonization by an endophytic, nonmycorrhizal fungus increased development and altered

the composition of the essential oils in *Mentha piperita*, grown in vitro and in vivo. The observed modification in the synthesis of some essential oils is considered a defense response to fungal colonization. Considering the fungicide properties of several essential oils (Simon et al. 1990; Wan et al. 1998; Griffin et al. 1999; Pascual-Villalobos and Ballesta-Acosta 2003) and that defense responses are observed in AM interactions, it may be that such a relation exists in the case of the AM symbiosis.

According to our data, *Gi. rosea* was the most useful fungus to increase *O. basilicum* var. *Genovese* biomass and essential oil production. The extension of such results to other *O. basilicum* cultivars should be evaluated because many different chemocultivars, varying in their aroma, were selected or bred by crossing with other cultivars or closely related species (Grayer et al. 1996; Simon et al. 1999; Labra et al. 2004). Such is the variety of commercially exploited chemocultivars that no single compound can be said to provide the characteristic basil aroma and taste (Lachowicz et al. 1997; Ioannidis et al. 2002). Further investigation is necessary to understand which AM fungi can be more useful to improve the production of essential oil by each chemocultivar.

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