CARYOLOGIA Vol. 60, no. 1-2: 106-110, 2007

Influence of arbuscular mycorrhizal fungi on growth and essential oil composition in *Ocimum basilicum* var. Genovese

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Abstract — Sweet basil is characterized by its essential oils, synthesized and stored in glandular hairs. Arbuscular mycorrhizal (AM) fungi colonize the root of most terrestrial plants, forming a very common symbiosis. In the present paper, the effects induced by three arbuscular mycorrhizal fungi (*Glomus mosseae* or *Gigaspora margarita* or *Gigaspora rosea*) on shoot growth and essential oil production of *Ocimum basilicum* var. Genovese were evaluated. *Gi. rosea* significantly increased internode number and decreased internode distance in comparison with control plants and the other fungal treatments. Furthermore, *Gi. margarita* and *Gi. rosea* modified essential oil content analyzed semi-quantitatively.

Key words: AM fungi, essential oil, internodes, sweet basil.

INTRODUCTION

Ocimum basilicum L. is a Mediterranean aromatic species that produces characteristic flavours and fragrances used in pharmaceutical, cosmetic, food and industrial products. Several authors have reported antioxidant, insecticidal, nematocidal, antifungal and antibacterial activity of basil essential oils (Sangwan *et al.* 1990; Wan *et al.* 1998; Griffin *et al.* 1999; Dorman *et al.* 2000; Javanmardi *et al.* 2003; Pascual-Villalobos and Ballesta-Acosta 2003). Essential oils are synthesized and stored in peltate glands especially; these are leaf structures responsible for oil production (Gang *et al.* 2001). Recent studies demonstrated that arbuscular mycorrhizal (AM) fungi, root symbionts that improve plant health and mineral nutrition (Smith and Read 1997), can modify the number of peltate trichomes within basil and oregano leaves, and increase the concentration of some essential oils (Copetta *et al.* 2006a; Khaosaad *et al.* 2006). Such modifications of the essential oil profile was confirmed by both NIR (Near Infra Red) Spectroscopy and Electronic Nose analyses (Copetta *et al.* 2006b).

Five different chemotypes of *O. basilicum* L have been identified depending on the relative abundance of aromatic compounds (Grayer *et al.* 1996). In *O. basilicum* var. Genovese the most abundant essential oils are eugenol and linalool, but this variety produces others phenyl-propanoids and terpenoids in different proportion.

The aim of the present work was to better characterize the effects of three AM fungi on the aromatic profile of *O. basilicum* var. Genovese, by means of chemical analyses concerning six, previously not considered, essential oils. In addition, also the impact of AM colonization on shoot morphology was considered.

MATERIALS AND METHODS

Sweet basil (*Ocimum basilicum* L., var. Genovese) seeds (Zorzi, Padova, Italy) were surface sterilized (Gamalero *et al.* 2004) and pre-germinated on moist sterile filter paper at 24°C in the dark for 3 days. They were then 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 200°C for 2 h. Four treatments were considered: control plants without mycorrhizae (C), plants inoculated with *Glomus*

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mosseae (Nicolson & Gerdemann) Gerd. & Trappe BEG 12 (*G.m*) or *Gigaspora margarita* Becker & Halle BEG 34 (*Gi.ma*) or *Gigaspora rosea* Nicolson & Schenck BEG 9 (*Gi.r*) plants. Inoculation of AM fungi was obtained by incorporating 30% (v/v) of the 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 $\mu Em^{-2}s^{-2}$ light irradiance at pot height (Sylvania 58W), and watered to saturation three times per week with a modified Long Ashton nutrient solution containing 32μM phosphate (Trotta *et al.* 1996). Plants were harvested at 21, 42 and 63 days from the sowing and processed as described below.

Root systems were fixed in 70% ethanol and stored at 4°C. Mycorrhizal colonization was estimated according to Trouvelot *et al.* (1986). Shoot length, node number, and the internode distances were determined.

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, weighed and the essential oils extracted in *n*-hexane (Sigma) and analyzed according to Copetta *et al*. (2006a). 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). Therefore, the analyses were semi-quantitative. Quantitative results for the 12 oils used as standard are published in Copetta *et al.* (2006a).

Mycorrhizal, shoot, leaf and essential oil data were statistically analysed by ANOVA followed by Fisher's PSLD test, with cut-off significance at $p \leq 0.05$.

RESULTS

The root system of basil was colonised in different ways by the three AM fungi (Fig. 1). *Gi. margarita* colonised less extensively basil root system than the other two fungi. Arbuscule density (A%) decreased with time in *G. mosseae* plants, while it was similar to mycorrhizal colonization (M%) in the roots of *Gi. rosea* and *Gi. margarita* samples. Control roots were not infected.

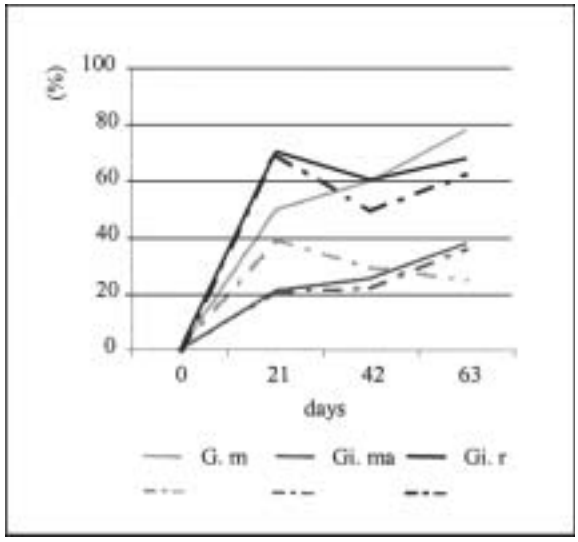

Fig. 1 — Mycorrhizal colonization (continuous lines) and arbuscular density (dotted lines) in the root systems, at the three harvests.

After 21 days of growth *G. mosseae* significantly increased the number of nodes, in comparison with the other three treatments (Table 1). After 63 days, *Gi. rosea* plants showed a significant increase of shoot length and number of nodes. Treatment with AM fungi also affected the distance of each node from the collar (i.e. the internode length) (Fig. 2): the distance between nodes and collar was often significantly lower in *Gi. rosea* plants than in the other treatments.

After 42 days of growth, no differences in essential oil could be observed between control and mycorrhizal plants or between the various fungal treatments (Table 2). At the last harvest, plants colonized by *Gi. margarita* showed a significant increased in the yield of methyl eugenol in com-

Table 1 — Shoot length and node number at the three harvests.

	Shoot length (cm)			Number of nodes		
	21st day	42nd day	63rd day	21st day	42nd day	63rd day
C	4.2 ± 0.3 ab[1]	26.0 ± 1.8 ab	46.9 ± 1.7 a	1.2 ± 0.2 a	4.9 ± 0.1 ac	7.7 ± 0.3 a
G. m	4.9 ± 0.6 a	27.6 ± 2.2 a	48.5 ± 2.2 a	1.8 ± 0.2 b	5.4 ± 0.2 b	8.2 ± 0.3 a
G. ma	3.9 ± 0.4 ab	23.0 ± 1.6 b	50.5 ± 1.6 ab	1.0 ± 0.0 a	4.8 ± 0.1 a	8.1 ± 0.2 a
G. r	3.6 ± 0.3 b	26.9 ± 1.4 ab	54.7 ± 1.4 b	1.0 ± 0.0 a	5.2 ± 0.1 bc	9.0 ± 0.2 b

[1] Different letters indicate statistically significant differences ($p<0.05$) comparing treatments (along the columns of the table)

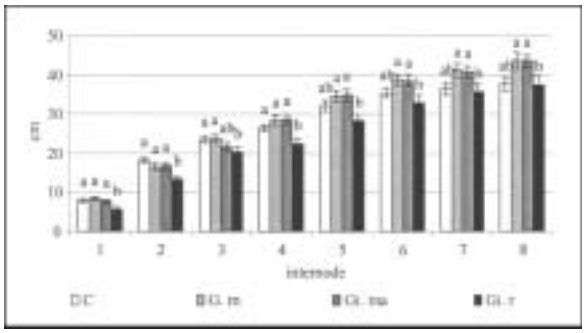

Fig. 2 — Distance between the collar and each leaf couple in basil plants inoculated with different fungal treatments. Different letters indicate statistically significant differences ($p<0.05$).

parison with all the other treatments, while those colonized by *Gi. rosea* increased bornil acetate content. In addition, plants colonized by *Gi. margarita* and *Gi. rosea* showed higher content in δ-cadinene.

DISCUSSION

Recent works (COPETTA *et al.* 2006a; COPETTA *et al.* 2006b) proved the influence of mycorrhizal colonization on plant growth, glandular tricome distribution in basil leaf and essential oil production. Theses modifications are not due to increased P nutrition. In the above mentioned papers, growth and development of mycorrhizal and non-mycorrhizal basil were compared, and the effects of different AM fungi on plant growth are described. In particular, growth results showed that *Gi. rosea* increased shoot height; the present data show that such increase is also associated to decreased internode length. The latter is a desirable trait because it enhances plant stability, therefore improving plant resistance to the wind and stem breaking (NICK 2000). Increased growth can be obtained by increasing either cell number or individual cell volumes. During most of their life cycle, plants exhibit indeterminate morphogenesis and grow predominantly by cell expansion. An extensive body of information demonstrate an intimate link between cortical microtubules and the preferential axis of growth. Hormones such as auxins, giberellins and abscissic acid can induce reorientation of microtubules (NICK 2000). Particularly, the giberellins (GAs) control stem elongation, internode length, leaf expansion and trichome development (DAVIES 1995; VOGLER *et al.* 2003). Several studies (DIXON *et al.* 1988; TORELLI *et al.* 2000) showed that AM fungi can alter the profile of these hormones. These alterations can be responsible for variations in both shoot morphogenesis and production of peltate glands (COPETTA *et al.* 2006a), the responsible for oil production (GANG *et al.* 2001).

The cultivar Genovese showed the linalool-eugenol profile, as observed also from MARITTO *et al.* (1996). Previous papers showed that after 63 days of growth, *Gi. rosea* significantly increased the concentration of camphor, α-terpineol, and the total amount of essential oils, while plants treated with *Gi. margarita* had significantly decreased eucalyptol, linalool, eugenol content, and the total concentration of essential oils (COPETTA *et al.* 2006a). In this work, semiquantitative analyses did not detect any difference after 42 days of growth. At last harvest, semiquantitave chemical analyses showed differences in essential oil composition among treatments: while *G. mosseae* did not alter content of examined compounds relative to control plants, *Gi. rosea* and *Gi. margarita* sig-

Table 2 — Essential oil concentrations in *O. basilicum* leaves (μg/g) according to semi-quantitative chemical analyses.

		C	*G.m*	*Gi.ma*	*Gi.r*
42 days	Borneol	3.32 ± 1.00 a[1]	2.38 ± 0.96 a	3.15 ± 0.68 a	2.46 ± 0.95 a
	Bornil acetate	1.53 ± 0.70 a	1.25 ± 0.62 a	1.43 ± 0.27 a	1.12 ± 0.48 a
	Elixene	0.56 ± 0.30 a	0.58 ± 0.39 a	1.23 ± 0.52 a	0.60 ± 0.46 a
	Methyl Eugenol	8.02 ± 4.57 a	10.11 ± 5.61 a	13.79 ± 7.17 a	19.07 ± 7.18 a
	α-Bergamotene	17.25 ± 5.01 a	17.05 ± 3.89 a	13.56 ± 2.52 a	13.99 ± 6.91 a
	δ-Cadinene	7.25 ± 1.24 a	10.86 ± 2.00 a	10.12 ± 2.56 a	5.88 ± 1.94 a
63 days	Borneol	3.81 ± 1.52 a	2.65 ± 1.17 a	3.39 ± 1.65 a	2.07 ± 0.62 a
	Bornil acetate	1.66 ± 0.53 a	0.78 ± 0.23 a	2.26 ± 0.75 ab	3.33 ± 0.49 b
	Elixene	1.11 ± 0.36 a	0.79 ± 0.29 a	1.30 ± 0.25 a	1.14 ± 0.13 a
	Methyl Eugenol	0.24 ± 0.15 a	0.15 ± 0.15 a	13.19 ± 5.26 b	1.00 ± 0.27 a
	α-Bergamotene	14.90 ± 4.38 a	8.87 ± 2.50 a	20.05 ± 5.27 a	17.36 ± 4.20 a
	δ-Cadinene	6.57 ± 0.77 a	7.10 ± 1.11 a	21.60 ± 2.99 b	17.05 ± 1.47 b

[1] Different letters indicate statistically significant differences ($p<0.05$) comparing treatments (along the lines of the table)

nificantly increased bornil acetate and methyl eugenol respectively; both the *Gigaspora* species increased δ-cadinene content.

MIELE *et al.* (2001) have shown that methyl eugenol concentration decreased with shoot length in *O. basilicum*. Similar results were obtained in our experiments, with the exception of the plants colonized by *Gi. margarita*. A *O*-methyltransferase catalyzed a specific methylation of eugenol for methyl eugenol production (WANG *et al.* 1997; LEWINSOHN *et al.* 2000). The activity of the enzyme decreases when plants grow (MIELE *et al.* 2001). We assumed that in *Gi. margarita* plants the activity of *O*-methyltransferase did not decrease. In the other treatments, the decrease of methyl eugenol content followed the increase of eugenol content. Although methyl eugenol is approved for commercial use in food, in perfumes, creams and detergents, this compound is a carcinogenic phenylpropanoid (MIELE *et al.* 2001). However, intake of this compound with the human diet is usually considered to be very low (MIELE *et al.* 2001). The use of AM fungi, increasing the speed of shoot growth, decreased the time and the production of methyl eugenol in *O. basilicum* var Genovese.

Results showed that different AM fungi can induce different effects very important in the same plant also for human health. These results are in agreement with NIR analyses and aromatic profiles obtained by EN in previous results by our group (COPETTA *et al.* 2006b).

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Received November 30th 2006; accepted January 30th 2007