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RESEARCH ARTICLE

The effect of arbuscular mycorrhizal fungal isolates on the development and oleoresin production of micropropagated *Zingiber officinale*

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We have investigated the effects of phosphate fertilization and inoculation with isolates of arbuscular mycorrhizal fungi *Scutellospora heterogama* SCT120E, *Gigaspora decipiens* SCT304A, *Acaulospora koskei* SCT400A, *Entrophospora colombiana* SCT115, and an assemblage (Mix) of all four isolates on growth, development and oleoresin production of micropropagated *Zingiber officinale*. After 120 and 210 d of growth, the Mix and phosphorus addition significantly increased shoot height relative to control plants. Phosphorus addition was the only treatment resulting in significantly large shoot dry biomass relative to control after 120 d. No statistical differences were observed between treatments for shoot dry biomass after 210 d and for fine and coarse root biomass at both harvests. Inoculation with *S. herogama* and *G. decipiens* resulted in larger yields of oleoresin, corresponding to 3.48% and 1.58% of rhizome fresh biomass respectively. Based on retention index and mass spectrometry, we have characterized the following constituents present in ginger rhizomes: ar-curcumene, zingiberene, γ-cadinene, bisabolene, δ- or α-cadinene and farnesol. Two other constituents were characterized as possible members of the gingerol class. Results suggest that the screening and inoculation of arbuscular mycorrhizal fungi in ginger plants is a feasible procedure to increase the oleoresin production of *Z. officinale* and consequently increase the aggregate value of ginger rhizome production.

Key words: fungal assemblage, ginger, oleoresin, secondary metabolites

Efeito de isolados de fungos micorrízicos arbusculares no desenvolvimento e produção de óleo-resina em plantas micropropagadas de *Zingiber officinale*: Avaliou-se o efeito da adubação fosfatada e da inoculação de isolados de fungos micorrízicos arbusculares [*Scutellospora heterogama* SCT120E, *Gigaspora decipiens* SCT304A, *Acaulospora koskei* SCT400A, *Entrophospora colombiana* SCT115 e uma mistura (Mix) de todos os quatro isolados] sobre o crescimento e desenvolvimento vegetativo e produção de óleo-resina em plantas micropropagadas de gengibre (*Zingiber officinale*). Verificou-se que, aos 120 e 210 d após o cultivo, as plantas dos tratamentos Mix e sob adição de fósforo tiveram maior altura em relação às plantas-controle. A adição de fósforo foi o único tratamento que resultou em aumento significativo na biomassa seca da parte aérea relativamente ao tratamento-controle, após 120 d de cultivo. A biomassa seca da parte aérea, aos 120 d, bem como a biomassa de raízes finas e grossas, aos 120 e 210 d, não responderam aos tratamentos aplicados. A inoculação com *S. herogama* e *G. decipiens* foram os únicos tratamentos que proporcionaram incrementos significativos de produção de óleo-resina, respectivamente 3,48 e 1,58% em relação à biomassa fresca de rizomas de gengibre. Baseado no índice de retenção e espectrometria de massa, caracterizaram-se os seguintes constituintes presentes nos rizomas de gengibre: ar-curcumeno, zingibereno, γ-cadineno, bisaboleno, δ- ou α-cadineno e farnesol. Outros dois constituintes foram caracterizados como possíveis membros da classe dos gingeróis.

Os resultados sugerem que a seleção e a inoculação de fungos micorrízicos arbusculares em plantas de gengibre é uma alternativa viável para aumentar a produção de óleo-resinas e, conseqüentemente, aumentar o valor agregado da produção de rizomas nessa espécie.
Palavras-chave: assembléia de fungos, gengibre, metabolismo secundário, óleo-resina

INTRODUCTION

Several aspects of the mycorrhizal symbiosis resulting from the biotrophic interaction between arbuscular mycorrhizal fungi (AMF) and roots of micropropagated plants have been reported in the literature. In this association, AMF improve uptake of soil nutrients such as P, N, Zn^{2+}, Cu^{2+}, K^{+}, thereby influencing plant growth and survival, plant nutrition, tolerance to water stress and to adverse environmental conditions (Smith and Read, 1997). For micropropagation systems, the acclimatization stage represents a developmental phase where plants are subject to environmental stress due to poor root, shoot and cuticular development. Inoculation with AMF represents a biological solution that can result in growth enhancement at this stage (Hooker et al., 1994). Several papers have reported the positive effect of AMF inoculation on growth parameters, root morphology and survival rates of micropropagated bananas (Declerck et al., 2002), potatoes (Vosátka and Gryndler, 2000), strawberries (Borkowska, 2002), grapevine (Schellenbaum et al., 1991), apple (Locatelli and Lovato, 2002), *Prunus* (Monticelli et al., 2000), and artichokes (Fortunato et al., 2005).

However, little is known about the effect of AMF upon either plant secondary metabolic pathways or the production and yield of secondary compounds of their hosts (Copetta et al., 2006). For instance, studies have demonstrated that AMF can influence phytohormone levels of jasmonate (Hause et al., 2002), terpenoids and carotenoids (Akiyama and Hayashi, 2002; Fester et al., 2002) and phenols (Zhu and Yao, 2004). In addition, the association with AMF has altered essential oil yield and quality of several plants. Kapoor et al. (2002) observed that inoculation with AMF *Glomus macrocarpum* and *G. fasciculatum* increased significantly the concentration of limonene and α-phellandrene, respectively, relative to non-mycorrhizal control plants of *Anethum graveolens* L. Kapoor et al. (2002b, 2004) also observed enhanced concentration and quality of essential oils on mycorrhizal *Coriandrum sativum* L (coriander) and *Foeniculum vulgare* Mill. (fennel). For *Mentha arvensis* L. (mint) mycorrhizal colonization significantly increases oil content and yield relative to non-mycorrhizal plants (Gupta et al., 2002). Freitas et al. (2004) also observed that inoculation with AMF resulted in increments of 89% in the essential oil and menthol contents of mint. To our knowledge, the influence of AMF inoculation on ginger oleoresin production and yield has not been studied.

Ginger (*Zingiber officinale* Roscoe) is an economically important plant largely cropped for its variety of uses, especially for its medicinal and flavoring potentials (Onyenekwe and Hashimoto, 1999). Ginger has been used as a spice since ancient times (Goyal and Korla, 1993) and among others its carminative, diuretic, and expectorant properties are well known in medical research (Cost, 1989). Ginger rhizomes contain both aromatic and pungent components responsible for its potent aroma and use in food and beverages, and these are mainly monoterpenoids such as geraniol, linalool and geranial (Sekiwa-Iijima et al., 2001). Concentration of oleoresins in the dry rhizome ranges from 1.5 to 3% (Onyenekwe and Hashimoto, 1999; Zancan et al., 2002) and the study of these oils is of paramount importance as they determine ginger flavor, which ultimately determines quality and international market price of ginger (Onyenekwe and Hashimoto, 1999; Sekiwa-Iijima et al., 2001).

In this investigation, the biotechnological processes of plant micropropagation and mycorrhizal inoculation were used to study the effect of AMF on ginger growth and oleoresin production. Ginger is an alternative crop for small and medium growers in the Itajai Valley in Santa Catarina state, in the South of Brazil, and oil extraction represents a means of increasing the aggregate value of this crop for small families. Our approach rests on the needs to produce homogenous, disease-free and high quality medicinal plants for essential oil exploitation on a commercial scale. This is particularly important for ginger as conventional plant propagation using rhizomes might transmit pathogens from one growth cycle to another

(EPAGRI, 1998; Debiasi et al., 2004). The hypothesis that different AMF isolates will influence yield and quality of oleoresin of micropropagated ginger plants under greenhouse conditions was tested.

MATERIAL AND METHODS

Plant material: In vitro ginger plants obtained from the Laboratory of Biotechnology and Plant Micropropagation of the Universidade Regional de Blumenau (Brazil) were used in the experiment. Buds were excised from commercially grown ginger rhizomes obtained from a field crop in the municipality of Ilhota, Santa Catarina state (Brazil) and superficially disinfected according to the methodology of Debiasi et al. (2004). After this process, buds were placed on Murashige-Skoog medium (Murashige and Skoog, 1962) supplemented with sucrose (30 g L^{-1}), agar (7 g L^{-1}) and 10 µM 6-benzylaminopurine, with pH adjusted to 5.8 before autoclaving. Plant cultures were maintained in growth chambers in 20 mL tubes for 50 d, at a temperature of 25 ± 2ºC and a light intensity of 50 µmol m^{-2} s^{-1}.

Mycorrhizal inoculation: Single cultures of four AMF species were obtained from the germplasm bank of the Laboratory of Botany of the Universidade Regional de Blumenau to bulk up inoculum for use in the plant growth experiment. Fungal isolates were *Scutellospora heterogama* (Nicol. & Gerdemann) Walker & Sanders (isolate SCT120E), *Gigaspora decipiens* Hall & Abbott (isolate SCT304A), *Acaulospora koskei* Blaszkowski (isolate SCT400A) and *Entrophospora colombiana* Spain & Schenck (isolate SCT115). Soil inoculum of each isolate (containing hyphae, spores and colonized pieces of root) was individually mixed with a substrate composed of a sterilized soil:sand mix (v/v, 1:1, pH 5.5), placed in plastic pots of 1.5 kg and seeded with *Sorghum bicolor* L. Pots containing only sterilized soil:sand mix were also set up to obtain appropriate non-mycorrhizal inoculum for use in the plant growth experiment. The soil:sand mix was sterilized twice (121ºC for 1 h each) with a 24-h interval between autoclaving sessions. Plants were grown under greenhouse conditions for three to four months [(16 h daylength, temperature between 20-25ºC, supplemented with fluorescent light (irradiance equivalent to 16.2 µmol m^{-2} s^{-1})] and watered daily or as needed. At this moment, the shoots were discarded and the soil together with the root ball was thoroughly homogenized to produce the mycorrhizal soil inoculum for each isolate.

Plant growth experiment: In vitro micropropagated ginger plants were transferred to plastic pots (400 mL) containing a sterilized mix of river sand:soil (1:1, pH 5.5). The substrate was previously sterilized twice at 121ºC for 1 h in an autoclave. At transplanting, one plant was established per pot and each pot was inoculated with 10 mL of AMF soil inoculum. Fungal treatments included *Scutellospora heterogama* (*Sh*), *Gigaspora decipiens* (*Gd*), *Acaulospora koskei* (*Ak*), *Entrophospora colombiana* (*Ec*) and an assemblage of all four isolates (Mix). Control (Ctl) and Phosphorus (P) treatments were inoculated with 10 mL of non-mycorrhizal soil inoculum from *Sorghum bicolor* pot culture. Phosphorus was added. The experiment was carried out in a greenhouse under growth conditions as described above for up to seven months. Plants were distributed in a completely randomized design, with seven treatments and 15 replicates.

Plant harvest and measurements: After 120 d of growth, five plants per treatment were used for measurement of shoot height (SH) and biomass production. Weight of shoot biomass (SB) was obtained by drying leaves at 50ºC for 4 d. Roots were first divided into fine roots and coarse roots (> 3 mm diameter). Half of the fresh weight of fine roots and coarse roots was dried to obtain root biomass (RB) and coarse root biomass (CB). The remaining half of fine roots was stained according to Koske and Gemma (1989) and percentage of mycorrhizal root colonization estimated by the grid line methods of Giovannetti and Mosse (1980). Spores (AMF) were extracted from a 30 mL soil subsample after wet sieving (Gerdemann and Nicolson, 1963) followed by centrifugation on a sucrose gradient (20%/60%) and counted under a compound microscope.

After 210 d, the measurements of SH, SB, RB, CB, and AMF root colonization and sporulation were repeated, except for rhizomes which were weighed fresh and used for the chemical evaluation of oleoresin.

Extraction and Chemical analysis of oleoresins: After 210 d of growth, rhizomes and shoots within each treatment were pooled to obtain oleoresin: nine replicates were used

for the Ctl, Mix, *Ec* and *Sh* treatments and 10 replicates for the P, *Gd* and *Ak* treatments. Rhizomes were first washed with distilled water, blot dried and weighed to obtain total fresh weight. After this procedure, they were sliced and dehydrated in an oven with air circulation (33 ± 3ºC, 48 h) to reduce the moisture content to 7-9 %, as determined by the azeotropic distillation method (Cecchi, 1999). Oleoresins were extracted from 2 g of rhizomes using 90 mL of acetone in a Soxhlet extractor for 3 h. The extracts were concentrated in a rotary evaporator at 55ºC under reduced pressure. The yield for each extraction was determined by the quotient of oleoresin mass and rhizome fresh weight. Shoots were weighed fresh and dried out to obtain SB before using the same procedures for oleoresin extraction.

The analysis was carried out by gas chromatography (GC) on a Shimadzu-14B instrument and by gas chromatography coupled to a mass spectrometer (GC-MS) using a HP5890A/5970 instrument. Gas chromatography was performed using the following conditions: sample injection (1 µL obtained by diluting *ca.* 10 µg of extract in 1 mL hexane); fused silica capillary column (DB-5, 30 m ´ 0.25 mm, 0.25 µm film thickness); helium as carrier gas, flow rate 1 mL min^{-1}; split mode 1:50; injector temperature 250ºC and FID 280ºC; oven temperature gradient programmed from 60ºC (5 min) to 190ºC (2 min) at 5ºC min^{-1}, then raised to 280ºC (15 min) at 10ºC min^{-1}. Coupled GC-MS at 70 eV was performed using a computerized system associated with a mass selective detector under the same analytical conditions as previously described. The identification of the components was made by a computer library search based on matching of MS spectra, followed by fragmentation pathway analysis and, when required, on the basis of retention indexes with reference to a homologous series of linear saturated hydrocarbons (C_{10}-C_{30}) (Adams, 2007).

Statistical analysis: All dependent variable data (spore counts, shoot height and biomass) were checked for homogeneity of variance according to Levene´s test. After that, ANOVA was carried out and if the *F* test was significant, treatment means were compared by the *ad hoc* Tukey's test with significance at ≤ 0.05. Percentage values of mycorrhizal colonization were transformed to arc sin square root prior to analyses. All statistical analyses were performed using the software JMP® (SAS Inst. Inc., 1995).

RESULTS

Vegetative development: After 120 d, shoot height (SH) of the ginger plants was 8.18 cm for P and 8.98 cm for Mix treatments and these were the only treatments differing statistically from Ctl plants (Table 1). At 210 d, plants inoculated with Mix and *Ak* and added P produced significantly longer shoots than Ctl plants. Plants associated with *Sh* produced the shortest shoots at both harvests (Table 1). Shoot biomass (SB) was significantly higher in plants of P treatment than for Ctl plants after 120 d, but no differences between treatments and Ctl were detected for SB after 210 d (Table 1). Fine and coarse root biomass did not differ significantly among treatments at both 120 and 210 d of growth (Table 1).

Mycorrhizal colonization and AMF sporulation: No mycorrhizal colonization was detected in Ctl and P roots while root colonization ranged from 5.4 to 59% in plants inoculated with Mix or individual AMF isolates (Table 2). The largest values observed for ginger mycorrhizal colonization was for Mix at 120 d and *Gd* at 210 d. Mycorrhizal colonization significantly decreased from 120 to 210 d for Mix and *Sh* and significantly increased between harvests for *Gd*. Ginger root colonization by *Ak* and *Ec* remained the same between harvests (Table 2).

Number of spores per 30 mL of soil for *Ak, Ec* and AMF present in the Mix tended to increase from 120 to 210 d, although the differences were not statistically significant (Table 2). Spore numbers of *Gd* slightly increased from 120 to 210 d while those of *Sh* decreased.

Chemical analysis of ginger rhizome oleoresins: Data related to oleoresin extraction from shoots are not shown as our procedure resulted in extraction of chlorophyll. After 210 d, plants grown with P and *Ak* produced the heaviest rhizomes, weighting 0.35 and 0.33 g, respectively (Table 3). The yield of oleoresin based on the rhizome fresh weight was < 1% for Ctl and *Ec* plants and the largest value (3.48%) was observed for plants associated with *Sh* (Table 3). For all other treatments, yield ranged from 1.02% to 1.58%.

The GC profile from the Mix treatment (Figure 1G) was selected to determine the retention index (RI) of the oleoresin main components from ginger rhizome (Table 4). We chose the Mix treatment as the standard due to the equilibrated presence of compounds in all regions of the chromatogram and therefore as representative of all

Table 1. Vegetative development of ginger submitted to the different treatments after 120 and 210 d of growth. Means followed by the same letter within columns are not statistically different (Tukey's test, $P < 0.05$). Ctl = Control; Mix = mix of all four isolates; P = Phosphorus; *Sh* = *Scutellospora heterogama*; *Gd* = *Gigaspora decipiens*; *Ak* = *Acaulospora koskei*; *Ec* = *Entrophospora colombiana*. Values are means ± SD.

Treatments	Shoot height (cm)		Shoot dry biomass (g)		Fine roots biomass (g)		Coarse root biomass* (g)	
	120 d	210 d	120 d	210 d	120 d	210 d	120 d	210 d
Ctl	3.38 ± 1.50 b	3.54 ± 1.36 c	0.056 ± 0.02 bc	0.061 ± 0.046 a	0.011 ± 0.005a	0.015 ± 0.010a	0.010 ± 0.017 a	0.225 ± 0.012a
P	8.18 ± 1.28 a	7.90 ± 2.32 ab	0.100 ± 0.019 a	0.085 ± 0.021 a	0.015 ± 0.004 a	0.020 ± 0.002 a	0.048 ± 0.029a	0.025 ± 0.374a
Mix	8.98 ± 1.36 a	9.22 ± 1.51 a	0.092 ± 0.17 ab	0.093 ± 0.038 a	0.012 ± 0.006 a	0.010 ± 0.006a	0.039 ± 0.016 a	0.137 ± 0.055 a
Sh	4.44 ±1.60 b	4.16 ± 1.17 c	0.054 ±0.012 c	0.048 ± 0.031 a	0.006 ± 0.004 a	0.007 ± 0.007 a	0.009 ± 0.002a	0.043 ±0.053 a
Gd	6.10 ± 1.85 ab	5.46 ± 1.58 bc	0.080 ± 0.029 abc	0.061 ± 0.002 a	0.007 ± 0.001 a	0.012 ± 0.006 a	0.021 ± 0.019 a	0.251 ±0.398 a
Ak	6.40 ± 2.48 ab	10.14 ± 2.35 a	0.082 ± 0.020 abc	0.092 ± 0.036 a	0,023 ± 0.03a	0.013 ± 0.007 a	0.038 ± 0.055a	0.173 ± 0.111 a
Ec	6.46 ± 0.68 ab	5.56 ± 0.74 bc	0.070 ± 0.000 abc	0.071 ± 0.003 a	0.011 ± 0.011 a	0.018 ± 0.010 a	0.029 ± 0.017 a	0.035 ± 0.011 a

*Coarse roots have diameter > 3mm.

samples. The different treatments could be grouped into three categories according to their chromatographic profiles: (i) Group 1 including treatments *Gd*, P, *Sh* and Ctl, (Figures 1A,B,D,E) characterized by the presence of two major resinous compounds with a calculated RI of 1977 and 2818 (Table 4); (ii) Group 2 including treatments *Ak* and Mix (Figures 1C,G) characterized by the presence of a single dominant resinous compound (calculated RI of 2598) associated with volatile constituents (calculated RI below 1703), the former corresponding to *ca.* 29% of the sample (Table 4); (iii) Group 3 including treatment *Ec* (Figure 1F) characterized by a complex mixture with dominance of volatile constituents (calculated RI $\leq$ 1703). Monoterpenes were not detected in any of the samples analyzed.

We determined the constituents of the rhizome extract using both the retention index and mass spectrometry of the treatment Mix (Table 4). The following ginger rhizome sesquiterpenes were identified: ar-curcumene, zingiberene, γ-cadinene, bisabolene, δ- or α-cadinene and farnesol. Two compounds were characterized as possible representatives of gingerols. We were not able to establish the molecular structure of some oleoresin compounds, but they are resinous compounds based on the calculated RI (Table 4).

DISCUSSION

In this study we have demonstrated that inoculation with AMF isolates and P addition influenced mainly the shoot height and dry biomass production of ginger microplants. Treatments P, Mix, and *Ak* improved height relative to control plants while the addition of P was the only treatment that influenced dry shoot biomass after 120 d. These results indicate that the use of mycorrhizal inoculation is a feasible approach to replace or decrease the use of phosphorus fertilizer during ginger plantlet production. Besides lowering cost of fertilizer consumption, inoculation with effective mycorrhizal fungal isolates represents a more ecologically sound approach to sustainable ginger production. Considering the production of shoot dry biomass in dill, Kapoor et al. (2002) observed that the addition of phosphate resulted in higher yield compared to inoculation with several species of *Glomus*. Similar results were observed by Taylor and Harrier (2001) evaluating the effect of nine species of AMF in microplants of strawberry where they

Table 2. Spore numbers and percentage of mycorrhizal root colonization in micropropagated ginger plants inoculated with different AMF isolates singly or in an assemblage (Mix) after 120 and 210 d. See further details in legend to Table 1.

Treatments	Mycorrhizal colonization (%)		Spore numbers (in 30 mL soil)	
	120 days	**210 days**	**120 days**	**210 days**
Mix	43.45 ± 21.30a	23.40 ± 8.46 b	154 ± 37.42a	260 ± 129.34 a
Sh	14.76 ± 9.04 a	5.75 ± 1.30 b	105 ± 121.24 a	60 ± 49.58 a
Gd	17.80 ± 10.00 b	58.95 ± 6.13 a	49 ± 32.22a	53 ± 41.42 a
Ak	29.82 ± 12.47 a	28.42 ± 16.04a	123 ± 90.76a	298 ± 166.45 a
Ec	26.50 ± 13.75 a	5.39 ± 3.85a	25 ± 37.14a	97 ± 136.02 a

Table 3. Rhizome fresh biomass and levels of oleoresin after 210 d of micropropagated ginger plants. See further details in legend to Table 1.

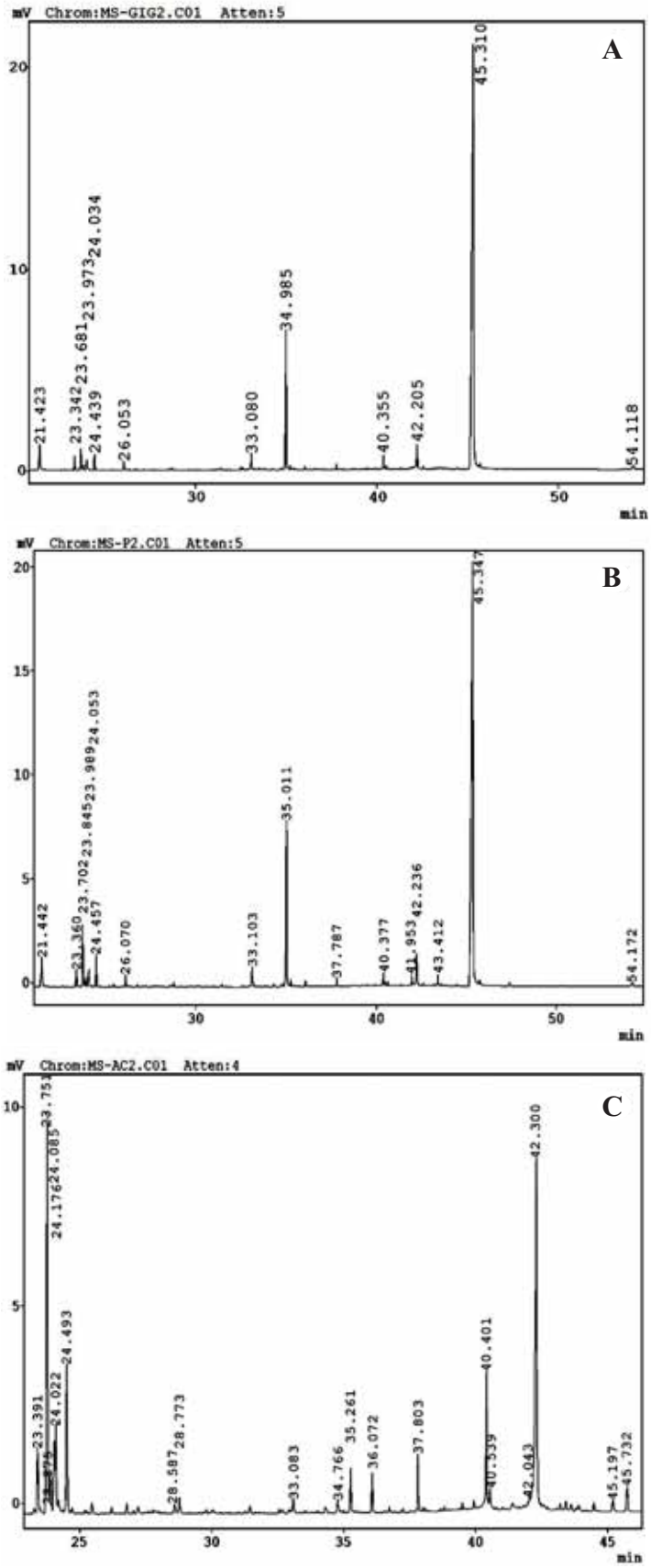
Treatments	Fresh biomass (g)	Content of total extracted oils (g)	Yield of oleoresin (%)
Ctl	0.1454 ± 0.2333	0.0130	0.99
P	0.3471 ± 0.1836	0.0469	1.35
Mix	0.2730 ± 0.1994	0.0251	1.02
Sh	0.1000 ± 0.0240	0.0348	3.48
Gd	0.2166 ± 0.2113	0.0340	1.58
Ak	0.3331 ± 0.2445	0.0344	1.02
Ec	0.1466 ± 0.1488	0.0096	0.72

Table 4. Ginger oleoresin chemical composition of the rhizome from the Mix treatment after 210 d. Mix = mix of all four isolates. RI = retention index.

Retention time (min)	Constituent	RI Calculated	RI Literature	Yield (%)
23.401	Ar-curcumene	1487	1479	4.8
23.744	Zingiberene	1500	1493	9.4
23.888	γ-Cadinene	1505	1513	2.1
24.095	Bisabolene	1514	$1505^{\beta}/1529^{\gamma}$	3.9
24.499	Cadinene	1529	$1522^{\delta}/1537^{\alpha}$	5.3
28.795	Farnesol	1703	1714	1.6
35.016	-	1977	-	2.0
35.276	-	1991	-	1.6
36.087	-	2042	-	1.5
37.816	-	2164	-	2.1
40.415	Gingerol [(1)]	2397	-	7.2
40.551	Gingerol [(1)]	2411	-	0.9
42.306	-	2598	-	29.1
45.268	-	2818	-	19.1

[(1)] Compound structurally related to gingerol.

found that some fungal species suppressed plant growth compared to non-mycorrhizal control plants. However, the levels of root mycorrhizal colonization indicate that all AMF isolates were able to establish a compatible symbiosis with the ginger root system that was not necessarily translated into larger height or shoot biomass yield. Despite the absence of specificity between fungus and host in the mycorrhizal symbiosis, the isolate efficiency is under genetic control, and is also affected by the plant species, fungal species and environmental conditions (Declerck et al., 1995). The soil pH could represent one of the environmental factors selecting AMF species; hence the substrate pH used in the experiment was 5.5, which is considered the optimum for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24.493
24.022
23.391
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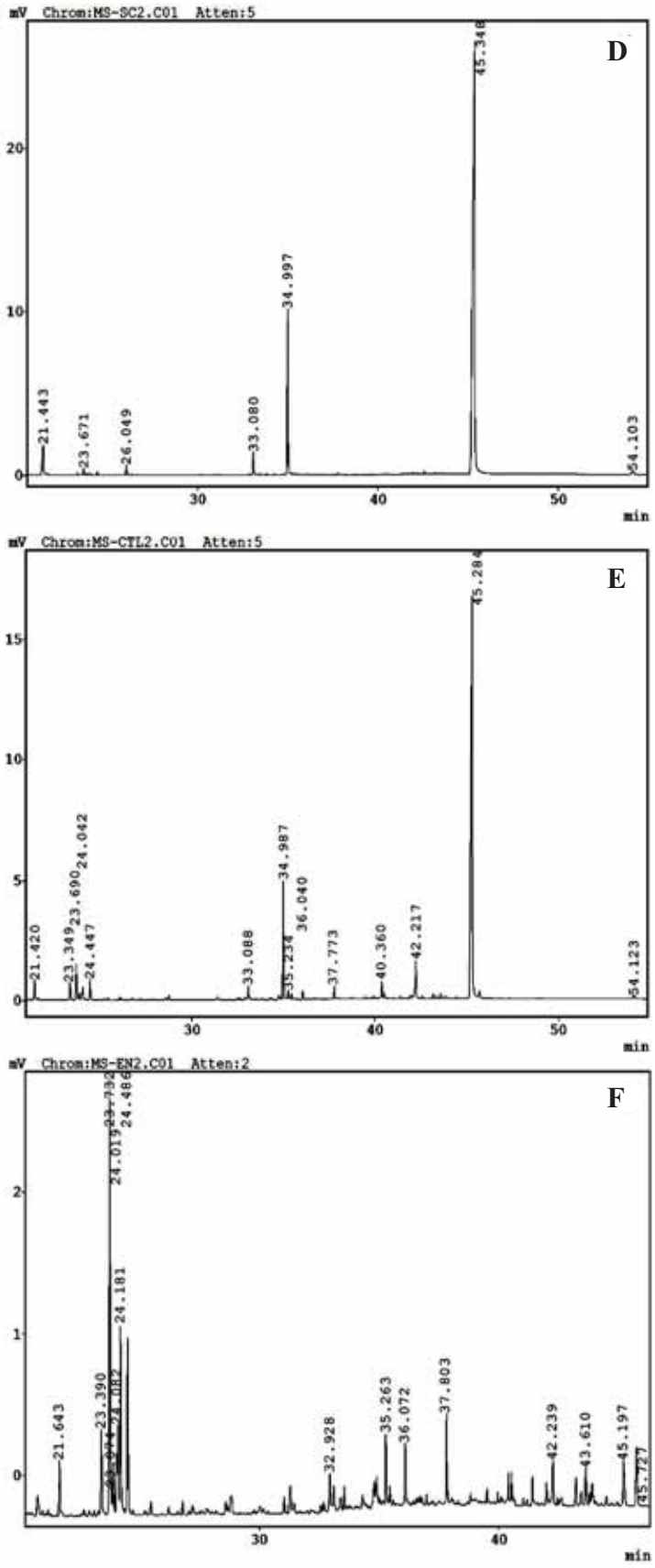
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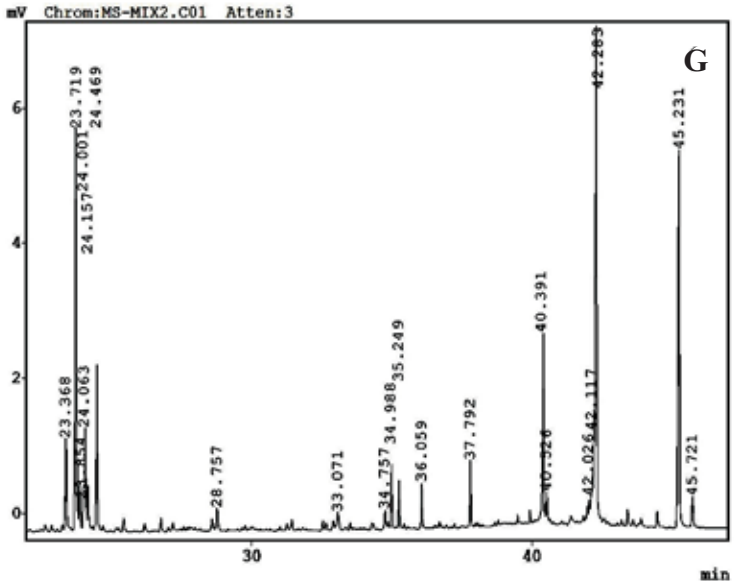

Figure 1. Chromatographic profiles obtained from oleoresin extracts of *Zingiber officinale* after gas chromatography. (**A**) = *Gigaspora decipiens*, (**B**) = Phosphorus, (**C**) *Acaulospora koskei*, (**D**) = *Scutellospora heterogama*, (**E**) = Control, (**F**) = *Entrophospora colombiana*, (**G**) = Mix. Note figures have different scales.

ginger crop (Epagri, 1998) and all isolates originated from soils with pH < 5.0. After 210 d, the production root biomass of mycorrhizal plants was not statistically different from the control plants. One explanation for this result is the natural dormancy period experienced by ginger during winter (Mello et al., 2000), where ginger shoots become senescent due to the low temperatures. The harvest of the experiment was coincident with wintertime, while the optimum growth of ginger occurs between 25 and 30ºC during summer time.

Mycorrhizal root colonization of ginger after 120 d was significantly higher than after 210 d except for plants inoculated with *Gd*. Variations in root colonization between harvest dates could also be related to ginger plant dormancy affecting fungal nutrition and root morphology. At this phase, plants loose their shoots thereby decreasing the production of carbon compounds by photosynthesis, the only source of carbon for fungal growth and development (Siqueira et al., 1985). Reduction of carbon allocation for fungal growth is perceived as a stressful condition that reduces mycorrhizal colonization and triggers sporulation (Pearson and Schwiger, 1993). Indeed, sporulation increased from 120 to 210 d for all isolates except *Sh*, although values were not statistically significant. Moreover, at this phenological stage, ginger allocates carbon to the production of coarse roots, which further help the formation of the rhizome, rather than to fine roots. It is well established that mycorrhizal colonization occurs in fine roots and arrest of fine root production leads to lower levels of mycorrhizal colonization. Although no comparison was performed between 120 and 210 d for root production, the overall biomass of coarse roots increased and the biomass of fine roots remained constant between harvests (Table 1).

Our hypothesis that different AMF isolates influence oleoresin yield was supported by the data obtained after growing ginger plants for 210 d. For most treatments the levels of total oils extracted were 2-4 fold higher than control and inoculation with *Sh* increased the oleoresin yield threefold compared to all other treatments. In comparison with P treatment, inoculation with *Gd* and *Sh* promoted a higher yield of oleoresin. Kapoor et al. (2002) also observed that inoculation with *Glomus macrocarpum* and *Glomus fasciculatum* significantly increased the concentration of essential oil in dill and carum relative to P fertilization and control plants. Copetta et al. (2006) tested three AMF isolates and observed that only one isolate (*Gigaspora rosea*) significantly increased the amount of essential oil in basil. Phosphorus is one of the main nutrients involved in

the synthesis of secondary metabolites as their production demands ATP (Sangwan et al., 2001). Although P shoot concentration was not measured, the increase in availability of P through mycorrhizal association would probably underlie the increase of secondary metabolites such as oleoresins. Maffei et al. (1989) state that the synthesis of secondary metabolites is also dependent on plant age and developmental stage.

Inoculation with different AMF isolates and addition of P resulted in different compounds being detected in the extracts. This finding lends support to our hypothesis that different AMF isolates would result in qualitative differences in ginger oleoresin production. We were able to distinguish three groups among treatments according to chromatographic profiles: group 1 characterized by the presence of two resinous compounds, group 2 characterized by the presence of one resinous compound plus a volatile compound and group 3 described as a complex mixture of volatile compounds. Similarly, Copetta et al. (2006) in their work on basil inoculated with *Glomus mosseae, Gigaspora margarita* and *Gigaspora rosea* found that essential oil concentration was modulated according to each fungal isolate. Camphor and á-terpineol concentrations were significantly higher in basil inoculated with *Gigaspora rosea* compared to the other two isolates. Differential production in quantity and quality of essential oils was also reported by Freitas et al. (2004), Kapoor et al. (2004) and Khaosaad et al. (2006), in other host plants. At the moment we are unable to propose a mechanism explaining how different AMF isolates influence ginger essential oil production. However, it is interesting to note that *Gd* and *Sh* pertaining to the family Gigasporaceae, and therefore closely related phylogenetically, resulted in similar HPLC profiles. The analysis of chromatograms obtained from rhizome extracts of mycorrhizal ginger plants also suggests that the isolate *Ak* had a more consistent influence on the composition of oleoresin obtained in the treatment Mix compared to the other fungal isolates. This is observed by the similarity existing between the chromatograms of *Ak* and Mix, except for the peak with a calculated RI of 2818, absent in the former (Figure 1); indeed *Ak* was the main sporulator in the Mix treatment (data not shown). Neither of these treatments show the first constituent with RI 1416 which is found in treatments *Sh*, *Gd* and *Ec*.

The concentration of compounds related to oleoresins of Mix plants indicated that two unidentified compounds (calculated RI of 2598 and 2818) were the major constituents followed by zingiberene (calculated RI of 1500) (Table 4). The essential oil of ginger is a mixture of monoterpenes and sesquiterpenes that have volatile compounds responsible for the aroma where zingiberene is the major component (Zancan et al., 2002). We did not observe the production of monoterpenes in this study despite the fact that these compounds are commonly found in the oleoresin of ginger rhizomes (Wu et al., 1990; Onyenekwe and Hashimoto, 1999; Jiang et al., 2006). According to Castro et al. (2004), some of the monoterpene compounds are chemical components developed by plants as defense mechanisms against herbivores and pathogens. However, in the present study, ginger plants were grown under greenhouse conditions in sterilized substrate and no signs of pathogenesis were detected during the experiment. Under these circumstances, it is possible that production of monoterpenes was not induced as the inoculum potential of soil pathogens was not high due to soil sterilization. The oleoresin contains volatile substances responsible for the pungency of ginger rhizome and some of the constituents are 4-, 6-, 8-, 10- and 12-gingerol. In our study, two compounds structurally related to gingerol were detected but we were not able to determine the exact nature of the constituent.

CONCLUSIONS

For a rhizome-producing plant such as ginger, the effect of AMF inoculation and phosphate addition on height and shoot biomass production has to consider the phenological state of the plant. Dormancy in ginger seems to influence carbon allocation to roots that in turn might affect AMF root colonization.

Different isolates of AMF were equivalent to P nutrition in terms of oleoresin yield, indicating that inoculation with AMF (i) can replace P fertilization when the aim is to produce oleoresin and (ii) is a feasible technique for the production of ginger plants with increased quantities of oleoresin and oleoresins with different composition, depending on the associated AMF isolate.

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