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SHORT NOTE

Horst Vierheilig · Hubert Gagnon · Dieter Strack
Walter Maier

Accumulation of cyclohexenone derivatives in barley, wheat and maize roots in response to inoculation with different arbuscular mycorrhizal fungi

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Abstract Colonization of barley, wheat and maize roots by different arbuscular mycorrhizal fungi, i.e. *Glomus intraradices*, *Glomus mosseae*, and *Gigaspora rosea* leads to the accumulation of cyclohexenone derivatives. Mycorrhizal roots of all plants accumulate in response to all three fungi blumenin [9-*O*-(2′-*O*-glucuronosyl)-*β*-glucopyranoside of 6-(3-hydroxybutyl)-1,1,5-trimethyl-4-cyclohexen-3-one], 13-carboxyblumenol C 9-*O*-gentiobioside, nicoblumin [9-*O*-(6′-*O*-*β*-glucopyranosyl)-*β*-glucopyranoside of 13-hydroxy-6-(3-hydroxybutyl)-1,1,5-trimethyl-4-cyclohexen-3-one] and another, as yet unidentified, cyclohexenone derivative. The accumulation of all four compounds in three tested mycorrhizal plants colonized by the three arbuscular mycorrhizal fungi indicates no fungus-specific induction of these compounds.

Key words Glomales · *Glomus intraradices* · *Glomus mosseae* · *Gigaspora rosea* · Isoprenoid metabolism

Introduction

After colonization of roots by arbuscular mycorrhizal fungi (AMF) various changes occur in secondary plant metabolism, e.g. the accumulation of secondary plant compounds, as recently reviewed by Morandi (1996) and Vierheilig et al. (1998a). Extensive studies on secondary compounds in roots of the Poaceae colonized by AMF showed various glycosylated C13 cyclohexenone derivatives (Maier et al. 1995, 1997; Peipp et al. 1997). The same was observed recently in the non-gramineous plant *Nicotiana tabacum* (Maier et al. 1999). AMF most likely induce the formation of root carotenoids via the alternative non-mevalonate deoxyxylulose phosphate pathway. The first evidence for this came from feeding mycorrhizal barley roots ^{13}C-labeled glucose followed by ^{13}C-NMR spectroscopy of the cyclohexenone derivatives (Maier et al. 1998).

Recently, it was suggested that AMF have genus- or even species-specific requirements for the establishment of the symbiosis (Vierheilig et al. 1998a). Therefore, it was tempting to speculate that the accumulation of secondary plant compounds varies with the root-colonizing AMF. In this work, we tested the effect of inoculation of gramineous plants (barley, wheat and maize) with different AMF (*Glomus mosseae*, *Glomus intraradices* or *Gigaspora rosea*) on the induction of secondary compounds in roots.

Material and methods

Biological material and growing conditions

Grains of barley (*Hordeum vulgare* L. cv. Salome), wheat (*Triticum aestivum* L. cv. Hatri) and maize (*Zea mays* L. cv. Garant) were surface-sterilized by soaking in 0.75% sodium hypochlorite for 5 min, rinsed with tap water and germinated in vermiculite.

After 5 days, the seedlings were transferred to a steam-sterilized (40 min, 120 °C) mixture of silicate sand, TurFace (baked clay substrate mechanically broken into particles with a diameter of 2–5 mm; Applied Industrial Materials, Corp.; Buffalo Grove, Ill., USA) and soil (v:v:v/2:2:1). Inoculated and non-inoculated plants were grown in a growth chamber (day/night cycle: 16 h 23 °C/8 h 19 °C; RH 50%) in the compartment system developed by Wyss et al. (1991). The three AMF tested were: *G. mosseae* (Nicolson & Gerdemann) Gerd. & Trappe (BEG 12; La Banque Européenne des Glomales; International Institute of Biotechnology, UK); *G. intraradices* Smith & Schenck (DAOM 197198;

H. Vierheilig (✉)
Centre de Recherche en Biologie Forestiére,
Pavillon C.-E.-Marchand,
Université Laval,
Quebec G1K 7P4, Canada
Fax: +1-418-656-7493
e-mail: nonhorst@rsvs.ulaval.ca

H. Gagnon
Department of Biochemistry, Université de Montréal,
Montréal H3C 3J7, Canada

D. Strack · W. Maier
Leibniz-Institut für Pflanzenbiochemie,
Abteilung Sekundärstoffwechsel, Weinberg 3,
D-06120 Halle (Saale), Germany

Department of Agriculture, Ottawa, Canada), and a recent newly classified *G. rosea* Nicolson & Schenck (Bago et al. 1998), which was formerly wrongly classified as *G. margarita* Becker & Hall (DAOM 194757). The experiment was repeated twice with 3 replicates per treatment.

Estimation of root colonization

Several fresh roots from each plant were cleared by boiling in 10% KOH and stained according to the method of Vierheilig et al. (1998b) by boiling in a 5% ink (Shaeffer black/household vinegar = 5% acetic acid) solution. Stained roots were observed with a light microscope and the percentage of root colonization was determined according to a modified method of Newman (1966).

Root extraction and high-performance liquid chromatography

Sixteen days after inoculation with the AMF, plants were harvested and roots were rinsed with tap water, stored at –20 °C and finally lyophilized. These roots were treated twice for about 1 min with an Ultra Turrax homogenizer (Janke & Kunkel, Staufen, Germany) in 5 ml 80% aqueous methanol. The mixture was centrifuged and the supernatant was used for HPLC analysis (20-µl aliquots).

The HPLC system (Waters 600, Milford, Mass., USA) equipped with a 5-µm Nucleosil C_{18} column (20×4 mm i.d.; Macherey-Nagel, Düren, Germany) was described previously (Maier et al. 1995). Separation of the cyclohexenone compounds was achieved using a linear gradient elution system at a flow rate of 1 ml/min within 30 min from 5 to 20% solvent B (acetonitrile) in solvent A (1.5% ortho-phosphoric acid in water). Compounds were photometrically detected (maxplot between 210 and 450 nm) by a Waters 996 photodiode array detector.

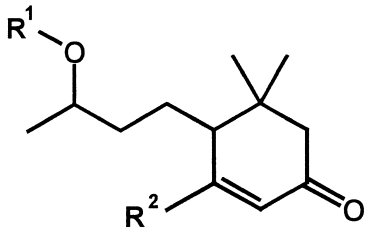
The cyclohexenone derivatives were identified by comparison (HPLC online UV-spectroscopy) with standards (Maier unpublished data).

Results and discussion

Roots of all three plants tested (barley, wheat and maize) were colonized by the different AMF and showed a fungus-induced formation of cyclohexenone derivatives (Fig. 1). Root colonization of barley was 70 ± 6% (mean ± SD) with *Gi. rosea*, 81 ± 5% with *G. intraradices* and 80 ± 4% with *G. mosseae*, of wheat was 63 ± 3% with *Gi. rosea*, 75 ± 5% with *G. intraradices* and 88 ± 2% with *G. mosseae*, and of maize was 70 ± 5% with *Gi. rosea*, 70 ± 5% with *G. intraradices* and 77 ± 3% with *G. mosseae*.

Vierheilig et al. (1998a) suggested recently that, apart from general signal requirements similar for all AMF, AMF might also have genus- or even species-specific requirements for successful establishment of the symbiosis. This could be reflected in different accumulation patterns of secondary compounds in roots colonized by different AMF.

As shown in Figure 2, all four cyclohexenone derivatives determined in this study (Fig. 1) were detected in all mycorrhizal roots, but never in non-mycorrhizal control roots: (1) blumenin [9-*O*-(2′-*O*-glucuronosyl)-*β*-glucopyranoside of 6-(3-hydroxybutyl)-1,1,5-trimethyl-4-cyclohexen-3-one] (Maier et al. 1995); (2)



	R^1	R^2
1	Glc(2)—GlcUA	CH_3
2	—— unknown ——	
3	Glc(6)—Glc	CH_2OH
4	Glc(6)—Glc	COOH

Fig. 1 Structures of the cyclohexenone derivatives accumulating in gramineous mycorrhizal roots (*Glc* glucose, *GlcUA* glucuronic acid)

unknown cyclohexenone derivative (tentatively identified by its HPLC retention behaviour and UV spectrum); (3) nicoblumin [9-*O*-(6′-*O*-*β*-glucopyranosyl)-*β*-glucopyranoside of 13-hydroxy-6-(3-hydroxybutyl)-1,1,5-trimethyl-4-cyclohexen-3-one] (Maier et al. 1999); (4) 13-carboxyblumenol C 9-*O*-gentiobioside (W. Maier, J. Schmidt, V. Wray, D. Strack, unpublished). This induction of all four compounds by all three AMF dis-

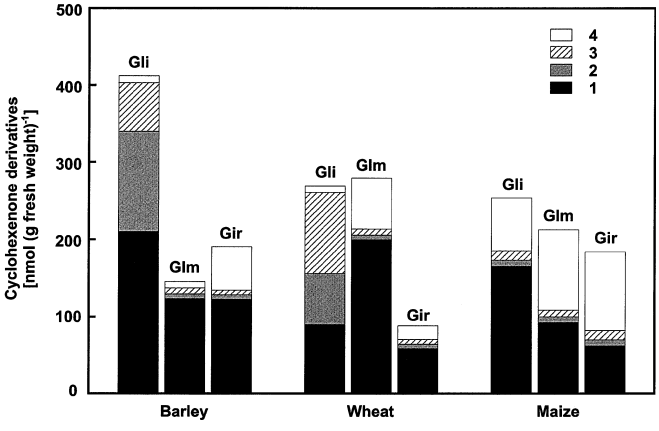


Fig. 2 Induction of cyclohexenone derivatives by the arbuscular mycorrhizal fungi *Glomus intraradices* (Gli), *Glomus mosseae* (Glm) and *Gigaspora rosea* (Gir) in roots of barley, wheat and maize. *1* blumenin, *2* unknown cyclohexenone derivative, *3* nicoblumin, *4* 13-carboxyblumenol C 9-*O*-gentiobioside. For structures see Figure 1

cards their fungus-specific induction; however, a fungus-specific accumulation of other compounds can not be excluded.

Recently Grandmaison et al. (1993) found different accumulation levels of some secondary plant compounds in roots colonized by *G. intraradix* or *G. versiforme*. Interestingly, we also detected differences in the accumulation levels of compounds. The concentration of compound *1* in wheat roots colonized by *G. mosseae* was more than doubled compared with roots colonized by the other two AMF. Compound *3* and the unknown compound *2* were detected in relatively large amounts in wheat and barley roots colonized by *G. intraradices* and in small amounts in the other plants with all three fungi. Compound *4* accumulated in significant amounts in all mycorrhizal maize roots, in wheat with *G. mosseae* and in barley with *Gi. rosea*. These accumulation patterns were consistent in the two experiments performed, indicating a possible quantitative but not a qualitative fungus-specific induction of the determined compounds.

In all previous studies of secondary compounds in members of the Poaceae, *G. intraradices* has been used for inoculation, resulting in an accumulation of compound *1* in the mycorrhizal roots of many species tested (Maier et al. 1995 1997; Peipp et al. 1997). We found that compound *1* also accumulates in maize (Maydeae), barley and wheat roots after colonization by the other two AMF tested.

The function of the compounds determined in this study in the formation of the AM symbiosis is still unknown. Most information is available about compound *1*. As this compound is not induced by fungal pathogens or endophytes (Maier et al. 1997), a specific role in the AM symbiosis seems likely. Recently, application of compound *1* to roots in the presence of an AMF resulted in reduced root colonization and arbuscule formation (Fester et al. 1999). With regard to this observation, it is interesting to note that a suppression of further colonization by AMF has been found in barley roots precolonized by AMF (Vierheilig et al. 2000). Moreover, application of root exudates of mycorrhizal plants to roots in the presence of AMF showed an inhibitory effect on root colonization (Pinior et al. 1999). These two observations point toward the presence of a factor in mycorrhizal roots that affects further root colonization by AMF negatively.

To summarize, our results suggest no qualitatively different induction patterns of cyclohexenone derivatives in roots colonized by different AMF; however, different quantitative fungus-specific accumulation patterns seem possible. Further studies are needed to elucidate whether these quantitative differences are of biological relevance for the symbiosis.

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