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SHORT NOTE

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Systemic inhibition of arbuscular mycorrhiza development by root exudates of cucumber plants colonized by *Glomus mosseae*

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Abstract The arbuscular mycorrhizal (AM) non-host plants mustard, sugar beet, lupin and the AM host plant cucumber were used as test plants. Cucumber plants were grown either in the absence of the AM fungus (AMF) *Glomus mosseae* or in a split-root system, with one side mycorrhizal and one side non-mycorrhizal. Root exudates of the AM non-host plants, the non-mycorrhizal cucumber plants and the mycorrhizal and the non-mycorrhizal side of the split-root system of mycorrhizal cucumber plants were collected and applied to cucumber plants inoculated with the AMF. Root exudates of non-mycorrhizal cucumber plants showed a significant stimulatory effect on root colonization, whereas root exudates from the mycorrhizal and the non-mycorrhizal sides of a split-root system of a mycorrhizal cucumber plant did not show this stimulatory effect and were even slightly inhibitory. Root exudates of the two AM non-host plants mustard and sugar beet significantly reduced root colonization in cucumber plants, whereas no such effect was observed when root exudates of the AM non-host plant lupin were applied.

Keywords Glomales · Mycorrhiza · Root exudates · Non-host · Host · Systemic

Introduction

Root exudates are the first plant signals arbuscular mycorrhizal fungi (AMF) perceive and, thus, are thought to play an important role in the establishment of the arbuscular mycorrhizal (AM) symbiosis. There is abundant information about the stimulatory effect of root exudates from AM host plants on the asymbiotic (germ tube) in vitro growth of AMF from spores (e.g. Graham 1982; Elias and Safir 1987; Becard and Piché 1989; Gianinazzi-Pearson et al. 1989; Tawaraya et al. 1996). In these experiments, sterile root exudates were used because other soil-inhabiting fungi and bacteria have higher growth rates than AMF. However, such sterile experimental set-ups do not reflect the complex situation in the rhizosphere, where there are many interacting microorganisms.

Recently, Tawaraya et al. (1998) have reported a test for the effect of non-sterile root exudates on the development of the AM symbiosis. Non-sterile root exudates were collected from onion plants and applied to onion plants inoculated with an AMF. Non-sterile root exudates of phosphorus (P)-deficient plants stimulated AM root colonization, whereas this effect was less evident when non-sterile root exudates of P-supplied plants were applied (Tawaraya et al. 1998). Although the test gave no indication of whether the root exudates affected the first stages of the AMF-plant interaction (i.e. contact and recognition between partners) and/or intraradical fungal spreading, it is a useful approach for evaluating the effect of different types of root exudates on the development of the AM symbiosis.

Recently, it has been suggested that plants, once colonized by an AMF, suppress further root colonization by AMF (Vierheilig and Piché 2002). Using the experimental system of Tawaraya et al. (1998), Pinior et al. (1999) reported that root exudation patterns differ to the mycorrhizal status of the plant. Root exudates from cucumber plants colonized by an AMF not only lost their stimulatory effect on root colonization, but inhibited root colonization. These results indicate that mycorrhization can alter the root exudation pattern of a host plant and thus affect AM development. Systemic suppression of further AM root colonization in mycorrhizal plants has been reported in several studies (Vierheilig et al. 2000a, 2000b); however, no data have been provided on the mechanisms involved.

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Although most land plants are AM host plants, some plant families, e.g. the Brassicaceae and the Chenopodiaceae, are in general non-AM (Smith and Read 1997). The mechanism responsible for the non-susceptibility of these plants to AMF is still controversial. The hypothesis has been put forward that root exudates of some AM non-host plants lack signals essential for root colonization by AMF, whereas root exudates of other AM non-host plants contain compounds inhibitory to AMF (reviewed by Giovannetti and Sbrana 1998; Giovannetti 2000; Vierheilig et al. 1998a).

Using the experimental system described by Tawaraya et al. (1998), we tested whether the suppressive effect of root exudates of mycorrhizal cucumber plants on root colonization by AMF is not only local (Pinior et al. 1999) but also systemic and whether root exudates of different AM non-host plants have a similar effect on root colonization.

Materials and methods

Biological material

Cucumber (*Cucumis sativus* L. cv. Straight Eight), mustard (*Brassica nigra* L.), sugar beet (*Beta vulgaris* L. cv. Red Ace) and lupin (*Lupinus albus* cv. Blanca; bitter) seeds were surface sterilized in 50% commercial bleach for 5 min, rinsed several times in sterile distilled water and germinated in autoclaved (40 min, 120°C) vermiculite.

Plants were inoculated with *Glomus mosseae* (Nicolson and Gerdemann) Gerd. & Trappe (BEG 12; La Banque Européenne des Glomales).

Collection of root exudates of non-host plants

Six days after planting, mustard, sugar beet and lupin seeds had germinated and were transferred into pots in a steam-sterilized (40 min, 120°C) mixture of silicate sand, TurFace (baked clay substrate that is mechanically broken into particles with a diameter of 2–5 mm) and a loamy field soil (v:v:v/2:2:1) in a growth chamber (day/night cycle: 16 h, 22°C/8 h, 20°C; RH 50%). Twenty-one days later, whole plants were harvested, roots were rinsed with tap water and placed in distilled water for 22 h in a growth chamber (for details see above) in order to obtain root exudates. After collection of the root exudates, the root fresh weight was determined.

Collection of root exudates from split-root systems of cucumber plants

Four days after planting, the main root was cut from germinated cucumber seeds. Thereafter, plants were grown for a further 6 days in autoclaved vermiculite, then transferred into a split-root compartment system (see details below) in the mixture of silicate sand, TurFace and soil described above. Plants were maintained in a growth chamber as above.

The split-root compartment system consisted of three compartments: an inoculum compartment and two split-root compartments. Three bean seedlings (*Phaseolus vulgaris* L. cv. Sun Gold) were planted into the inoculum compartment in the silicate sand, TurFace, soil substrate with an inoculum of *G. mosseae* (3:1/v:v). The inoculum consisted of the substrate in which inoculated beans had been grown for 3 months. After 1 month, the AM symbiosis was well established (>50% root colonization) in the inoculum compartments and the compartments were ready for use.

The two split-root compartments were glued together, but separated by a PVC plate. The root systems of cucumber plants were each divided into two equal parts and planted in the two split-root compartments. As the PVC plate separating such two split-root compartments is impermeable for roots and hyphae, one part of the root system can be mycorrhizal, whilst the other part remains uncolonized.

The glued split-root compartments were joined on one side with the inoculum compartment (containing the growing beans), from which they were separated by a nylon screen (60 μm mesh size) that can be penetrated by fungal hyphae but not by roots. Joining the inoculum compartment to the split-root plant compartments thus results in fast root colonization of one side of the split-root system (for further details see Vierheilig et al. 2000a).

Twenty days after joining the split-root compartment to the inoculum compartment, intact cucumber plants were harvested and their roots rinsed with tap water. Roots from the mycorrhizal and non-mycorrhizal sides of the split-root system were placed in distilled water in separate beakers for 22 h (see above) to obtain root exudates. Root exudates of non-mycorrhizal cucumber plants, grown in the same way but without *G. mosseae* in the inoculum compartment, were also collected.

The percent colonization of mycorrhizal and non-mycorrhizal roots from the split-root systems and the root fresh weight of all plants were determined.

Treatment of root exudates

The crude, non-concentrated root exudate solutions were filtered (Whatman 1). In order to standardize root exudate concentrations, the ratio root fresh weight/root exudate solution (w/v) was adjusted to 1 g root fresh weight equivalent to 22 ml of exudate solution by adding distilled water as necessary. The pH of the non-host plant solutions and of the cucumber solutions was measured. The pH of the distilled H_20 (water control, pH 6.4) and the exudate solutions in the AM non-host root exudates (pH 5.2–5.5) was adjusted with HCl or KOH to 6, and in the split-cucumber root exudates (pH 6.6–6.7) to 6.6. Exudates were stored at −20°C until use.

Estimation of root colonization

To visualize AMF colonization, roots were cleared by boiling for 4 min in 10% KOH, rinsed three times with tap water and stained by boiling for 4 min in a 5% ink (Shaeffer; black)/household vinegar (5% acetic acid) solution (Vierheilig et al. 1998b). After staining, the percent root colonization was determined according to the method of Newman (1966) modified by Giovannetti and Mosse (1980).

Testing of the effect of root exudates on root colonization

The effect of collected root exudates on root colonization of cucumber plants by *G. mosseae* was tested in 70-ml pots containing a mixture of silicate sand, TurFace and soil as described above. Cucumber plants (5 days old) were transferred to the pots (one plant/pot) and inoculated with *G. mosseae* by mixing an inoculum of the fungus with growth substrate (1:1, v:v). Root exudate solutions of non-host plants or of the mycorrhizal and non-mycorrhizal sides of the split-root system of cucumber plants (4 ml per plant) were applied daily to each cucumber plant. Distilled H_20 was used as a control. Eleven days later, cucumber plants were harvested and root colonization was determined.

The experiments were repeated twice using five replicate pots per treatment. Experimental data were analyzed with ANOVA and a post-hoc LSD test (P=0.05).

The experiments with root exudates of non-host plants and of cucumber plants were performed independently. As root coloniza-

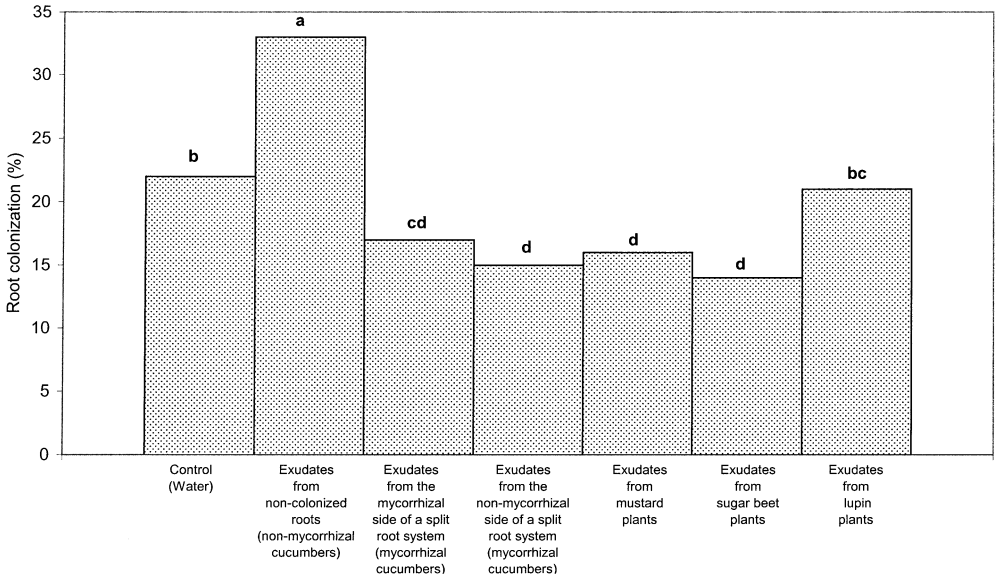

Fig. 1 Effect on root colonization of cucumber plants of root exudates from the AM non-host plants mustard, sugar beet and lupin and from the mycorrhizal and non-mycorrhizal sides of a cucumber split-root system 11 days after inoculation with *Glomus mosseae*. *Bars with the same letter* are not significantly different (P=0.05; ANOVA and post-hoc LSD test)

tion was similar in the water control treatments in both experiments, the data of the two experiments were combined in Fig. 1.

Results and Discussion

It has been reported that root exudates of several AM non-host plants, e.g. different Brassicaceae (El-Atrach et al. 1989; Schreiner and Koide 1993), sugar beet (Chenopodiaceae) (Nagahashi and Douds 2000) and lupin (Oba et al. 2002), inhibit asymbiotic hyphal growth of AMF from spores. Studies in soil compartment systems have also demonstrated that roots of the AM non-host plants rape (Brassicaceae), spinach (Chenopodiaceae) and stinging nettle (Urticaceae) negatively affect the spread of extraradical hyphae (symbiotic fungal growth) of *G. mosseae* (Vierheilig et al. 1995, 1996). The results from the present study show that the inhibitory effect of root exudates of Brassicaceae (mustard) and Chenopodiaceae (sugar beet) on AMF is also at the level of root colonization (Fig. 1).

Moreover, root exudates from different non-host plants exhibited different effects on root colonization. Whereas mustard and sugar beet apparently reduced root colonization by AMF in cucumber at least partially through inhibitory compounds in their exudates, root exudates of lupin had no effect on cucumber root colonization (Fig. 1). This points towards a difference in the mechanism controlling non-susceptibility in lupins and other AM non-host plants. Interestingly, *L. albus* seems to be atypical of the genus *Lupinus*. Whereas compounds released by roots of *L. albus* affect neither spore germination (Avio et al. 1990) nor asymbiotic hyphal growth (Oba et al. 2002), compounds released by roots of other *Lupinus* species such as *L. luteus*, *L. cosentini* and *L. aridus* clearly inhibited asymbiotic hyphal growth (Oba et al. 2002). The data presented here indicate that there is no general mechanism for the non-susceptibility of AM non-host plants to AMF and that inhibitory compounds in root exudates play a role in the expression of the non-host status of some plants (Vierheilig et al. 1998a).

Root exudates of AM host plants affect root colonization differently than root exudates of AM non-host plants. A stimulatory effect of root exudates of non-mycorrhizal AM host plants on root colonization was found by Tawaraya et al. (1998) with onion, and by Pinior et al. (1999) and in our study with cucumber. Recently, the flavonoid isovitexin 23-*O*-β-glucoside was detected in non-mycorrhizal but not in mycorrhizal roots of melon (Akiyama et al. 2002). Application of this flavonoid to AMF-inoculated melon plants enhanced root colonization. Melon (*Cucumis melo*) belongs to the same genus as cucumber. This suggests that, similar to mycorrhizal melon roots, root colonization-stimulating compounds are lower or absent in mycorrhizal cucumber roots, and thus are also reduced or absent in root exudates. This should result in a reduced stimulatory effect on root colonization or a root colonization similar to the water control plants.

In our study, the effect of root exudates from the mycorrhizal (63%±7 root colonization) and the non-mycorrhizal (0%±0 root colonization) sides of a cucumber split-root system and of root exudates from non-mycorrhizal cucumber plants (0%±0 root colonization) on root colonization of cucumber plants by *G. mosseae* was studied. Root exudates of non-mycorrhizal cucumber plants clearly stimulated root colonization of cucumber plants, whereas root exudates of the mycorrhizal side of the split-root system plants had an inhibitory effect on colonization (Fig. 1). This points towards the exudation of an inhibitory compound(s) by mycorrhizal cucumber roots. The accumulation of a broad range of plant secondary metabolites in mycorrhizal roots is well documented and the involvement of some of these compounds in the establishment of AM symbiosis has been suggested. However, only few data are available on the effect of accumulated compounds on

AMF (reviewed by Morandi 1996; Vierheilig et al. 1998a) and there are no data on their presence in root exudates of mycorrhizal plants.

Looking at our results, it is tempting to speculate that alterations in the root exudation of a mycorrhizal plant affect the susceptibility of the plant not only to AMF but also to other soil-borne fungi (see Vierheilig and Piché 2002). Norman and Hooker (2000) have recently reported that sporulation of the fungal pathogen *Phytophthora fragariae* is stimulated more by exudates of non-mycorrhizal than mycorrhizal strawberry plants.

A systemic effect has been suggested in the autoregulation of mycorrhization. When one side of a split root system was colonized by an AMF, further root colonization of the other side by an AMF was suppressed (Vierheilig et al. 2000a, 2000b). Interestingly, we found that root exudates from the non-mycorrhizal half of the split-root system of a mycorrhizal cucumber plant did not have the expected stimulatory effect on root colonization (similar to root exudates of non-mycorrhizal cucumber plants), but were even inhibitory (Fig. 1). This indicates that root exudates in mycorrhizal plants are systemically altered and, thus, are at least partially involved in the systemic suppression of further AM root colonization in mycorrhizal plants. Further studies are needed to elucidate whether these systemic alterations in root exudates are also involved in the systemic resistance of mycorrhizal plants to soil-borne fungal pathogens (Cordier et al. 1998; Pozo et al. 2002).

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