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Short communication

Exudates of dark septate endophyte (DSE) modulate the development of the arbuscular mycorrhizal fungus (AMF) *Gigaspora rosea*

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ABSTRACT

Exudates of a dark septate endophyte (DSE) identified as *Dreschlera* sp., a common endophyte isolated by the inner cortical cells of the grass *Lolium multiflorum*, were put in contact with the arbuscular mycorrhizal fungus (AMF) *Gigaspora rosea*. These exudates stimulated the hyphal length and the hyphal branching of the AMF. A negative effect on the extramatrical phase of the AMF was detected. This is the first report to show how exudates of DSE can affect the development of AMF. These results show that DSE could be modifying the mycorrhizal status of the plants, modulating a different symbiosis in the rhizosphere.

Vascular plants host a great variety of microorganisms. Colonization of roots by fungal endophytes or mycorrhizal fungi is a common feature in the plant kingdom. The arbuscular mycorrhizal fungi (AMF) are important components of the rhizospheric microbial community. Another type of root-dwelling symbiotic fungi, the so-called dark septate endophyte (DSE) fungi, has recently been identified as frequent colonizers of plant root. These fungi with regularly septate and melanised hyphae probably constitute the most abundant and most widespread group of colonizer endophytes.

Arbuscular mycorrhizal fungi (AMF) are known to be influenced by the activities of microorganisms in the soil (Bagyaraj, 1990; Andrade et al., 1997). Mycorrhiza formation can affect the microbial population in the rhizosphere directly or indirectly through changes in root exudation patterns, or through fungal exudates (Linderman, 1992). In this way AMF are known to interact with other soil microorganism such as yeast or saprophytic fungi (Fracchia et al., 2004; Scervino et al., 2008) and are common components in the rhizosphere and share the ecological niches with DSE.

Like mycorrhizal associations, DSE associations can either stimulate or reduce host plant growth (Caldwell et al., 2000). They are found worldwide, and often coexist with different mycorrhizal fungi (Muthukumar et al., 2006; Menoyo et al., 2007). The function of DSE is controversial, they have been found to be pathogenic to mutualistic symbionts (Jumpponen and Trappe, 1998). The physiology of this tripartite association has not been studied before.

Soil microorganisms mainly influence AM fungi when these are in the extramatrical phase or, as was recently shown (Scervino et al., 2008), when the AMF fungi are in the presymbiotic stage. Soluble exudates produced by soil microorganisms are involved in these effects (Sampedro et al., 2004). Nevertheless, in contrast to the large number of studies involving the interaction between AMF and saprophytic fungi, no studies on the influence of DSE on the presymbiotic stage of AMF interactions have been conducted.

Presently we are studying the effect of exudates produced by a typical DSE fungus on hyphal growth, hyphal tips and extramatrical phase of the AM fungi *Gigaspora rosea* (BEG no. 9).

The culture and isolation of DSE fungi from roots of *Lolium multiflorum* was undertaken as described by Silvani et al. (2008). Healthy roots with visible intraradical structures were selected, surface-sterilized and cut into pieces. Each root piece was transferred to drops of Gel-Gro medium and incubated at 25 °C in the dark. After re-growth took place, segments with DSE fungi were plated onto malt extract agar (MEA).

In order to verify that the isolate was the same as those seen in plant roots, resynthesis studies (Schadt et al., 2001) were performed in *L. multiflorum* and *Solanum lycopersicum*. The resynthesis studies confirmed, on one hand, that the isolate is the same as those originally develop in plant roots samples. On the other hand, the resynthesis surveys showed a typical DSE development in both,

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S. lycopersicum and *L. multiflorum* (Fig. 1), growing with hyphaes running parallels within intercellular spaces and the typical formation of microsclerotia of DSE.

The isolated DSE fungus was inoculated on a Petri dish containing MEA and incubated 25 days at 25 °C in the darkness. Aerial mycelium was harvested, and the genomic DNA was extracted as described by Grünig et al. (2001). The strain was molecularly identified using nucleotide sequence data from the ITS1–5.8S–ITS2 (ITS) region of the nuclear ribosomal DNA. Conditions for PCR amplification were adopted from Gottlieb et al. (2005). Both strands of the complete ITS region (ITS 1–5.8S–ITS 2) were sequenced. Boundaries of the coding and spacer regions were determined by comparison with published sequences of endophytic fungi. The ITS sequence was deposited in GenBank (FJ868975). The BlastN search of the complete ITS nucleotide sequence (627 bp) of the DSE yielded 145 hits, being *Dreschlera* sp. (AY336133, Deacon et al., unpublished) the best score (99% of query coverage and 98% of identity). The genus *Dreschlera* has been isolated from grasses (Ellis, 1971) and aforementioned as endophyte of root (AY336133 NCBI date base, Deacon et al., unpublished). The loss of reproductive abilities complicates the morphological identification of asexual isolates and has been related to the acquisition of mutualistic habit (Law and Lewis, 1983).

Dreschlera sp. exudates were obtained after 10 days of incubation in minimal medium (Fracchia et al., 2003), at which time stationary growth was observed. The culture medium was filtered and centrifuged at 3000 rpm and the supernatant was sterilized by filtration through a 0.20 μm Millipore membrane. The effect of *Dreschlera* sp. exudates (0.05, 1.5 and 3% (V/V)) on hyphal length of AMF *G. rosea* was tested according to Scervino et al. (2008). These concentrations were used to fill 5 cm of Petri dishes with 10 ml of 4% Gel-Gro in 10 mM MES buffer (pH 7) containing 10 surface-sterilized spores (Mosse, 1962). The Petri dish contained semi-solid media (Gel-Gro) amended with the filtered exudates. The viscosity of this semi-solid media allowed optimal spore germination and contact of the spores with the exudates. A treatment without exudates but with spores was used as control. Ten replicates were used for each treatment. The plates were incubated at 25 °C for 15 days and hyphal length and the hyphal branched (measure as hyphal tips) was determined (Marsh, 1971).

Different results have been shown in the last few years. For instance, the percentage of spore germination was increased by exudates of saprobe fungi (Fracchia et al., 2004), whereas exudates of the yeast showed no effect on this parameter (Scervino et al., 2008). The exudates of our strain of *Dreschlera* increased the hyphal length and the hyphal tips of *G. rosea* spores except at high doses (Fig. 2A–B). One of the concentrations (1.5%) increased the hyphal length of the *G. rosea* spores (Fig. 2A). By contrast, the number of hyphal tips increased when the spores were in contact with either 0.05 or 1.5% of *Drechslera* sp. exudates (Fig. 2B). Similar studies in agreement with experiments reported here indicate that the nature of the compound affecting AMF became important when microorganisms of soil, as yeast or saprophytic fungi, achieve the exponential growth phase (Fracchia et al., 2004; Scervino et al., 2008). The results of these experiments suggest that exudates of *Drechslera* sp. can be considered as hyphal "modulators", stimulating or inhibiting hyphal growth of AMF depending on their concentration (Fracchia et al., 2004; Scervino et al., 2008).

In a second step, an experiment to evaluate the effect on the extramatrical mycelium development of AMF *G. rosea* was done as described by González-Guerrero et al. (2008). For this experiment spores of *G. rosea* were produced by using in vitro cultures of carrot (*Daucus carota*) hairy roots in medium M (Bécard and Fortin, 1988). The medium M was supplied with the same DSE exudate concentrations of the first experiment (i.e., 0; 0.05; 1.5; 3%). The dishes were incubated (25 °C) for a month and the extramatrical phase was measured after 14, 21 and 30 days using the gridline intersect method (Newman, 1966).

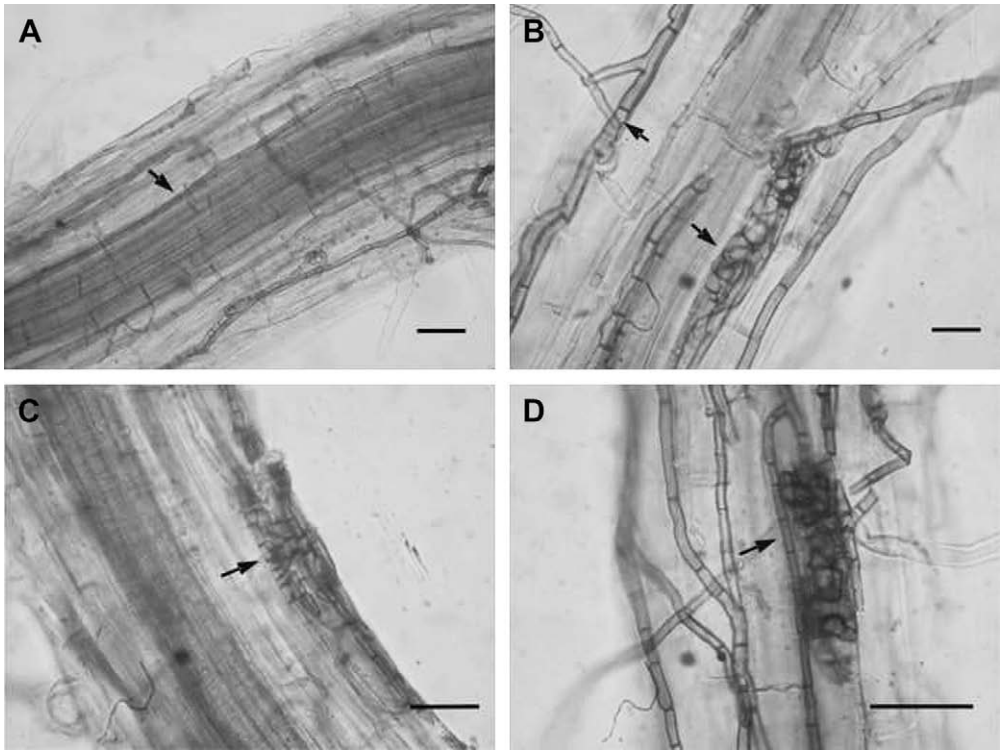

Fig. 1. (A–D) Typical root colonization by the DSE (*Drechslera* sp.) is showed in the resynthesis experiments on *Solanum lycopersicum* (A, C) and *Lolium multiflorum* (B, D). The arrows indicate hyphaes running parallels within intercellular spaces and the typical formation of microsclerotia. *Bars* 100 μm.

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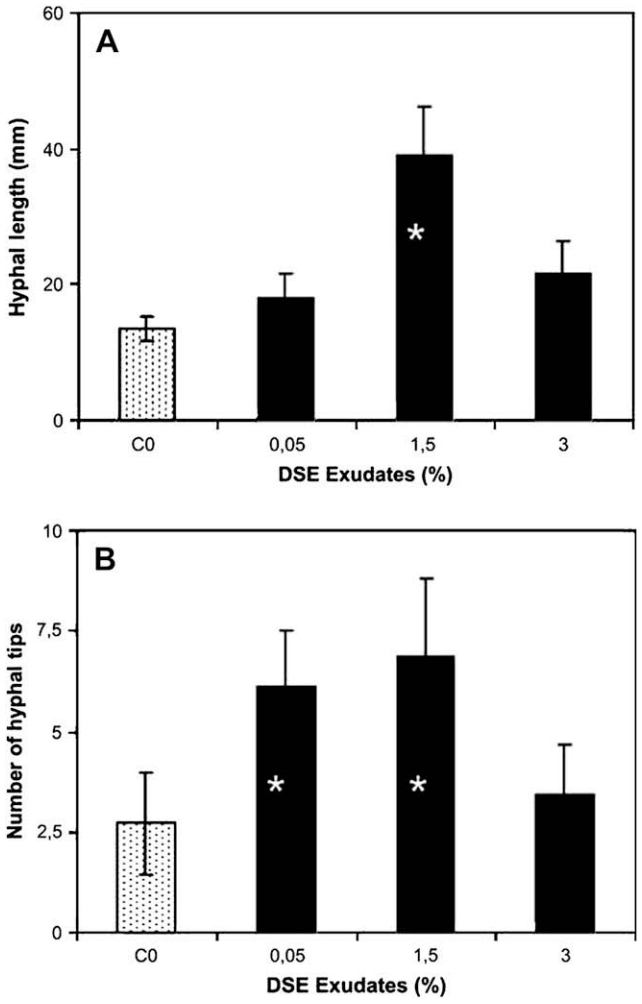

Fig. 2. (A and B) Effect of various concentrations of DSE (*Drechslera* sp.) exudates on the presymbiotic stage of *G. rosea*. (A) Effect of the DSE exudates on the hyphal length of germinated spores of *G. rosea*. (B) Effect of the DSE exudates on the hyphal tips development of mycelium of *G. rosea*. Vertical bars are the standard deviation ($p = 0.05$). (*) Significantly different from control. Each value represents the mean of ten replicates.

Once the AMF infected the root, the hyphal development was inhibited by the exudates of the DSE fungus (Table 1). The lowest exudate concentration (0.05%) negatively affected the extramatrical phase of the AMF interaction at day 30th, while concentrations (0.5, 1.5 and 3%) did not affect hyphal development (Table 1). The extramatrical phase was negatively affected by the exudates of the DSE. These results could not entirely be explained. However, recent work showed that compounds produced by one microorganism can affect different parameters of AM interaction (Scervino et al., 2006, 2007). Our experiment indicated that the extramatrical phase and the presymbiotic stage of AMF can be affected in different ways for the same microorganism. Similar results seem to show that soil microorganisms mainly influence AM fungi when they are in the extramatrical phase (McAllister et al., 1994). Different experiments should be conducted to evaluate the effect of DSE exudates on AM interaction independently of the root exudate influence.

Table 1
Effect of DSE (*Drechslera* sp.) exudates on the hyphal length of extramatrical phase of *G. rosea*.

Hyphal length (mm)[a]	Measure time		
Exudate %	14 days	21 days	30 days
0% (control)	44.09[a]	105.93[b]	452.56[c]
0.05%	38.27[a]	90.95[b]	292.83[d]
1.5%	34.94[a]	101.49[b]	468.08[c]
3%	45.43[a]	102.05[b]	420.94[c]

[a] Column values with the same letter are not significantly different as determined by Tukey's test ($p = 0.05$).

Infection and symbiosis development might be two independent processes. The fungus physiology changed once the symbiosis was established, however these processes should be investigated more deeply.

In conclusion, the AM interaction can be affected by exudates of endophytic fungi. Based on the fact that *Dreschlera* sp. (DSE fungi) can also act as saprobes (Caldwell et al., 2000; Law and Lewis, 1983), it is therefore likely that this fungus might modulate the growth of other rhizospheric microorganisms. It is proposed that an increase in the presymbiotic stage of AMF could be translated in an increase in root colonization. However, experiments in glasshouse pot trials should be conducted in order to test this hypothesis.

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References

Andrade, G., Mihara, K.L., Linderman, R.G., Bethlenfalvay, G.J., 1997. Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. Plant and Soil 192, 71–79.
Bagyaraj, D.A., 1990. Ecology of vesicular-arbuscular mycorrhizae. In: Arora, D.K., Rai, B., Mukerjii, K.G., Knudsen, G.R. (Eds.), Handbook of Applied Mycology. Soil and Plants. Marcel Dekker, Inc., New York, pp. 3–34.
Bécard, G., Fortin, J.A., 1988. Early events of vesicular-arbuscular mycorrhiza formation on Ri T-DNA transformed roots. New Phytologist 108, 211–218.
Caldwell, B.A., Jumpponen, A., Trappe, J.M., 2000. Utilization of major detrital substrates by dark-septate, root endophytes. Mycologia 92, 230–232.
Ellis, M.B., 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, pp. 403–452.
Fracchia, S., Godeas, A., Scervino, J.M., Sampedro, I., Ocampo, J.A., Garcia-Romero, I., 2003. Interaction between soil yeast *Rhodotorula mucilaginosa* and the arbuscular mycorrhizal fungi *Glomus mosseae* and *Gigaspora rosea*. Soil Biology & Biochemistry 35, 701–707.
Fracchia, S., Sampedro, I., Scervino, J.M., Garcia-Romero, I., Godeas, A., Ocampo, J.A., 2004. Influence of saprobe fungi and its exudates on the arbuscular mycorrhizal symbiosis. Symbiosis 36, 161–182.
González-Guerrero, M., Lewis, H.M., Ferrol, N., Lott, J.N.A., Azcón-Aguilar, C., Petersen, L., 2008. Ultrastructural localization of heavy metals in the extraradical mycelium and spores of the arbuscular mycorrhizal fungus *Glomus intraradices*. Canadian Journal of Microbiology 54, 103–110.
Gottlieb, A.M., Giberti, G.C., Poggio, L., 2005. Molecular analyses of the genus *Ilex* (Aquifoliaceae) in southern South America, evidence from AFLP and ITS sequence data. American Journal of Botany 92, 352–369.
Grünig, C.R., Sieber, T.N., Holdenrieder, O., 2001. Characterisation of dark septate endophytic fungi (DSE) using inter-simple-sequence-repeat-anchored polymerase chain reaction (ISSR-PCR) amplification. Mycological Research 105, 24–32.
Jumpponen, A., Trappe, J.M., 1998. Dark-septate root endophytes: a review with special reference to facultative biotrophic symbiosis. New Phytologist 140, 295–310.
Law, R., Lewis, D.H., 1983. Biotic environments and the maintenance of sex – some evidence from mutualistic symbioses. Biological Journal of the Linnean Society 20, 249–276.
Linderman, R.G., 1992. Vesicular-arbuscular mycorrhizae and soil microbial interactions. In: Bethlenfalvay, G.J., Linderman, R.G. (Eds.), Mycorrhizae in Sustainable Agriculture. ASA Spec. Publ., pp. 45–70. Madison, Wisconsin.
Marsh, B.A.B., 1971. Measurement of length in random arrangements of lines. Journal of Applied Ecology 8, 265–270.
McAllister, C.B., García-Romera, I., Godeas, A., Ocampo, J.A., 1994. Interaction between *Trichoderma koningii*, *Fusarium solani* and *Glomus mosseae*: effect on plant growth, arbuscular mycorrhizas and saprophytic population. Soil Biology & Biochemistry 26, 1363–1367.
Menoyo, E., Becerra, A.G., Renison, D., 2007. Mycorrhizal associations in *Polylepis* woodlands of Central Argentina. Canadian Journal of Botany 85, 526–531.

Mosse, B., 1962. The establishment of vesicular arbuscular mycorrhiza under aseptic conditions. Journal of General Microbiology 27, 509–520.

Muthukumar, Senthilkumar, M., Rajangam, M., Udaiyan, K., 2006. Arbuscular mycorrhizal morphology and dark septate fungal associations in medicinal and aromatic plants of Western Ghats, Southern India. Mycorrhiza 17, 11–24.

Newman, E.I., 1966. A method of estimating the total length of root in a sample. Journal of Applied Ecology 3, 139–145.

Sampedro, I., Aranda, E., Scervino, M., Fracchia, S., Garcia-Romera, I., Ocampo, J.A., Godeas, A., 2004. Improvement by soil yeasts of arbuscular mycorrhizal symbiosis of soybean (*Glycine max*) colonised with *Glomus mosseae*. Mycorrhiza 14, 229–234.

Scervino, J.M., Ponce, M.A., Erra-Bassells, R., Bompadre, J., Vierheilig, H., Ocampo, J.A., Godeas, A., 2007. The effect of flavones and flavonols on colonization of tomato plants by arbuscular mycorrhizal fungi of the genera *Gigaspora* and *Glomus*. Canadian Journal of Microbiology 53, 702–709.

Scervino, J.M., Ponce, M.A., Erra-Bassels, R., Vierheilig, H., Ocampo, J.A., Godeas, A., 2006. Glycosidation of apigenin results in a loss of its activity on different growth parameters of arbuscular mycorrhizal fungi from the genus *Glomus* and *Gigaspora*. Soil Biology & Biochemistry 38, 2919–2922.

Scervino, J.M., Sampedro, I., Ponce, M.A., Rodríguez, M.A., Ocampo, J.A., Godeas, A., 2008. Rhodotorulic acid enhances root colonization of tomato plants by arbuscular mycorrhizal (AM) fungi due to its stimulatory effect on the pre-symbiotic stages of the AM fungi. Soil Biology & Biochemistry 40, 2474–2476.

Schadt, C.W., Mullen, R.B., Schmidt, S.K., 2001. Isolation and phylogenetic identification of a dark-septate fungus associated with the alpine plant *Ranunculus adoneus*. New Phytologist 150, 747–755.

Silvani, V.A., Fracchia, S., Fernandez, L., Pergola, M., Godeas, A., 2008. A simple method to obtain endophytic microorganisms from field-collected roots. Soil Biology & Biochemistry 40, 1259–1263.

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