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Alteration of plant metabolites and root exudate-mediated interactions by pathogenic and mycorrhizal fungi in tomato

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ABSTRACT

Over the past years, the knowledge on signaling in plant–microbe interactions has increased to a great extent. However, the signal communication of roots is still not satisfactorily understood. Our work was initiated to elucidate the plant response in a biological system consisting of the crop plant tomato, the arbuscular mycorrhizal fungus *Glomus mosseae* and the soil borne tomato pathogen *Fusarium oxysporum* sp. *lycopersici* in monoculture and mixed cultivation. Among the effects of mycorrhizal and pathogenic fungi on the growth and development of tomato in single and combined inoculations we will point out the response of soilborne fungi on root exudates. Moreover, based on chemical analyses of the above- and belowground plant organs by HPLC-PDA and quadrupol GC-MS we will provide data on alterations in the profile of plant metabolites in various organs and root exudates which are specifically caused by pathogenic and mycorrhizal fungi. The main classes of focused metabolites include sugars, sugar alcohols, organic and amino acids, and secondary metabolites, such as phenolic acids, flavonoids, and terpenoids. Our research will provide improved insights about metabolic dynamics in leaves, roots, and root exudates of tomato. The extent of correlation of detectable in the metabolite profiles in various organs of tomato to the inoculation with a specific mycorrhizal and pathogenic fungus, or a combination of both, will be explored.
KEYWORDS: Tomato, *Fusarium oxysporum*, mycorrhiza, plant metabolites, root exudates

1. INTRODUCTION

Root exudates play an important role in plant–pathogen interactions in soil. Due to their different compounds like sugars, sugar alcohols, organic and amino acids, and secondary metabolites (Bertin et al., 2003, Nelson, 1991), they can act as signals in plant–pathogen interactions. However, these interactions are not satisfactorily understood yet. Further knowledge of these interactions can provide new ideas for the control of soilborne fungi.

To get more insight in such interactions we work with a biological model system consisting of the crop plant tomato, the arbuscular mycorrhizal fungus *Glomus mosseae* (AMF) and the soil borne tomato pathogen *Fusarium oxysporum* sp. *lycopersici* (*Fol*).

2. MATERIAL AND METHODS

2.1. Plant material

Tomato seedlings (cv. “Tiny Tim”, 14 days old) were potted in a mixture of potting soil, perlite and sand (1:1:1 v/v/v). The experiment consisted of 5 treatments: control, phosphorus, AMF, AMF & *Fol*, and *Fol* alone. Except the phosphorus-treatment all plants were irrigated with a nutrient solution with a low phosphorus content.

2.2. Collection of root exudates

After 6 and 10 weeks, respectively, the roots of the plants were gently washed and submerged in a buffer solution (pH=5.5) for 6 h. The obtained exudates were adjusted with the buffer solution to 10 ml/g root fresh weight, passed through sterile filters (0.2 µm) and stored at – 20°C.

2.3. Fungal spore germination rate and degree of mycorrhization

Aliquots of 500 µl of sterilized Czapek Dox broth (Duchefa Biochemie, Haarlem, NL) or root exudates were mixed with 100 µl of a *Fol*-suspension (*(*10^7 microconidia/ml) in 24-well-culture plates and incubated at 24°C in the dark while shaking. After 20 h 200 spores in each well were checked for germination under the microscope.

The degree of mycorrhization was estimated according to the staining method of Vierheilig et al. (1998) and Giovanetti and Mosse (1980).

2.4. HPLC-DAD analyses

The lipophilic compounds of the root exudates were injected into a Dionex Summit HPLC. The detection wavelength was 229 nm and UV spectra were recorded from 220–595 nm. Identification was carried out on basis of comparison to reference compounds and an in-house UV spectra library.

3. RESULTS

3.1. Spore germination rate

In Figure 1 the germination rate of *Fol* in the different root exudates relative to the standard Czapek Dox broth is shown. These preliminary results show no differences in *Fol* germination between exudates of 6- and 10-week-old tomato plants, respectively. Only for the *Fol* treatment the germination rate increased in exudates of 10-week-old plants. In general the control, the AMF and the AMF + *Fol* treatment tend to trigger equal germination rates of *Fol*, apart from the phosphorus treatment, where the germination rate is increased.

The degree of mycorrhization was 1 % and 3 % for the AMF treatment and 5 % and 14 % for the AMF and *Fol* treatment after 6 and 10 weeks respectively.

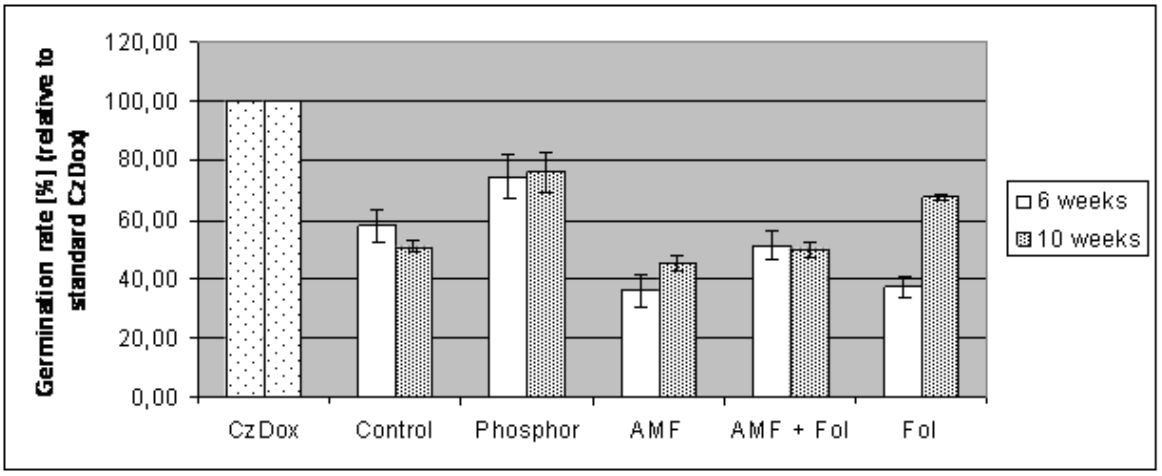

Figure 1. Germination rate of *Fol* incubated in Czapek Dox and root exudates of tomato plants inoculated with AMF, *Fol* or AMF and *Fol*. The germination rate is presented relative to the standard medium CzDox. Bars indicate standard deviation.

3.2. HPLC analyses

In **Figure 2** the preliminary results of the first sampling (after 6 weeks) of the root exudates are shown. All treatments except the phosphorus treatment showed a characteristic peak of salicylic acid. Besides, benzoic acid was detected in all treatments. Furthermore a hitherto unidentified derivative of benzoic acid was detected in all treatments except in the phosphorus treatment.

The later part of the chromatogram showed various peaks (not identified yet) which varied strongly between the single treatments.

The analyses of the second sampling (after 10 weeks) of the root exudates (data not shown) indicate that *Fol* stimulates the exudation of cinnamic acid, some of its derivatives and salicylic acid, regardless of the presence of AMF. In AMF exudates alone, these metabolites could not be detected until now.

In the exudates of both sampling dates (6 and 10 weeks) the amino acid tryptophan and one derivative occurred in variable amounts in all analyzed exudates.

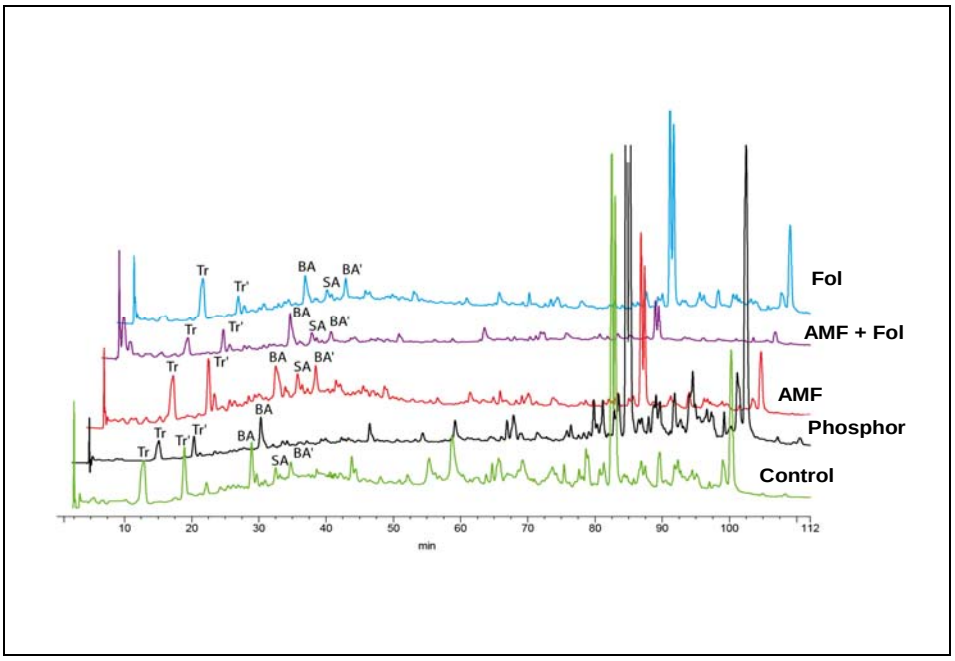

Figure 2. Chromatogram of the HPLC analyses of the collected root exudates (6 weeks). BA = benzoic acid; SA = salicylic acid; ChA = chlorogenic acid; CiA = cinnamic acid; ‘ = derivative, Tr = tryptophan.

4. CONCLUSION

Our first results show differences in the production of metabolites in tomato root exudates, which are linked with plant defence, especially in exudates of tomato plants inoculated with *Fol*. However, in the germination assay the *Fol* and AMF + *Fol* treatments do not show similar development. Further experiments are under way to elucidate metabolites in root exudates as well as in above- and belowground tomato organs, which might be involved in these interactions and in the different developing stages of *Fol*. We will not only focus on mycorrhizal and pathogenic fungi in tomato monoculture, we are also interested in effects based on mixed cultivation.

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