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Effects of pathogenic and symbiotic fungi on root exudation of tomato in intercropping systems

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Zusammenfassung

Die Rhizosphere ist ein sehr dynamischer Lebensraum, wo vielfältige Interaktionen zwischen Pflanzen und Mikroorganismen und zwischen Pflanzen bzw. Mikroorganismen untereinander stattfinden. Diese Interaktionen spielen eine wichtige Rolle sowohl in der Entstehung von Infektionen, hervorgerufen durch Pathogene, als auch in der Etablierung von günstigen Kolonisierungen der Wurzeln durch Mikroorganismen. Es ist weiters bekannt, dass pflanzenspezifische Signalstoffe an diesen Interaktionen beteiligt sind, dennoch gibt es noch einige ungeklärte Fragen dazu. Aus diesem Grund wurde ein Modellsystem entwickelt bestehend aus der Tomate (*Solanum lycopersicum* L.), dem Lauch (*Allium porrum* L.) als Mischkulturpartner, dem bodenbürtigen Pathogen *Fusarium oxysporum* f.sp. *lycopersici* (FOL) und dem arbuskulären Mykorrhizapilz *Glomus mosseae* (AMF).

Die Tomate/Lauch Kombinationen wurden in Töpfen kultiviert und den folgenden Behandlungen unterzogen: FOL, AMF, FOL und AMF in Kombination. Wurzelgewichte, AMF Kolonisierungsgrade und FOL Infektionsraten wurden untersucht. Weiters wurden mit Wurzelexsudaten in-vitro Tests mit FOL durchgeführt, um Effekte auf Sporenkeimrate und Myzelentwicklung untersuchen zu können. Zusätzlich wurden die Wurzelexsudate auf ihre Inhaltsstoffe (z.B. Zucker, Sekundärmetaboliten) untersucht, um Schlüsselkomponenten identifzieren zu können.

Schlagwörter: Fusarium oxysporum f.sp. *lycopersici, Glomus mosseae,* arbuskuläre Mykorrhizapilze, Sekundärmetaboliten, root exudates

Summary

The rhizosphere is a very dynamic environment where several plant–microbe, plant–plant and microbe–microbe interactions take place. These interactions play an important role in the development of pathogenic infection as well as in beneficial colonization of plant roots by microorganisms. It is also known that signal compounds of plants are involved in these interactions. However, the picture of these signals is not clear yet. Therefore a model system was created consisting of tomato (*Solanum lycopersicum* L.), the intercropping partner leek (*Allium porrum* L.), the soilborne tomato pathogen *Fusarium oxysporum* f.sp. *lycopersici* (FOL) and the arbuscular mycorrhizal fungus *Glomus mosseae* (AMF).

Tomato/leek combinations were grown together in pots and received the following treatments: FOL, AMF, FOL and AMF in combination. Root weights, AMF colonization rates and FOL infection rates were assessed. Furthermore, root exudates were collected for in vitro tests of FOL to determine their effects on germination rate and mycelial development. Additionally, root exudates were analysed for their components (e.g. sugars, secondary metabolites) to identify key compounds.

Keywords: Fusarium oxysporum f.sp. *lycopersici, Glomus mosseae,* arbuscular mycorrhizal fungi, plant metabolites, root exudates

Introduction

The rhizosphere is a very dynamic environment where several plant–microbe, plant–plant and microbe–microbe interactions take place. These interactions play an important role in the development of pathogenic infection as well as in beneficial colonization of plant roots by microorganisms. It is also known that signal compounds are involved in these interactions. Root exudates, as signals from the plant side (NELSON 1991), offer a wide range of different compounds such as sugars, sugar alcohols, organic and amino acids, and secondary metabolites, such as phenolic acids and flavonoids (BERTIN et al. 2003). However, it is not clear yet how compounds in root exudates affect the development of pathogenic fungi in the rhizosphere. A better knowledge of the role of root exudates in plant–pathogen interactions would provide new prospects for control strategies against soilborne fungi. To get more insights in such interactions we studied a model system consisting of tomato (*Solanum lycopersicum* L.), the intercropping partner leek (*Allium porrum* L.), the soilborne tomato pathogen *Fusarium oxysporum* f.sp. *lycopersici* (FOL) and the arbuscular mycorrhizal fungus *Glomus mosseae* (AMF).

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Material and methods

For this experiment 21-day-old tomato (cv. "Tiny Tim") and 63-day-old leek (cv. "Golem") seedlings were potted together in a mixture of potting soil, perlite and sand (1:1:1 v/v/v). The plants received the following treatments: FOL, AMF and AMF+FOL. For FOL inoculation tomato seedlings were dipped for 5 min in a spore suspension (1 x 10^5 microconida/ml) of *Fusarium oxysporum* f.sp. *lycopersici* isolate "Fol 007". For AMF inoculation a commercially available inoculum of *Glomus mosseae* (BEG 12, Biorize/ Agrauxine, Quimper, France) was used. Uninoculated tomato and leek and tomato and tomato plants were added as control treatments to the experimental set-up. Plants were irrigated with a nutrient solution and cultivated for 10 weeks under greenhouse conditions.

After 10 weeks root exudates were collected. The roots of the plants were washed and submerged in an acetate buffer solution (25 mmol/L, pH=5.5) for 6 h. The obtained exudates were adjusted with the acetate buffer solution to 10 ml/g root fresh weight, passed through millipore filters (0.2 µm) and stored at – 20°C.

FOL disease incidence, i.e. precentage of infected plants, was determined according to the symptoms described in WELLMAN (1939). The degree of mycorrhization was estimated according to the staining method of VIERHEILIG et al. (1998) and the gridline intersect method of GIOVANETTI and MOSSE (1980).

For the determination of fungal activity and spore germination rates aliquots of 170 µl of root exudates (adjusted to a final concentration of 20 ml/g root fresh weight) were mixed with 30 µl of a FOL-suspension (1 x 10^7 microconidia/ml) in 96-well-culture plates and incubated at 24°C in the dark while shaking for 6 h 10 min. After incubation 10 µl of resazurin (fluorescent dye) were added and incubated for further 50 mins. Afterwards fluorescence (Ex: 540 nm, Em: 590 nm) was determined using a fluorescence reader (Fluostar, Omega). Furthermore, spore germination rates were determined.

To analyse chemical compounds in root exudates GC-MS analyses were performed with an AutoSystem XL gas chromatograph. Compounds were detected in a coupled mass spectrometer equipped with a mass selective flam ionization detector. Mass spectra were identified by comparison with spectra from commercial databases.

Results and discussion

In the AMF treatment an increased root fresh weight compared to other treatments could be observed. The AMF+FOL treatment did not differ from the other treatments. In general was the AMF colonization rate (*Glomus mosseae*) of the tomato plants cv. "Tiny Tim" quite low. Furthermore, in the AMF+FOL treatment no reduction of disease incidence could be observed. However, in other intercropping systems with different AMF species and isolates the situation can be different (AKKÖPRÜ and DEMIR 2005).

Looking on the fungal activity our assays show no differences between the different treatments. The chemical analyses revealed dynamics in the root exudate composition. Differences in fructose, glucose, malate and citrate levels could be detected according to the different treatments. These preliminary results can only give an idea about these dynamics. Further tests need to be done to get more insights.

Acknowledgements

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