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Root exudates as important factor in the *Fusarium* – host plant interaction

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Abstract: *Fusarium oxysporum* strains are known as major pathogens on tomato, an important vegetable crop worldwide. Chemical signals from plants and/or microbes seem to have an important function in determining the positive or negative outcome of interactions in the rhizosphere. However, there is still a lack in the understanding of how root exudates affect the development of fungal propagules in the rhizosphere. We found that tomato root exudates were modified by different *Fusarium oxysporum* strains. The results indicate that the *F. oxysporum* strains pathogenic on tomato Fol 007 and Forl 101587 and the non pathogenic *F. oxysporum* strains Fo 135 and Fo 47 trigger different responses in tomato plants.

Key words: root exudates, *Fusarium oxysporum*, microconidia germination, tomato

Introduction

Secreted chemical signals from plants and/or microbes have an important function in determining the positive or negative outcome of interactions in the rhizosphere. Although our understanding of plant–plant and plant–microbe interactions has increased to a great extent, the signal communication of plants is still far from being satisfactorily understood (Bais *et al*. 2006).

Recently, it has been shown that root exudates of tomato stimulate the microconidia germination of the tomato pathogen *Fusarium oxysporum* (Steinkellner *et al*., 2005). However, host specific signals may not be the major factor in the microconidia germination of these fungi, since similar pattern of microconidia germination was found in the presence of root exudates from non-host plants, such as sweet pepper, bean, barley, tobacco and cucumber (Steinkellner *et al*., 2005). Kamilova *et al*. (2006) reported on changes of the composition of organic acids and sugars in tomato root exudates due to the presence of *F. oxysporum* f. sp. *radicis-lycopersici*. However, there is still a lack in the understanding of how root exudates affect the development of fungal propagules in the rhizosphere.

The objective of the present study was to investigate the response of microconidia of pathogenic and non-pathogenic *F. oxysporum* strains to root exudates from tomato plants inoculated with pathogenic and non-pathogenic *F. oxysporum* strains.

Material and methods

Plant material

Surface sterilized seeds of tomato (*Solanum lycopersicum* L. cv. Micro Tom) were sown in pots containing steam sterilized perlite. Sixteen days after seeding, the plantlets were removed by gently washing the perlite off the roots with tap water. Thereafter the roots were dipped in

a microconidia suspension (1 x 10^7 microconidia/ml) of *F. oxysporum* f. sp. *lycopersici* strain Fol 007, *F. oxysporum* f.sp. *radicis-lycopersici* strain Forl 101587, the biocontrol strain *F. oxysporum* Fo 47 and the unspecific *F. oxysporum* strain Fo 135, respectively, for five minutes. Non-inoculated plants treated the same way as inoculated plants were used as control. Each treatment consisted of 20 plantlets. The plantlets were transplanted in plastic pots (volume 630 ml) containing moist, sterilized perlite. The transplanted plants were cultivated for further 24 days and watered with a nutrient solution (Steinkellner *et al*., 2005) throughout the experiments. All the experiments were performed in a plant growth chamber at 24 °C with a photoperiod of 16 h light/8 h dark.

Collection of root exudates

After the total growth period of 50 days plants were removed by gently washing the perlite off the roots with tap water. The plants were placed in a beaker containing sterile distilled water, such that the roots were completely submerged. The plants were placed in a plant growth chamber for 24 h at 24°C, thereafter removed from the beaker. Subsequently the fresh weight of the root was determined. The volume of exudate obtained was adjusted with sterilized water to 20 ml per g root fresh weight. The exudates were passed through 0.22 µm sterilfilters and stored at -20°C for further investigation.

Germination experiments

The microconidia germination in presence of root exudates collected from non inoculated tomato plants and plants inoculated with different *Fusarium oxysporum* strains was tested. Czapek Dox broth was used as a positive control and sterile, distilled water alone as a negative control. The germination assay was performed in sterile 24-well culture plates. Aliquots of 500 µl of root exudate were mixed with 100 µl of microconidia suspension and incubated for 20 h at 24°C in the dark with shaking. Lactophenol cotton blue was added and the microconidia germination was determined microscopically by counting 200 spores. A microconidium was considered germinated if the germ tube length was at least as long as the spore. The germination experiments were performed with at least two exudate batches and with three replicates each time.

Statistical analysis

Analysis of variance was performed after a variance check by the Levene's test. Mean values were compared using Fisher's least significant difference (LSD= 0.05). All analyses were performed using Statgraphics Plus version 5.0.

Results and discussion

The highest microconidia germination of Forl 101587 compared to the water control was observed in root exudates from plants inoculated with Forl 101587 and Fol 007 and from non-inoculated plants (Figure 1). Root exudates from plants inoculated with Fo 135 and Fo 47 showed a significantly lower germination rate. The effects of tomato root exudates on microconidia germination of the tomato wilt pathogen *F. oxysporum* f.sp. *lycopersici* strain Fol 007 is shown in Figure 2. Microconidia germination in all root exudates was significantly higher than in water and lower than in Czapek Dox broth. The highest stimulating effect was found in root exudates collected from tomato plants inoculated with Fol 007. Exudates from plants inoculated with the biocontrol strain Fo 47 and the unspecific strain Fo 135 showed a similar effect than exudates from non-inoculated plants. Exudates from plants inoculated with strain Forl 101587 resulted in reduced microconidia germination compared to exudates from non-inoculated control plants.

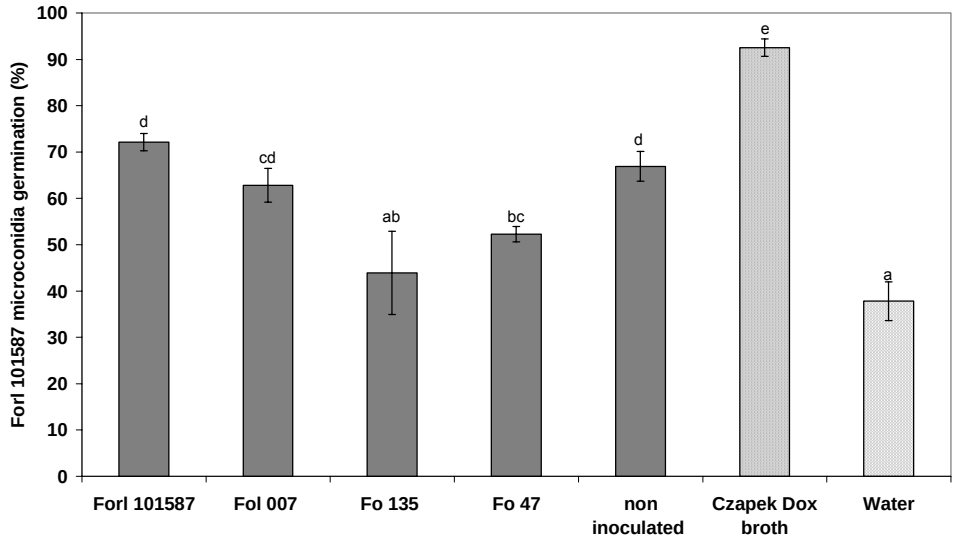

Figure 1. Germination of *Fusarium oxysporum* Forl 101587 in root exudates collected from tomato plants non inoculated and inoculated with *F. oxysporum* strains, Czapek Dox broth and water. Values are the means ± standard error. Bars with different letters are significantly different according to Fisher's LSD test ($p< 0.05$).

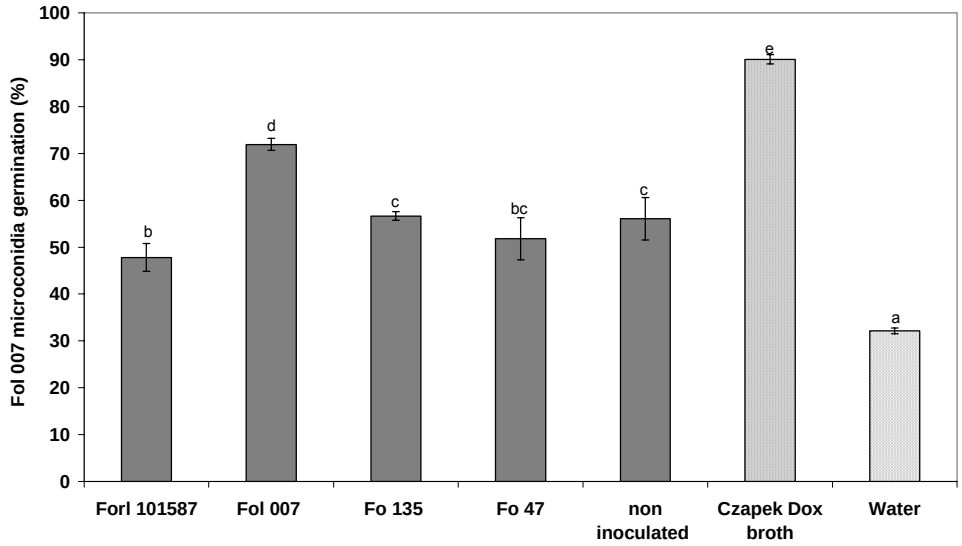

Figure 2. Germination of *Fusarium oxysporum* Fol 007 microconidia in root exudates collected from tomato plants non inoculated and inoculated with *F. oxysporum* strains, Czapek Dox broth and water. Values are the means ± standard error. Bars with different letters are significantly different according to Fisher's LSD test ($p<0.05$).

Relatively scarce information is available on the effect of tomato root exudates on *F. oxysporum*. Bolwerk *et al*. (2005) studied the spore germination of a Forl strain and Fo 47 in tomato root exudate and two of its major known compounds, glucose and citric acid. In contrast to our results they found a higher germination of Fo 47 spores than of *Forl* spores in tomato root exudates. This differing effect could be due to a certain *F. oxysporum* strain- and tomato cultivar-specificity. Previous studies have shown a great diversity in mycelial

development among strains of *F. oxysporum* in the vicinity of tomato root exudates (Steinberg *et al*., 1999).

Our results indicate that in the plant-Fol 007 interaction, the pathogen improves via an altered root exudation the conditions to further establish, whereas the pathogenic competitor, Forl 101587, impairs the germination conditions for the tomato wilt pathogen Fol 007. Moreover, there is an indication that an infection with these two pathogenic strains does not result in alterations of the root exudates affecting the microconidia germination of Forl 101587. Thus Forl 101587 did not develop the same strategy as Fol 007 to improve its competitive strength at the level of microconidia germination.

In conclusion, tomato root exudates were modified by different *Fusarium oxysporum* strains. The results indicate that the pathogenic Fusarium strains Fol 007 and Forl 101587 and the non pathogenic strains Fo 135 and *Fo* 47 trigger different responses in tomato plants. However, a possible diversity among strains of *Fusarium oxysporum* formae speciales has to be considered. Further studies are desirable to characterize the signaling compounds in tomato root exudate involved in the tomato - Fusarium wilt interaction.

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