Influence of the fungal root endophyte *Piriformospora indica* on tomato growth and spread of *Pepino mosaic virus*

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Introduction

Piriformospora indica (Fig.1) is a root endophytic fungus belonging to the *Sebacinales* (*Basidiomycota*) and has been discovered in the Indian Thar desert. It possesses plant-promoting properties in numerous plants and induces resistance against root and shoot pathogens in barley, wheat and Arabidopsis.

Pepino mosaic virus (PepMV) (Fig.2) belonging to the genus Potexvirus family *Flexiviridae* was first identified in 1974 in pepino plants (*Solanum muricatum* Ait.) in Peru. PepMV was rapidly distributed in the world after it appeared in the protected production of tomato in greenhouses in The Netherlands and in Great Britain in 1999. A few years later this pathogen affected tomato greenhouse production in most European countries, USA, Canada and China. The virus cause great losses in tomato production that were up to 30% in the yield and even up to 50% concerning the quality of the fruits. The only method to control the viruses in greenhouse is the disinfection of all tools.

The aim of the present work to establish the interaction between *P. indica* and tomato in soilless culture systems and to analyse, if the spread of PepMV in leaves is influenced by fungal colonisation of the roots. Second the impact of *P. indica* on tomato fruit biomass in a hydroponic system was determined.

Fig. 3 Experiment Setup for tomato plants in hydroponic culture (gullies system)

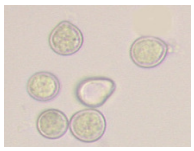

Fig.1 Chlamydospores of *Piriformospora indica* 400x

Fig.2: PepMV Particle: Length 500 nm, width 12 nm)

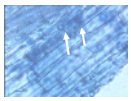

Fig.4 Roots of tomatoes in hydroponic culture were coloured with trypan blue after *Piriformospora indica* arrows inoculation 400x.

Materials and Methods

Tomato plants (cultivar Hildares) were grown in nutrient solution in a recirculating hydroponic system (Fig. 3) under standard conditions in gullies at leaf stage 8-9 in a group of experiment that were achieved in winter 2006, summer 2007,and summer 2008. The plants were inoculated with spores (Fig.1) and mycelium suspensions of the fungus that was pre-cultured on Potato Dextrose Broth for four weeks. Colonisation of roots with *P. indica* was detected after staining with Trypan blue (Fig.4). Three weeks later after controlling fungal colonisation of the roots, two young tomato leaves were inoculated with PepMV-France Isolate. The spread of the virus was controlled using DAS-ELISA test system with the specific antibody AS-0554 (DSMZ, Braunschweig, Germany). At the end of the experiment plant growth parameters were measured.

Young leaf samples for investigations of gene expression were collected 47 days after PepMV inoculation and frozen in liquid nitrogen and total RNA was extracted. Tomato genes were selected from data bases (TIGR, EMBL) because they are known to be differentially expressed after virus infection of plants: 1) ***LeTV***, Tomato tobamovirus-induced, 2) ***LePRP***, pathogenesis-related protein, and 3) ***LeGST***, glutathione S-transferase. The other genes were selected because they are known to be involved in plant resistance or defence mechanisms: 4) ***LeTRP***, Tomato tospovirus resistance protein, 5) ***LeSLC***, Cystatin, and 6) ***LePVP*** (tomato potyvirus VPg interacting protein). Primers were designed based on the sequences of the genes and used for semi quantitative RT-PCR.

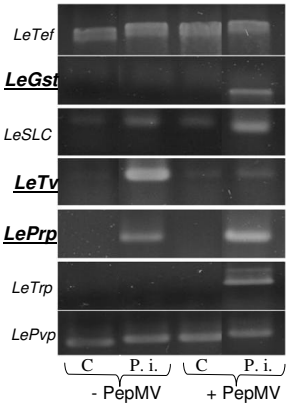

Fig.7:RT-PCR results show at all searched genes were induced with *P. indica* + PepMV treatment.

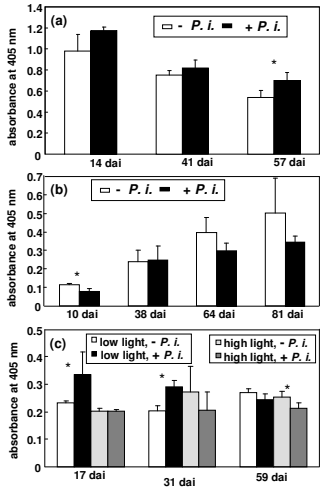

Fig.5 Influence of *Piriformospora indica* on *Pepino mosaic virus* spread. Tomato plants were grown in nutrient solution in three consecutive years (a: winter 2006; b: summer 2007, c: late summer 2008 under two light regimes)

Results

PepMV spread (Fig.5):The concentrations of PepMV particles decreased over time in the upper leaves, but were always between 10% and 20% higher in tomato plants colonised by *P. indica* than in non-colonised controls (Fig. 5a). The difference was significant at the latest date. In experiment summer 2007, the virus responded opposed (Fig. 5b). First, virus concentration increased during the course of the experiment. Secondly, at all dates except the first the virus was detected at the same concentration in plants which were inoculated with the root endophyte.

At the first date the concentration was even reduced. In order to find out the differences between the two experiments, climate conditions during the cultivation were compared. Light intensity revealed as the major difference between the two experiments. Consequently, half of the plants were shaded in experiment summer 2008 (Fig. 5c). In these shaded plants, *P. indica* inoculation led to a significantly increased spread of PepMV at the first two dates. In plants however, which obtained higher light intensities, more virus particles were detected in the leaves at the last two dates when the roots were colonised by the endophytic fungus (significant at 59 dai).

Influence on plant growth (Fig.6):In all experiments, higher numbers of flowers or setting of fruits were observed. Plants of experiment Summer 07 and 08 were therefore used to harvest and to analyse the fruits (Fig. 6 shows results of experiment summer 08). This revealed a significant influence of *P. indica* on fruit biomass. At the date of harvest, tomato fresh weights per plant were increased between 50% and 100% and dry matter content between 10% and 20%. The increases in fresh weights were not due to differences in the single fruit, but due to higher numbers of fruits. Significant differences were also obtained in experiment 3 with fresh weight increases between 40% and 50% and a 7% higher dry matter content

RNA accumulation (Fig.7): Genes encoding are differentially expressed, while a gene encoding a potyvirus interacting protein (PVP) seems not to be regulated. There is however no clear correlation between gene expression and spread of the PepMV. The gene for the translation elongation factor EF-1α (TEF) was used as constitutively expressed control. Some of the selected genes are induced when roots are colonized by the fungi *P. indica* and infected by PepMV (induction of systemic resistance?).

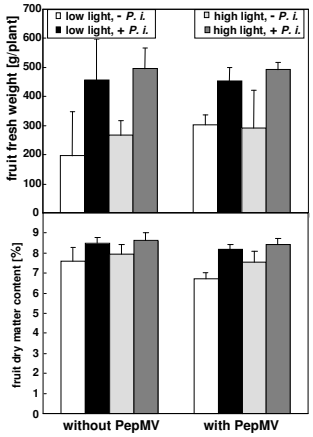

Fig.6: Influence on fruit fresh weight and dry matter content. Tomato plants were grown in nutrient solution under two light regimes and inoculated or not with *Piriformospora indica* and *Pepino mosaic virus*. It showed significant influence on fresh weight for *P. indica* and on dry weight for all three factors.

Conclusion

Piriformospora indica is able to repress the spread of *Pepino mosaic virus* provided that light intensities exceed a particular level. Tomato plants colonised by the endophyte show only slightly enhanced vegetative development, but fruit biomass is strongly increased. More research is necessary to further optimize the application of *P. indica* and to ensure that quality of fruits concerning taste- and health-related compounds are not negatively affected. The presented results however let us already suppose that the plant-protecting and development-promoting abilities of *P. indica* could be used to improve the production of tomatoes in hydroponic cultures.

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