Can root endophytic fungi confine spread of *Pepino mosaic virus* in tomatoes?

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Background

Pepino mosaic virus (PepMV, Fig. 1) may infect several *Solanaceae*, tomato, pepper, potato, weeds. Although infection can be symptomless the damage caused by the virus was reported to be up to 90 % of collapsed plants. At the moment the virus can only be controlled by comprehensive disinfection and care in the greenhouse. Our goal was to find out if biological agents can confine the virus. Three endophytic fungi were tested:
- *Piriformospora indica* (Fig. 2). All plants inoculated and tested so far responded with an enhanced growth of shoot and root. It induced resistance in barley against *Fusarium* spp. and *Blumeria graminis*.
- *Pythium oligandrum* (Fig. 3). The oomycete, a soil born fungus, induced systemic resistance in tomato leaves against *Botrytis cinerea.*
- *Pythium aphanidermatum* (Fig. 4). An important pathogen in soilless systems, causing root rot. When pre-inoculated before PepMV a delayed infection and distribution of the virus up to eight weeks was found.

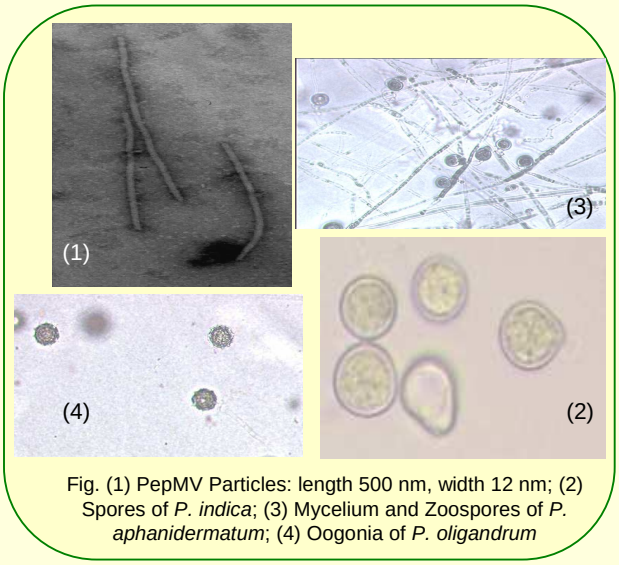


Fig. (1) PepMV Particles: length 500 nm, width 12 nm; (2) Spores of *P. indica*; (3) Mycelium and Zoospores of *P. aphanidermatum*; (4) Oogonia of *P. oligandrum*

Fig. 5.

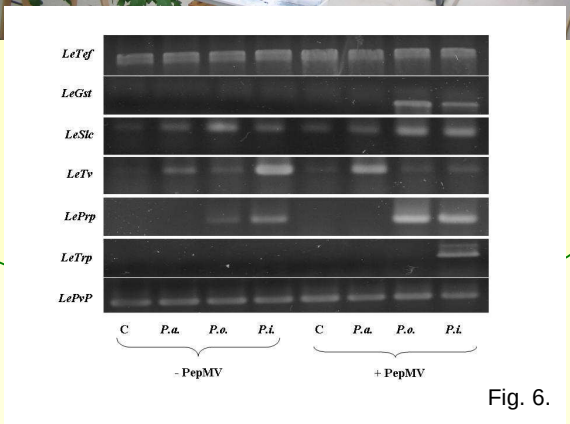


Fig. 6.

Material and Methods

Tomato (cv. Hildares) were grown hydroponically in independent gullies for 84 days in a greenhouse: eight treatments, two replications, 10 plants per replication (Fig. 8): **1**) *P. indica*, **2**) *P. aphanidermatum*, **3**) *P. oligandrum*, **4**) no fungus, **5**) PepMV, **6**) PepMV+*P. indica*, **7**) PepMV+*P. aphanidermatum*, **8**) PepMV+*P. oligandrum*. Day/night temperature was 23.3 /20.0 ℃, relative humidity 76.6 %, and daily g lobal radiation 4 MJ m-2. One week after adjusting, plants were inoculated with the three fungi. Two weeks later, leaves of 50 % of the plants were mechanically inoculated with PepMV (French-isolate E 397/1). Infection was confirmed and quantified with DAS-ELISA. Gene expression was analysed seven weeks after PepMV inoculation using semiquantitative PCR (Qiagen, Hilden, Germany). Tomato genes were selected from data bases (TIGR, The Computational Biology and Functional Genomics Laboratory; EMBL, European Molecular Biology Laboratory). a-c) induced after virus infection: a) LeTV, Tomato tobamovirus-induced; b) LePRP, pathogenesis-related protein; c) LeGST, glutathione S-transferase. d-f) involved in plant defense mechanisms: d) LeTRP, Tomato tospovirus resistance protein; e) LeSLC, Cystatin; f) LePVP, Tomato potyvirus VPg, virus-interacting protein. g) LeTef, control. with the cDNAs from the different samples.

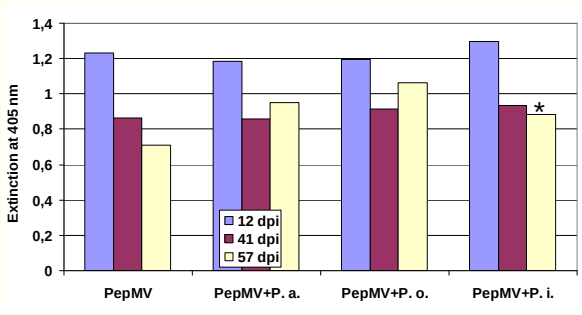
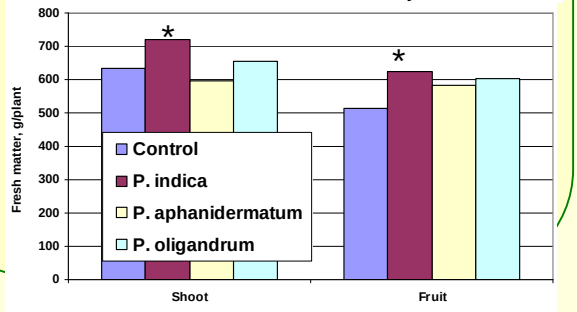


Fig. 7. Fungi affected spread and propagation of PepMV in tomato 12, 41 and 57 days after infection (dpi). DAS-ELISA.

Fig. 8. Effect of fungal microorganism on shoot fresh weight and yield of tomato.



Result

- Tomato inoculated only with *P. indica* resulted in a significant enhancement of growth and fruit setting, for the first time shown in nutrient solution (Fig. 7). *P. oligandrum* and *P. aphanidermatum* did not affect the growth significantly.
- All fungi promoted the distribution of PepMV in the tomato shoot organs (Fig. 8).
- No correlation was found between gene expression patterns and spread of PepMV.
- Genes selected were always induced significantly when tomato were infected by both *P. indica* and PepMV (Fig. 6).
- Mechanisms leading to the higher susceptibility of tomatoes against PepMV when co-inoculated with fungi must be explained in further experiments.