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ORIGINAL PAPER

# Impact of *Piriformospora indica* on tomato growth and on interaction with fungal and viral pathogens

**Ahmad Fakhro · Diana Rocío Andrade-Linares · Susanne von Bargen · Martina Bandte · Carmen Büttner · Rita Grosch · Dietmar Schwarz · Philipp Franken**

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**Abstract** *Piriformospora indica* is a root endophytic fungus with plant-promoting properties in numerous plant species and induces resistance against root and shoot pathogens in barley, wheat, and Arabidopsis. A study over several years showed that the endophyte *P. indica* colonised the roots of the most consumed vegetable crop tomato. *P. indica* improved the growth of tomato resulting in increased biomass of leaves by up to 20%. Limitation of disease severity caused by *Verticillium dahliae* by more than 30% was observed on tomato plants colonised by the endophyte. Further experiments were carried out in hydroponic cultures which are commonly used for the indoor production of tomatoes in central Europe. After adaptation of inoculation techniques (inoculum density, plant stage), it was shown that *P. indica* influences the concentration of Pepino mosaic virus in tomato shoots. The outcome of the interaction seems to be affected by light intensity. Most importantly, the endophyte increases tomato fruit biomass in hydroponic culture concerning fresh weight (up to 100%) and dry matter content (up to 20%). Hence, *P. indica* represents a suitable growth promoting endophyte for tomato which can be applied in production systems of this important vegetable plant not only in soil, but also in hydroponic cultures.

A. Fakhro · D. R. Andrade-Linares · R. Grosch · D. Schwarz · P. Franken (✉)
Leibniz-Institute for Vegetable and Ornamental Crops,
Theodor-Echtermeyer-Weg,
14979 Grossbeeren, Germany
e-mail: franken@igzev.de

A. Fakhro · S. von Bargen · M. Bandte · C. Büttner
Faculty of Agriculture and Horticulture, Section Phytomedicine,
Humboldt-University Berlin,
Lentzeallee 55/57,
14195 Berlin, Germany

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## Introduction

*Piriformospora indica* was originally isolated from the spore of an arbuscular mycorrhizal (AM) fungus, but inoculation experiments showed its ability to colonise plant roots (Verma et al. 1998). It is an anamorphic strain of the Sebacinales (Basidiomycota), a group with many plant-interacting organisms including ecto-, ericoid, and orchid mycorrhiza (Weiss et al. 2004; Selosse et al. 2007). The endophyte *P. indica* possesses positive influence on growth and development of many different plant species like AM fungi. Inoculation leads to increased fresh weights (Varma et al. 1999), supports the establishment of micro propagated plantlets (Sahay and Varma 1999), enhances flower and seed production (Rai et al. 2001; Barazani et al. 2005; Shahollari et al. 2007), promotes the rooting from cuttings (Drüge et al. 2007), and results in higher yield (Waller et al. 2005). It could be also shown that *P. indica* induces tolerance against salt stress and resistance against root and shoot pathogens (Waller et al. 2005; Serfling et al. 2007; Deshmukh and Kogel 2007; Sherameti et al. 2008; Baltruschat et al. 2008; Stein et al. 2008). In contrast to AM fungi however, the endophytic fungus colonises the roots and promotes the development of the model plant *Arabidopsis thaliana* (Peskan-Berghöfer et al. 2004). In this experimental system, a number of plant proteins and genes were identified which are important for the interaction between the endophyte and the plant root (Shahollari et al. 2005, 2007; Sherameti et al. 2005, 2008), and the basis for induced resistance was analysed (Stein et al. 2008). However, no data are available for the interaction between

*P. indica* and plants of the *Solanaceae* which contain important crop species and models like potato, tomato, tobacco, eggplant, or petunia.

Tomato is the most commonly grown fresh market vegetable worldwide, but various pathogens can lead to dramatic losses in yield. One widespread disease of field grown tomato plants is verticillium wilt caused by the soilborne fungus *Verticillium dahliae* (Pegg and Brady 2002). The disease symptoms are characterised by V-shaped yellowing of the leaves, browning of vascular bundles, and wilting. The fungus infects the root system directly or through wounds, invades the xylem, and moves upward. Once in plant tissues, it produces toxins (Mansoori et al. 1995), and in course of the interaction, the disease symptoms progress up the stem, and the plant becomes stunted (Gold et al. 1996). Control strategies are soil fumigation, crop rotation, and growing cultivars with the *Ve* resistance gene (Talboys 1984; Huisman and Ashworth 1976; Ligoxigakis and Vakalounakis 1994). However, these strategies are limited by the longevity of the *V. dahliae* microsclerotia in soil (Pegg 1974; Talboys 1984), by the economically unpractical long rotation cycles (Harrington and Dobinson 2000) and by the appearance of new race 2 strains which cause typical disease symptoms also on resistant *Ve* cultivars (Dobinson et al. 1996)

Besides production in the field, the importance of soilless cultivation of tomato using open or closed hydroponic systems has been increasing worldwide during the last three decades (Savvas 2003). In these production systems, plants are usually supplied with a nutrient solution circulating to allow a more accurate control of the root environment. This can result in an optimal use of water and nutrients, and thus, in higher yields and better fruit quality. However, recirculation of the nutrient solution and high plant density facilitates the rapid and efficient spread of pathogens and may, thus, increase the risk for epidemics if not managed well (Stanghellini and Rasmussen 1994). Since 1999, Pepino mosaic virus (PepMV) has attracted much attention because it is found widely in tomato greenhouses in many European countries, in Morocco, South and North America, and in China (see references in Spence et al. 2006). The origin of its sudden occurrence is not clear. However, rapid transmission and spread of PepMV within and between greenhouses can be mechanically caused by tools, clothes, and the hands of workers during crop handling (Jones et al. 1980). Typical symptoms of infected leaves are rolling, light yellow mosaics, dark green discolouration, and leaf distortion (Jordá et al. 2001; Van der Vlugt et al. 2002). Fruits may show yellow blotches, necrotic or yellow spots, and irregular ripening (French et al. 2008). Symptom expression can be affected by the tomato cultivar, the genotype of the virus, and the environmental conditions (French et al. 2008; Hanssen et al. 2009). There are conflicting reports on yield losses due to PepMV infection varying from low up to the collapse of the crop (Soler-Aleixandre et al. 2005). Apart from total yield losses, significant decreases in fruit quality, and thus, marketable yield reductions up to 40% are reported (Spence et al. 2006; Schwarz et al. 2009).

The present investigation was carried out first to analyse, if the endophyte *P. indica* is able to reduce the symptoms of verticillium wilt in substrate-grown tomato. Secondly, it was aimed to establish the interaction between *P. indica* and tomato in hydroponic culture systems for analysing, if the accumulation of PepMV within the apical shoot is influenced by fungal colonisation of the roots. At third, the impact of *P. indica* on tomato fruit biomass in the hydroponic system was determined.

## Materials and methods

### Cultivation of fungi and Pepino mosaic virus

The endophyte *P. indica* was propagated on potato dextrose agar (PDA, VWR, Berlin, Germany) at 28°C. Chlamydospores were collected after 2 weeks from the agar plate and used to inoculate 100 ml potato dextrose broth (PDB, VWR) in 300-ml Erlenmeyer flasks. These flasks were further incubated at 28°C and 90 rpm for 4 weeks. Mycelium and spores were filtered through gauze and mixed with a blender (Model D72, Moulinex, Leipzig, Germany) for 2 min at lowest speed in 100 ml of sterile tap water. Propagule (chlamydospores, hyphal fragments) concentration was examined in a Thoma chamber and the number of viable propagules by plating on PDA.

*V. dahliae* (kindly provided by Valerie Grimault, GEVES, Angers, France) was grown in 150-ml sucrose sodium nitrate liquid medium (Sinha and Wood 1968) at 28°C and 100 rpm. After 1 week, the culture was transferred to 200-ml fresh medium and further incubated for 2 weeks. The culture was mixed by a blender (Model D72, Moulinex) for 40 s at minimal speed. The mixed solution was filtrated and washed once by centrifugation. The number of conidia was estimated by counting in a Thoma chamber, and their viability was checked by plating on PDA. For inoculation, the suspensions were adjusted with sterile tap water to a concentration of $10^5$ conidia per millilitre based on counted colonies.

The virus isolate Pepino mosaic virus-Sav E397 used in all experiments was obtained from tomatoes purchased in a German supermarket that were labelled to be imported from France. The virus isolate was recovered from these tomato fruits by maceration of crude fruit in ELISA sample buffer (10% phosphate-buffered serum (PBS) buffer, 2% polyvinylpyrrolidone) followed by

mechanical inoculation using 0.05% Celite as an abrasive to tobacco plants (*Nicotiana benthamiana*) on which it was further propagated as described (Schwarz et al. 2009).

### Cultivation and inoculation of tomato plants

For the experiment with the pathogen *V. dahliae*, surface disinfected tomato seeds (*Lycopersicon esculentum* Mill. cv. Hildares) were germinated on 0.8% water agar for 1 week and subsequently transplanted into pots containing 1 l of the substrate "Fruhstorfer Erde Typ P" (Archut, Vechta, Germany). These pot cultures were placed for the whole experiment in a greenhouse at day/night temperature of 25°C/19°C, relative humidity 54%/69%, and a mean daily radiation of 362 Mol $m^{-2}day^{-1}$. The plants were watered twice per week with 40 ml of nutrient solution (De Kreij et al. 1997; pH=5.5; electric conductivity (EC)= 2 dS $m^{-1}$). Half of the plants (36) were inoculated with *P. indica* before planting into "Fruhstorfer Erde Type P" by dipping the roots overnight in a suspension of 105 cfu/ml of tap water. In addition, fresh *P. indica* mycelium was mixed 1/100 (*w*/*w*) with this substrate to achieve heavily colonised plants. Two weeks after inoculation with *P. indica*, half of the plants (18 colonised by *P. indica* and 18 controls) were drenched with 30 ml of the conidia suspension of *V. dahlia* ($10^5$ conidia per millilitre).

For all other experiments (Table 1), seeds were germinated for 2 weeks in sterilised sand and further grown in pots filled with 0.5 l sterilised sand (*v*/*v*: 1 (0.2–1 mm)/ 1 (2–3 mm); Euroquarz, Laußnitz, Germany) fertilised with the nutrient solution mentioned above. One to 4 weeks later, plants were inoculated by dipping the roots for 2 h in a *P. indica* suspension or a control solution (Table 1). Thereafter, inoculated plants and controls were transferred to buckets or gullies (ten plants per biological replicate), containing the nutrient solution, sand (as above), or substrate (Fruhstorfer Erde Type P). Twice a week, nutrient solution was renewed or added to sand or substrate until it drained off the buckets. In gullies, nutrient solution was applied as described (De Kreij et al. 1997). Daily climate data averages are shown in Table 1. For ensuring root colonisation by *P. indica*, five to six fragments (3–4 cm) of plant roots were sampled 10 days after inoculation, incubated on PDA, and analysed for the appearance of chlamydospores with characteristic morphology (Verma et al. 1998) by means of light microscopy. Two weeks after inoculation with *P. indica*, when tomato plants had developed nine to ten leaves, half of the *P. indica*-inoculated or the control plants were mechanically inoculated on leaf number 8 or 9 by abrading a homogenate containing PepMV which was obtained from leaves of host plants (*N. benthamiana*) using 0.05% Celite as an abrasive. These leaves were, thereafter, washed with sterile water. Successful infection was determined 1 week later by double antibody sandwich (DAS)-ELISA (see below).

### Analysis of plants

Disease severity in *V. dahliae*-inoculated plants was assessed at harvest (8 weeks after sowing, 6 weeks after *P. indica* inoculation, and 4 weeks after pathogen inoculation) based on an arbitrary scale of disease classes: 0=no symptoms, 1=slight yellowing of leaf, stunting, or wilting, 2=moderate yellowing of leaf, stunting, or wilting, 3=severe yellowing of leaf, stunting, or wilting, and 4=leaf death. Values were estimated for the different set of plants using the formula $(\Sigma\, n^{\circ}\ of\ leaves_{disease\ class} \times\ disease\ class)\ /\ total\ number\ of\ leaves$. The value obtained for the control plants infected with *V. dahliae* was set at 100%. In parallel, pieces of the stem base were placed on PDA, and the outgrowth was microscopically confirmed. Fresh and dry weights of shoots were estimated at harvest time.

PepMV infection of tomato plants was determined by testing the upper most leaves in DAS-ELISA (modified after Clark and Adams 1977) using commercially available polyclonal antibodies (immunoglobulin IgG) according to the instructions provided (AS-0632; DSMZ, Braunschweig, Germany). Each ELISA test included a negative and a positive control. Samples were rated positive if the absorbance measured at 405 nm was greater than twice the level obtained from healthy controls (Cordoba-Selles et al. 2007).

Fresh and dry weights of shoots were measured at the end of all experiments, while fresh and dry weights of fruits at the end of experiments 4 and 5 (Tab. 1). Dry matter content was calculated.

### Statistical analysis

Disease severity after *V. dahliae* infection was analysed by the nonparametric Kruskal–Wallis test, while all other experimental data were processed by analysis of variance procedures. Means at the different measurement dates were separated by Tukey's test procedure at $P=0.05$. Significant differences are presented by different letters, standard deviation bars are added in the figures, and significant interactions between factors are mentioned in the legends. STATISTICA 6.0 software (2003) was used for all statistical analyses indicated in the figure legends.

## Results

### Impact of *P. indica* on verticillium wilt

Tomato plants grown in substrate and infected with the pathogen *V. dahliae* showed typical symptoms as leaf

**Table 1** Conditions of tomato growth in soilless cultures

| Experiment number | 1 | 2 | 3 | 4 | 5a | 5b |
|---|---|---|---|---|---|---|
| Date of *P. indica* inoculation (weeks after sowing; developmental stage[a]) | 3–6; 102–110 | 4; 104–105 | 4; 104–105 | 4; 104–105 | 4; 104–105 | 4; 104–105 |
| Inoculum density ($10^5$ cfu/ml) | 3 | 3 or 9 | 3 | 3 | 3 | 3 |
| Substrate | Sand | Sand, substrate, nutrient solution | Nutrient solution | Nutrient solution | Nutrient solution | Nutrient solution |
| Containment | 1 l buckets | 2 l buckets | Gullies | Gullies | 10 l buckets | 10 l buckets |
| Pathogen | No | No | PepMV | PepMV | PepMV | PepMV |
| Plants per treatment | 6 | 6 | 2×10 | 4×10 | 4 | 4 |
| Temperature (day/night; °C) | 21.0/17.6 | 22.2/19.2 | 21.7/20.0 | 23.4/18.9 | 25.8/20.8 | 25.8/20.8 |
| Humidity (day/night; %) | 63.2/63.8 | 59.1/57.4 | 78.2/75.3 | 76.3/79.9 | 67.4/78.8 | 67.4/78.8 |
| Mean daily radiation (Mol $m^{-2}d^{-1}$) | 18.5 | 7.8 | 9.0 | 12.8 | 11.0 | 6.0 |
| $CO_2$ concentration (ppm) | 479.6 | 454.5 | 365.7 | 396.3 | 452.0 | 452.0 |
| End of the experiment (weeks after sowing) | 9 | 11 | 10 | 13 | 12 | 12 |

[a] According to Feller et al. (1995)

yellowing, stunted growth, wilting, and death of the plant. Fresh weights were significantly reduced in the pathogen-infected control plants, while the dry matter content was only slightly different (Fig. 1a). Tomato plants inoculated with the endophyte *P. indica* showed a significant increase in fresh weights. Moreover, the negative effect of the pathogen on plant growth was alleviated when the plants were colonised by the root endophytic fungus. Differences in fresh weight and dry matter content between *P. indica*-inoculated plants and the corresponding controls were higher, when the plants were infected with *V. dahliae*. This was mirrored by the result of a two-way analysis of variance (ANOVA) showing a significant interaction between the two factors "pathogen" and "endophyte." Estimation of the disease symptoms also showed the protecting effect of *P. indica* (Fig. 1b). The disease severity was reduced by 32% in plants colonised by the endophytic fungus.

## Establishment of *P. indica* inoculation in a soilless system

In order to reveal the best conditions for *P. indica* infection in soilless systems, at first, the optimal plant stage of inoculating the endophyte was determined (experiment 1 in Table 1). For this purpose, tomato plants were inoculated at different stages (Fig. 2). Significant positive effects were only detected when the fungal inoculum was added 4 weeks after sowing (stage 104–105 according to Feller et al. 1995). This experiment showed in addition that the medium used for growing *P. indica* had no influence on tomato biomass as it has been seen before in soil cultures (Varma et al. 1999).

The second experiment (Table 1) was carried out for analysing the effects of growth medium and inoculum density on tomato growth (Fig. 3). Comparison of substrate, sand, and nutrient solution showed a significant positive effect of *P. indica* on shoot fresh weight with a low density of inoculum only in sand. Significant negative effects were obvious with a high inoculum dosage in nutrient solution. No differences were detected for the dry matter content and interaction between the two factors "inoculum density" and "growth medium" could not be observed.

## Impact of *P. indica* on Pepino mosaic virus spread

Experiments 3–5 (Table 1) were carried out to analyse the influence of *P. indica* on the concentration of PepMV. Typical symptoms of PepMV infection were observed in all experiments, as it has been described by Jordá et al. (2001) and Van der Vlugt et al. (2002), but not quantified. The concentration of PepMV decreased over time (14, 41, and 57 days after inoculation (dai)) in the upper most leaves in experiment 3, but was always between 10% and 20% higher in tomato plants colonised by *P. indica* than in noncolonised controls (Fig. 4a). The difference was significant at the latest date (57 dai). The virus responded

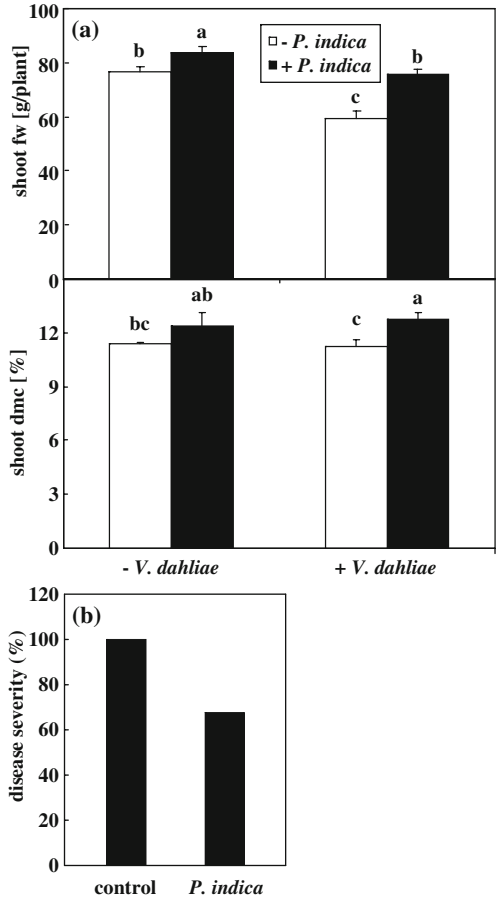


**Fig. 1** Influence of *P. indica* on tomato–*V. dahliae* interaction. Tomato plantlets were transferred to pots containing substrate supplemented or not with *P. indica*. After 2 weeks, half of the controls and the inoculated plants were infected or not with the pathogen *V. dahliae*. Four weeks later, shoot fresh weights (*fw*) and dry weights were measured, and dry matter content (*dmc*) was calculated (**a**). Significant different values are indicated by *different letters above the columns*. The factors *P. indica* and *V. dahliae* showed a significant interaction for both parameters (two-way ANOVA; $P=0.05$; $n=18$). In addition, disease severity was estimated and set for the control plants as 100% (**b**). Statistical analysis showed that disease severity was significantly different (Kruskal–Wallis test; $P=0.05$; plants)

opposed in experiment 4 (Fig. 4b). First, virus concentration increased during the course of the experiment (10, 38, 64, and 81 dai). Secondly, the virus was detected at all dates except the second (38 dai) with lower concentrations in plants, which were inoculated with the root endophyte, than in the controls. This reduction of virus spread was significant at the first date of sampling (10 dai). In order to find out the differences between the two experiments, climate conditions during the cultivation were compared, and light intensity was revealed as the major variation between the two experiments (Table 1). Consequently, half of the plants were shaded in experiment 5 (Fig. 4c). In these shaded plants, *P. indica* inoculation led to a significantly increased content of PepMV in the apical leaves at the first two dates (17 and 31 dai). In plants however, which obtained higher light intensities, a decreased virus concentration was detected in the leaves at the last two dates (31 and 59 dai) when the roots were colonised by the endophytic fungus (significant at 59 days after inoculation).

### Impact of *P. indica* on fruit biomass

Higher numbers of flowers or setting of fruits were observed in the plants inoculated with *P. indica* in all experiments. Plants of experiment 4 and 5 were, therefore, used to harvest and to analyse the fruits (Fig. 5 shows results of experiment 5). This revealed a significant influence of *P. indica* on total fruit biomass. (Yield of marketable fruits was not determined). At the date of harvest, tomato fruit fresh weights per plant were increased between 50% and 100% and dry matter content between 10% and 20%. The increase in fresh weights was not due to differences in the single fruit, but due to higher numbers of fruits. Significant differences were also obtained in experiment 4 with fresh weight increases between 40% and 50% and a 7% higher dry matter content (data not shown).

## Discussion

*P. indica* is a root-endophytic fungus with plant growth-promoting abilities. The increase of fresh weights was in some studies between 20% and 40% (Peskan-Berghöfer et al. 2004; Barazani et al. 2005) while it could reach in others up to 100% (Varma et al. 1999; Waller et al. 2005; Serfling et al. 2007). This characteristic could be confirmed in the present experiments using tomato as a plant host. Fresh weight of *P. indica*-colonised plants were, however, in the best case not more than 20% higher than in controls and reached although significant in most experiments increases of only 10%. This is probably on the one hand dependent on the plant species. For instance, rooting of cuttings was strongly enhanced in *Euphorbia pulcherrima* and *Pelargonium* x *hortorum*, while no effect could be observed in *Petunia hybrida* although cultivated under the same conditions (Drüge et al. 2007). On the other hand, conditions of inoculation (plant stage, substrate, and inoculum density) and growth clearly played an important role. Best results for tomato were obtained in sand compared to substrate and nutrient solution. A similar result comparing sand and soil has been obtained with wheat (Serfling et al. 2007). Nutrient poor conditions in the sand compared to the substrate cannot be the reason, since nutrient supply was optimal, and *P. indica* does not improve at least P and N content in tobacco (Barazani et al. 2005) or in barley (Achatz et al. unpublished). Hence, an explanation for the influence of the substrate has for the moment to be

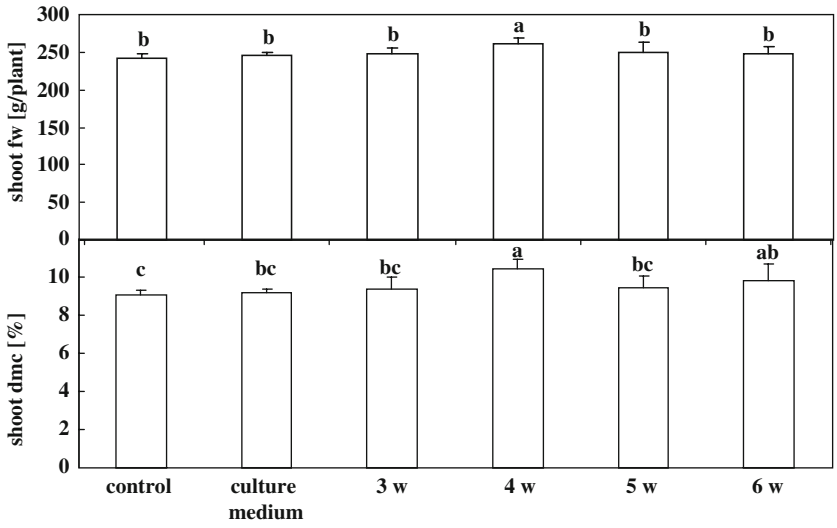


**Fig. 2** Influence of inoculation date. Tomato plantlets (3 weeks after sowing) were transferred to pots containing a nutrient solution and supplemented with the *P. indica* inoculum immediately (3 weeks after sowing) or after 7, 14, or 21 days (4–6 weeks after sowing). Control plants obtained no supplement (control) or culture medium without the fungus. Nine weeks after sowing, shoot fresh weights (*fw*) and dry weights were measured, and dry matter content (*dmc*) was calculated. Significant differences between different types of inoculum are indicated by *different letters above the columns* (one-way ANOVA; $P=0.05$; $n=6$)

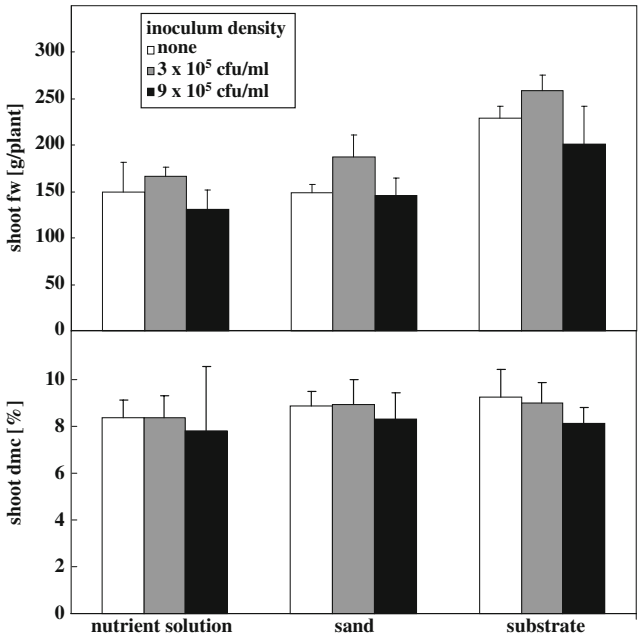


**Fig. 3** Influence of cultivation system and *P. indica* inoculum density. Tomato plantlets were transferred into buckets containing a nutrient solution, sand, or a commercial garden substrate. Each cultivation system was supplemented with no, with 3, or with $9\times10^5$ cfu/ml of *P. indica* inoculum. Shoot fresh weights (*fw*) and dry weights were measured, and dry matter content (*dmc*) was calculated 8 weeks after inoculation. Two-way ANOVA ($P=0.05$; $n=6$) revealed significant differences in shoot fw for the influence of inoculum density and of the cultivation system, but not for the interaction of the two factors. No significant differences were revealed for shoot dmc

left open. The other conditions tested for tomato were the inoculum density and the plant stage of inoculation. This showed that the amount of fungus should not exceed a certain extent, because negative effects on plant growth were obtained. Such negative effects with high amounts of inoculum of *P. indica* have been also seen in an experiment with tomato using field soil as substrate (data not shown). Another study using poplar as host in a Petri dish system also revealed negative effects of the endophyte (Kaldorf et al. 2005). This was not dependent on inoculum amount, but on the mode of cultivation of the fungus. If *P. indica* was grown on media containing ammonium as N source, the fungus started to colonise not only the cortex, but also the vascular cylinder of the roots, and necrotic lesions occurred. Positive effects were observed, if the endophyte derived from cultures with nitrate. Negative effects could be based on the mode of colonisation. The fungus is not a biotroph as e.g., the arbuscular mycorrhizal fungi, but increases the number of dead cells in the root (Franken et al. 2000). Interestingly, *P. indica* seems to induce the programmed cell death of plants as numerous pathogens do, but in contrast to these pathogens, the colonisation of the root by the endophyte depends on the cell death programme of the plant (Deshmukh et al. 2006). If the number of dead cells in the root exceeds a particular threshold, *P. indica* could exert a negative influence on plant growth and development. This might be the case, if the amount of inoculum is too high and the colonisation is from the beginning too intense. An optimal balance between positive and negative effects of *P. indica* could also explain the observation that best effects were obtained, if the fungus

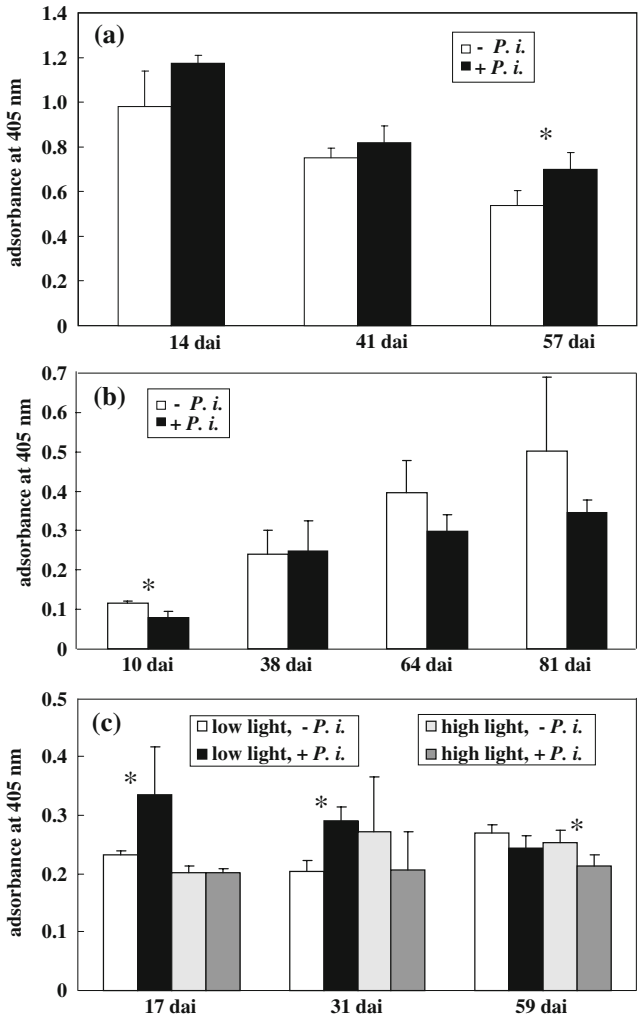


**Fig. 4** Influence of *P. indica* on Pepino mosaic virus spread. Tomato plants were grown in nutrient solution in three consecutive years and inoculated or not with *P. indica*. **a** Winter 2006. **b** Summer 2007. **c** Late summer 2008 under two light regimes When roots were colonised, youngest leaves of half of the plants were inoculated with PepMV. At different days after inoculation (*dai*), youngest leaves were harvested, and PepMV colonisation was measured by ELISA. Significant differences between plants inoculated or not by *P. indica* are indicated by *asterisks*. (One-way (**a**, **b**) and two-way (**c**) ANOVA; $P=0.05$; $n=2$ (**a**) or 4 (**b**, **c**)). An interaction between light and *P. indica* was detected at 31 dai (**c**)

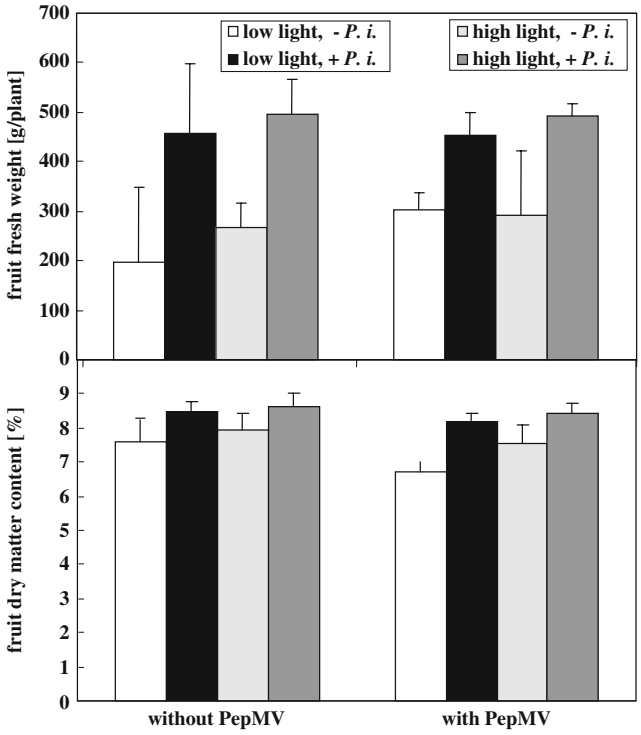


**Fig. 5** Influence of *P. indica* on fruit fresh weight and dry matter content. Tomato plants were grown in nutrient solution under two light regimes and inoculated or not with *P. indica* and Pepino mosaic virus. Twelve weeks after germination, fruits were harvested, fresh and dry weights measured, and dry matter content calculated. A three-way ANOVA ($P=0.05$; $n=4$) showed significant influence on fresh weight for *P. indica* and on dry weight for all three factors (light, PepMV, and *P. indica*). Interactions between any of the factors were not detected

was applied at the four to five leaf stages. At the earlier date, the percentage of dying cells could reach the point, where positive and negative effects are in equilibrium, while at the later dates, the root is not susceptible anymore for the positive activity of the fungus. Variable effects of *P. indica* on vegetative growth are probably not simply due to the extent of colonisation, because plants showing between 10% and 50% colonisation intensities were not different in their shoot fresh weights (data not shown). All this indicates that the outcome of the interaction between tomato and *P. indica* depends on experimental conditions, and future experiments will be directed to proof more variables in this respect. Preliminary experiments indicate for instance that the culture medium for growing the fungus also seems to influence the effect of the endophyte on plant performance. In addition, different isolates of *Sebacina vermifera*, a close relative of *P. indica*, have to be tested, if they promote tomato growth even more as it has been shown for tobacco and barley (Barazani et al. 2005; Deshmukh et al. 2006). The mechanisms behind the growth-promoting effects of *P. indica* are a matter of debate. Phytohormones as ethylene, auxin, and cytokinin seem to play a role as different analyses of the fungal culture filtrate and particular plant mutants indicate (Barazani et al. 2007; Sirrenberg et al. 2007; Vadassery et al. 2008). To make things even more complicated, a recent analysis has shown that *P. indica* contains bacteria inside its cytoplasm which show similar effects on plant growth and defence reactions as the fungus (Sharma et al. 2008).

In addition to promoting vegetative growth, *P. indica* also exerts a positive influence on the generative organs of plants. In contrast to the relatively low enhancement of

shoot fresh weights, the increase in tomato fruit biomass was surprisingly high. This was not due to increased fresh weight of single fruits, but the fruit number was larger than in control plants. Higher number of inflorescences and fruit settings was already observed in the experiments 1–4 (data not shown). In addition to the fresh weight, *P. indica* also enhanced the dry matter content of the fruits. This indicates that more biomass was transported into the generative organs during the growth period of the plants. Because the vegetative organs were not reduced in size, there must have been a higher production of these biomasses during growth of the tomatoes in interaction with the endophyte. In barley, were yield increases up to 11% were observed in open door experiments (Waller et al. 2005), different parameters have been tested (Achatz et al. submitted). While improved mineral nutrition or protection against pathogens did not play a role, enhanced $CO_2$ assimilation under low light conditions was observed. Indeed, in the last experiment without the virus, fresh weights were increased by a factor of 2.3 under low light and by a factor of 1.9 under high light. This difference was not significant as no interaction could be observed between the two factors endophyte and light. Nevertheless, further experiments will be carried out with higher differences in the light intensities and measurements of C assimilation and total C in the different organs of the tomato plant in order to better elucidate the basis for the increased biomasses. In addition, the total yield until the last fruit setting has to be monitored for excluding the possibility that *P. indica*-colonised plants are just developmentally progressed compared to controls. Such an accelerated development was indicated by particular gene expression patterns in barley roots (Waller et al. 2008). However, yield of seeds in tobacco, barley, and Arabidopsis was not only increased at a particular date, but also overall at the end of the whole growth period (Barazani et al. 2005; Waller et al. 2005; Shahollari et al. 2007).

In barley, in wheat, and in Arabidopsis, it has been shown that *P. indica* is able to alleviate the symptoms after attack of the plants by fungal pathogens (Waller et al. 2005; Serfling et al. 2007; Stein et al. 2008). It was, therefore, not surprising to find that the endophyte is also reducing the symptoms of verticillium wilt in tomato. The disease severity was lowered by more than 30%, and *P. indica* balanced the fresh weight decrease caused by the pathogen. This was in the range what has been observed in the other systems mentioned in the introduction. A similar effect on verticillium wilt has been also observed by using the nonvirulent isolate Dvd-E6 of *V. dahliae* as a competitor (Chen et al. 2004). In this case, the colonisation of Dvd-E6 nearly totally excluded the infection by the virulent race (Shittu et al. 2009). If this is also the case for *P. indica* remains to be analysed by assessing the biomass of the pathogen in the plant. Such resistance reactions induced by *P. indica* have been observed in case of powdery mildew in barley, wheat, and Arabidopsis (Waller et al. 2005; Serfling et al. 2007; Stein et al. 2008). The increased production of antioxidants in roots and shoots of *P. indica*-colonised plants were discussed as one reason for the induced resistance (Waller et al. 2005; Serfling et al. 2007), and also, particular genes known to be involved in plant defence reactions were shown to be systemically induced after *P. indica* inoculation (Waller et al. 2008). Such type of studies could be also carried out for tomato, where many defence and pathogenesis-related genes are known.

Although not significant at all dates of investigation, the overall picture of the last three experiments suggested that *P. indica* interferes with Pepino mosaic virus accumulation in the apical shoot and that the outcome of this interference is dependent on light intensities during tomato cultivation. Such interactions between fungal root endophytes and viral pathogens were up to now only reported for arbuscular mycorrhizal fungi and the tobacco mosaic virus (Dehne 1982; Shaul et al. 1999). In both cases, viral occurrence and resulting symptoms were increased in leaves. In contrast, when tomatoes were coinoculated with PepMV and a fungal pathogen, such as *Verticillium* spp. (Spence et al. 2006) or *Pythium aphanidermatum* (Schwarz et al. 2009), the virus colonisation of the plant was inhibited. The root necrosis caused by the fungal pathogens could perhaps induce resistance mechanisms affecting virus multiplication and spread (Van Loon 1997), and/or biochemical and structural changes in root architecture reduced the efficiency of PepMV uptake of roots through the nutrient solution. Similar to the AM fungi, *P. indica* might increase under low light conditions the carbohydrate content of cells due to a higher photosynthetic rate and in this way, stimulates the number of virus particles in the tissues as detected by ELISA. Increases in C assimilation of *P. indica*-colonised plants have been observed in barley (Achatz et al. submitted) and in *P.* x *hortorum* (unpublished). The difference in carbohydrate contents between control and endophyte-colonised plants would be lower under high light conditions, and another mechanism would become evident. Such a mechanism could be similar to the systemic induced resistance (SIR) against viruses which was obtained with particular plant growth-promoting rhizobacteria (Raupach et al. 1996; Jetiyanon and Kloepper 2002) and results in a decrease of the accumulation of PepMV in tomato. It has to be mentioned that usually systemic acquired resistance and not SIR is acting against viruses (Ton et al. 2002). However, an SIR-similar mechanism dependent on jasmonate signalling was revealed as being responsible for the reduction of powdery mildew in *P. indica*-colonised Arabidopsis plants (Stein et al. 2008).

## Conclusion

*P. indica* reduces the disease symptoms caused by the fungal pathogen *V. dahliae* and is able to repress the amount of Pepino mosaic virus provided that light intensities are high. Tomato plants colonised by the endophyte show only slightly enhanced vegetative development, but fruit biomass is strongly increased. More research is necessary to further optimise 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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