22 Analysis of the Plant Protective Potential of the Root Endophytic Fungus *Piriformospora indica* in Cereals

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22.1 Introduction

Piriformospora indica is a recently discovered basidiomycete that infests roots of a large variety of mono- and dicotyledonous plants (Verma et al. 1998; Pham et al. 2004). Endophytic growth of this fungus in roots leads to enhanced plant growth (Varma et al. 1999), reminiscent of the beneficial effects of arbuscular mycorrhiza in host plants. We have recently shown that *P. indica* – upon successful establishment in the roots – reprogrammes barley to salt stress tolerance, resistance to diseases and higher yield. Successful powdery mildew infections in barley leaves are reduced by this root endophyte, due to a yet unknown mechanism of induced resistance (IR) (Waller et al. 2005). As *P. indica* can easily be cultured without a host plant (Varma et al. 1999), it is suitable both as a model system for research and for future applications in agriculture. Here, we present approaches and methods to study the mechanisms behind the observed pathogen resistance induced by *P. indica*. These methods should provide valuable tools for studying the effect of root-interacting fungi on IR in cereals.

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22.2 Plant Responses and Resistance to Pathogens

22.2.1 Local Reactions

Plants are continuously defending themselves against a plethora of attacking viruses, bacteria, fungi and invertebrates. Each plant cell has both preformed and inducible defence capabilities. Among the preformed defences are physical barriers, such as the cell wall or, for example, secondary metabolites with antimicrobial properties. After recognition of the pathogen, induced defence responses may comprise of local cell wall fortifications, the production or activation of secondary metabolites, the localized release of antimicrobial compounds at sites of attack or a hypersensitive reaction (HR), leading to localized cell death which limits the spread of the pathogen in the plant. Local defence responses are accompanied by an enhanced expression of defence-related genes: pathogenesis-related genes (PR genes) respond rapidly to challenges by pathogens and have been widely used as markers for defence reactions in plants.

22.2.2 Systemic Reactions and Resistance in Cereals

A prior activation of plant defence that leads to resistance against pathogens is termed induced resistance (IR; Sticher et al. 1997). IR has been studied extensively in the case of salicylic acid (SA)-mediated *systemic acquired resistance* (SAR) in dicotyledonous plants: Micro-lesions induced by necrotizing pathogens trigger a local accumulation of salicylic acid, with mitogen-activated protein kinases, H_2O_2 and other signals being involved. Whereas the mobile signal leading to systemic responses is still a matter of debate, it is clear that the expression of SAR marker genes, the PR genes, is controlled by the protein NPR1, which is necessary for SAR (Mou et al. 2003; Dong 2004). Another major type of IR is *induced systemic resistance* (ISR) which is triggered by non-pathogenic rhizobacteria. ISR depends on both NPR1 and the jasmonic acid/ethylene pathway, but not on SA (Pieterse et al. 1998). Cereals share many components of resistance pathways with dicotyledonous plants: SA derivatives induce, for example, resistance in cereals (Kogel et al. 1994), and NPR 1 homologues have been shown to be functional in rice (Chern et al. 2005). However, specific IR signalling components in cereals have yet to be characterized in detail (Kogel and Langen 2005).

22.2.3
Beneficial Microbial Endophytes Protecting Cereals from Pathogens

Micro-organisms growing inside of plants are referred to as endophytes. A large number of these are known to protect plants against pathogens. For example, grasses (Poaceae) are frequently associated with fungi of the Clavicipitaceae (Ascomycota), with interactions ranging from mutualism to antagonism (Schardl et al. 2004). In these interactions, the endophyte is strictly confined to upper parts of the plant, grows only intercellularly and has a rather narrow host range. The beneficial effect of these endophytes has been shown to result from a direct antimicrobial and insecticidal activity of alkaloids. Another group of endophytic fungi, the arbuscular mycorrhiza (AM; Glomeromycota; Schüssler et al. 2001) protect plants from various stresses, including root diseases (Dehne 1987; Azcón-Aguilar and Barea 1996; Borowicz 2001; Harrison 2005; Hause and Fester 2005). An improved defence status of mycorrhizal roots is associated with an increased expression of the H_2O_2 scavengers catalase, peroxidase and superoxide dismutase (Blee and Anderson 2000; Pozo et al. 2002). Such an elevated antioxidant activity could protect roots from cell death mediated by necrotrophic root pathogens, which require killing of host cells for a successful infection. Using a split root technique, it has been demonstrated that AM induce systemic protection against root pathogens (Cordier et al. 1998; Pozo et al. 2002). This systemic effect of mycorrhization is restricted to the root and does not protect plants from leaf diseases, but rather increases susceptibility to them (Shaul et al. 1999; Gernns et al. 2001). In addition to this agronomic drawback of the AM symbiosis, AM cannot be cultured axenically, limiting a wide-spread field application. Since a biological approach to protect cereals from pathogens has a significant impact for modern plant production systems, exploiting an axenically cultivatable endophyte with the ability to protect all plant parts from pathogens would be an important step towards a feasible broad-range application of biological measures in agriculture.

22.3
Interaction of *P. indica* with Cereals

P. indica is a basidiomycete fungus from the newly defined order Sebacinales (Hymenomycetes; Verma et al. 1998; Weiß et al. 2004). This endophyte infests roots of a large variety of mono- and dicotyledonous plants and can be axenically cultured (Verma et al. 1998, Pham et al. 2004). It has been shown that hyphae of *P. indica* develop both inter- and intracellular in the root cortex of a number of different plant species, thereby improving plant growth and stress tolerance (Varma et al. 1999, 2000). As the fungus' broad host range and easy cultivation

could be valuable for agricultural applications, we tested *P. indica* for the ability to protect barley from abiotic stress and pathogens (Waller et al. 2005).

22.3.1 *P. indica* Colonizes Root Cortical Cells in Barley

We analysed fungal growth in barley roots grown in *P. indica*-inoculated substrate upon staining with 0.01% acid fuchsin lactic acid (Kormanik and McGraw 1982). For microscopy, whole roots as well as longitudinal and cross-sections produced by a cryo-microtome were used. Hyphae develop a dense mesh on the surface of the roots (Fig. 22.1a). Both hyphae and typical pear-shaped chlamydospores were localized intracellularly in the first few cell layers of the root (Fig. 22.1b), but could not be detected in the central root tissues beyond the endodermis.

22.3.2 *P. indica* Enhances Biomass and Yield in Barley

For growth experiments, barley seedlings were planted into pots with *P. indica*-inoculated soil (see Section 22.4.1). After five weeks of cultivation in the greenhouse, the fresh weight of shoots was evaluated. Shoot fresh weight was up to 1.65 times higher than that of control plants grown in soil without *P. indica* (Waller et al. 2005). Tests under field conditions, using Mitscherlich pots with

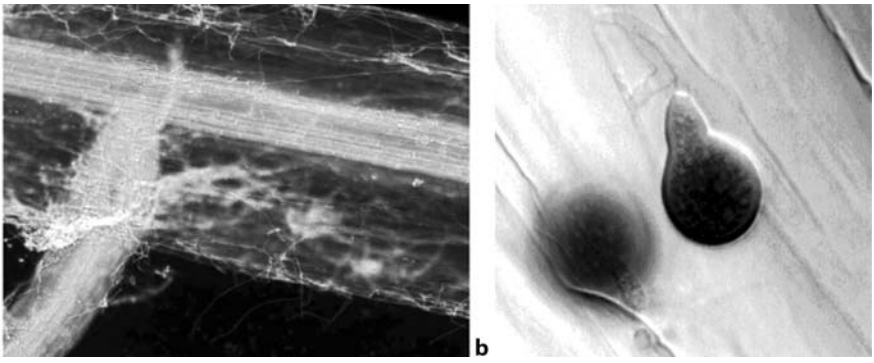

Fig. 22.1 *P. indica* hyphae and spores on a barley root. Two weeks after inoculation of barley roots with *P. indica*, acid fuchsin lactic acid staining reveals a mesh of hyphae surrounding the root (central part and emerging lateral root exhibit autofluorescence; **a**, fluorescence microscopy), as well as typical pear-shaped chlamydospores (**b**, bright-field image)

six plants per pot, revealed that a beneficial effect of *P. indica* on plant growth is present in plants until harvest: Grain yield increased by 11% in the barley elite cultivar *Annabell* as compared with control plants (Waller et al. 2005). This increase was mainly due to a higher number of ears per plant.

22.4 Approaches to Study the Mechanism of *P. indica*-Induced Pathogen Resistance

22.4.1 *P. indica* Induces Disease Resistance Against Root Pathogens

To assess whether *P. indica*-infested plants are more resistant to biotic stress, barley roots were inoculated with macroconidia of the necrotrophic fungal pathogen *Fusarium culmorum* (causing root rot). In the presence of *P. indica*, the devastating effect of *F. culmorum* infection was strongly diminished: Root and shoot fresh weight was reduced only 2-fold in *P. indica*-infested plants as compared with the 12-fold decrease in controls with *F. culmorum* alone. Similar results were obtained when resistance to the root-pathogenic fungus *Cochliobolus sativus* (hemibiotrophic life style) was tested. In axenic culture, *P. indica* did not exhibit antifungal activity to *F. culmorum* nor to *C. sativus*, indicating that the protective potential of the endophytic fungus does probably not rely on antibiosis (Waller et al. 2005).

1. Method for infestation of barley with *P. indica* and cultivation of plants:
   Barley was grown in pots with a 2:1 mixture of expanded clay (Seramis; Masterfoods, Verden, Germany) and Oil-Dri (Damolin, Mettmann, Germany) in an incubator with a 22 °C/18 °C day/night cycle, a photoperiod of 16 h (240 μmol m^{-2} s^{-1} photon flux) and 60% relative humidity. Plants were fertilized weekly with 20 ml of a 0.1% Wuxal top N solution (N/P/K: 12/4/6; Schering). For inoculation with *P. indica*, 2 g of crushed mycelium were added to 300 g of substrate before sowing. *P. indica* was propagated in liquid *Aspergillus* minimal medium (Peskan-Berghöfer et al. 2004) on a horizontal rotary shaker at 18–22 °C. Mycelium from liquid culture was washed with water to remove remaining traces of medium and crushed using a Waring Blendor (VWR International, Darmstadt, Germany) before adding it to the substrate. For yield evaluations, barley was sown in soil containing *P. indica* mycelium (4 g per 300 g of substrate) and grown for 4 weeks in a growth chamber after which six plantlets were transplanted into 6-l Mitscherlich pots (Stoma, Siegburg, Germany) filled with a mixture of a loam soil and sand (1:2). Soil nutri-

ent additives were 0.25 g of N, 0.4 g of P, 1.6 g of K, and 0.2 g of Mg; N was applied a second time at a rate of 0.25 g per pot, 2 weeks after planting.

2. Method for testing *Fusarium culmorum* in barley:
   To test the effect of *F. culmorum*, barley was grown as described above. Two weeks after planting into *P. in*dica containing soil, plants were transferred into pots containing macroconidia of *F. culmorum*. Root and shoot fresh weight was measured two weeks after inoculation with *F. culmorum*.

22.4.2 *P. indica* Induces Systemic Disease Resistance

We recorded the effect of *P. indica* infestation on leaf infections by the biotrophic barley powdery mildew fungus, *Blumeria graminis* f.sp. *hordei*. A reduction in powdery mildew infection on leaf segments of *P. indica*-infested plants could be observed. Frequencies of mildew colonies decreased by 48% in second youngest leaves and by 58% in youngest leaves of 3-week-old *P. indica* infested plants (Waller et al. 2005).

Beside a reduction in pustule number, we frequently observed a smaller size and a reduced density of pustules. We quantified colonies belonging to three categories "large compact white colonies" (cat. I), "smaller, less dense colonies" (cat. II), and "colonies smaller than 0.3 mm in diameter" (cat. III; Fig. 22.2). In *P. indica*-infested plants, a shift towards smaller colonies was observed. This indicates a resistance mechanism that is limiting the development of the fungus after successful penetration. One possible explanation could be a reduced supply of nutrients to the fungus.

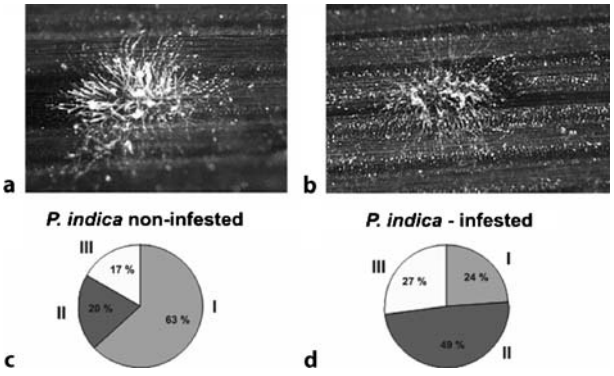

Fig. 22.2 Phenotype of *Blumeria graminis* pustules on barley leaves of *P. indica* infested plants. Shown are pustules on barley leaves 6 days after inoculation with *Blumeria graminis* f.sp. *hordei* (**a**, **b**). We quantified the percentage of colonies belonging to three categories: large, compact white colonies (as can be seen in **a**; cat. I), smaller, less dense colonies (as in **b**; cat. II) and colonies smaller than 0.5 mm in diameter (cat. III). In *P. indica*-infested plants, a shift towards smaller colonies, as compared with *P. indica* non-infested plants, was observed (**c**, **d**)

Microscopic analysis of powdery mildew on barley leaves revealed higher frequencies of HR as well as a cell wall-associated defence visible as cell wall appositions. These observations confirmed that the pathogen is arrested by an active plant response. As *P. indica* grows only in the outer cell layers of the host root and does not infest barley leaves, these data demonstrate a systemic plant response mediated by an endophytic fungus.

1. Method for leaf segment test:
   To assess powdery mildew resistance, leaf segments 7 cm in length were cut about 1 cm distal from the leaf sheath. Leaf segments were placed on 0.7% agar plates containing 40 mg l^{-1} benzimidazol (to inhibit leaf senescence). Inoculation was performed by shaking barley leaves heavily infected with *B. graminis* f.sp. *hordei*, race A6 (Wiberg 1974) in an inoculation tower about 1 m above the plates and manually circulating the air to ensure equal distribution of the spores. Inoculation density was checked by counting the number of spores per square millimetre, using a counting plate of defined size placed beside the plates with the leaf segments and counting the spores in this plate using a microscope. For counting the number of successful interaction sites, an inoculation density of 8–20 spores mm^{-2} was used. Plates were placed in an incubator at 18 °C with a 16 h /8 h light/dark cycle. After 6 days, pustules were visible and could be counted on a defined leaf segment, e.g. 3 cm or 5 cm in length. The severity of powdery mildew infection (disease index) was calculated as colonies produced by *B. graminis* on a defined leaf area. Generally, at least nine leaves were used per experiment and standard deviation as well as significance level calculated (unpaired Student's *t*-test).
2. Method for microscopic classification of interactions with the powdery mildew fungus:
   For cytological analysis, youngest leaves of three-week-old barley plants were inoculated with *B. graminis* f.sp. *hordei* (A6) as described above. Then whole plants were incubated in an incubator at 18 °C with a 16 h/8 h light/dark cycle.
   For H_2O_2 detection, a histochemical staining method using 3,3-diaminobenzidine (DAB) was used (Thordal-Christensen et al. 1997). After inoculation of the whole plant with powdery mildew and incubation for 27–43 h (depending on which stage of infection is visualized), leaves were cut and placed with the cut side in a solution of 1 mg ml^{-1} DAB for approximately 5 h. Subsequently, the leaves were destained [0.15% trichloroacetic acid (w/v) in ethylalcohol/chloroform (4:1 (v/v)]. The solution was changed once during the next 48 h of incubation. Leaf segments were stored in 50% glycerol.
   Staining of fungal structures and microscopy was done as described by Hückelhoven and Kogel (1998): To stain fungal structures for bright-field microscopy, leaves were incubated in 10% blue ink (v/v, Pelikan 4001; Pelikan, Hannover, Germany) in 25% acetic acid for 1 min followed by a washing step to remove excess ink. Autofluorescence was observed by fluorescence microscopy (excitation wavelength 485 nm). For cytological studies, an Axioplan microscope (Zeiss, Jena, Germany) was used. For quantification of interaction types, one hundred or more attacked short cells (cell type A and B of

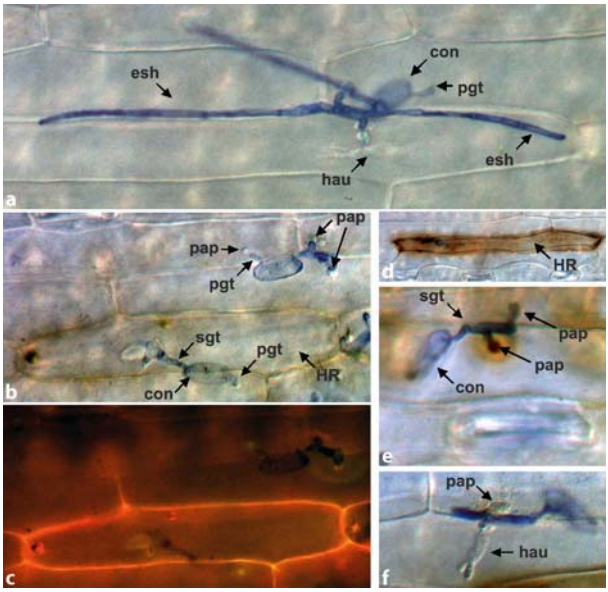

Fig. 22.3 Interaction of the powdery mildew fungus *B. graminis* f. sp. *hordei* with its host plant *Hordeum vulgare*. Shown are interaction sites at 32 h (**d**, **e**, **f**), 48 h (**b**, **c**) and 72 h (**a**) after inoculation with the pathogen *B. graminis* f.sp. *hordei*. After formation of a primary germtube (*pgt*) on the surface of the leaf, conidia (*con*) of the pathogen form a secondary germtube (*sgt*) that penetrates the epidermal leaf cell (**a**, **b**). **a** Overview of a successful penetration, with the fungus developing its nutrition organ, the haustorium (*hau*) and elongated secondary hyphae (*esh*) spreading on the leaf surface; **b** and **c** show the same cell as bright-field (**b**) and fluorescence (**c**) images. Active responses of the plant can stop the biotrophic pathogen from spreading through the plant, either by local cell death, resulting from a hypersensitive reaction (HR) of the penetrated cell (**b**, **c**, **d**) or by local fortifications of the cell wall at the site of attempted penetration (papilla = *pap*; **b**, **e**). Sites of H_2O_2 accumulation are detected by staining with 3,3-diaminobenzidine (DAB), as can be seen in **b** and **d** as the brown staining of the attacked cell and in **e** as the brown stain surrounding the papillae. Autofluorescence is visible at sites of HR (**c**), as phenolic cell wall components accumulate

the epidermis, according to Koga et al. 1990) were scored per leaf. Cellular responses to powdery mildew attack were categorized by counting cells showing (1) an active defence response, (HR, visible as whole cell autofluorescence, DAB staining), (2) a local defence stopping a penetration attempt (non-penetrated cell, visible as the formation of cell wall appositions), or (3) a successful penetration (formation of a haustorium; Fig. 22.3).

22.4.3 Assessment of the Antioxidant Capacity of *P. indica*-Infested Roots

The protective activity by *P. indica* against root pathogens with necrotrophic nourishment strategies prompted us to analyse the antioxidant status of infested

roots. Ascorbate levels were consistently higher at one, two and three weeks after root infestation with *P. indica*, while levels of dehydroascorbate (DHA) were reduced. At the same time, activity of ascorbate recycling dehydroascorbate reductase (DHAR) increased. Concomitantly, slightly enhanced total glutathione concentrations and glutathione reductase activities were observed (Waller et al. 2005). It can be reasoned that higher antioxidant levels protect roots from cell death provoked by the root pathogens *F. culmorum* and *C. sativus*. Because production of reactive oxygen species and host cell killing is a prerequisite for successful fungal development and pathogenesis of necrotrophic fungi (Govrin and Levine 2000), we hypothesize that higher antioxidant capacity, such as elevated ascorbate levels, could cause the observed reduction of necrotrophic pathogens in the barley root.

22.4.4 Gene Expression Induced by *P. indica* in Barley Leaves

To gain information on the nature of *P. indica*-induced systemic protection of leaves against powdery mildew infection, we analysed the expression of "marker genes", indicative of specific resistance pathways. Interestingly, a number of genes typically associated with IR are not induced in the interaction with *P. indica*. Genes tested include *pathogenesis-related protein* 1 (*PR 1*), *pathogenesis-related protein* 5 (*PR 5*), *barley chemical induced protein* 1 (*BCI 1*; Beßer et al. 2000), and *jasmonate induced protein* 23 (*JIP 23*; Hause et al. 1996). As barley leaves do not show a constitutive up-regulation of typical marker genes for SA and JA, it is possible that other signalling pathways are involved in inducing systemic resistance after *P. indica* infestation of the roots (Waller et al. 2005). To elucidate the *P. indica* mediated IR mechanism, future strategies include screening of the Affymetrix Barley 1 gene chip (Close et al. 2004; Affymetrix, Santa Clara, Calif., USA) and custom-made microarrays with subtracted cDNA libraries enriched in *P. indica*-induced transcripts.

22.5 Conclusions

Cereals provide the staple crops for feeding a growing world population. Different approaches have to be taken to provide a stable harvest of these crops. Along with high yields, resistance against abiotic stress and pathogens is a prime goal. Identification of *P. indica*, an axenically cultivatable endophyte with the ability to protect the plant systemically from pathogens is an important step towards a broad-range application of more efficient biological measures in agriculture. Understanding the molecular mechanism mediated by *P. indica* will enable us to

envisage new approaches to ensure healthy plants producing stable harvests. The methods presented in this chapter provide the means to analyse these mechanisms in all interactions of cereals with beneficial microbes.

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References

Azcón-Aguilar C, Barea JM (1996) Arbuscular mycorrhizas and biological control of soil-borne plant pathogens – an overview of the mechanisms involved. Mycorrhiza 6:457–464

Beßer K, Jarosch B, Langen G, Kogel K-H (2000) Expression analysis of genes induced in barley after chemical activation reveals distinct disease resistance pathways. Mol Plant Pathol 1:277–286

Blee KA, Anderson AJ (2000) Defence responses in plants to arbuscular mycorrhizal fungi. In: Podila GK, Douds DD (eds) Current advances in mycorrhizae research. APS, St. Paul, pp 27–44

Borowicz VA (2001) Do arbuscular mycorrhizal fungi alter plant–pathogen relations? Ecology 82:3057–3068

Chern MS, Fitzgerald HA, Canlas PE, Navarre DA, Ronald PC (2005) Over-expression of a rice NPR1 homologue leads to constitutive activation of defense response and hypersensitivity to light. Mol Plant Microbe Interact 18:511–520

Close TJ, Wanamaker SI, Caldo RA, Turner SM, Ashlock DA, Dickerson JA, Wing RA, Muehlbauer GJ, Kleinhofs A, Wise RP (2004) A new resource for cereal genomics: 22K barley genechip comes of age. Plant Physiol 134:960–968

Cordier C, Pozo MJ, Barea JM, Gianinazzi S, Gianinazzi Pearson V (1998) Cell defense responses associated with localized and systemic resistance to *Phytophthora parasitica* induced in tomato by an arbuscular mycorrhizal fungus. Mol Plant Microbe Interact 11:1017–1028

Dehne H-W (1987) Zur Nutzung der VA Mykorrhiza als Antistressfaktor. Angew Bot 61:135–143

Dong X (2004) NPR1, all things considered. Curr Opin Plant Biol 7:547–552

Gernns H, Alten HV, Poehling HM (2001) Arbuscular mycorrhiza increased the activity of a biotrophic leaf pathogen – is a compensation possible? Mycorrhiza 11:237–243

Govrin EM, Levine A (2000) The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea*. Curr Biol 10:751–757

Harrison MJ (2005) Signaling in the arbuscular mycorrhizal symbiosis. Annu Rev Microbiol 59:19–42

Hause B, Fester T (2005) Molecular and cell biology of arbuscular mycorrhizal symbiosis. Planta 221:184–196

Hause B, Demus U, Teichmann C, Parthier B, Wasternack C (1996) Developmental and tissue-specific expression of JIP-23, a jasmonate-inducible protein of barley. Plant Cell Physiol 37:641–649

Hückelhoven R, Kogel K-H (1998) Tissue-specific superoxide generation at interaction sites in resistant and susceptible near-isogenic barley lines attacked by the powdery mildew fungus (*Erysiphe graminis* f. sp. *hordei*). Mol Plant Microbe Interact 11:292–300

Koga H, Bushnell WR, Zeyen RJ (1990) Specificity of cell type and timing of events associated with papilla formation and the hypersensitive reaction in leaves of *Hordeum vulgare* attacked by *Erysiphe graminis* f.sp. *hordei*. Can J Bot 68:2344–2352

Kogel K-H, Langen G (2005) Induced resistance and gene expression in cereals. Cell Microbiol 7:1555–1564

Kogel K-H, Beckhove U, Dreschers J, Münch S, Rommé Y (1994) Acquired resistance in barley. Plant Physiol 106:1269–1277

Kormanik PP, McGraw A-C (1982) Quantification of vesicular–arbuscular mycorrhizae in plant roots. In: Schenck NC (ed) Methods and principles of mycorrhizal research. American Phytopathological Society, St. Paul, pp 37–45

Mou Z, Fan W, Dong X (2003) Inducers of plant systemic acquired resistance regulate npr1 function through redox changes. Cell 113:935–944

Peskan-Berghöfer T, Markert C, Varma A, Oelmüller R (2004) Association of *Piriformospora indica* with *Arabidopsis thaliana* roots represents a novel system to study beneficial plant–microbe interactions and involves early plant protein modifications in the endoplasmic reticulum and at the plasma membrane. Physiol Plant 122:465–477

Pham GH, Singh A, Malla R, Kumari R, Prasad R, Sachdev M, Rexer K-H, Kost G, Luis P, Kaldorf M, Buscot F, Herrmann S, Peskan T, Oelmüller R, Saxena AK, Declerck S, Mittag M, Stabentheimer E, Hehl S, Varma A (2004) Interaction of *Piriformospora indica* with diverse microorganisms and plants. In: Varma A, Abbott LK, Werner D, Hampp R (eds) Plant surface microbiology. Springer, Berlin Heidelberg New York, pp 237–265

Pieterse CMJ, Van Wees SCM, Van Pelt JA, Knoester M, Laan R, Gerrits H, Weisbeek PJ, Van Loon LC (1998) A novel signaling pathway controlling induced systemic resistance in *Arabidopsis*. Plant Cell 10:1571–1580

Pozo MJ, Cordier C, Dumas-Gaudot E, Gianinazzi S, Barea JM, Azcon-Aguilar C (2002) Localized versus systemic effect of arbuscular mycorrhizal fungi on defence responses to *Phytophthora* infection in tomato plants. J Exp Bot 53:525–534

Schardl CL, Leuchtmann A, Spiering MJ (2004) Symbioses of grasses with seedborne fungal endophytes. Annu Rev Plant Biol 55:315–340

Schüssler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol Res 105:1413–1421

Shaul O, Galili S, Volpin H, Ginzberg I, Elad Y, Chet I, Kapulnik Y (1999) Mycorrhiza-induced changes in disease severity and PR protein expression in tobacco leaves. Mol Plant Microbe Interact 12:1000–1007

Sticher L, Mauch-Mani B, Métraux JP (1997) Systemic acquired resistance. Annu Rev Phytopathol 35:235–270

Thordal-Christensen H, Zhang ZG, Wei YD, Collinge DB (1997) Subcellular localization of H2O2 in plants – H_2O_2 accumulation in papillae and hypersensitive response during the barley–powdery mildew interaction. Plant J 11:1187–1194

Varma A, Verma S, Sudha, Sahay N, Bütehorn B, Franken P (1999) *Piriformospora indica*, a cultivable plant-growth-promoting root endophyte. Appl Environ Microbiol 65:2741–2744

Varma A, Singh A, Sudha, Sahay NS, Sharma J, Roy A, Kumari M, Rana D, Thakran S, Deka D, Bharti K, Franken P, Hurek T, Blechert O, Rexer K-H, Kost G, Hahn A, Maier W, Walter M, Strack D, Kranner I (2000) *Piriformospora indica*: an axenically cultureable mycorrhiza-like endosymbiotic fungus. In: Hock B (ed) The Mycota IX. Springer, Berlin Heidelberg New York, pp 125–150

Verma S, Varma A, Rexer K-H, Hassel A, Kost G, Sarbhoy A, Bisen P, Bütehorn B, Franken P (1998) *Piriformospora indica*, gen. et sp. nov., a new root-colonizing fungus. Mycologia 90:896–903

Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M, Heier T, Hückelhoven R, Neumann C, von Wettstein D, Franken P, Kogel K-H (2005) The endophytic fungus *Piriformospora indica* reprograms barley to salt stress tolerance, disease resistance and higher yield. Proc Natl Acad Sci USA 102:13386–13391

Weiß M, Selosse M-A, Rexer K-H, Urban A, Oberwinkler F (2004) Sebacinales: a hitherto overlooked cosm of heterobasidiomycetes with a broad mycorrhizal potential. Mycol Res 108:1003–1010

Wiberg A (1974) Genetical studies of spontaneous sources of resistance to powdery mildew in barley. Hereditas 77:89–148