Mutualism or parasitism: life in an unstable continuum. What can we learn from the mutualistic interaction between *Piriformospora indica* and *Arabidopsis thaliana*? - Review

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Abstract

Almost all plants live in interactions with fungi, where they express different lifestyles ranging from mutualism (beneficial for both partners) through commensalism (beneficial for the microbe, while the host species is neither positively nor negatively affected) to parasitism (the host is noticeably harmed or deprived at the expense of the fungus). Mutualism represents a balanced stage of plant/microbe interactions. However, depending on the partner combination, the genetic constitutions of both partners, the developmental stage and the life-history of the symbiosis, the physiological status of both partners, the colonization pattern of the host, the ability of the microbe to produce toxins and of the host to defend them, the evolutionary status of the symbiosis, environmental and habitat-specific conditions and the stress situation, a mutualistic inter-action can become unstable and shift towards commensalism or parasitism. Genetic studies with mycorrhizal and endophytic fungi discovered genes which are crucial for determining a beneficial symbiotic stage. Recent progress in this field is summarized in this review. In addition, the interaction of the endophytic fungus *Piriformospora indica* with *Arabidopsis* as a novel model system in this field is introduced.

Introduction

The vast majority of the plants live in symbiotic interactions with fungi, where they can express different lifestyles ranging from mutualism *via* commensalism to parasitism (SCHULZ and BOYLE 2005; KOGEL et al. 2006; PASZKOWSKI 2006; RODRIGUEZ and REDMAN 2008; http://en.wikipedia.org/wiki/symbiosis). In mutualistic interactions, the fungi confer fitness benefits to the plants which can include biotic and abiotic stress tolerance, growth enhancement, and increased reproductive success. Many plants are unable to survive in their native habitats without beneficial symbionts. The mutualistic lifestyle is also beneficial for the microbes, because it assures a permanent supply with carbon from the autotrophic plant and shelter from most biotic stresses. Arbuscular mycorrhizal fungi are widespread and form the best studied mutualistic interactions. The mycorrhizal symbiosis is more than 460 million years old and thus represents one of the evolutionarily oldest beneficial symbioses of plants, which was established when the land was colonized by the plants (REDECKER et al. 2000). This required an efficient access of the plants to nutrients and water in the soil, a process for which root-associated fungi were either helpful or necessary. Mutant analyses have uncovered that the signaling pathway leading to the establishment of mycorrhiza shares many components with that leading to nodulation of legumes by rhizobacteria. This demonstrates that the younger rhizobial symbiosis has evolved from the mycorrhizal symbiosis (ANÉ et al. 2004; LÉVY et al. 2004). Arbuscular mycorrhizal interactions are normally restricted to a few plant species and rely on the formation of an intimate relationship between fungi of the *Glomeromycota*

and roots of legumes, while fungal endophytes have a broad host range encompassing both monocots and eudicots. Many benefits for the plants known from mycorrhizal interactions have also been described for the symbiosis with endophytes, such as improved growth (JUMPPONEN 2001; MUCCIARELLI et al. 2002; ERNST et al. 2003; PEŠKAN-BERGHÖFER et al. 2004; SHERAMETI et al. 2005, 2008a and b), the induction of defense metabolites potentially active against pathogens (SCHULZ et al. 1999; MUCCIARELLI et al. 2003; ARNOLD and HERRE 2003), secretion of phytohormones by the microbe (HOLLAND 1997; REY et al. 2001; RÖMMERT et al. 2002; TUDZYNSKI and SHARON 2002; SIRRENBERG et al. 2007; VADASSERY et al. 2008), mobilization of nutrients and water from the rhizophere (JUMPPONEN and TRAPPE 1998; CADWELL et al. 2000; USUKI et al. 2002; SHAHOLLARI et al. 2007), stimulation of disease resistance (PICARD et al. 2000; BENHAMOU and GARAND 2001), alterations of the host metabolism (JALLOW et al. 2004; SHERAMETI et al. 2005), abiotic stress tolerance (BARROW and AALTONEN 2001; BARROW 2003; SHERAMETI et al. 2008a) and protection against pathogens and insects (SCHULZ et al. 1995; HALLMANN and SIKORA 1996; SCHULZ et al. 2002; MILLER et al. 2002; SELOSSE et al. 2004; BARAZANI et al. 2005; SEGARRA et al. 2009).

Mutualism is defined as an interaction that is beneficial for both partners, and - together with the commensalisms - represents a balanced stage of plant/microbe interactions. Commensalism provides benefit to the endophyte by enabling an undisturbed existence and nutrient supply without affecting the host. In contrast, parasitism is characterised by the dominance of the microbe, which harms the plant, by producing various cellulytic and proteolytic enzymes, of toxin, unbalanced growth hormones, inactivation/suppression of various plant defense compounds, and might ultimately lead to the death of the plant. These three types of symbiotic interactions are often unstable, depending on the interacting partners, genetic parameters, environmental conditions and in particular nutrient supply or habitat-specific stress conditions, etc. (KOGEL et al. 2006; PASZKOWSKI 2006; RODRIGUEZ and REDMAN 2008). Many examples in nature demonstrate that a mutualistic interaction can shift to commensalism or even parasitism when environmental conditions change. This has been observed for mycorrhizal and endophytic interactions, and several studies demonstrate that mutation of a single gene of either plant or fungal origin can change the stage of the symbiotic interaction from parasitism to mutualism (cf. below). Many signaling components which are involved in beneficial plant/fungus interactions are also involved in antagonistic plant symbioses (PARNISKE 2000). This raises the question how the appropriate response patterns are activated in the three different types of symbioses.

The symbiotic continuum

Analysis of *Colletotrichum* species have demonstrated that individual isolates can express either parasitic or mutualistic lifestyles depending on the host genotype, i.e. colonization of different hosts by the same fungus can establish different lifestyles (FREEMAN et al. 2001). *Colletotrichum* species, which are known to be virulent pathogens, can form mutualistic symbioses in non-disease hosts and confer benefits to these plants including disease resistance, growth enhancement, or drought tolerance (REDMAN et al. 2001, 2002a, b). *C. magna*, e.g., causes anthracnose in cucurbit, but exerts an endophytic lifestyle on various non-cucurbit species. Several *C. magna* mutant isolates can colonise different cucurbit cultivars which are resistant to the *C. magna* wild-type and do not elicit disease symptoms in these cultivars. They can establish even mutualistic interactions with these cultivars and have developed a broader host range (FREEMAN et al. 1993; REDMAN et al. 1999a, b). For instance, the

non-host tomato can be colonized by these mutualistic *C. magna* isolates (REDMAN et al. 2001) and they express either mutualistic, commensalistic or parasitic lifestyles depending on the tomato cultivars. In most cases, the genetic basis of the symbiotic communication is not known, however, the results show that small differences in the genomes of a fungal cultivar can determine the mode of interaction. A non-pathogenic mutant of a virulent *C. magna* isolate, obtained after UV mutagenesis, can colonize host plants symbiotically without producing any deleterious symptoms (FREEMAN and RODRIGUEZ 1993) and the symbiosis shows all features of a mutualistic interaction where it confers fitness benefits, disease and drought resistance and growth enhancement to the tested host plants. Genetic experiments support the idea that a switch between the different lifestyles can be controlled by a single genetic fungal locus. REDMAN et al. (2002a) proposed that inactivation of an extracellular serine protease could be responsible for such a shift. Since a non-pathogenic *C. magna* isolate (REDMAN et al. 1999a, b) loses the ability to switch between lifestyles and forms either mutualistic or commensalistic symbioses, a parasitic symbiosis for instance, can easily be inactivated, by a single point mutation in a gene. This shows that all processes required for the establishment of a beneficial interaction are already present in such a pathogenic fungal isolate, but they are apparently masked or not expressed because of the dominance of the events leading to a pathogenic interaction. Obviously, there must be a substantial overlap in the three different types of symbioses. Similar results have been observed with other endophytes. The endophytic genus *Epichloë*, for instance, comprises species that express either mutualistic or parasitic lifestyles (SCHARDL and LEUCHTMANN 2005; SCHULZ et al. 1999; SCOTT 2001).

Besides genetic determinants, endophytes can become also parasites under changing environmental conditions. This further supports the idea that both genetic programs are present in the fungus and that the expression pattern leading to one of the lifestyles can be changed or adjusted. Based on these observations it has been proposed that host/microbe interactions range from mutualism through commensalism to parasitism in a continuous manner (REDMAN et al. 2001b; CARROLL 1988; JOHNSON et al. 1997; SAIKKONEN et al. 1998; SCHULZ et al. 1999; SCHARDL and LEUCHTMANN 2005). Many groups of fungal symbionts contain isolates or species that span the entire symbiotic continuum and express all three lifestyles, while others are more restricted to one of the lifestyles with little flexibility. Further support for this "symbiotic continuum" hypothesis comes from evolution: mutualistic *Clavicipitaceous* endophytes might have evolved from pathogenic ancestors (SCHARDL and LEUCHTMANN 2005). A balanced interaction with the potential for variability and shifts between the different lifestyle might be a driving force in evolutionary development: a symbiosis can evolve in the direction of a more specialized mutualism or a more specialized parasitism (SCHULZ and BOYLE 2005).

As mentioned above, genetic studies have demonstrated that the type of interaction can be determined by a single fungal gene (product) (MÜLLER and KRAUS 2005; SCHULZ and BOYLE 2005). Also plant mutants have been isolated in which the mutualistic interaction with mycorrhizal or endophytic fungi is shifted towards the activation of defense genes (see below). Thus processes in the plants which are controlled by a single plant gene product can also contribute to the decision of the lifestyle. Changes in the habitat environment, stress situations and nutrient limitations are well characterized factors which can induce a shift from mutualism to parasitism. This is often associated with reprogramming of the plant expression pattern. Quite often, a combination of unfavourable environmental condi-

tions and unstable genetic backgrounds favour shifts from mutualistic to parasitic lifestyles. Another important factor is the life-history of the symbiosis. During early phases of the interaction of two symbionts, the microbe is often considered as a foe, presumably because the benefits for the plants are not yet established, e.g. by an imbalance of nutrient exchange. During that period, arbuscular mycorrhizal fungi build up their extraradical mycelium without providing mineral nutrients to the plants, and the plant respond to the microbe by activating a mild defense response. The same has been observed for endophytic plant/fungus interactions, as long as the mycelium has not yet reached a certain size that allows the plant to recognize the microbe as a helpful partner. During later stages of the symbiosis, the interaction can shift to mutualism, e.g. after an arbuscular system has been established that allows an efficient nutrient and information exchange among the partners. This is associated with the down-regulation of defense responses against the invader.

It is likely that proteins which cause a shift in the lifestyle are often related to each other (e.g. defense proteins or signal transduction components which activate the corresponding defense genes in response to fungal signals). The task of the future will be to identify processes which determine a lifestyle, maintain it in a stable mode or are responsible for a shift to another lifestyle. Since *Arabidopsis thaliana* is not a host of mycorrhizal fungi, endophytes interacting with this model system might also be useful to understand these symbiotic interactions. It remains to be determined whether the mutualistic interaction established by mycorrhizal fungi induces the same or similar signal pathways and responses in plants as endophytic fungi.

As mentioned above, a mutation in a single (fungal or plant) gene can induce a shift in the lifestyle. If we assume that the three genetic programs for mutualistic, commensalistic and parasitic lifestyles are established in the symbiontic interactions, the role of the individual genes which determine the lifestyle need to be defined. A shift from parasitism to mutualism can easily be explained by the loss of a fungal gene function (e.g. for a virulence factor) which causes toxic effects in the plant. A similar shift in the lifestyle can be achieved by a plant mutation which leads to resistance against pathogens. A shift from mutualism to parasitism is probably more difficult to explain. Recent studies have demonstrated that the degree of root colonization is a crucial parameter (e.g. TANAKA et al. 2006; SHERAMETI et al. 2008b). In a mutualistic interaction, the host restricts root colonization by a mild activation of defense responses against the invader. (Partial) inactivation of this defense machinery allows the microbe to grow faster and eventually to overcolonize the host, which in turn results in stimulation of the defense responses by the plant against the invader. Recently, it was shown that the fungus itself can also restrict its own growth in the host, for instance by the production of reactive-oxygen species (ROS, TANAKA et al. 2006; see below).

A suitable model system to study a shift from a mutualistic to a less beneficial lifestyle might be the interaction between the *Piriformospora indica* and *A. thaliana*. *P. indica*, a basidiomycete of the *Sebacinaceae* family, interacts with many plant species including *Arabidopsis*. Like other members of the *Sebacinaceae*, *P. indica* colonizes the roots, grows inter- and intracellularly and forms pear-shaped spores which accumulate in the roots as well as on the root surface. The endophyte promotes nutrient uptake, allows plants to survive under water and salt stress, confers resistance to toxins, heavy metal ions and pathogenic organisms and stimulates growth and seed production (cf. VERMA et al. 1998; VARMA et al. 1999, 2001; SAHAY and VARMA 1999; OELMÜLLER et al. 2004, 2005; PHAM et al. 2004; PEŠKAN-BERGHÖFER et al. 2004; SHAHOLLARI et al. 2005, 2007; SHERAMETI et al. 2005; WALLER et al. 2005; VARMA and

OELMÜLLER 2007; OELMÜLLER et al. 2009). *P. indica* is a cultivable fungus and can grow on synthetic media without a host (VARMA et al. 2001; PEŠKAN-BERGHÖFER et al. 2004). The host range includes trees, agri-, horticultural and medicinal plants, mono- and dicots and mosses (VARMA et al. 2001; GLEN et al. 2002; PEŠKAN-BERGHÖFER et al. 2004; WEIß et al. 2004; BARAZANI et al. 2005; SHAHOLLARI et al. 2005, 2007; SHERAMETI et al. 2005; WALLER et al. 2005) suggesting that the interaction is based on general recognition and signalling processes.

We have isolated *Arabidopsis* mutants which do not respond to *P. indica* with regard to growth promotion, the expression of *P. indica*-specific marker genes in the roots and shoots, the modification of plasma membrane associated marker proteins, resistance to abiotic and biotic stresses, seed yield, *etc*. Interestingly, the ethyl-methane sulfonate-induced point mutants can be put into three classes: P*. indica-insensitive* (*pii*) mutants, i.e. mutants which grow like uncolonized wild-type mutants in the presence of the fungus; *P. indica-repressed* (*pir*) mutants, i.e. mutants which grow slower in the presence of the fungus and produces less seeds compared to the uncolonized *pir* plants; and *P. indica-retarded* (*pit*) mutants, which are retarded in their response to the fungus (Figure 1). The second group of mutants clearly demonstrate that single gene mutations can result in a shift from a mutualistic interaction to an interaction in which the fungus inhibits rather than promotes growth and in which several *P. indica*-induced processes do no longer occur. The identification of the mutated genes will help to understand which plant proteins are required to maintain the interaction between the two symbionts in a mutualistic state.

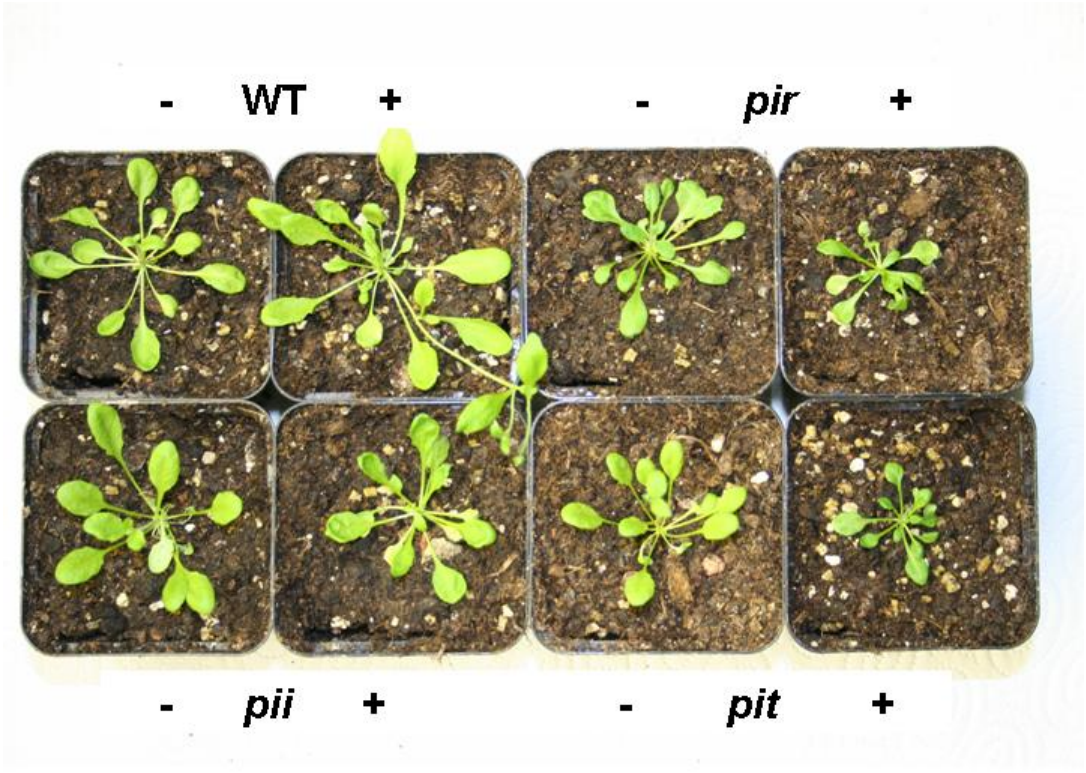

Figure 1: *Arabidopsis* plants on soil which were either co-cultivated with *P. indica* (+) or mock-treated (-). The figure shows wild-type plant (WT) and mutants (*pii, pir*, *pit*), as described in the text.

Beneficial and pathogenic interactions have many things in common

1. Host recognition

Strigolactones are molecules from plants that induce branching of the hyphae of mycorrhizal fungi (AKIYAMA et al. 2005) and thus function as host-recognition compounds. Strigolactones also stimulate seed germination of parasitic plants, such as the parasitic weeds *Striga* (BIDARTONDO et al. 2002) and *Orobanche*. The mycorrhizal fungi and the parasitic plants produce very small seeds, which barely contain storage material for the survival after germination. It is important for them to recognize the presence of a host plant in their environment which they can rapidly colonized to gain access to a carbon source (BIDARTONDO et al. 2002). Thus, it has been postulated that perception of strigolactones by mycorrhizal fungi induces a metabolic switch (BECARD et al. 2004; TAMASLOUKHT et al. 2003). This example demonstrates that similar or even the same molecules released by the host can play a role in beneficial and pathogenic interactions. This is not surprising because efficient recognition of a near-by root is common in both interactions.

2. Appressoria formation and penetration

Most of plant/microbe interactions have also in common that the microbes form appressoria on the root surface and haustoria after the penetration into the eukaryotic cell. The basic mechanisms for these processes are conserved in beneficial and pathogenic interactions. Therefore, the actin cytoskeleton and its reorganisation in response to a fungal contact play important roles and are very similar among parasites and mutualists. Several groups have shown that the plant cells organizes networks of microfilaments around fungal feeding structures (TAKEMOTO and HARDHAM 2004; GENRE and BONFANTE 2002; OPALSKI et al. 2005; VENUS et al., in preparation). Cytoskeleton structures control the establishment of any kind of symbiotic interactions, in particular during the entry process of the fungal hyphae into the plant cell (PERFECT and GREEN 2001; MENDGEN and HAHN 2002; DITENGOU et al. 2003; STRACK et al. 2003; GENRE et al. 2005; KWON et al. 2008). The infection process that leads to compatible interactions between plants and biotrophic pathogens is similar to the development of an arbuscular mycorrhizal symbiosis. It starts with the adhesion of the spore to the cell surface, the formation of appressoria and the initiation of the penetration process (LIPKA and PANSTRUGA 2005). The haustoria formation and the establishment of the perihaustorial interface of pathogens and of beneficial fungi share many morphological similarities (PARNISKE 2000). Recently, a fungal-induced prepenetration apparatus consisting of cytoskeleton and endoplasmic reticulum was discovered in *Medicago truncatula*, which builds a cytoplasm bridge for plasma membrane invagination and subsequent fungal invasion (GENRE et al. 2005, reviewed in KOGEL et al. 2005; PASZKOWSKI 2006). Upon appressorium formation and prior to fungal ingress, the nucleus becomes repositioned next to the appressorium. During this migration process through the cell, the nucleus produces a hollow column through which the fungal hyphae trespasses the rhizodermal cell after penetration. Cytoskeleton re-arrangements and nuclear movement have been observed in compatible and incompatible interactions and in cells after infection with beneficial fungi, and this leads to the establishment of cell polarization (HEATH et al. 1997; SKALAMERA and HEALTH 1998; SCHMELZER 2002). By contrast, a symbiosis-defective mutant of *Lotus japonicus* shows incompatible cytoskeleton reorganization and cell death when confronted with the *Gigaspora margarita* (GENRE and BONFANTE 2002).

3. Receptors and events at the plasma membrane

Cell wall penetration of beneficial and patho-

genic microbes is accompanied by the release of plant-derived molecules. Hence, endophytes must avoid or overcome non-specific resistance responses to achieve successful penetration by reprogramming the invaded cell to accommodate infection structures and to maintain host cell integrity for a long-lasting interaction (PASZKOWSKI 2006). Accommodation requires sophisticated recognition of the endophyte as a friendly intruder; for mycorrhiza, this recognition is realized by host receptor-kinase-mediated transmembrane signalling (STRACKE et al. 2002). Receptor-kinase-mediated recognition is also involved in non-specific recognition of pathogens (ZIPFEL and FELIX 2005; PASZKOWSKI 2006). Thus, the similarity of recognition of endophytes and parasites by plants indicates that a potential common basis might have been specified during the evolution of symbioses.

Recognition of Pathogen or Microbe Associated Molecular Patterns (PAMPs and MAMPs) at the plant cell surface occurs through several classes of receptor(s) (kinases) which are highly conserved among eukaryotes (NÜRNBERGER 1994; NÜRNBERGER and KEMMERLING 2006). The signal transduction in microbial/rhizobial interactions is initiated by two receptor-like kinases, NOD-FACTOR RECEPTOR1 (NFR1) and NFR5, with LysM domains, which perceive the Nod-factor released by rhizobacteria at the root plasma membrane (MADSEN et al. 2003; RADUTOIU et al. 2003). An equivalent receptor-like kinase is presumed to exist for the recognition of a mycorrhizal signal. DOES not MAKE INFECTIONS (DMI) 2/SYMBIOSIS RECEPTOR-LIKE KINASE (SYMRK) is an additional receptor-like kinase, with three leucine-rich repeat domains that is a component of the common symbiotic pathway (ENDRÉ et al. 2002; LIMPENS et al. 2003). Similarly, signal transduction induced by pathogenic microorganisms activates basal defense mechanisms in plants to potential pathogens through receptor-mediated recognition of PAMPs/MAMPs and downstream signalling to activation of innate immunity responses. During compatible interactions, pathogens possess effector(s)/virulence molecule(s) that suppresses the PAMP-induced responses, thus overcoming the basal resistance and are able to infect the plant (ESPINOSA et al. 2003; HE et al. 2006; KIM et al. 2005). Whether such mechanisms also exist in beneficial plant/microbe interactions, is unclear. Pep13, a 13-amino-acid motif from an oomycete transglutaminase, elicits defense responses in parsley and potato and exhibits all the features required for the classification as a PAMP (BRUNNER et al. 2002). Pep13 is perceived by a ~100 kDa plasma-membrane-localized receptor protein in parsley (NÜRNBERGER et al. 1995). Similar recognition events have been reported for other systems, for example the cyptogein-tobacco (GARCIA-BRUGGER et al. 2006), the ß-heptaglucan-soybean (EBEL 1998) or the flg22-*Arabidopsis* (ZIPFEL et al. 2004) systems. For the last system, the flg22 receptor, FLS2, has been cloned (GOMEZ-GOMEZ and BOLLER 2000). Taken together, in spite of the specificity of the individual receptors or receptor combinations for a given cell response, the basic mechanism of recognition follows the same principles in mutualistic and pathogenic interactions.

A similar recognition process has been proposed for *P. indica* and *Arabidopsis*. Two leucine-rich repeat proteins, the atypical receptor kinase LRR1 and a small LRR2 protein, which are located in lipid rafts/plasma membrane microdomains of the roots, maybe required for the interaction (SHAHOLLARI et al. 2005, 2007). Inactivation of *LRR2* completely prevents the beneficial interaction and the *LRR1* mRNA is transiently up-regulated in response to *P. indica*. The integrity of lipid rafts appears to be crucial for the interaction, since partial inactivation of a sphingosine kinase, which is required for the biosynthesis of sphinolipids found in plasma membrane microdomains, also affects the *Arabidopsis*/*P.*

indica interaction (SHAHOLLARI et al. 2005, 2007). Lipid rafts also play important roles in pathogenic plant/microbe interactions (BHAT et al. 2005; ZAPPEL and PANSTRUGA 2008). It is also likely that *P. indica* releases a factor(s) that induces the cellular responses in the host root. This is based on the observations that *P. indica*-inducible genes are up-regulated in *Arabidopsis* roots before the colonization occurs, and that culture filtrates and extracts from the fungal cell wall promotes growth (VADASSERY et al. 2009a). The fastest response that has been observed so far, is the modification of a plasma-membrane associated meprin and TRAF-C homology (MATH) protein, which does not occur in *Arabidopsis* mutants, which do not interact with the fungus (PEŠKAN-BERGHÖFER et al. 2004; OELMÜLLER et al. 2005; SHAHOLLARI et al. 2007). The role of MATH proteins in plant/microbe interactions is unknown at present. They are located at the extracellular site of the plasma membrane and might be involved in binding signalling molecules from microbes (Drzewiecki et al., in preparation).

The similarities between the different response patterns of plants to beneficial or pathogenic microbes are further supported by the observation that the *Lotus japonicus SYMRK* pathway required for both arbuscular mycorrhizal and the nitrogen-fixing symbiosis with rhizobia (STRACKE et al. 2002) is also involved in early stages of infection of roots by root-knot nematodes (WEERASINGHE et al. 2005). Similar to beneficial and pathogenic fungi, the nematodes enter the root cells by mechanical penetration, and they grow intercellularly. During the pre-symbiotic phase, wild type, but not *symrk* root-hairs respond to nematode-released signals by enhanced branching. Since perception of signals from beneficial fungi, rhizobia and nematodes share the same receptor/signaling components, not only rhizobacteria, but also other microbes with similar penetration mechanisms take advantage of already established recognition/signaling pathways (WEERASINGHE et al. 2005). Nematodes have a large host range including *Arabidopsis*. Thus, analogous proteins/signaling pathways can be involved in multiple plant/microbe interactions in different species.

4. Ca^{2+}

A central player downstream of receptor activation in beneficial and pathogenic plant/microbe interactions is Ca^{2+}. In mycorrhizal interactions, the phosphorylation cascade at the plasma membrane induces Ca^{2+} changes in the cytoplasm and nucleus. This probably involves a secondary messenger that might be the product of phospholipases C and D. These phospholipases could be regulated by phosphorylation and the activity of the cation channel formed by DMI1/POLLUX and CASTOR (ANÉ et al. 2004; IMAIZUMI-ANRAKU et al. 2005). The Ca^{2+} spikes in the nucleoplasm and nuclear-associated cytoplasm activate a Ca^{2+}/calmodulin-dependent kinase (CCaMK), which is located in the nucleus (MITRA et al. 2004; LÉVY et al. 2004; KALO et al. 2005). This Ca^{2+}-activated kinase regulates nodulation-induced gene expression *via* the transcriptional regulators NSP1 and NSP2 (KALO et al. 2005; SMIT et al. 2005), GRAS proteins as well as ERN, an ERF transcription factor. Thus, Ca^{2+} signaling starts at the plasma membrane and might end in the nucleus with the activation of specific transcription factors. The involvement of Ca^{2+} spiking and Ca^{2+}/calmodulin-dependent protein kina-ses in beneficial plant/microbe interactions appear to be a general phenomenon (LÉVY et al. 2004; PARNISKE 2004; IMAIZUMI-ANRAKU et al. 2005; IVASHUTA et al. 2005; VADASSERY et al. 2009a). IVASHUTA et al. (2005) used RNA-interference-mediated knockdown of CDPK1 in *Medicago* and demonstrated that the plants have defects in root development and do not from mycorrhiza. Their actin skeleton is disrupted and defense genes become activated.

The signal events leading to defense-

related gene activation and phytoalexin accumulation in pathogenic interactions consists also of ion fluxes at the plasma membrane (H^+/Ca^{2+} influxes, K^+/Cl^- effluxes) and a phospholipase-C-mediated phosphatidic acid accumulation (Dierk Scheel, unpublished). Although these processes resemble each other, increase in cytoplasmic Ca^{2+} constitutes a signal pathway that is conserved to a variety of processes including pathogenic interactions (RUDD and FRANKLIN-TONG 1999), but must also differ substantially from the pathway which leads to mutualistic interactions. The specificity of this universal second messenger may lie in the magnitude, frequency and duration - the so-called Ca^{2+} signature (TREWAVAS 1999). Another important parameter is the subcellular localisation of Ca^{2+} that is released in response to different stimuli in the cell. In the case of the pathogenic parsley system, the Ca^{2+} response consists of a rapid peak followed by a lower but sustained plateau of elevated cytoplasmic Ca^2, and it is the sustained plateau of this biphasic signature that is required for the downstream defense reactions (BLUME et al. 2000).

Ca^{2+} signaling is also an early event in the *P. indica*/*Arabidopsis* interaction. A cell wall extract from the fungus promotes growth of wild-type seedlings, but not of seedlings from *P. indica*-insensitive mutants (Figure 1). The extract and the fungus also induce a similar set of genes in *Arabidopsis* roots, among them genes with Ca^{2+} signalling-related functions. The cell wall extract induces a transient cytosolic Ca^{2+} ($[Ca^{2+}]_{cyt}$) elevation in the roots of *Arabidopsis* and tobacco plants, as well as in tobacco BY-2 suspension cultures expressing the Ca^{2+} bioluminescent indicator aequorin. Nuclear Ca^{2+} transients were also observed in tobacco BY-2 cells. The Ca^{2+} response was more pronounced in roots than in shoots and involved Ca^{2+} uptake from the extracellular space as revealed by inhibitor studies. Inhibition of the Ca^{2+} response by staurosporine and the refractory nature of the Ca^{2+} elevation suggest that a receptor may be involved. The cell wall extract does not stimulate H_2O_2 production and the activation of defense gene expression, although it led to phosphorylation of mitogen-activating protein kinases (MAPKs) in a Ca^{2+}-dependent manner. The involvement of MAPK6 in the mutualistic interaction was shown for an *mpk6* line, which did not respond to *P. indica*. Thus, Ca^{2+} is likely to be an early signalling component in the mutualistic interaction between *P. indica* and *Arabidopsis* or tobacco. Identification of the active compound in the cell wall extract of the fungus and of the responsive receptor in the plasma membrane of the root will be first step to understand, whether Ca^{2+} triggers defense responses in the beneficial system, or whether this is a novel Ca^{2+}-dependent pathway that leads to the beneficial interaction (VADASSERY et al. 2009a). It is unlikely that Ca^{2+} activates a mycorrhiza-type pathway in *Arabidopsis*, since the major signaling components are not present.

5. Signaling, kinases

A novel defense pathway in pathogenic plant/pathogen interactions includes the kinase PDK1/OXI1/PTI1/MAPK3/6. Interestingly, PDK1, OXI1, MAPK3 and in particular MAPK6 are also involved in the beneficial interaction between *P. indica* and *Arabidopsis* (cf. chapter: Defense responses in beneficial plant/microbe interactions).

6. Gene expression pattern

RNA profiling was performed for rice seedlings exposed to mutualistic (*Glomus intraradices*) and pathogenic (*Magnaporthe grisea*, a hemibiotroph, and *Fusarium moniliforme*, a necrotroph) fungi (GÜIMIL et al. 2005). This uncovered that 43% of the genes that respond to mycorrhizal fungi, also respond to the two pathogenic fungi. The initial root infection pattern of *M. grisea* resembles that of arbuscular mycorrhizal fungi, while the pathogenic fungus *F. moniliforme* follows another strategy,

mainly by disrupting the root cells. Twice as many genes responded similarly to the biotroph and the hemi-biotroph compared to the necrotroph. This suggests that the overlapping genes are mainly involved in controlling the invasion process in rice (GÜIMIL et al. 2005).

Defense responses in beneficial plant/ microbe interactions

Initial infestation of roots by mycorrhizal fungi or endophytes is accompanied by a sophisticated balance between the defense responses of the plant and the nutrient demands of the endophytes. However, also during later stages, defense genes can be activated, in particular under stress or unfavourable conditions for the plants. In general, it appears that defense genes become activated in established mutualistic interactions when the balance between the responses of the partners is disturbed and the microbes become dominant, e.g. because the hosts are weakened due to external conditions (nutrient limitations, biotic or abiotic stress), metabolic inconsistencies, genetic deficiencies or senescence. The role of defense gene activation is unknown; it may play a role in the maintenance of a stable status of the association or control fungal growth. How these defenses affect the functioning and development of the symbiosis is also unclear. The best studied systems are again mycorrhizal interactions, where most defense genes are up-regulated during the early stages of infestation (GARCIA-GARRIDO and OCAMPO 2002) and during arbuscule development (GRUNWALD et al. 2004). Arbuscule formation within the plant cell is particularly interesting since the entire development occurs within a few days. It is likely that the different stages (establishment of the arbuscular structure, fully developed and functional arbuscule, and degradation) are accompanied by substantial changes in the expression pattern of the host´s genes. This also includes defense responses.

Compared to pathogenic interaction, defense gene activation in mutualistic interactions is normally low, only transient and/or restricted to certain developmental periods. The induction of defense responses may be caused through similar signaling processes known from plant–pathogen interactions. SALZER and BOLLER (2000) suggest that mycorrhizal fungi can secrete chitin elicitors, which are similar to those from pathogens. A MAMP from the mycelium of the root-colonizing *Glomus intraradices* induces phytoalexin synthesis in soybean cotyledons (LAMBAIS 2000). In *Medicago truncatula* chalcone synthase expression is activated during early phases of the contact of the roots with *M. truncatula*. This gene codes for the first enzyme of the flavonoid and glucosinolate biosynthesis pathway (BONANOMI et al. 2001). Furthermore, weak hypersensitive-like responses become activated in mutualistic interactions, e.g. in incompatible interactions between mycorrhizal fungi and non-host plants or mycorrhizal mutants exposed to mycorrhizal fungi (ALLEN et al. 1989). A local oxidative burst was observed at the penetration sites of fungal hyphae from *G. intraradices* into *M. truncatula* root cells (SALZER et al. 1999). In general, activation of defense responses, such as the deposition of callose, the synthesis of PR-1 proteins or phenolic compounds, are typical when mycorrhiza mutants are exposed to mycorrhizal fungi (GOLLOTTE et al. 1993). Moreover, several reports demonstrate that cell necrosis and cell death occur also in mutualistic interactions, e.g. at the infection site of *Gigaspora margarita* on *Medicago sativa* roots (DOUDS et al. 1998). A detailed analysis confirmed the importance of cell-death strategies also for the beneficial fungus *P. indica*: The root tip meristem of colonized barley plants showed no colonization, and the elongation zone showed mainly intercellular colonization. In contrast, the differentiation zone was heavily infested by inter- and intra-

cellular hyphae and intracellular chlamydospores. The majority of hyphae were present in dead rhizodermal and cortical cells that became completely filled with chlamydospores. In some cases, hyphae penetrated cells and built a meshwork around plasmolyzed protoplasts, suggesting that the fungus either actively kills cells or senses cells undergoing endogenous programmed cell death. Fungal proliferation was strongly inhibited in transgenic barley over-expressing the BAX inhibitor-1, an inhibitor of plant cell death, which shows that *P. indica* requires host cell death for proliferation in differentiated barley roots. DESHMUKH et al. (2006) suggest that the endophyte interfers with the host cell death program to form a mutualistic interaction with plants.

Based on those observations it is likely that beneficial fungi release MAMPs which induce the same signal pathways leading to defense as pathogenic fungi. During the first contact of mycorrhizal fungi with tobacco, onion and bean root cells, and during appressoria formation and fungal penetration into the root cells, transient increases in catalase and peroxidase activities were observed (BLILOU et al. 2000a; SPANU and BONFANTE-FASOLO 1988; LAMBAIS 2000). In tobacco, this increase was accompanied by the accumulation of salicylic acid (SA) (BLILOU et al. 2000a). In the rice/*Glomus mosseae* interaction, expression of genes for the lipid transfer protein and for phenylalanine ammonia-lyase was also accompanied by higher SA levels (BLILOU et al. 2000b). This suggests that SA fulfils a similar function in activating defense responses in beneficial and pathogenic plant/microbe interactions.

In fully established beneficial interactions, defense gene expression is often low or at the detection limit. This has been shown for individual genes and, more generally, by transcriptome analyses. Monitoring the complete rice genome in fully established mycorrhizal symbiosis uncovered no or only low levels of defense gene expression. *Arabidopsis* microarray analyses for roots colonized by the endophytic fungus *P. indica* demonstrate that defense genes are not activated as long as the mutualistic interaction is not disturbed (OELMÜLLER, unpublished). Many studies on mycorrhizal and endophytic symbioses have shown that defense processes become weakly induced during early stages of the symbiosis and are subsequently down-regulated as development of the symbioses progresses (HARRISON 2005).

If the induction of defense gene expression is caused by a fungal MAMP and the activation of a signal transduction pathway which is similar or identical to that of pathogenic fungi, the weak and transient character of the defense response could have several reasons: beneficial fungi could produce only low amounts of the MAMPs, they could produce MAMPs which are less effective in binding to plant receptors compared to those of pathogenic fungi, fungal MAMPs could become inactivated or even degraded by plant enzymes, such as proteases, or the signaling in the plant cells could be actively repressed by other factors from either fungal or plant origin (see below). This raises also another important question: is the absence of plant defense during later phases of the symbiosis caused by an active suppression or by the avoidance of defense induction. It has been postulated for mycorrhizal symbiosis that a suppression mechanism becomes activated during the formation of haustoria (HARRISON 2005). However, the absence of host defense responses in plant interactions that do not include the formation of haustoria, such as that between grasses and *Epichloë* endophytes (SCOTT 2001), suggests that also haustoria-independent mechanisms must exist (GARCIA-GARRIDO and OCAMPO 2002). Furthermore, whether the suppression of defense processes is fungus-mediated or plant-induced needs to be determined. A possible scenario could be that a MAMP-induced defense re-

sponse is down-regulated during later phases fungus-derived effector molecules which interfere with specific plant signal transduction components activating defense gene expression (OELMÜLLER et al. 2009). However, the existence of such a scenario has not yet been shown for any beneficial plant/microbe interaction system.

1. Radical oxygen species (ROS)

All roots produce H_2O_2, since it is required for root elongation growth (KWAK et al. 2003; LISZKAY et al. 2004). *A. thaliana rhd2* (root hair defective) mutants are defective in Ca^{2+} uptake and cell expansion. These mutants also have short root hairs and stunted roots. *Rhd2* encodes an NADPH oxidase, a protein that transfers electrons from NADPH to an electron acceptor leading to the formation of ROS. ROS accumulate in growing root hairs and their levels are markedly decreased in *rhd2* mutants. Blocking the activity of the NADPH oxidase with diphenylene iodonium also inhibits ROS formation. Treatment of *rhd2* roots with ROS partly suppresses the mutant phenotype and stimulates the activity of plasma membrane localized Ca^{2+} channels, which are required for root hair growth. This indicates that NADPH oxidases control root development by producing ROS that regulate plant cell expansion through the activation of Ca^{2+} channels (FOREMAN et al. 2003).

Thus, it is difficult to define a specific role of ROS in beneficial plant/microbe interactions, although it has been shown that ROS accumulate in mycorrhizal interactions (SALZER et al. 1999; GÜIMIL et al. 2005; HOHNJEC et al. 2005; FESTER and HAUSE 2005; BAPTISTA et al. 2007; SCOTT and EATON 2008). SALZER et al. (1999) demonstrated H_2O_2 production in arbuscular mycorrhizal symbiosis by diaminobenzidine staining. HAUSE and FESTER (2005) proposed that at least two phenomena specific to arbuscular mycorrhizal roots might be connected to the accumulation of H_2O_2: the induction of carotenoid biosynthesis (STRACK et al. 2003), which is induced by ROS during chromoplast differentiation in *Capsicum annuum* (BOUVIER et al. 1998), and bioprotection (DUMAS-GAUDOT et al. 2000; LINDERMAN 2000). H_2O_2 generated by a fungal superoxide dismutase or various other antioxidative enzymes might also contribute to the beneficial interaction, as known for arbuscule formation (LANFRANCO et al. 2005; ARINES et al. 1994; BLILOU et al. 2000a; LAMBAIS et al. 2003). An isolate of *Paxillus involutus* form ectomycorrhizal symbiosis with hybrid poplar and displayed strong H_2O_2 accumulation in the outer hyphal mantle. GAFUR et al. (2004) suggests that H_2O_2 might regulate growth of the host's roots, activate defense against other invading microbes, or increases plant-innate immunity. Interestingly, during a comparative transcriptome analyse of rice roots infected with *Glomus interradices* and two pathogenic fungi, 12 genes were identified which are specifically up-regulated in response to mycorrhizal fungi, and thus they were considered as marker genes. Among them were genes for H_2O_2 producing and scavenging enzymes (GÜIMIL et al. 2005).

It is long known that arbuscule-containing cells have specific cytoskeletal structures and accumulate ROS during the establishment of these structures. Once an endophyte has entered a plant cell, cellular integrity has to be maintained for the period of interaction. The plant cell reacts to fungal invasions by ROS production which can lead to a subsequent hypersensitive cell death reaction, if the fungus is aggressive and its growth can no longer be controlled by the host. It is conceivable that similar scenarios occur also after the infection of a root cell by a beneficial fungus. Again, which signaling events ultimately prevent a hypersensitive response, remains unknown. A recently detected fungal H_2O_2 generation that is mediated by superoxide dismutase in arbuscules could be involved either in removing

the superoxide anion or in provoking an antioxidative plant response to H_2O_2 (LANFRANCO et al. 2005).

A switch from mutualism to parasitism occurs by a mutation in a single microbial gene in the mutualistic interaction of the clavicipitaceous fungal endophyte *Epichloë festucae* with its ryegrass host *Lolium perenne*. Molecular analysis of a fungal mutant uncovered a plasmid insertion into the *NoxA* gene, which encodes an NADPH oxidase. Plants that were inoculated with the *noxA* mutant became strongly infected, lost apical dominance, became severely stunted, showed precocious senescence, and eventually died. Cytochemical analysis proved that production of ROS was reduced in the mutant. TANAKA et al. (2006) suggest a symbiosis mechanism in which ROS production by the *E. festucae* NoxA *in planta* negatively regulates fungal development and hyphal tip growth, thereby preventing excessive colonization of the plant tissue. Also in rhizobial interactions, the ROS levels increase during the early and late phases (MATAMOROS et al. 2003). During early phases, ROS accumulation is modulated by Nod factors (RAMU et al. 2002; SHAW and LONG 2003). Whether this is required for nodule formation or a site-effect leading to an oxidative burst that has a similar function as described for the infection process by pathogenic bacteria (SANTOS et al. 2001) is not known. Accumulation of ROS during later phases might be associated with nodule senescence (PUPPO et al. 2005).

ROS synthesized by plants play an important role in pathogenic plant/microbe interactions (BORDEN and HIGGINS 2002; TORRES et al. 2002, 2005; HÜCKELHOVEN and KOGEL 2003). It is produced during the oxidative burst, one of the first responses to pathogen attack, and the amount of H_2O_2 during oxidative burst is much higher than the levels required for root development. Furthermore, while ROS produced for root growth accumulates mainly at the root hair tips, the pathogenic response is characterized by a fast and massive accumulation of H_2O_2 in and around the infected roots cells. Pathogen-induced ROS production might have two main functions: H_2O_2 can stimulate cross-linking processes in the cell wall and thus contribute to the induced defense response against the invader, and it might function as a signaling molecule within and between the cells. Whether this is also true for beneficial interactions, albeit at much lower levels, is unknown. It is conceivable that a beneficial fungus induces similar defense processes *via* a mild oxidative burst as long as the plant does not yet recognized the microbe as a friend. In any case, an efficient control of the amount of H_2O_2 in the roots might be crucial for the establishment of a beneficial interaction, in order to avoid an oxidative burst. Very little is known about these processes. In the future, biochemical or genetic evidence will unravel the molecular mechanisms that control ROS production in beneficial interactions. The interaction of endophytes with *Arabidopsis* might contribute to these studies. Obvious questions are: How do endophytes interact with the *rhd2* mutant or other mutants defective in ROS production? Is ROS production necessary for the beneficial interaction, or the restriction of the growth of the endophyte in the host root? What is the role of the mild hypersensitive response? Is a shift from a mutualistic to a parasitic lifestyle accompanied by an increase in ROS production?

2. PDK1/OXI1/PTI1-2/MAPK defense pathway

OXI1 is a serine/threonine kinase necessary for oxidative burst-mediated signalling in *Arabidopsis* roots (ANTHONY et al. 2004; RENTEL et al. 2004). The enzyme is a member of the AGC protein kinase family and was originally identified because its expression was induced by H_2O_2 *in vivo* (RENTEL et al. 2004). OXI1 is required for full activation of MAPK3 and MAPK6 after treatment with ROS or elici-

tors and for different ROS-mediated processes including basal resistance to *Peronospora parasitica* infection and root hair growth (RENTEL et al. 2004). Besides ROS, OXI1 is also activated by the phospholipid-binding kinase PDK1 (Anthony et al. 2004). The active OXI1 phosphorylates and thus activates the downstream serine/threonine kinase PTI1-2 in response to ROS and phospholipid signals (ANTHONY et al. 2006), and many of these signals derive from microbial pathogens or elicitors, such as cell wall fragments or specific protein factors released by pathogens (VAN DER LUIT et al. 2000; YAMAGUCHI et al. 2005).

PDK1 and OXI1 control the colonization of *Arabidopsis* roots by *P. indica* (CAMEHL et al., in preparation) and this is crucial for the mutualistic interaction and the repression of defense responses against the fungus. Furthermore, MAPK6 becomes phosphorylated in response to signals from the fungus (VADASSERY et al. 2009a), similar to signals from pathogenic fungi or PAMP applications. The major question is how specificity in the response pattern can be achieved if these kinases are involved in beneficial and pathogenic responses. Neither *P. indica* itself nor the cell wall extract from this fungus induces significant defense responses as judged by H_2O_2 production in *Arabidopsis* (Figure 2) or tobacco roots or phytoalexin synthesis in parsley cell suspension cultures (Figure 3). Nevertheless, we cannot exclude that a low and constant activation of this defense kinase pathway is permanently required for restricting root colonization and preventing the roots from over-colonization. This is supported by the observation that mutants impaired in PDK1, OXI1 or MAPK6 still respond to *P. indica* at the seedlings level. However, long-term harmony requires these kinases, since adult plants fail to respond to the fungus (CAMEHL et al., in preparation).

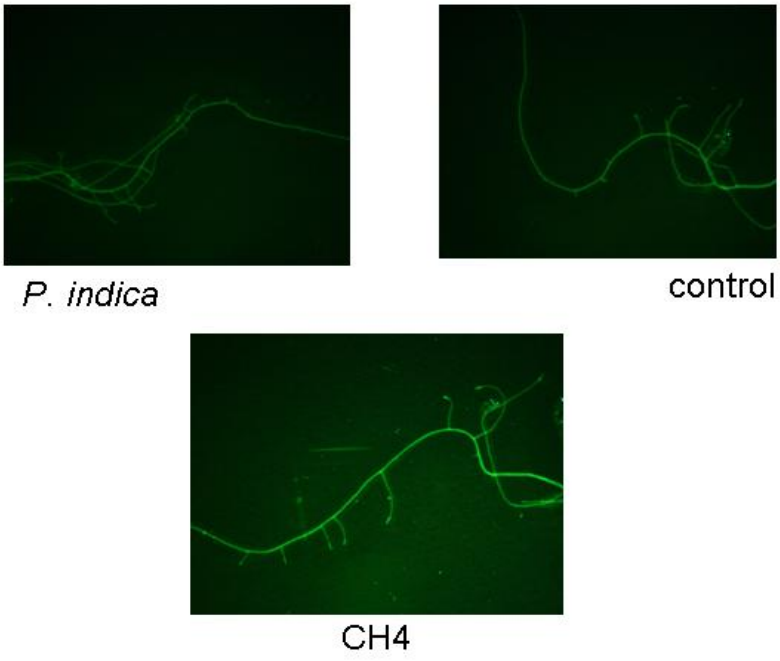

Figure 2: Intracellular accumulation of hydrogen peroxide was measured using the molecular probe 2′,7′-dichlorofluorescein diacetate (H2DCF-DA). This chemical can cross the plasma membrane freely, and is then cleaved to its impermeable counterpart, dichlorofluorescein (H2DCF) by endogenous esterases. H2DCF functions as a reporter of cytoplasmic hydrogen peroxide by converting it to its fluorescent form, dichlorofluorescein (DCF), upon oxidation. Seedlings were incubated for 10 min in 50 µm H2DCF-DA solution and elicitors added for 10 minutes and photographed. Data from VADASSERY, J. (Jena).

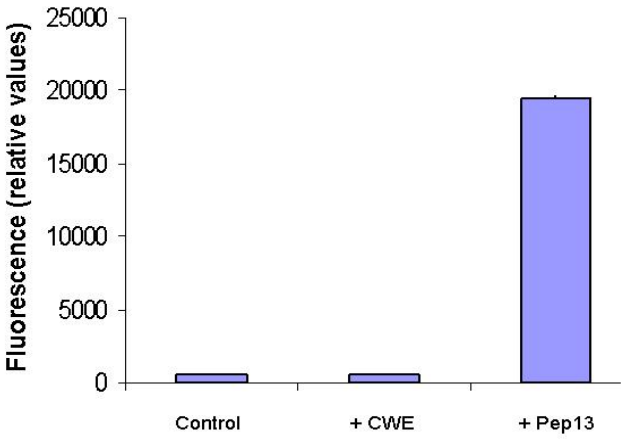

Figure 3: Induction of phytoalexin in parsley cell suspension cultures 4 h after the application of a cell wall extract (CWE) from *P. indica* or the application of the pathogenic elicitor Pep13. Phytoalexin levels are expressed as relative fluorescence units. Control: untreated. Data from RANF, S. (IPB Halle).

3. Glucosinolates in plant/microbe interaction

Brassicales differ from many other plant species in that they synthesize huge amount of glucosinolates, S-rich metabolites that function in the defense of plants against pests and pathogens. In particular under sulfur limitation conditions, synthesis of sulfur-containing metabolites and storage compounds (such as glucosinolates) is down-regulated and sulfur is released from these compounds through active breakdown processes (HIRARI et al. 1995, 2005; KUTZ et al. 2002). In Brassicales, up to 30% of the sulfur is stored in glucosinolates (FALK et al. 2007). Under sulfur deficiency, down-regulation of glucosinolate biosynthesis genes is accompanied by an up-regulation of genes controling glucosinolate breakdown. Activation of sulfate acquisition and repression of glucosinolate production may occur in parallel in response to sulfur limitation (HIRARI et al. 1995, 2004, 2005; MARUYAMA-NAKASHITA et al. 2003, 2005). Thus, glucosinolates may be considered a potential source of sulfur for other metabolic processes under sulfur limitation (FALK et al. 2007; HALKIER and GERSHENZON 2006; GRUBB and ABEL 2006). Active defense compounds are released from the glucosinolates after enzymatic cleavage by myrosinases, and several of these enzymes play important functions in plant/microbe interactions. The availability of sulfur and the regulation of its metabolism might have an important influence on the balance between mutualism and parasitism in Brassicales. Stimulation of growth promotion and metabolism by beneficial fungi depends on the availability of sulfur and on enzymes involved in sulfate uptake from the soil and reduction in the plastids. This also leads to higher glucosinolate biosynthesis and thus more efficient protection of the plants against microbes or pests. Reprogramming of the sulfur metabolism under sulfur limitations including the degradation of glucosinolates leaves the plants less protected against microbe or pests, and/or allows an uncontrolled hyphal growth of an otherwise beneficial fungus in the roots, a shift from mutualism to a less beneficial symbiosis and eventually to the activation of defense processes.

Several lines of evidence support the important role of glucosinolates for beneficial interactions of microbes with *Arabidopsis*. A

mutant screen for plants, which do not show benefits when grown in the presence of the endophyte *P. indica* uncovered several genes of the glucosinolate biosynthesis pathway (SHERAMETI et al. 2008b; OELMÜLLER, unpublished). At least two of the identified genes code for enzymes/myrosinases potentially involved in glucosinolate cleavage and the release of defense-active compounds. The best studied example is PYK10, a potential myrosinase abundantly expressed in roots (NITZ et al. 2001). The enzyme restricts root colonization by *P. indica*, which results in the repression of defense responses and the up-regulation of responses leading to a mutualistic interaction between the two symbiotic partners (SHERAMETI et al. 2008b). PYK10 exhibits striking sequence similarities to PEN2, a glycosyl hydrolase, which restricts pathogen entry of two ascomycete powdery mildew fungi into *Arabidopsis* leaf cells (LIPKA et al. 2005). Like PEN2, PYK10 belongs to the class of glycosyl hydrolase family 1, both proteins are located in intracellular organellar structures (PYK10 in ER bodies and PEN2 in peroxisomes), and both proteins share a high degree of sequence similarities. The catalytic domains of both proteins contain two conserved nucleophilic glutamates. LIPKA et al. (2005) have shown that glutamate^{183} is required for PEN2 function *in vivo*, which suggests that PEN2 catalytic activity is required for restricting pathogen entry. Since the function of PEN2 in pathogenic plant/microbe interactions has now been identified as a beta-thioglucoside glucohydrolase (BEDNAREK et al. 2009), it is conceivable that PYK10 might have a similar biological function in beneficial plant/microbe interactions, by releasing antifungal toxins from glucosinolates.

The perception of PAMPs/MAMPs by plants triggers a basal defense response analogous to animal innate immunity and is defined partly by the deposition of the glucan polymer callose at the cell wall at the site of pathogen contact. BENDAREK et al. (2009) identified a metabolic pathway for glucosinolates that is active in living plant cells, may contribute to glucosinolate turnover, and has been recruited for broad-spectrum antifungal defense responses. A P450 monooxygenase (encoded by the *Arabidopsis CYP81F2* gene) is essential for the pathogen-induced accumulation of 4-methoxyindol-3-ylmethylglucosinolate, which in turn is activated by the atypical PEN2 myrosinase for antifungal defense. The authors propose that reiterated enzymatic cycles, controlling the generation of toxic molecules and their detoxification, enable the recruitment of glucosinolates in defense responses. Similarly, CLAY et al. (2009) have identified major roles in pathogen response for the plant hormone ethylene and the secondary metabolite 4-methoxy-indol-3-ylmethyl glucosinolate. PEN2 and PEN3, are necessary for resistance to pathogens and required for both callose deposition and glucosinolate activation, suggesting that the pathogen-triggered callose response is required for resistance to microbial pathogens. Thus, well-studied plant metabolites, previously identified as important in avoiding damage by herbivores, are also required as a component of the plant defense response against microbial pathogens (CLAY et al. 2009). Since increasing evidence points to the important function of glucosinolates in the restriction of colonization by pathogenic fungi, it is conceivable that a similar mechanism is also functional in beneficial plant/microbe interactions. Over-colonization results in less benefit for the plants and – as a consequence – the activation of defense responses against the invader. The availability of mutants impaired in various aspects of glucosinolate biosynthesis, degradation or cleavage allows to test this hypothesis.

4. Antioxidants and redox balance

Microarray analyses have uncovered that beneficial fungi can substantially change the expression patterns for enzymes involved in

antioxidative function (WALLER et al. 2005; VADASSERY et al. 2009b, and references therein). Both plant and fungal antioxidants might contribute to the protection of invaded cells against defense-associated ROS production. Like mycorrhizal fungi, *P. indica* enhances the antioxidative capacities of barley (WALLER et al. 2005; BALTRUSCHAT et al. 2008) and *Arabidopsis* (VADASSERY et al. 2009b). Whether stimulation of the antioxidative capacity in *P. indica*-infested plants reflects a defense reaction of the host and/or if it is part of creating a friendly environment for the fungus (KOGEL et al. 2006), is unknown.

However, besides controlling ROS accumulation, antioxidants and antioxidant enzymes might be involved in a number of other processes in beneficial interactions, which are not stimulated in pathogenic symbioses. For instance, ascorbate is one of the major redox buffer in plants (PIGNOCCHI and FOYER 2003), a cofactor of many enzymes (SMIRNOFF and WHEELER 2000), a regulator of cell division and growth (KERK and FELDMAN 1995) and a molecule for signal transduction (NOCTOR et al. 2000). Most of the ascorbate is localized in the cytoplasm (PIGNOCCHI et al. 2003) but some has also been identified in the apoplast (NOCTOR and FOYER 1998). In many organisms, the ascorbate–glutathione cycle plays a major role in the protection of the organism against ROS, because it maintains a high level of ascorbate in the different cell compartments (ASADA 1997). In this cycle, H_2O_2 is reduced to H_2O by ascorbate peroxidase using ascorbate, which generates monodehydroascorbate. Monodehydroascorbate is a radical and reduced back to ascorbate by monodehydroascorbate reductase (MDAR). If not reduced rapidly, monodehydroascorbate is disproportionated into ascorbate and dehydroascorbate. Dehydroascorbate will then be reduced to ascorbate by dehydroascorbate reductase (DHAR) using reduced glutathione. Oxidized glutathione is in-turn reduced by glutathione reductase using NADPH. Thus, MDAR and DHAR are the two enzymes of the ascorbate-glutathione cycle, which maintain ascorbate in its reduced state. The *MDAR* mRNA level is up-regulated in response to oxidative stress (YOON et al. 2004). Furthermore, transgenic *Arabidopsis* plants expressing the rice *DHAR* gene are resistant to salt stress (USHIMARU et al. 2006). YOSHIDA et al. (2006) have shown that the cytosolic DHAR is important for ozone tolerance in *Arabidopsis*. The *MDAR2* (At3g09940) and the *DHAR5* (At1g19570) mRNA levels are up-regulated in *Arabidopsis* roots, which are colonized by the beneficial endophytic fungus *P. indica*. This demonstrates that the fungus specifically targets individual members of a gene family. Insertional inactivation of the two genes shows that they are crucial for maintaining the interaction between *P. indica* and *Arabidopsis* in a mutualistic state, in particular under drought stress. Besides potential roles in controlling ROS accumulation or creating a friendly atmosphere for the invader, antioxidants and antioxidant enzymes might also participate in signal transduction processes, direct or indirect control of cell elongation and division or resistance against abiotic stress such as heavy metals (SCHÜTZENDÜBEL and POLLE 2002) in symbiotic interactions. The latter two processes are not stimulated by pathogenic fungi.

Glutathione (GSH) is upgraded with increasing sulfate supply and may establish resistance to stress in different plant species. GSH is a major redox buffer and protects the cell against ROS. An *Arabidopsis* mutant lacking the gamma-glutamylcysteine ligase 1 (GSH1), the rate-limiting enzyme for GSH synthesis (BALL et al. 2004), is impaired in defense reactions against pathogens. WALLER et al. (2005) and BALTRUSCHAT et al. (2008) have shown that GSH plays a crucial role in *P. indica* induced resistance of barley plants against pathogens. In the ascorbate–GSH-cycle, the function of GSH is linked to ascorbic acid and the electron flow from NADPH.

DHAR and glutathione reductase activities are stimulated by *P. indica* in salt-stress barley (BALTRUSCHAT et al. 2008). MDAR2 and DHAR 5 are crucial for a mutualistic interaction between *P. indica* and *Arabidopsis* under drought stress (VADASSERY et al. 2009b). Many glutathione *S*-transferases (GST) are crucial for detoxification mechanisms, and GSH is the precursor of phytochelatins, cysteine-rich peptides synthesized via phytochelatin synthase (COBBETT and GOLDSBROUGH 2002). Genes for the latter examples are rapidly (< 1h) up-regulated when *Arabidopsis* roots are exposed to *P. indica*.

GSH may also be responsible for the activation of the Nonexpressor of PR Genes (NPR1). NPR1 affects transcription of SA-induced genes for pathogenesis-related proteins (DONG 2004). In the oxidized, non-induced plants, NPR1 is cross-linked by intermolecular disulfide bridges and localized in aggregated form in the cytosol. Upon infection, NPR1 becomes reduced and the monomers are translocated to the nucleus. Inactivation of *npr1* does not affect the beneficial interaction at the seedling´s stage, however, adult plants become over-colonized and thus, the interaction shifts to parasitism (CAMEHL and OELMÜLLER, unpublished).

In summary, a survey of the literature clearly demonstrates that control of the defense responses is crucial for all beneficial interactions. Defense genes might control hyphal spread and arbuscule formation in the root. Earlier studies have demonstrated that the formation of arbuscular mycorrhiza in transgenic plants constitutively expressing several of the classical PR proteins was not affected (VIERHEILIG et al. 1993, 1995). However, experiments with the *P. indica*/*Arabidopsis* symbiosis demonstrate that detailed studies are required to fully understand the role of defense in mutualism. Quite often, inactivation of one of the defense genes has no immediate effect on the beneficial interactions, while the analysis of the symbiosis over the entire life of the plant demonstrates that defense processes are required for a long-term harmony and long-term benefits for the two symbionts. The role of defense responses in mutualistic interactions is not yet understood. It is important to understand whether MAMPs from beneficial fungi induce the same signaling cascades leading to defense responses as PAMPs from pathogens, albeit at lower rates. Furthermore, how defense responses are kept at a low level or turned off is also a matter of debate. Many different scenarios have been proposed, which can be tested with the molecular tools available now: Beneficial fungi may also induce less defense responses, because fungal-released PAMPs/MAMPs are degraded (SALZER et al. 2000; SALZER and BOLLER 2000). For instance, secreted plant hydrolases could degrade proteinous PAMPs/MAMPs. The *sym* genes could play a role in restricting plant defense responses induced by the beneficial symbionts (GIANIANZZI-PEARSON 1996; BLILOU et al. 1999). Or, *sym* genes could either produce (a) negative regulator(s) of plant defense responses or (a) specific suppressor(s) (GIANINAZZI-PEARSON 1996). Plant defense responses could be blocked by an effector molecule from the fungus. Finally, H_2O_2 and other ROS could be degraded by the up-regulation of catalases or other ROS-degrading enzymes to avoid the activation of defense genes (BLEE and ANDERSON 2000).

Conclusions

During the last decades, *Arabidopsis* has become a model system to study pathogenic plant/microbe interactions and many root and shoot pathogens have been brought together with this model plant. In contrast, much less is known about the interaction of *Arabidopsis* with beneficial microbes (bacteria and fungi). Using easy co-cultivation systems (e.g. PEŠKAN-BERGHÖFER et al. 2004) for *Arabidopsis* seedlings and adult plants, many obvious questions can now be addressed. This model

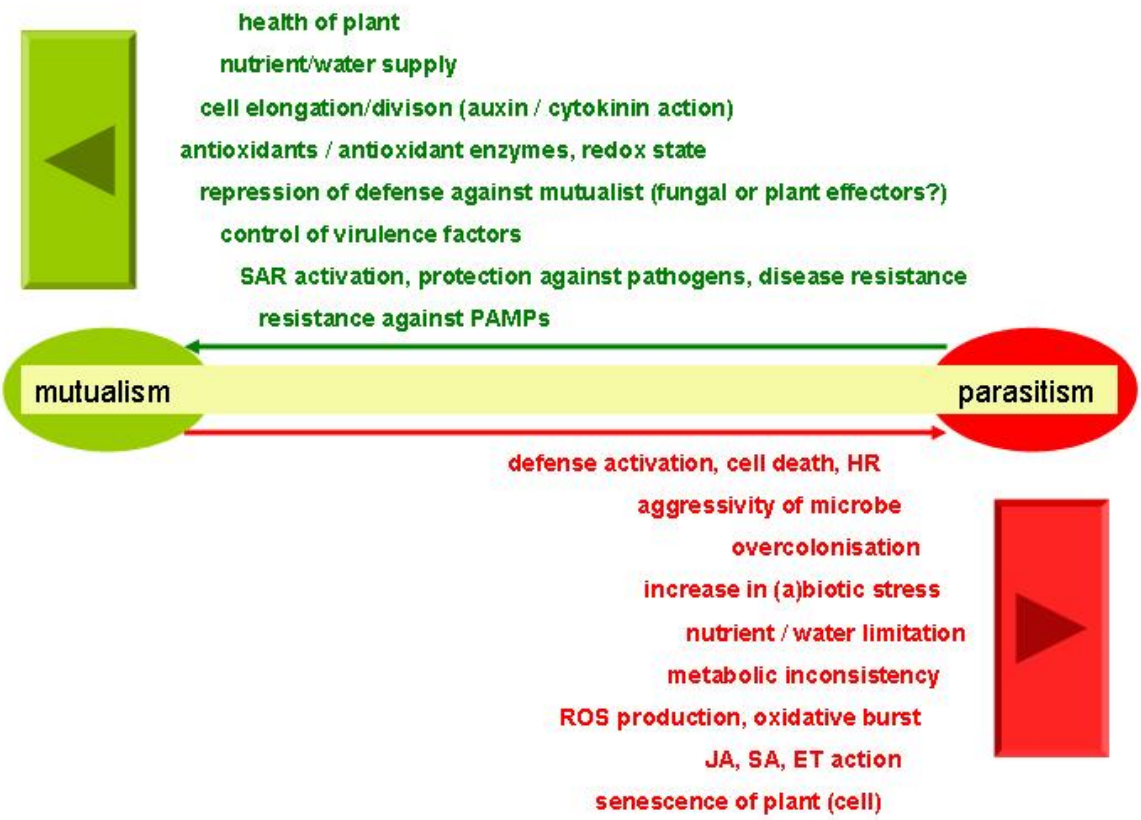

Figure 4: Factors determining the lifestyle of symbionts in a "symbiotic continuum". Factors shifting the symbiosis towards mutualism (parasitism) are in green (red). The balance between all of these factors ultimately determine the lifestyle between the two symbionts. HR, hypersensitive response, SA, salicylic acid; JA, jasmonic acid; ET, ethylene.

plant might contribute to the identification of components which determine the lifestyle between two symbionts (Figure 4). How are MAMPs perceived by the plant cell, and which are the signaling events leading to a beneficial interaction? The receptors need to be identified. Do they also form dimers, e.g. with BAK1 (BOLLER 2008), as known from PAMP receptors? Is there an overlap with mycorrhizal signaling? How do MAMPs differ from PAMPs? Do beneficial fungi produce the same or similar elicitors (MAMPs) as pathogenic fungi (PAMPs) to induce defense responses, and are they perceived by the same plant receptors in the plasma membrane?

The role of phospholipids in both beneficial and pathogenic interactions is barely understood. For both systems, evidence has accumulated that fungal contact to the plant cells or applications of PAMPs/MAMPs result in changes of those phospholipids which are involved in signaling (MUNNIK and TESTERING 2008). One major target that has been identified to play a role in pathogenic interactions as well as the *P. indica*/*Arabidopsis* symbiosis is PDK1. The availability of k.o. lines impaired in specific phospholipids biosynthesis branches will help to understand their role in the interactions.

Only very little is known about signaling molecules/compounds involved in endophytic interactions. A mutant screen as outlined above (cf. Figure 1) might help to elucidate some of the major components. These studies should also include hormones and redox-signaling events in the cytoplasm. It needs to

be clarified how specificity can be achieved if signaling components are involved in the establishment of different lifestyles (e.g. for Ca^{2+}). Again, a screen for mutants which are defective in cytosolic Ca^{2+} elevation in response to *P. indica* is required to understand its role in the beneficial interaction.

The signaling events lead to the establishment of cellular structures which are similar in mutualistic and parasitic interactions, but must also be different, at least during later stages of the infestation. Within this scenario, the cytoskeleton plays an important role. Mutant analyses will help to unravel the cellular events and the compounds which are necessary for the establishment of a certain lifestyle.

As outlined above, many genes and proteins involved in plant defense against pathogens might also play a role in mutualistic interactions. It is not clear, whether they are required only during certain stages of the mutualistic symbiosis. Inactivation of those components could demonstrate their role in mutualistic interactions, in particular for long-term harmony between the symbionts, and in the control of root colonization. One should also keep in mind that Brassicales might be different in their response to beneficial or pathogenic fungi, since they produce high amounts of glucosinolates. Thus, the role of these secondary metabolites and their specific involvement in Brassicales/fungi interactions needs to be addressed.

The mutant screen performed for the *P. indica*/*Arabidopsis* symbiosis uncovered that restriction of root colonization is apparently quite important for the establishment of a mutualistic interaction and that gene (products) from quite diverse pathways are involved in this process. What is the role of these different components in restricting root colonization? Do they cooperate or do they function independently of each other? Is there any organ-specificity? Is it possible to compensate the absence of one of these compounds by overproducing another one? This might have important biotechnological implications. Do components which restrict entry of pathogens into *Arabidopsis* cells also restrict the entry of beneficial fungi? PEN2 (LIPKA et al. 2005) is probably a good candidate to address this questions for root-interacting fungi, because PEN2 is also expressed in roots.

Finally, the role of phytohormones in beneficial plant/endophyte interactions is controversial. Some microbes clearly synthesize auxins or cytokinins which influence the plant metabolism. Since cell elongation and/or division involve the action of phytohormones, it is likely that they play a role in these symbioses. Cytokinins are important for different types of beneficial interactions (MURRAY et al. 2007; TIRICHINE et al. 2007; VADASSERY et al. 2008). A change in the lifestyle should be accompanied by changes in the action of the phytohormones. While auxins, cytokinins and gibberellins are mainly involved in growth and differentiation, jasmonic acid, SA and ethylene are crucial hormones in plant defense regulation. However, jasmonic acid and ethylene is also involved in systemic acquired resistance induced by root-colonizing fungi and bacteria (VAN WEES et al. 2008).

Model plants like *Arabidopsis*, for which many molecular, biochemical, physiological and bioinformatics tools are available, might help to identify some of the components which allow the plant to distinguish between friends and foes, and which determine the mode of symbiosis and the shifts that may occur under unfavourable conditions. However, since almost all plant species interact with fungi, these studies will only be the tip of the iceberg.

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References

AKIYAMA, K., MATSUZAKI, K. and HAYASHI, H. (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature **435,** 824-827

ALLEN, M.F., ALLEN. E.B. and FRIESE, C.F. (1989) Responses of the non-mycotrophic plant *Salsola kali* to invasion by vesicular-arbuscular mycorrhizal fungi. New Phytol. **111,** 45-49

ANÉ, J.M., KISS, G.B., RIELY, B.K., PENMETSA, R.V., OLDROYD, G.E., AYAX, C., LÉVY, J., DEBELLE, F., BAEK, J.M., KALO, P., ROSENBERG, C., ROE, B.A., LONG, S.R., DENARIE, J. and COOK, D.R. (2004) *Medicago truncatula* DMI1 required for bacterial and fungal symbioses in legumes. Science **303,** 1364-1367

ANTHONY, R.G., HENRIQUES, R., HELFER, A., MÉSZÁROS, T., RIOS, G., TESTERINK, C., MUNNIK, T., DEÁK, M., KONCZ, C. and BÖGRE, L. (2004) A protein kinase target of a PDK1 signalling pathway is involved in root hair growth in *Arabidopsis*. EMBO J. **23,** 572-581

ANTHONY, R.G., KHAN, S., COSTA, J., PAIS, M.S. and BÖGRE, L. (2006) The *Arabidopsis* protein kinase PTI1-2 is activated by convergent phosphatidic acid and oxidative stress signaling pathways downstream of PDK1 and OXI1. J. Biol. Chem. **281,** 37536-37546

ARINES, J., QUINTELA, M., VILARINO, A. and PALMA, J.M. (1994) Protein patterns and superoxide dismutase activity in non-mycorrhizal and arbuscular-mycorrhizal *Pisum sativum* L. plants. Plant Soil **166,** 37-45

ARNOLD, A.E. and HERRE, E.A. (2003) Canopy cover and leaf age affect colonisation by tropical fungal endophytes: ecological pattern and process in *Theobroma cacao* (Malvaceae). Mycologia **95,** 388-398

ASADA, K. (1997) The role of ascorbate peroxidase and monodehydroascorbate reductase in H_2O_2 scavenging in plants. In: Scandalios, J.G. (ed) Oxidative stress and the molecular biology of antioxidant defenses. Cold Spring Harbor Laboratory Press, New York, pp 715-735

BALL, L., ACCOTTO, G.P., BECHTOLD, U., CREISSEN, G., FUNCK, D., JIMENEZ, A., KULAR, B., LEYLAND, N., MEJIA-CARRANZA, J., REYNOLDS, H., KARPINSKI, S. and MULLINEAUX, P.M. (2004) Evidence for a direct link between glutathione biosynthesis and stress defense gene expression in *Arabidopsis*. Plant Cell **16,** 2448-2462

BALTRUSCHAT, H., FODOR, J., HARRACH, B.D., NIEMCZYK, E., BARNA, B., GULLNER, G., JANECZKO, A., KOGEL, K.H., SCHÄFER, P., SCHWARCZINGER, I., ZUCCARO, A. and SKOCZOWSKI, A. (2008) Salt tolerance of barley induced by the root endophyte *Piriformospora indica* is associated with a strong increase in antioxidants. New Phytol. **180,** 501-510

BAPTISTA, P., MARTINS, A., PAIS, M.S., TAVARES, R.M. and LINO-NETO, T. (2007) Involvement of reactive oxygen species during early stages of ectomycorrhiza establishment between *Castanea sativa* and *Pisolithus tinctorius*. Mycorrhiza **17,** 185-193

BARAZANI, O., BENDEROTH, M., GROTEN, K., KUHLEMEIER, C. and BALDWIN, I.T. (2005) *Piriformospora indica* and *Sebacina vermifera* increase growth performance at the expense of herbivore resistance in *Nicotiana attenuata*. Oecologia **146,** 234-243

BARROW, J.R. (2003) A typical morphology of dark septate fungal root endophytes of *Bouteloua* in arid southwestern USA rangelands. Mycorrhiza **13,** 239-247

BARROW, J.R. and AALTONEN, R.E. (2001) Evaluation of the internal colonisation of *Atriplex canescens* (Pursh) Nutt. Roots by dark septate fungi and the influence of host physiological activity. Mycorrhiza **11,** 199-205

BÉCARD, G., KOSUTA, S., TAMASLOUKHT, M., SÉJALON-DELMAS, N. and ROUX, C. (2004) Partner communication in the arbuscular mycorrhizal interaction. Can. J. Bot. **82,** 1186-1197

BEDNAREK, P., PISLEWSKA-BEDNAREK, M., SVATOS, A., SCHNEIDER, B., DOUBSKY, J., MANSUROVA, M., HUMPHRY, M., CONSONNI, C., PANSTRUGA, R., SANCHEZ-VALLET, A., MOLINA, A. and SCHULZE-LEFERT, P. (2009) A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. Science **323,** 101-106

BENHAMOU, N. and GARAND, C. (2001) Cytological analysis of defense-related mechanisms induced in pea root tissues in response to colonisation by nonpathogenic *Fusarium oxysporum* Fo47. Phytopathology **91,** 730-740

BHAT, R.A., MIKLIS, M., SCHMELZER, E., SCHULZE-LEFERT, P. and PANSTRUGA, R. (2005) Recruitment and interaction dynamics of plant penetration resistance components in a plasma membrane micro-

domain. Proc. Natl. Acad. Sci. USA **102,** 3135-3140

BIDARTONDO, M.I., REDECKER, D., HIJRI, I., WIEMKEN, A., BRUNS, T.D., DOMINGUEZ, L., SERSIC, A., LEAKE, J.R. and READ, D.J. (2002) Epiparasitic plants specialized on arbuscular mycorrhizal fungi. Nature **419,** 389-392

BLEE, K.A. and ANDERSON, A.J. (2000) Defense responses in plants to arbuscular mycorrhizal fungi. In: Podila, G.K. and Douds, D.D. (eds) Current advances in mycorrhizae research. Minnesota, USA: The American Phytopathological Society, pp. 27-44

BLILOU, I., BUENO, P., OCAMPO, J.A. and GARCÍA-GARRIDO, J.M. (2000a) Induction of catalase and ascorbate peroxidase activities in tobaccco roots inoculated with the arbuscular mycorrhizal fungus *Glomus mosseae*. Mycol. Res. **104,** 722–725

BLILOU, I., OCAMPO, J.A. and GARCÍA-GARRIDO, J.M. (1999) Resistance of pea roots to endomycorrhizal fungus or *Rhizobium* correlates with enhanced levels of endogenous salicylic acid. J. Exp. Bot. **50,** 1663-1668

BLILOU, I., OCAMPO, J.A. and GARCÍA-GARRIDO, J.M. (2000b) Induction of *Ltp* (Lipid transfer protein) and *Pal* (phenylalanine ammonia-lyase) gene expression in rice roots colonized by the arbuscular mycorrhizal fungus *Glomus mosseae*. J. Exp. Bot. **51,** 1969-1977

BLUME, B., NÜRNBERGER, T., NASS, N. and SCHEEL, D. (2000) Receptor-mediated rise in cytoplasmic free calcium required for activation of pathogen defense in parsley. Plant Cell **12,** 1425-1440

BOLLER, T. (2008) Stabbing in the BAK – an original target for avirulence genes of plant pathogens. Cell Host Microbe **4,** 5-7

BONANOMI, A., OETIKER, J.H, GUGGENHEIM, R., BOLLER, T., WIEMKEN, A. and VÖGELI-LANGE, R. (2001) Arbuscular mycorrhizas in mini-mycorrhizotrons: first contact of *Medicago truncatula* roots with *Glomus intraradices* induces chalcone synthase. New Phytol. **150,** 573-582

BORDEN, S. and HIGGINS, V. J. (2002) Hydrogen peroxide plays a critical role in the defence response of tomato to *Cladosporium fulvum*. Physiol. Mol. Plant Pathol. **61,** 227−236

BOUVIER, F., BACKHAUS, R.A. and CAMARA, B. (1998) Induction and control of chromoplast-specific carotenoid genes by oxidative stress. J. Biol. Chem. **273,** 30651-30659

BRUNNER, F., ROSAHL, S., LEE, J., RUDD, J.J., GEILER, C., KAUPPINEN, S., RASMUSSEN, G., SCHEEL, D. and NÜRNBERGER, T. (2002) Pep-13, a plant defense-inducing pathogen-associated pattern from *Phytophthora transglutaminases*. EMBO J. **21,** 6681-6688

BÜTEHORN, B., RHODY, D. and FRANKEN, P. (2000) Isolation and characterization of *Pitef1* encoding the translation elongation factor EF-1a of the root endophyte *Piriformospora indica*. Plant Biol. **2,** 687-692

CALDWELL, B.A., JUMPPONEN, A. and TRAPPE, J.M. (2000) Utilization of major detrital substrates by dark-septate, root endophytes. Mycologia **92,** 230-232

CARROLL, G. (1988) Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. Ecology **69,** 2-9

CLAY, N.K., ADIO, A.M., DENOUX, C., JANDER, G. and AUSUBEL, F.M. (2009) Glucosinolate metabolites required for an *Arabidopsis* innate immune response. Science **323,** 95-101

COBBETT, C. and GOLDSBROUGH, P. (2002) Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu. Rev. Plant Biol. **53,** 159-182

DESHMUKH, S., HÜCKELHOVEN, R., SCHÄFER, P., IMANI, J., SHARMA, M., WEIß, M., WALLER, F. and KOGEL, K.H. (2006) The root endophytic fungus *Piriformospora indica* requires host cell death for proliferation during mutualistic symbiosis with barley. Proc. Natl. Acad. Sci. USA **103,** 18450-18457

DITENGOU, F.A., RAUDASKOSKI, M. and LAPEYRIE, F. (2003) Hypaphorine, an indole-3-acetic acid antagonist delivered by the ectomycorrhizal fungus *Pisolithus tinctorius*, induces reorganisation of actin and the microtubule cytoskeleton in Eucalyptus globulus ssp bicostata root hairs. Planta **218,** 217-225

DONG, X. (2004) NPR1, all things considered. Curr. Opin. Plant Biol. **7,** 547-552

DOUDS D.D., GALVEZ, L., BÉCARD, G. and KAPULNIK, Y. (1998) Regulation of arbuscular mycorrhizal development by plant host and fungus species in alfalfa. New Phytol. **138,** 27-35

DUMAS-GAUDOT, E., GOLLOTTE, A., CORDIER, C., GIANINAZZI, S. and GIANINAZZI-PEARSON, V. (2000) Modulation of host defence systems. In: Kapulnik, Y. and Douds, D.D. (eds) Arbuscular mycorrhizas:

physiology and function. Kluwer, Dordrecht, pp. 173–200

DUNAND, C., CRÈVECOEUR, M. and PENEL, C. (2007) Distribution of superoxide and hydrogen peroxide in *Arabidopsis* root and their influence on root development: possible interaction with peroxidases. New Phytol. **174,** 332-341

EBEL, J. (1998) Oligoglucoside elicitor-mediated activation of plant defense. Bioessays **20,** 569-576

ENDRE, G., KERESZT, A., KEVEI, Z., MIHACEA, S., KALO, P. and KISS, G.B. (2002) A receptor kinase gene regulating symbiotic nodule development. Nature **417,** 962-966

ERNST, M., MENDGEN, K.W. and WIRSEL, S.G. (2003) Endophytic fungal mutualists: seed-borne *Stagonospora* spp. enhance reed biomass production in axenic microcosms. Mol. Plant-Microbe Interact. **16,** 580-587

ESPINOSA, A., GUO, M., TAM, V.C., FU, Z.Q. and ALFANO, J.R. (2003) The *Pseudomonas syringae* type III-secreted protein HopPtoD2 possesses protein tyrosine phosphatase activity and suppresses programmed cell death in plants. Mol. Microbiol. **49,** 377-387

FALK, K.L., TOKUHISA, J.G. and GERSHENZON, J. (2007) The effect of sulfur nutrition on plant glucosinolate content: physiology and molecular mechanisms. Plant Biol. **9,** 573-581

FESTER, T. and HAUSE, G. (2005) Accumulation of reactive oxygen species in arbuscular mycorrhizal roots. Mycorrhiza **15,** 373-379

FOREMAN, J., DEMIDCHIK, V., BOTHWELL, J.H., MYLONA, P., MIEDEMA, H., TORRES, M.A., LINSTEAD, P., COSTA, S., BROWNLEE, C., JONES, J.D., DAVIES, J.M. and DOLAN, L. (2003) Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature **422,** 442−446

FREEMAN, S. and RODRIGUES, R.J. (1993) Genetic conversion of a fungal plant pathogen to a nonpathogenic, endophytic mutualist. Science **260,** 75-78

FREEMAN, S., HOROWITZ, S. and SHARON, A. (2001) Pathogenic and nonpathogenic lifestyles in *Colletotrichum acutatum* from strawberry and other plants. Phytopathology **91,** 986-992

GAFUR, A., SCHÜTZENDÜBEL, A., LANGENFELD-HEYSER, R., FRITZ, E. and POLLE, A. (2004) Compatible and incompetent Paxillus involutus isolates for ectomycorrhiza formation in vitro with poplar (Populus x canescens) differ in H2O2 production. Plant Biol. **6,** 91-99

GAO, L.L., KNOGGE, W., DELP, G., SMITH, F.A. and SMITH, S.E. (2004) Expression patterns of defense-related genes in different types of arbuscular mycorrhizal development in wild-type and mycorrhiza-defective mutant tomato. Mol. Plant-Microbe Interact. **17,** 1103-1113

GARCIA-BRUGGER, A., LAMOTTE, O., VANDELLE, E., BOURQUE, S., LECOURIEUX, D., POINSSOT, B., WENDEHENNE, D. and PUGIN, A. (2006) Early signaling events induced by elicitors of plant defenses. Mol. Plant-Microbe Interact. **19,** 711-724

GARCIA-GARRIDO, J.M. and OCAMPO, J.A. (2002) Regulation of the plant defence response in arbuscular mycorrhizal symbiosis. J. Exp. Bot. **53,** 1377-1386

GENRE, A. and BONFANTE, P. (2002) Epidermal cells of a symbiosis-defective mutant of *Lotus japonicus* show altered cytoskeleton organisation in the presence of a mycorrhizal fungus. Protoplasma **219,** 43-50

GENRE, A., CHABAUD, M., TIMMERS, T., BONFANTE, P. and BARKER, D.G. (2005) Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. Plant Cell **17,** 3489-3499

GIANINAZZI-PEARSON, V. (1996) Plant cell responses to arbuscular mycorrhizal fungi: getting to the roots of the symbiosis. Plant Cell **8,** 1871-1883

GLEN, M., TOMMERUP, I.C., BOUGHER, N.L. and O'BRIEN, P.A. (2002) Are *Sebacinaceae* common and widespread ectomycorrhizal associates of Eucalyptus species in Australian forests? Mycorrhiza **12,** 243-247

GOLLOTTE, A., GIANINAZZI-PEARSON, V., GIOVANNETTI, M., SBRANA, C., AVIO, L. and GIANINAZZI, S. (1993) Cellular localization and cytochemical probing of resistance reactions to arbuscular mycorrhizal fungi in a 'locus a' mutant *Pisum sativum* L. Planta **191,** 112–122

GOMEZ-GOMEZ, L. and BOLLER, T. (2000) FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in *Arabidopsis*. Mol. Cell **5,** 1003-1011

GRUBB, C. and ABEL, S. (2006) Glucosinolate metabolism and its control. Trends Plant Sci. **11,** 89-100

GRUNWALD, U., NYAMSUREN, O., TARNASLOUKHT, M., LAPOPIN, L., BECKER, A., MANN, P., GIANINAZZI-PEARSON, V., KRAJINSKI, F. and FRANKEN, P. (2004) Identification of mycorrhiza-regulated genes with arbuscule development-related expression profile. Plant Mol. Biol. **55,** 553-566

GÜIMIL, S., CHANG, H.S., ZHU, T., SESMA, A. OSBOURN, A., ROUX, C., IOANNIDIS, V., OAKELEY, E.J., DOCQUIER, M., DESCOMBES, P. et al. (2005) Comparative transcriptomics of rice reveals an ancient pattern of response to microbial colonization. Proc. Natl. Acad. Sci. USA **102,** 8066-8070

HAHLBROCK, K., SCHEEL, D., LOGEMANN, E., NÜRNBERGER, T., PARNISKE, M., REINOLD, S., SACKS, W.R. and SCHMELZER, E. (1995) Oligopeptide elicitor-mediated defense gene activation in cultured parsley cells. Proc. Natl. Acad. Sci. USA **9,** 4150-4157

HALKIER, B.A. and GERSHENZON, J. (2006) Biology and biochemistry of glucosinolates. Annu. Rev. Plant. Biol. **57,** 303-333

HALLMANN, J. and SIKORA, R.A. (1996) Toxicity of fungal endophyte secondary metabolites to plant-parasitic nematodes and soil-borne plant-pathogenic fungi. Eur. J. Plant Pathol. **102,** 155-162

HANCOCK, J.T., DESIKAN, R. and NEILL, S.J. (2001) Role of reactive oxygen species in cell signalling pathways. Biochem. Soc. Trans. **29,** 345−350

HARRISON, M.J. (1999) Molecular and cellular aspects of the arbuscular mycorrhizal symbiosis. Annu. Rev. Plant Physiol. Plant Mol. Biol. **50,** 361-389

HARRISON, M.J. (2005) Signaling in the arbuscular mycorrhizal symbiosis. Annu. Rev. Microbiol. **59,** 19-42

HAUSE, B. and FESTER, T. (2005) Molecular and cell biology of arbuscular mycorrhizal symbiosis. Planta **221,** 184-196

HE, P., SHAN, L., LIN, N.C., MARTIN, G.B., KEMMERLING, B., NÜRNBERGER, T. and SHEEN, J. (2006) Specific bacterial suppressors of MAMP signaling upstream of MAPKKK in *Arabidopsis* innate immunity. Cell **125,** 563-575

Heath, M., Nimchuk, Z. and Xu, H. (1997) Plant nuclear migration as indicators of critical interactions between resistant or susceptible cowpea epidermal cells and invasion hyphae of the cowpea rust fungus. New Phytol. **135**, 689–700

HIRAI, M.Y., KLEIN, M., FUJIKAWA, Y., YANO, M., GOODENOWE, D.B., YAMAZAKI, Y., KANAYA, S., NAKAMURA, Y., KITAYAMA, M., SUZUKI, H., SAKURAI, N., SHIBATA, D., TOKUHISA, J., REICHELT, M., GERSHENZON, J., PAPENBROCK, J. and SAITO, K. (2005) Elucidation of gene-to-gene and metabolite-to-gene networks in *Arabidopsis* by integration of metabolomics and transcriptomics. J. Biol. Chem. **280,** 25590-25595

HIRAI, M.Y., FUJIWARA, T., CHINO, M. and NAITO, S. (1995) Effects of sulfate concentrations on the expression of a soybean seed storage protein gene and its reversibility in transgenic *Arabidopsis thaliana*. Plant Cell Physiol. **36,** 1331-1339

HIRAI, M.Y., YANO, M., GOODENOWE, D.B., KANAYA, S., KIMURA, T., AWAZUHARA, M., ARITA, M., FUJIWARA, T. and SAITO, K. (2004) Integration of transcriptomics and metabolomics for understanding of global responses to nutritional stresses in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **101,** 10205-10210

HOHNJEC, N., VIEWEG, M.E., PUHLER, A., BECKER, A. and KUSTER, H. (2005) Overlaps in the transcriptional profiles of *Medicago truncatula* roots inoculated with two different *Glomus* fungi provide insights into the genetic program activated during arbuscular mycorrhiza. Plant Physiol. **137,** 1283-1301

HOLLAND, M.A. (1997) Occam`s razor applied to hormonology. Are cytokinins produced by plants? Plant Physiol. **115,** 865-868

HÜCKELHOVEN, R. and KOGEL, K.H. (2003) Reactive oxygen intermediates in plant-microbe interactions: who is who in powdery mildew resistance? Planta **216,** 891-902

IMAIZUMI-ANRAKU, H., TAKEDA, N., CHARPENTIER, M. PERRY, J., MIWA, H., UMEHARA, Y., KOUCHI, H., MURAKAMI, Y., MULDER, L., VICKERS, K. et al. (2005) Plastid proteins crucial for symbiotic fungal and bacterial entry into plant roots. Nature **433,** 527-531

IVASHUTA, S., LIU, J. LIU, J., LOHAR, D.P., HARIDAS, S., BUCCIARELLI, B., VANDENBOSCH, K.A., VANCE, C.P., HARRISON, M.J. and GANTT, J.S. (2005) RNA interference identifies a calcium-dependent protein kinase involved in *Medicago truncatula* root development. Plant Cell **17,** 2911-2921

JALLOW, J.F.A., DIGASSA-GOBENA, D. and VIDAL, S. (2004) Indirect interaction between an unspecialized endophytic fungus and a polyphagous moth. Basic Appl. Ecol. **5,** 183-191

JOHNSON, N.C., GRAHAM, J.H. and SMITH, F.A. (1997) Functioning of mycorrhizal associations along the mutualism–parasitism continuum. New Phytol. **135,** 575-586

JUMPPONEN, A. (2001) Dark septate endophytes – are they mycorrhizal? Mycorrhiza **11,** 207-211

JUMPPONEN, A. and TRAPPE, J.M. (1998) Performance of *Pinus contorta* inoculated with two strains of root endophytic fungus, *Phialocephala fortinii*: effects of synthesis system and glucose concentration. Can. J. Bot. **76,** 1205-1213

KALO, P., GLEASON, C., EDWARDS, A., MARSH, J., MITRA, R.M., HIRSCH, S., JAKAB, J., SIMS, S., LONG, S.R., ROGERS, R. et al. (2005) Nodulation signaling in legumes requires NSP2, a member of the GRAS family of transcriptional regulators. Science **308,** 1786-1789

KERK, N.M. and FELDMAN, L.J. (1995) A biochemical model for the initiation and maintenance of the quiescent centre: implication for organization of root meristems. Development **121,** 2825-2833

KIM, M.G., DA CUNHA, L., MCFALL, A.J., BELKHADIR, Y., DEBROY, S., DANGL, J.L. and MACKEY, D. (2005) Two *Pseudomonas syringae* type III effectors inhibit RIN4-regulated basal defense in *Arabidopsis*. Cell **121,** 749-759

KWON, C., PANSTRUGA, R. and SCHULZE-LEFERT, P. (2008) Les liaisons dangereuses: immunological synapse formation in animals and plants. Trends Immunol. **29,** 159-166

KOGEL, K.H., FRANKEN, P. and HÜCKELHOVEN, R. (2006) Endophyte or parasite-what decides? Curr. Opin. Plant Biol. **9,** 358-363

KUTZ, A., MÜLLER, A., HENNIG, P., KAISER, W.M., PIOTROWSKI, M. and WEILER, E.W. (2002) A role for nitrilase 3 in the regulation of root morphology in sulphur-starving *Arabidopsis thaliana*. Plant J. **30,** 95-106

KWAK, J.M., MORI, I.C., PEI, Z.M., LEONHARDT, N., TORRES, M.A., DANGL, J.L., BLOOM, R.E., BODDE, S., JONES, J.D. and SCHROEDER, J.I. (2003) NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in *Arabidopsis*. EMBO J. **22,** 2623–2633

LAMBAIS, M.R. (2000) Regulation of plant defence-related genes in arbuscular mycorrhizae. In: Podila, G.K. and Douds, D.D. (eds) Current Advances in Mycorrhizae Research, Minnesota, USA, The American Phytopathological Society, pp. 45–59

LAMBAIS, M.R., RÍOS-RUIZ, W.F. and ANDRADE, R.M. (2003) Antioxidant responses in bean (*Phaseolus vulgaris*) roots colonized by arbuscular mycorrhizal fungi. New Phytol. **160,** 421-428

LANFRANCO, L., NOVERO, M. and BONFANTE, P. (2005) The mycorrhizal fungus *Gigaspora margarita* possesses a CuZn superoxide dismutase that is up-regulated during symbiosis with legume hosts. Plant Physiol. **137,** 1319-1330

LÉVY, J., BRES, C., GEURTS, R., CHALHOUB, B., KULIKOVA, O., DUC, R., JOURNET, E.P., ANÉ, J.M., LAUBER, E., BISSELING, T., DENARIE, J., ROSENBERG, C. and DEBELLE, F. (2004) A putative Ca^{2+} and calmodulin-dependent protein kinase required for bacterial and fungal symbioses. Science **303,** 1361–1364

LIMPENS, E., FRANKEN, C., SMIT, P., WILLEMSE, J., BISSELING, T. and GEURTS, R. (2003) LysM domain receptor kinases regulating rhizobial Nod factor-induced infection. Science **302,** 630-633

LINDERMAN, R.G. (2000) Effects of mycorrhizas on plant tolerances to diseases. In: Kapulnik, Y. and Douds, D.D. (eds) Arbuscular mycorrhizas: physiology and function. Kluwer, Dordrecht, pp. 345-365

LIPKA, V., DITTGEN, J., BEDNAREK, P. et al. (2005) Pre- and postinvasion defenses both contribute to nonhost resistance in *Arabidopsis*. Science **310,** 1180–1183

LIPKA, V. and PANSTRUGA, R. (2005) Dynamic cellular responses in plant–microbe interactions. Curr. Opin. Plant Biol. **8,** 625–631

LISZKAY, A., VAN DER ZALM, E. and SCHOPFER, P. (2004) Production of reactive oxygen intermediates ($O_2^{\cdot-}$, H_2O_2, and $^{\cdot}OH$) by maize roots and their role in wall loosening and elongation growth. Plant Physiol. **136,** 3114–3123

MADSEN, E.B., MADSEN, L.H., RADUTOIU, S., OLBRYT, M., RAKWALSKA, M., SZCZYGLOWSKI, K., SATO, S., KANEKO, T., TABATA, S., SANDAL, N. and STOUGAARD, J. (2003) A receptor kinase gene of the LysM type is involved in legume perception of rhizobial signals. Nature **425,** 637-640

MARUYAMA-NAKASHITA, A., INOUE, E., WATANABE-TAKAHASHI, A., YAMAYA, T. and TAKAHASHI, H. (2003) Transcriptome profiling of sulfur-responsive genes in *Arabidopsis* reveals global effect on sulfur nutrition on multiple metabolic pathways. Plant Physiol. **132,** 597-605

MARUYAMA-NAKASHITA, A., NAKAMURA, Y., WATANABE-TAKAHASHI, A., INOUE, E., YAMAYA, T. and

TAKAHASHI, H. (2005) Identification of a novel *cis*-acting element conferring sulfur deficiency response in *Arabidopsis* roots. Plant J. **42,** 305-314

MATAMOROS, M.A., DALTON, D.A., RAMOS, J., CLEMENTE, M.R., RUBIO, M.C. and BECANA, M. (2003) Biochemistry and molecular biology of antioxidants in the rhizobia–legume symbiosis. Plant Physiol. **133,** 499-509

MENDGEN, K. and HAHN, M. (2002) Plant infection and the establishment of fungal biotrophy. Trends Plant Sci. **7,** 352–356

MILLER, J.D., MACKENZIE, S., FOTO, M., ADAMS, G.W. and FINDLAY, J.A. (2002) Needles of white spruce inoculated with rugulosin-producing endophytes contain rugulosin reducing spruce budworm growth rate. Mycol. Res. **106,** 471-479

MITRA, R.M., GLEASON, C.A., EDWARDS, A., HADFIELD, J., DOWNIE, J.A., OLDROYD, G.E. and LONG, S.R. (2004) A Ca^{2+}/calmodulin-dependent protein kinase required for symbiotic nodule development: gene identification by transcript-based cloning. Proc. Natl. Acad. Sci. USA **101,** 4701-4705

MUCCIARELLI, M., SCANNERINI, S., BERTEA, C. and MAFFEI, M. (2002) An ascomycetous endophyte isolated from *Mentha pipertia* L.: biological features and molecular studies. Mycologia **94,** 28-39

MUCCIARELLI, M., SCANNERINI, S., BERTEA, C. and MAFFEI, M. (2003) In vitro and in vivo peppermint (*Mentha piperita*) growth promotion by nonmycorrhizal fungal colonisation. New Phytol. **158,** 579-591

MÜLLER, C.B. and KRAUSS, J. (2005) Symbiosis between grasses and asexual fungal endophytes. Curr. Opin. Plant Biol. **8,** 450-456

MUNNIK, T. AND TESTERINK, C. (2008) Plant phospholipid signaling - 'in a nutshell'. J. Lipid Res. [Epub ahead of print]

MURRAY, J.D., KARAS, B.J., SATO, S., TABATA, S., AMYOT, L. and SZCZYGLOWSKI, K. (2007) A cytokinin perception mutant colonized by Rhizobium in the absence of nodule organogenesis. Science **315,** 101-104

NITZ, I., BERKEFELD, H., PUZIO, P.S. and GRUNDLER, F.M. (2001) Pyk10, a seedling and root specific gene and promoter from *Arabidopsis thaliana*. Plant Sci. **161,** 337-346

NOCTOR, G. and FOYER, C.H. (1998) Ascorbate and glutathione: keeping active oxygen under control. Annu. Rev. Plant Physiol. Plant Mol. Biol. **49,** 249-279

NOCTOR, G., VELJOVIC-JOVANIVIC, S. and FOYER, C.H. (2000) Peroxide processing in photosynthesis: antioxidant coupling and redox signalling. Phil. Trans. R Soc. Lond. B. **355,** 1465-1475

NÜRNBERGER, T. and KEMMERLING, B. (2006) Receptor protein kinases-pattern recognition receptors in plant immunity. Trends Plant Sci. **11,** 519-522

NÜRNBERGER, T., NENNSTIEL, D., HAHLBROCK, K. and SCHEEL, D. (1995) Covalent cross-linking of the *Phytophthora megasperma* oligopeptide elicitor to its receptor in parsley membranes. Proc. Natl. Acad. Sci. USA **92,** 2338-2342

NÜRNBERGER, T., NENNSTIEL, D., JABS, T., SACKS, W.R., HAHLBROCK, K. and SCHEEL, D. (1994) High affinity binding of a fungal oligopeptide elicitor to parsley plasma membranes triggers multiple defense responses. Cell **12,** 449-460

OELMÜLLER, R., PEŠKAN-BERGHÖFER, T., SHAHOLLARI, B., SHERAMETI, I. and VARMA, A. (2005) MATH-domain containing proteins represent a novel gene family in *Arabidopsis thaliana* and are involved in plant/microbe interactions. Physiol. Plant. **124,** 152-166

OELMÜLLER, R., SHAHOLLARI, B., PEŠKAN-BERGHÖFER, T., TREBICKA, A., GIONG, P.H., SHERAMETI, I., OUDHOFF, M., ALTSCHMIED, L. and VARMA, A. (2004) Molecular analyses of the interaction between *Arabidopis* roots and the growth-promoting fungus *Piriformospora indica*. Endocytobiosis Cell Res. **15,** 639-352

OELMÜLLER, R., SHERAMETI, I., TRIPATHI, S. and VARMA, A. (2009) *Piriformospora indica*, a cultivable root endophyte with multiple biotechnological applications. Mycorrhiza (in press)

OPALSKI, K.S., SCHULTHEISS, H., KOGEL, K.-H. and HÜCKELHOVEN, R. (2005) The receptor-like MLO protein and the RAC/ROP family G-protein RACB modulate actin reorganization in barley attacked by the biotrophic powdery mildew fungus *Blumeria graminis* f. sp. *Hordei.* Plant J. **41,** 291-303

PARNISKE, M. (2000) Intracellular accommodation of microbes by plants: a common developmental program for symbiosis and disease? Curr. Opin. Plant Biol. **3,** 320-328

PARNISKE, M. (2004) Molecular genetics of the arbuscular mycorrhizal symbiosis. Curr. Opin. Plant Biol. **7,** 414-421

PASZKOWSKI, U. (2006) Mutualism and parasitism: the yin and yang of plant symbioses. Curr. Opin. Plant Biol. **9,** 364-370

PERFECT, P. and GREEN, J. (2001) Infection structures of biotrophic and hemibiotrophic fungal plant pathogens. Mol. Plant Pathol. **2,** 101–108

PEŠKAN-BERGHÖFER, T., SHAHOLLARI, B., GIONG, P.H., HEHL, S., MARKERT, C., BLANKE, V., VARMA, A.K. and 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 endoplasmatic reticulum and at the plasma membrane. Physiol. Plant. **122,** 465-477

PHAM, G.H., SINGH, A.N., MALLA, R., KUMARI, R., PRASAD, R., SACHDEV, M., REXER, K.-H., KOST, G., LUIS, P., KALDORF, M., BUSCOT, F., HERRMANN, S., PEŠKAN, T., OELMÜLLER, R., SAXENA, A.K., DECLERCK, S., MITTAG, M., STABENTHEINER, E., HEHL, S. and VARMA, A. (2004) Interaction of *Piriformospora indica* with diverse microorganisms and plants. In: Plant Surface Microbiology. Varma, A., Abbott, L., Werner, D., and Hampp, R. (eds) Springer-Verlag New York Inc., New York, pp. 237–265

PICARD, K., PONCHET, M., BLEIN, J.-P., REY, P., TIRILLY, Y. and BENHAMOU, N. (2000) Oligandrin. A proteinaceous molecule produced by the mycoparasite *Pythium oligandrum* induces resistance to *Phytophthora parasitica* infection in tomate plants. Plant Physiol. **124,** 379-395

PIGNOCCHI, C. and FOYER, C.H. (2003) Apoplastic ascorbate metabolism and its role in the regulation of cell signalling. Curr. Opin. Plant Biol. **6,** 379-389

PIGNOCCHI, C., FLETCHER, J.M., WILKINSON, J.E., BARNES, J.D. and FOYER, C.H. (2003) The function of ascorbate oxidase in tobacco. Plant Physiol. **132,** 1631-1641

PUPPO, A., GROTEN, K., BASTIAN, F., CARZANIGA, R., SOUSSI, M., LUCAS, M.M., DE FELIPE, M.R., HARRISON, J., VANACKER, H. and FOYER, C. (2005) Legume nodule senescence: roles for redox and hormone signalling in the orchestration of the natural aging process. New Phytol. **165,** 683-701

RADUTOIU, S., MADSEN, L.H., MADSEN, E.B., FELLE, H.H., UMEHARA, Y., GRØNLUND, M., SATO, S., NAKAMURA, Y., TABATA, S., SANDAL, N. and STOUGAARD, J. (2003) Plant recognition of symbiotic bacteria requires two LysM receptor-like kinases. Nature **425,** 585-592

RAMU, S.K., PENG, H.-M. and COOK, D.R. (2002) Nod factor induction of reactive oxygen species production is correlated with expression of the early nodulin gene *rip1* in *Medicago truncatula*. Mol. Plant-Microb. Interact. **15,** 522-528

REDECKER, D., KODNER, R. and GRAHAM, L.E. (2000) Glomalean fungi from the Ordovician. Science **289,** 1920-1921

REDMAN, R.S., DUNIGAN, D.D. and RODRIGUEZ, R.J. (2001) Fungal symbiosis from mutualism to parasitism: who controls the outcome, host or invader? New Phytol. **151,** 705-716

REDMAN, R.S., FREEMAN, S., CLIFTON, D.R., MORREL, J., BROWN, G. and RODRIGUEZ, R.J. (1999a) Biochemical analysis of plant protection afforded by a nonpathogenic endophytic mutant of *Colletotrichum magna*. Plant Physiol. **119,** 795-804

REDMAN, R.S., RANSON, J.C. and RODRIGUEZ, R.J. (1999b) Conversion of the pathogenic fungus *Colletotrichum magna* to a nonpathogenic, endophytic mutualist by gene disruption. Mol. Plant-Microbe Interact. **11,** 969-975

REDMAN, R.S., ROSSINCK, M.R., MAHER, S., ANDREWS, Q.C., SCHNEIDER, W.L. and RODRIGUEZ, R.J. (2002a) Field performance of cucurbit and tomato plants infected with a nonpathogenic mutant of *Colletotrichum magna* (teleomorph: *Glomerella magna*; Jenkins and Winstead). Symbiosis **32,** 55-70

REDMAN, R.S., SHEEHAN, K.B., STOUT, R.G., RODRIGUEZ, R.J. and HENSON, J.M. (2002b) Thermotolerance conferred to plant host and fungal endophyte during mutualistic symbiosis. Science **298,** 1581

RENTEL, M.C., LECOURIEUX, D., OUAKED, F., USHER, S.L., PETERSEN, L., OKAMOTO, H., KNIGHT, H., PECK, S.C., GRIERSON, C.S., HIRT, H. and KNIGHT, M.R. (2004) OXI1 kinase is necessary for oxidative burst-mediated signalling in *Arabidopsis.* Nature **427,** 858-861

REY, P., LEUCART, S., DÉSILETS, H., BÉLANGER, R.R., LARUE, J.P. and TIRILLY, Y. (2001) Production of indole-3-acetic acid and tryptophol by *Pythium* group F: possible role in pathogenesis. Eur. J. Plant Pathol. **107,** 895-904

RODRIGUEZ, R. and REDMAN, R. (2008) More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. J. Exp. Bot. **59,** 1109-1114

RÖMMERT, A.K., OROS-SICHLER, M., AUST, H.-J., LANGE, T. and SCHULZ, B. (2002) Growth promoting effects of endophytic colonisation of the roots of larch (*Larix decidua*) with *Cryptosporiopsis* sp. and *Phialophora* sp., 7th International Mycological Congress, Oslo, Norway, p. 309

RUDD, J.J. and FRANKLIN-TONG, V.E. (1999) Calcium signaling in plants. Cell Mol. Life Sci. **55,** 214-232

SAHAY, N.S. and VARMA, A. (1999) *Piriformospora indica*: a new biological hardening tool for micropropagated plants. FEMS Microbiol. Lett. **181,** 297-302

SAIKKONEN, K., FAETH, S.H., HELANDER, M. and SULLIVAN, T.J. (1998) Fungal endophytes: a continuum of interactions with host plants. Annu. Rev. Ecology and Systematics **29,** 319-343

SALZER, P. and BOLLER, T. (2000) Elicitor-induced reactions in mycorrhizae and their suppression. In: Podila, G.K. and Douds, D.D. (eds) Current Advances in Mycorrhizae Research, Minnesota, USA, The American Phytopathological Society, pp. 1–10

SALZER, P., BONANOMI, A., BEYER, K., VÖGELI-LANGE, R., AESCHBACHER, R.A., LANG, J., WIEMKEN, A., KIM, D., COOK, D.R. and BOLLER, T. (2000) Differential expression of eight chitinase genes in *Medicago truncatula* roots during mycorrhiza formation, nodulation and pathogen infection. Mol. Plant–Microbe Interact. **13,** 763-777

SALZER, P., CORBIÈRE, H. and BOLLER, T. (1999) Hydrogen peroxide accumulation in *Medicago truncatula* roots colonized by the arbuscular mycorrhiza-forming fungus *Glomus mosseae*. Planta **208,** 319-325

SANTOS, R., HEROUART, D., SIGAUD, S., TOUATI, D. and PUPPO, A. (2001) Oxidative burst in alfalfa–*Sinorhizobium meliloti* symbiotic interaction. Mol. Plant-Microbe Interact. **14,** 86-89

SCHARDL, C. and LEUCHTMANN, A. (2005) The epichloe endophytes of grasses and the symbiotic continuum. In: The fungal community: its organization and role in the ecosystem. Dighton, J., White, J.F. and Oudemans, P. (eds) Boca Raton, FL, Taylor and Francis, pp. 475-503

SCHMELZER, E. (2002) Cell polarization, a crucial process in fungal defence. Trends Plant Sci. **7,** 411-415

SCHULZ, B. and BOYLE, C. (2005) The endophytic continuum. Mycol. Res. **109**, 661-686

SCHULZ, B., BOYLE, C., DRAEGER, S., RÖMMERT, A.-K. and KROHN, K. (2002) Endophytic fungi: a source of biologically active secondary metabolites. Mycol. Res. **106,** 996-1004

SCHULZ, B., ROMMERT, A.K., DAMMANN, U., AUST, H.J. and STRACK, D. (1999) The endophyte–host interaction: a balanced antagonism? Mycol. Res.**10,** 1275-1283

SCHULZ, B., STUCKER, J., AUST, H.-J., KROHN, K., LUDEWIG, K., JONES, P.G. and DÖRING, D. (1995) Biologically active secondary metabolites of endophytic *Pezicula* species. Mycol. Res. **99,** 1007-1015

SCHÜTZENDÜBEL, A. and POLLE, A. (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J. Exp. Bot. **53,** 1351-1365

SCOTT, B. (2001) Epichloë endophytes: fungal symbionts of grasses. Curr. Opin. Microbiol. **4,** 393-398

SCOTT, B. and EATON, C.J. (2008) Role of reactive oxygen species in fungal cellular differentiations. Curr. Opin. Microbiol. **11,** 488-493

SEGARRA, G., VAN DER ENT, S., TRILLAS, I. and PIETERSE, C.M. (2009) MYB72, a node of convergence in induced systemic resistance triggered by a fungal and a bacterial beneficial microbe. Plant Biol. **11,** 90-96

SELOSSE, M.A., BAUDOI, E. and VANDENKOORHUYSE, P. (2004) Symbiotic microorganisms, a key for ecological success and protection of plants. C. R. Biol. **327,** 639-648

SHAHOLLARI, B., VADASSERY, J., VARMA, A. and OELMÜLLER, R. (2007) A leucine rich repeat protein is required for growth promotion and enhanced seed production mediated by the endophytic fungus *Piriformospora indic*a in *Arabidopsis thaliana*. Plant J. **50,** 1-13

SHAHOLLARI, B., VARMA, A. and OELMÜLLER, R. (2005) Expression of a receptor kinase in *Arabidopsis* roots is stimulated by the basidiomycete *Piriformospora indica* and the protein accumulates in Triton X-100 insoluble plasma membrane microdomains. J. Plant Physiol. **162,** 945-958

SHAW, S.L. and LONG, S.R. (2003) Nod factor inhibition of reactive oxygen efflux in a host legume. Plant Physiol. **132,** 2196-2204

SHERAMETI, I., SHAHOLLARI, B., VENUS, Y., ALTSCHMIED, L., VARMA, A. and OELMÜLLER, R. (2005)

The endophytic fungus *Piriformospora indica* stimulates the expression of nitrate reductase and the starch-degrading enzyme glucan-water dikinase in tobacco and *Arabidopsis* roots through a homeodomain transcription factor that binds to a conserved motif in their promoters. J. Biol. Chem. **280,** 26241-26247

SHERAMETI, I., TRIPATHI, S., VARMA, A. and OELMÜLLER, R. (2008a) The root-colonizing endophyte *Piriformospora indica* confers drought tolerance in *Arabidopsis* by stimulating the expression of drought-stress related genes in leaves. Mol. Plant-Microbe Interact. **21,** 799-807

SHERAMETI, I., VENUS, Y., DRZEWIECKI, C., TRIPATHI, S., DAN, V.M., NITZ, I., VARMA, A., GRUNDLER, F.M. and OELMÜLLER, R. (2008b) PYK10, a β-glucosidase located in the endoplasmatic reticulum, is crucial for the beneficial interaction between *Arabidopsis thaliana* and the endophytic fungus *Piriformospora indica*. Plant J. **50,** 1-17

SIRRENBERG, A., GÖBEL, C., GROND, S., CZEMPINSKI, N., RATZINGER, A., KARLOVSKY, P., SANTOS, P., FEUSSNER, I. and PAWLOWSKI, K. (2007) *Piriformospora indica* affects plant growth by auxin production. Physiol. Plant. **131,** 581-589

SKALAMERA, D. and HEATH, M. (1998) Changes in the cytoskeleton accompanying infection-induced nuclear movements and the hypersensitive response in plant cells invaded by rust fungi. Plant J. **16,** 191–200

SMIRNOFF, N. and WHEELER, G.L. (2000) Ascorbic acid in plants: biosynthesis and function. Crit. Rev. Plant Sci. **19,** 267-290

SMIT, P., RAEDTS, J., PORTYANKO, V., DEBELLE, F., GOUGH, C., BISSELING, T. and GEURTS, R. (2005) NSP1 of the GRAS protein family is essential for rhizobial nod factor-induced transcription. Science **308,** 1789–1791

SPANU, P. and BONFANTE-FASOLO, P. (1988) Cell wall-bound peroxidase activity in roots of mycorrhizal *Allium porrum*. New Phytol. **109,** 119–124

STRACK, D., FESTER, T., HAUSE, B., SCHLIEMANN, W. and WALTER, M.H. (2003) Arbuscular mycorrhiza: biological, chemical, and molecular aspects. J. Chem. Ecol. **29,** 1955-1979

STRACKE, S., KISTNER, C., YOSHIDA, S., MULDER, L., SATO, S., KANEKO, T., TABATA, S., SANDAL, N., STOUGAARD, J., SZCZYGLOWSKI, K. et al. (2002) A plant receptor-like kinase required for both bacterial and fungal symbiosis. Nature **417,** 959-962

TAKEMOTO, D. and HARDHAM, A.R. (2004) The cytoskeleton as a regulator and target of biotic interactions in plants. Plant Physiol. **136,** 3864-3876

TAMASLOUKHT, M., SEJALON-DELMAS, N., KLUEVER, A. JAUNEAU, A., ROUX, C., BECARD, G. and FRANKEN, P. (2003) Root factors induce mitochondrial-related gene expression and fungal respiration during the developmental switch from asymbiosis to presymbiosis in the arbuscular mycorrhizal fungus *Gigaspora rosea*. Plant Physiol. **131,** 1468–1478

TANAKA, A., CHRISTENSEN, M.J., TAKEMOTO, D., PARK, P. and SCOTT, B. (2006) Reactive oxygen species play a role in regulating a fungus–perennial ryegrass mutualistic interaction. Plant Cell **18,** 1052-1066

TORRES, M.A., DANGL, J.L. and JONES, J.D. (2002) *Arabidopsis* gp91phox homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defense response. Proc. Natl. Acad. Sci. USA **99,** 517-522

TORRES, M.A., JONES, J.D. and DANGL, J.L. (2005) Pathogen-induced, NADPH oxidase-derived reactive oxygen intermediates suppress spread of cell death in Arabidopsis thaliana. Nat. Genet. **37,** 1130-1134

TREWAVAS, A. (1999) Le calcium, C'est la vie: calcium makes waves. Plant Physiol. **120,** 1-6

TIRICHINE, L., SANDAL, N., MADSEN, L.H., RADUTOIU, S., ALBREKTSEN, A.S., SATO, S., ASAMIZU, E., TABATA, S. and STOUGAARD, J. (2007) A gain-of-function mutation in a cytokinin receptor triggers spontaneous root nodule organogenesis. Science **315,** 104-107

TUDZYNSKI, B. and SHARON, A. (2002) Biosynthexis, biological role and application of fungal phytohormones. In: Osiewacz, H.D. (ed.) The Mycota X. Industrial Applications. Springer, Berlin, Heidelberg, New York, pp. 183-212

USHIMARU, T., NAKAGAWA, T., FUJIOKA, Y., DAICHO, K., NAITO, M., YAMAUCHI, Y., NONAKA, H., AMAKO, K., YAMAWAKI, K. and MURATA, N. (2006) Transgenic *Arabidopsis* plants expressing the rice dehydroascorbate reductase gene are resistant to salt stress. J. Plant Physiol. **163,** 1179-1184

USUKI, F., NARISAWA, K., YONEZAWA, M., KAKISHIMA, M. and HASHIBA, T. (2002) An efficient method for colonisation of Chinese cabbage by the root endophytic fungus *Heteroconium chaetospira*. J. Gen. Plant Pathol. **68,** 326-332

VADASSERY, J., RANF, S., DRZEWIECKI, C., MITHÖFER, A., MAZARS, C., SCHEEL, D., LEE, J. and OELMÜLLER, R. (2009a) A cell wall extract from *Piriformospora indica* promotes growth of *Arabidopsis* seedlings and induces intracellular calcium elevation in roots. Plant J. (in press)

VADASSERY, J., RITTER, C., VENUS, Y., CAMEHL, I., VARMA, A., SHAHOLLARI, B., NOVÁK, O., STRNAD, M., LUDWIG-MÜLLER, J. and OELMÜLLER, R. (2008) The role of auxins and cytokinins in the mutualistic interaction between *Arabidopsis* and *Piriformospora indica*. Mol. Plant-Microbe Interact. **21,** 1371-1383

VADASSERY, J., TRIPATHI, S., PRASAD, R., VARMA, A. and OELMÜLLER, R. (2009b) Monodehydroascorbate reductase 2 and dehydroascorbate reductase 5 are crucial for the mutualistic interaction between *Piriformospora indica* and *Arabidopsis*. J. Plant Physiol. (in press)

VAN DER LUIT, A.H., PIATTI, T., VAN DOORN, A., MUSGRAVE, A., FELIX, G., BOLLER, T. and MUNNIK, T. (2000) Elicitation of suspension-cultured tomato cells triggers the formation of phosphatidic acid and diacylglycerol pyrophosphate. Plant Physiol. **123**, 1507-1516

VAN WEES, S.C., VAN DER ENT, S. and PIETERSE, C.M. (2008) Plant immune responses triggered by beneficial microbes. Curr. Opin. Plant Biol. **11,** 443-448

VARMA, A. and OELMÜLLER, R. (2007) Soil Biology. Advanced Techniques in Soil Microbiology. Springer Heidelberg, Berlin

VARMA, A., SINGH, A., SUDHA, SAHAY, N., 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., HOCK, B., MAIER, W., WALTER, M., STRACK, D. and KRANNER, I. (2001) *Piriformospora indica*: A cultivable mycorrhiza-like endosymbiotic fungus. In: Mycota IX, Springer Series, Germany, pp. 123-150

VARMA, A., VERMA, S., SUDHA, SAHAY, N.S., BÜTEHORN, B. and FRANKEN, P. (1999) *Piriformospora indica*, a cultivable plant growth promoting root endophyte. Appl. Environm. Microbiol. **65,** 2741-2744

VERMA, S.A., VARMA, A., REXER, K.-H., HASSEL, A., KOST, G., SARBHOY, A., BISEN, P., BÜTEHORN, B. and FRANKEN, P. (1998) *Piriformospora indica*, gen. et sp. nov., a new root-colonizing fungus. Mycologia **90,** 898-905

VIERHEILIG, H., ALT, M., LANGE, J., GUT-RELLA, M., WIEMKEN, A. and BOLLER, T. (1995) Colonization of transgenic tobacco con stitutively expressing pathogenesis-related proteins by vesicular-arbuscular mycorrhizal fungus *Glomus moseae*. Appl. Environm. Microbiol. **61,** 3031-3034

VIERHEILIG, H., ALT, M., NEUHAUS, J.M., BOLLER, T. and WIEMKEN, A. (1993) Colonization of transgenic *Nicotiana sylvestris* plants, expressing different forms of *Nicotiana tabacumm* chitinase, by root pathogen *Rhizoctonia solani* and by the mycorrhizal symbiont *Glomus mosseae*. Mol. Plant–Microbe Interact. **6,** 261-264

WALLER, F., ACHATZ, B., BALTRUSCHAT, H. et al. (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

WEERASINGHE, R.R., BIRD, D.M. and ALLEN, N.S. (2005) Root-knot nematodes and bacterial Nod factors elicit common signal transduction events in *Lotus japonicus.* Proc. Natl. Acad. Sci. USA **102,** 3147-3152

WEIß, M., SELOSSE, M.A., REXER, K.H., URBAN, A. and OBERWINKLER, F. (2004) Sebacinales: a hitherto overlooked cosm of heterobasidiomycetes with a broad mycorrhizal potential. Mycol. Res. **108,** 1003-1010

YAMAGUCHI, T., MINAMI, E., UEKI, J. and SHIBUYA, N. (2005) Elicitor-induced activation of phospholipases plays an important role for the induction of defense responses in suspension-cultured rice cells. Plant Cell Physiol. **46,** 579-587

YOON, H., LEE, H., LEE, I., KIM, K. and JO, J. (2004) Molecular cloning of monodehydroascorbate reductase gene from *Brassica campestris* and analysis of its mRNA level in response to oxidative stress. Biochem. Biophys. Acta **1658,** 181-186

YOSHIDA, S., TAMAOKI, M., SHIKANO, T., NAKAJIMA, N., OGAWA, D., IOKI, M., AONO, M., KUBO, A., KAMADA, H., INOUE, Y. and SAJI, H. (2006) Cytosolic dehydroascorbate reductase is important for ozone tolerance in *Arabidopsis thaliana*. Plant Cell Physiol. **47,** 304-308

ZAPPEL, N.F. and PANSTRUGA, R. (2008) Heterogeneity and lateral compartmentalization of plant plasma membranes. Curr. Opin. Plant Biol. **11,** 632-640ZIPFEL, C. and FELIX, G. (2005) Plants and animals: a different taste for microbes? Curr. Opin. Plant Biol. **8,** 353-360

ZIPFEL, C. and FELIX, G. (2005) Plants and animals: a different taste for microbes? Curr. Opin. Plant Biol. **8,** 353-360

ZIPFEL, C., ROBATZEK, S., NAVARRO, L., OAKELEY, E.J., JONES, J.D., FELIX, G. and BOLLER, T. (2004) Bacterial disease resistance in *Arabidopsis* through flagellin perception. Nature **428,** 764-767