

Article Addendum

Induced resistance triggered by *Piriformospora indica*

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The root endophytic Basidiomycete *Piriformospora indica* forms a specific type of mycorrhiza symbiosis with a broad spectrum of plant species, including the Brassicaceae. A recent report on the interaction of *P. indica* with *Arabidopsis thaliana* suggests that the fungus induces a mode of resistance to microbial pathogens reminiscent of Induced Systemic Resistance (ISR) first discovered with non-pathogenic rhizobacteria. The characteristics of *P. indica* mediated resistance are the dependency on JA-signalling and the cytosolic function of the master regulator protein Non-expressor-of-PR-genes 1 (NPR1), a low level of altered systemic gene expression in leaves before pathogen challenge, the induction of the JA-inducible marker gene *vegetative storage protein 1 (VSP1)* after pathogen challenge, and an independency of the resistance phenotype from salicylate biosynthesis and signalling. We discuss here two more factors regarding the *P. indica*-mediated ISR response: the role of the plant hormone ethylene as well as a possible contribution of the recently discovered close association of *P. indica* with the α -proteobacterium *Rhizobium radiobacter*.

Introduction

In addition to innate immunity and R gene-based resistance, induced resistance is one of the main mechanisms utilized by plants to protect themselves against a broad range of microbial pathogens. Certain biological or chemical agents can trigger this kind of resistance. In general, two forms of induced resistance are distinguished. The pathogen induced Systemic Acquired Resistance (SAR) refers to the case, in which non-infected parts of locally infected plants become more resistant to further infection.¹ Induced Systemic Resistance (ISR) on the other hand is triggered by strains of non-pathogenic rhizobacteria.²⁻⁴ Next to rhizobacteria also certain fungi can systemically protect plants against infections by pathogens.^{5,6} Whereas SAR is based on salicylic acid (SA) synthesis and signal-

ling, ISR by contrast often relies on an enhancement of jasmonate (JA)- and/or ethylene (ET)-dependent defence.^{7,8} Both mechanisms require the key regulator Non-expressor-of-PR-genes 1 (NPR1) though the biochemical mechanisms involving this protein are different in SAR and ISR.⁹

The here described root endophytic fungus *Piriformospora indica* belongs to the order Sebaciniales (Basidiomycota)¹⁰ and forms a mutualistic symbiosis with a broad spectrum of host plants, such as barley, maize, *Arabidopsis*, tomato and tobacco.¹¹⁻¹³ In barley the fungus induces resistance to root diseases and leads to systemic protection against powdery mildew caused by *Blumeria graminis* f. sp. *hordei*.^{14,15} Lately, we showed that *P. indica* similarly induces systemic resistance to *Golovinomyces orontii* in *Arabidopsis*.¹⁶

P. indica Induced Resistance Resembles ISR

P. indica-mediated resistance in *Arabidopsis* against the powdery mildew *G. orontii* shows clear parallels to JA and ET requiring ISR.¹⁶ The jasmonate-insensitive mutants *jasmonate-resistant 1 (jar1-1)*¹⁷ and *jasmonate-insensitive 1 (jin 1)*¹⁸ as well as the null mutant *npr1-1* [Nonexpressor of pathogenesis-related (PR) genes 1, also known as NIM1]¹⁹ are compromised in *P. indica*-mediated resistance. All these mutants define genes known to be involved in JA signaling. By contrast, *NahG* plants expressing a bacterial salicylate-hydroxylase²⁰ and the *npr1-3* mutant, lacking the nuclear-localisation signal, were not affected in *P. indica* mediated resistance to *G. orontii*. Both of them are defective in salicylate-governed SAR. Hence, *P. indica* induced systemic resistance against powdery mildew requires the transcriptional regulator JIN1/AtMYC2, the JA signalling component JAR1 (a JA-amino synthetase)²¹ and the cytosolic function of NPR1, but does not require elevated SA levels nor the nuclear function of NPR1 (which is compromised in *npr1-1* but not in *npr1-3*). Thus, the mutational analysis suggests that *P. indica* exploits mechanism known for ISR.

ISR is accompanied by a rather weak or even not detectable systemic up or downregulation of transcripts in the absence of a challenging pathogen.^{22,23} Accordingly, leaves of *P. indica* colonized and non-colonized plants showed comparable, non-induced levels of SA-, JA- and ET-responsive genes.¹⁶ Only after powdery mildew challenge a stronger expression of the JA-inducible vegetative storage protein gene *VSP1* was observed exclusively in *P. indica* colonized plants. A similar response of *Arabidopsis* was described for a vegetative storage protein during rhizobacteria-induced ISR.²⁴ The stronger *VSP1* induction in *P. indica* colonized plants after pathogen challenge not only substantiates the role of JA in *P. indica* induced resistance. It also

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indicates a potentiated defence response of these plants, suggesting that “priming” is also associated with the *P. indica* symbiosis.

***P. indica* Induced Resistance is not Strongly Dependent on Ethylene Signalling**

Ethylene has been shown to play a role during ISR for a variety but not all interactions between resistance-inducing bacteria and plants. While a requirement of the ethylene pathway has been reported for ISR conferred by *Pseudomonas fluorescens* WCS417r,^{7,25} ISR mediated by *P. fluorescens* CHA0r against *Peronospora parasitica* is independent of the ethylene receptor ETR1 and the downstream signalling component EIN2.²⁶ Mutants defective in *ETR1* or *EIN2* are impaired in ethylene signalling.^{27,28} Preliminary results indicate that *P. indica*-mediated resistance might also be independent of ethylene signalling since the ISR response against *G. orontii* was not fully compromised in *ein2-1* and *etr1-3*. In these experiments, the mutants showed a slight reduction of *G. orontii* conidiophores per mycelium in *P. indica* colonized plants. Moreover, the amount of *G. orontii* conidia formed 10 days after powdery mildew inoculation per mg of leaf fresh weight was also reduced. The interpretation of these data is complex since the penetration process and subsequent colonization of Arabidopsis roots is strongly influenced by the plant's ethylene biosynthesis and signalling (Schäfer et al., in preparation). An observed lower colonisation level in these mutants might lead to reduced ISR, which is consistent with the finding that the biological effects conferred to host plants is dependent on the concentration of *P. indica* inoculum (Jakobs S, Molitor A, Waller F unpublished).

Working in Concert: Bacterial Associations

The interpretation of the biological activity conferred by *P. indica* to host plants is further complicated by the fact that all Sebaciniales so far investigated are associated with bacteria.²⁹ For *P. indica* a close association to the α -proteobacterium *Rhizobium radiobacter* has been demonstrated. Although all attempts to cure *P. indica* from these bacteria failed, it was possible to produce *R. radiobacter* in pure culture. In experiments examining the biological activity of *R. radiobacter* in barley, Sharma et al.²⁹ proved the potential of the bacteria to induce growth promotion and systemic resistance to barley powdery mildew. In addition, the bacterium induced systemic resistance in Arabidopsis against *G. orontii*.³⁰ Consistent with the results obtained for *P. indica*-mediated resistance, a screen of Arabidopsis mutants indicated a requirement of JA and the cytosolic function of NPR1 but no requirement for SA-signalling during *R. radiobacter*-mediated resistance.³⁰ Comparing fungal and bacterial effects the biological activity exerted by *P. indica* in association with *R. radiobacter* as compared with the pure bacterium could hardly be distinguished. Especially the individual impact of the fungus and the bacterium on the observed ISR responses, the interplay between the two microorganisms, and their additive impact on their host remain to be elucidated.

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