Taylor & Francis
Taylor & Francis Group

RESEARCH PAPER

Check for updates

Piriformospora indica-induced phytohormone changes and root colonization strategies are highly host-specific

Huichun Liu [a], Rajendran Senthilkumar[b,c], Guangying Ma[a], Qingcheng Zou[a], Kaiyuan Zhu[a], Xiaolan Shen[a], Danqing Tian[a], Moda Sang Hua[b], Ralf Oelmüller[d], and Kai Wun Yeh[b]

[a]Research & Development Center of Flower, Zhejiang Academy of Agricultural Sciences, Hangzhou, China; [b]Institute of Plant Biology, College of Life Science, National Taiwan University, Taipei, Taiwan; [c]Academia Sinica-Biotechnology Center in Southern Taiwan, Tainan, Taiwan; [d]Matthias-Schleiden Institute, Plant Physiology, Friedrich-Schiller-University Jena, Jena, Germany

ABSTRACT

Piriformospora indica, an endophytic fungus of Sebacinales, has a wide host range and promotes the performance of mono- and eudicot plants. Here, we compare the interaction of P. indica with the roots of seven host plants (Anthurium andraeanum, Arabidopsis thaliana, Brassica campestris, Lycopersicon esculentum, Oncidium orchid, Oryza sativa, and Zea mays). Microscopical analyses showed that the colonization time and the mode of hyphal invasion into the roots differ in the symbiotic interactions. Substantial differences between the species were also observed for the levels and accumulation of jasmonate (JA) and gibberellin (GA) and the transcript levels for genes involved in their syntheses. No obvious correlation could be detected between the endogenous JA and/or GA levels and the time point of root colonization in a given plant species. Our results suggest that root colonization strategies and changes in the two phytohormone levels are highly host-specific.

Introduction

Piriformospora indica, an endophytic fungus of Sebacinales, was first isolated from the rhizosphere of two woody shrubs in the Indian Thar Desert.[1,2] The root-colonizing fungus has a broad host range, which includes bryophytes, pteridophytes, gymnosperms, and all tested monocot and eudicot plants.[3–13] P. indica colonizes the roots, grows inter- and intracellularly, forms pear-shaped and auto-fluorescent spores in the cortex and rhizosphere zone, but does not enter the endodermis and aerial parts of the plants.[2] Root colonization does not cause pathogenic symptoms in the roots and aerial parts of the plants. Conversely, P. indica improves nutrition uptake into the host plant,[14–17] promotes biomass production,[7,18–20] induces early flowering[19,20] and stimulates the accumulation of secondary metabolites.[17,21] Furthermore, the endophyte also enhances the plant´s resistance to biotic and abiotic stresses such as pathogens, insects, nematodes, high-temperature stress, salinity, drought, and radiation.[4,19,22–24]

Because of these benefits, many researchers use P. indica as a model system to understand the molecular, biochemical and cellular mechanisms leading to a beneficial symbiotic interaction.[25–31] However, huge differences in the responses to P. indica are described for different plant species, such as Chinese cabbage,[4] maize,[17] orchid,[31] tomato,[3] Arabidopsis[7] or barley.[32] Furthermore, the interaction of P. indica with A. thaliana or barley roots results in a quite different regulation of genes involved in phytohormone metabolism,[33,34] and the role of phytohormones in the symbiotic interaction is

a matter of debate.[4,34–36] The broad host range of P. indica suggests that the mutualist has evolved highly effective interaction and colonization strategies. Therefore, it is conceivable that the fungus recruits similar or identical hormone and signaling pathways from the plants for the establishment of a successful symbiosis. Successful root colonization requires also the repression of the host innate immunity.[26] Beneficial microbes manipulate the levels of the plant hormones jasmonic acid (JA), abscisic acid (ABA), salicylic acid (SA), gibberellic acid (GA) and ethylene (ET) in order to balance innate immune responses and the progression of root colonization. The quite different reports on the involvement of hormones in the colonization process in different host plants make it difficult to understand which primary targets are used by P. indica. Furthermore, the fungus protects Arabidopsis seedlings against pests, such as powdery mildew via JA, but not SA signaling.[37] Zarea et al.,[38] as well as Molitor et al.[5] reported that P. indica induces different biotic and abiotic stress tolerance and hormone responses in different host species. Barley plants impaired in GA synthesis and perception showed reduced mutualistic colonization, which was associated with an elevated expression of defense-related genes. GA and ET are also recruited by P. indica for correct root colonization and growth promotion in Arabidopsis.[26,33,34,36,39,40] These often conflicting reports raise the question whether the described differences are caused by different experimental conditions or reflect differences in the host response to the endophyte. The goal of this study was to compare the interaction of P. indica with different host species under

comparable growth conditions. We focus on root colonization, changes in the phytohormone levels and a few phytohormone-related mRNA levels to understand principles in the symbiotic interaction of the fungus with different host species.

Results

P. indica *colonizes the roots of different plant species at different time points and with different colonization strategies*

In order to investigate how *P. indica* colonizes the roots of different plant species, the efficiency and velocity of root colonization of seven different plant species were compared. The roots were exposed to the same amount of fungal hyphae, and the distance between the hyphal plaque and the roots of the maturation zone was the same for all plant species.[41] Progression

of colonization was detected microscopically (Figure 1) and by qPCR analyses (Figure 2). Microscopic observations were used to monitor the invasion and propagation of hyphae in the roots, and to detect the earliest root colonization time point and number of hyphae that enters the root cells. Criteria for a successful interaction were the initiation of root hair development after hyphae were detected around the primary and lateral roots. Following this, we monitored the progression of hyphal growth that started to invade between individual epidermal cells. Finally, root cells were scored based on the enlarged cortex and thick epidermal and cortex layers. According to Figure 1 and Table 1, extracellular colonization of *A. thaliana* roots is already visible 2 days after infection (dai). At this time point, hyphae can be detected around the primary and lateral roots and started to invade into the root tissue. The hyphae can be detected between individual epidermal cells but did not invade the root cells yet. Only 4 dai, we observe a few roots cells in which hyphae are
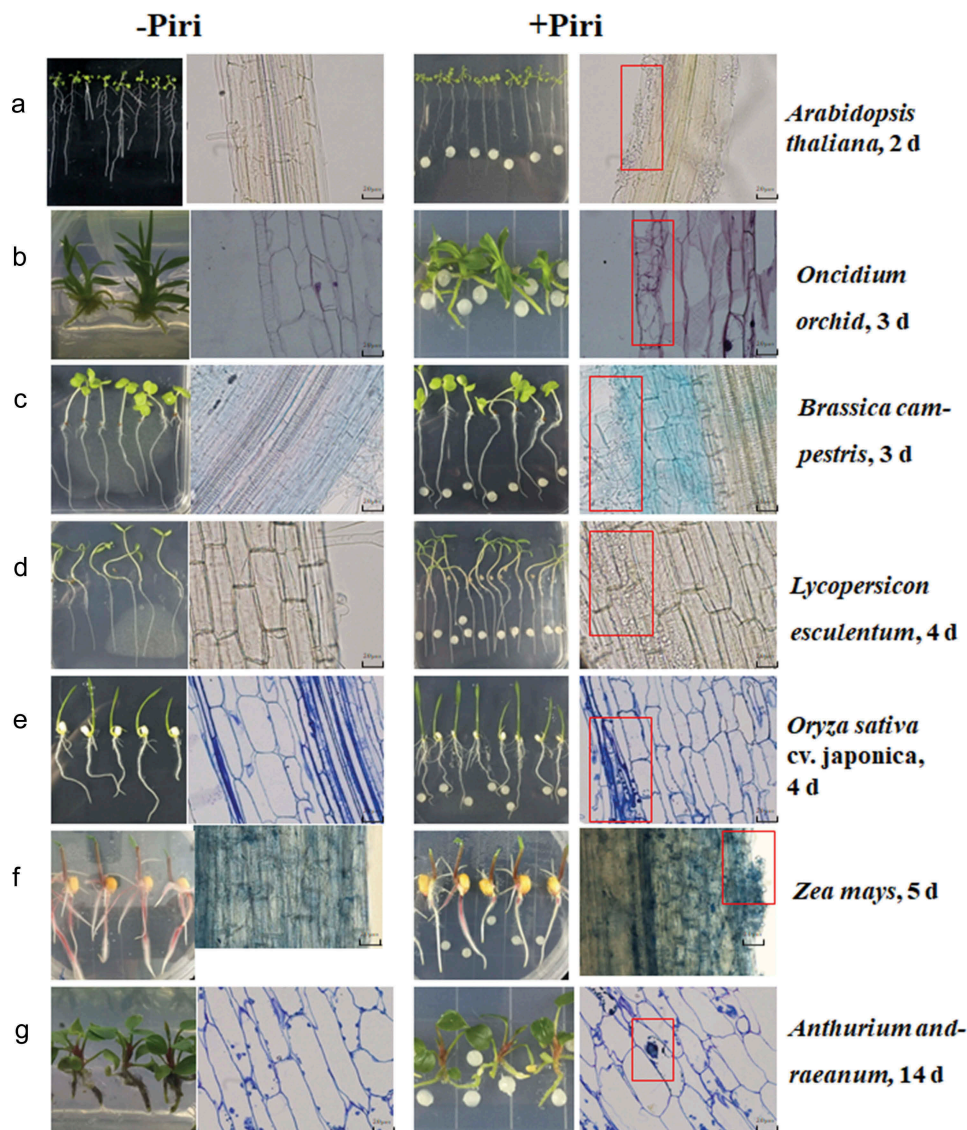

Figure 1. Microscopic detection of root colonization of different host species by *P. indica*. Root segments, starting 3 cm away from the root tip, were cut into 2 cm sections for microscopic analyses. Left panel: mock treatment, right panel: co-cultivation with P. indica. The pictures show root section from the seven investigated plant species stained with lactophenol or trypan blue between 2 and 14 dai. (a) *A. thaliana*, 2 dai; (b) *Oncidium* orchid, 3 dai; (c) *Brassica campestris*, 3 dai; (d) *Lycopersicon esculentum*, 4 dai; (e) *Oryza sativa cv. japonica*, 4 dai; (f) *Zea mays*, 5 dai; (g) *A. andraeanum*, 14 dai. Bar = 20 um. Red boxes highlight sections colonized by P indica in root tissues.
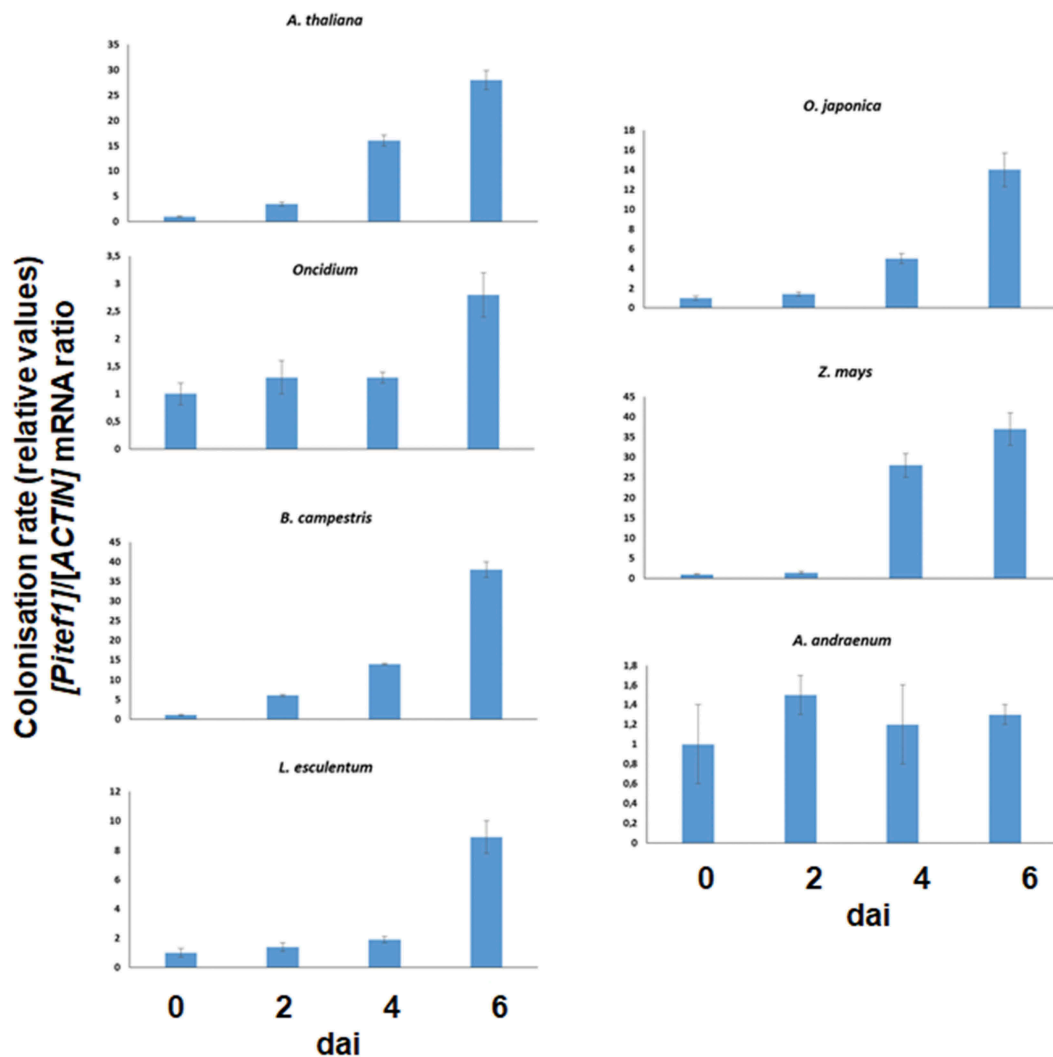
Figure 2. qPCR analyses for root colonization of different host species by *P. indica*. The plants were co-cultivated with *P. indica* for 0, 2, 4 and 6 days and the relative colonization rate determined as described in the Methods and Material chapter. The data are based on three independent experiments, and bar represents SEs. The values for the time points 0 were set as 1.0, and the other values were expressed relative to them.

Table 1. Earliest time point in days at which a successful colonization of the roots of various plant species by *P. indica* can be detected.

Plant type	Species	Successful colonization
Eudicot	*Arabidopsis thaliana*	2 dai
Monocot	*Oncidium* orchid	3 dai
Eudicot	*Brassica campestris*	3 dai
Eudicot	*Lycopersicon esculentum*	4 dai
Monocot	*Oryza sativa* cv. Japonica	4 dai
Monocot	*Zea mays*	5 dai
Monocot	*Anthurium andraeanum*	14 dai

invaded. In contrast to Arabidopsis, only 3 dai, the first hyphae are visible around the epidermal cells of *B. campetris* and *Oncidium* orchid, and single rhizodermal cells have already penetrated into the cells of the host roots without the formation of specific penetration organs (Figure 1 and Table 1). Therefore, while root colonization and the rarely occurring invasion of the hyphae into the root cells can be separated temporarily in Arabidopsis, it appears that in *B. campetris* and *Oncidium* orchid roots, hyphal invasion into root cells occurs almost immediately after the contact is established. *Lycopersicon esculentum* and *Oryza sativa* cv. Japonica appear to follow the Arabidopsis

colonization strategy, and we observed first the formation of a hyphal network on the root surface and between the epidermal cells. The first hyphae which invaded the root cells were only detected one day later. The establishment of the physical contact between hyphae and roots of *L. esculentum* and *O. sativa* cv. Japonica is slower, and the first hyphae attached to their root surfaces can only be detected 4 dai. Immediately after the attachment, the hyphae start to form intra-cellular hyphae in *L. esculentum* roots, whereas in case of *O. sativa* cv. Japonica, the vast majority of the *P. indica* mycelium remains on the root surface (Figure 1 and Table 1). Entry into the root cells is rare. Thus, the time points of extracellular root colonization, invasion into the root tissue and entry into root cells appear to be quite different for the different plant species: e.g., 4 dai, when Arabidopsis, *B. campestris* and *Oncidium* orchid roots are already massively colonized, the colonization of *L. esculentum* and *O. sativa* cv. Japonica roots is still developing. The colonization process for *Zea mays* plants is even slower. Three dai, no physical contact between the hyphae and roots can be detected yet. Having established the contact, a rapid progression of the symbiotic interaction is visible. Between the 5th and the 6th day,

a massive propagation of hyphae in the intracellular space of the roots is visible, which is also accompanied by the entry of hyphal inside of the *Z. mays* root cells (Figure 1 and Table 1). Thus, compared to the other described plant species, the establishment of the symbiotic interaction starts later but progresses fast once the contact is established. At the 6th day, the massive growth of the fungus results in a hyphal network that is found throughout the analyzed root sections, and such a massive hyphal growth within 2 days after the establishment of the first contact was never observed for the other plant species. Figure 1(f) demonstrates that the colonization pattern of the maize roots is quite different from the patterns observed for the other plant species, while hyphae can be detected almost everywhere in the maize roots, the fungal propagation in the other species is more focused on specific root sections which are highly colonized while other sections remain initially uncolonized.

Within the first 6 dai with *P. indica*, fungal sporulation could not be seen in any of the six host plants described so far. Furthermore, during the entire period (6 days), we did not observe significant changes in growth rates, leaf sizes, or morphology of the areal parts of the investigated plant species between *P. indica*-colonized and mock-treated plants. The first morphological changes, that can be detected, occur between 5 and 7dai, and the roots become visibly bushier. This phenotypic development is again very host-specific. While the bushy phenotype is most obvious for Chinese cabbage, it is less developed in Arabidopsis, followed by maize, rice, tomato and finally *Oncidium*. Apparently, the velocity of root colonization does not translate into corresponding alterations in root development. Finally, the symbiotic interaction between *P. indica* and *Anthurium andraeanum* starts only 14 dai. Once the hyphae reach the roots, a hyphal network around the roots and between the epidermal and subepidermal root cells become rapidly established, and the first few hyphae can be detected in the root epidermis of the host, almost immediately after the colonization occurred (Figure 1 and Table 1). Within the experimental period of time, we did not observe any visible alterations of the root phenotype by the fungus. These results demonstrate that the onset of root colonization (hyphae found on the surface of the primary and secondary roots as well as root hairs, entry of the hyphae into the root tissue, and occasional entry of the hyphae into the root cells) and reprogramming of root development by the fungus are quite different among the seven plant species.

Quantification of root colonization using the fungal RNA for the translation elongation factor 1 (*Pitef1*) relative to the plant RNA for actin basically confirms these results, although the differences observed by microscopy cannot be resolved by this technique (Figure 2). Overall, except for *A. andraeanum*, we observed an increase in the fungal RNA within the first 6 dai. As expected the kinetics are different for the seven plant species tested.

The JA and GA levels in the roots of the hosts respond differently to root colonization by P. indica

Previous studies have shown that symbiotic interactions are often associated with changes in the host's phytohormone levels, including JA and GA. In order to test whether co-cultivation of the roots of the different plant species affects

the host´s phytohormone levels, the JA and GA concentrations were determined in the roots of *P. indica*-exposed plants 0, 2, 4, and 6 dai.

Interestingly, we observed remarkable differences among the seven plant species (Figure 3). In *A. thaliana*, the JA levels remained low, while the GA level strongly increased 2 dai and dropped again until the end of experimentation. In Chinese cabbage roots, we observed an increase in the JA level which reached its maximum 6 dai, while the GA level remained more or less constant over the period of experimentation. In *Anthurium* roots, the JA level did not respond to *P. indica*, while a strong, more than 100-fold increase was observed for the GA level 6 dai. The JA level in rice roots increased 3–5 times 2 and 4 dai and dropped again, while the GA level did not change at all. In *Oncidium* roots, the JA level initially dropped, but the hormone started to re-accumulate at the end of the experiment, while the GA level is initially high and decreased dramatically 4 and 6 dai. In tomato roots, both phytohormones started to accumulate at the end of the experiment, while in maize roots, only the GA, but not the JA level showed a transient, but significant increase 2 and 4 dai. In addition, linear regression analysis showed that there is a negative correlation between *P. indica* colonization period and the JA hormone level for Chinese cabbage ($R^2 = 0.21$, $p > .05$). However, the relationship between *P. indica* colonization and JA was positively correlated for Arabidopsis ($R^2 = 0.024$, $p > .05$) and tomato ($R^2 = 0.21$, $p > .05$). Similarly, for monocots, only maize ($R^2 = 0.025$) showed a positive correlation for *P. indica* colonization (Figure 4). Interestingly, a positive correlation was noted for the GA levels and root colonization for dicots (Arabidopsis ($R^2 = 0.001$, $p > .05$), Chinese cabbage ($R^2 = 0.046$, $p > .05$) and tomato ($R^2 = 0.16$, $p > .05$), although the correlation was weak in significance. For the monocots *Oncidium* ($R^2 = 0.051$, $p > .05$), rice ($R^2 = 0.038$, $p < .05$), maize ($R^2 = 0.075$, $p < .05$) and *Anthurium* ($R^2 = 0.071$, $p < .05$)), the correlation was negative for *P. indica* colonization, but the analyses showed a strong significance (Figure 4). Based on these results, it is obvious that different plants show quite different responses in the regulation of their phytohormone levels in response to *P. indica* colonization. An obvious correlation between root colonization and changes in the phytohormone levels has been detected for the individual hosts, and it appears that the fungus has to cope with quite different JA and GA levels in the roots of the different host species.

P. indica induces host genes involved in the biosynthesis of JA, GA, and ethylene (ET)

To further investigate the role of phytohormones during early phases of root colonization, the expression of marker genes for JA and GA synthesis (*LOX1, AOC1, OPR3*, and *GA20-oxidase*) were analyzed by qRT-PCR. The ET-responsive *ERF1* gene was also included in our study.

Again, the gene expression pattern differed for different hosts (Figure 5). For instance, the *LOX1, AOC1* and *OPR3* mRNA levels representing JA biosynthesis remained relatively constant for *A. thaliana*, while an increase can be detected for Chinese cabbage within the first 4 dai. Furthermore, while the *GA20* RNA level increased in both species, the *AOC1* RNA level
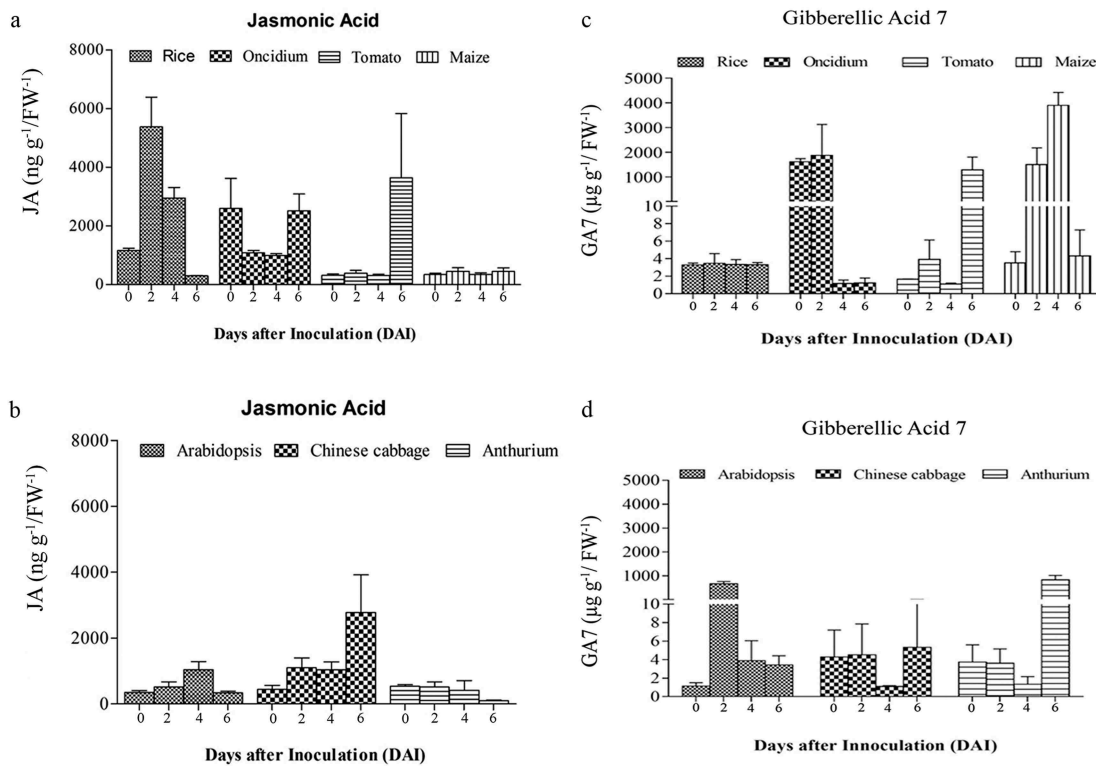
Figure 3. JA and GA7 levels in the roots of seven plant species after infection with *P. indica*. The roots of A. *thaliana, Oncidium* orchid, *Brassica campestris, Lycopersicon esculentum, Oryza sativa* cv. Japonica, *Zea mays* and *Anthurium andraeanum* were harvested 0, 2, 4, and 6 dai for the determination of the JA (a and b) and GA7 levels (c and d). For details, see Methods and Materials. The values are means ± SDs of three independent biological replications.

increased in Chinese cabbage roots, but not in *A. thaliana* roots. In tomato, only the *OPR3* RNA level appears to increase initially. In rice and maize, the *AOC1, OPR3* and *GA20* RNA levels appear to increase slightly during early colonization stages. Finally, in orchid and *A. andraeanum* roots, all transcript levels decrease after the 2 dai. The *ERF1* mRNA level was initially high in the seven species (Figure 5). In Chinese cabbage, it increased significantly until the 4th dai and decreased thereafter. In Arabidopsis, tomato, rice, and *A. draeanum*, the level decreased in response to *P. indica* infection. In Oncidium orchid, the level increased until 2nd dai and decreased dramatically during the later co-cultivation stages. In contrast, the *ERF1* mRNA level in maize decreased initially until 2nd dai but increased strongly again. We also analyzed the expression levels of these genes at 8 and 10 dai in *A. andraenum* roots, because they became colonized much later than the other species. The mRNA levels for the JA- and GA-related genes still declined, and the *AOC1* expression level was too low to be detected by our means (Supplementary Figure 1). Interestingly, the ET-related *ERF1* mRNA level increased and was higher 8 and 10 dai when compared to 6 dai suggesting that the *ERF1* mRNA level appears to respond to root colonization (Figure 5). Linear regression analyses for *P. indica* colonization and the expression of the tested genes often showed a good relationship across the plant species (Figure 6). For instance, *LOX1* expression in Arabidopsis ($R^2 = 0.067$, $p < .05$) and Chinese cabbage ($R^2 = 0.32$, $p > .05$) and *AOC1* expression in Arabidopsis ($R^2 = 0.51$, $p < .05$) and Chinese cabbage ($R^2 = 0.08232$, $p > .05$) showed

a positive correlation, whereas *LOX1* ($R^2 = 0.22$, $p > .05$) and *AOC1* ($R^2 = 0.64$, $p < .05$) expression were negatively correlated to root colonization in tomato. Also, *GA20* expression in Arabidopsis ($R^2 = 0.83$, $p > .05$), Chinese cabbage ($R^2 = 0.15$, $p > .05$) and tomato ($R^2 = 0.083$, $p > .05$) as well as *OPR3* expression in Arabidopsis ($R^2 = 0.043$, $p > .05$), Chinese cabbage ($R^2 = 0.15$, $p > .05$) and tomato ($R^2 = 0.033$, $p > .05$) showed a positive relationship to root colonization (Figure 6), while *ERF1* expression was only positively related to root colonization in Chinese cabbage ($R^2 = 0.24$, $p > .05$) (Figure 6). Interestingly, while the expressions of *LOX1, AOC1, OPR3, GA20*, and *ERF3* exhibited a strong positive linear relationship to *P. indica* colonization in maize and rice (Figure 6), this was not the case for *Oncidium* and *Anthurium*. Taken together, our results demonstrate that various hormone-related mRNA levels respond quite differently to the presence of *P. indica*.

Discussion

We demonstrate that the time point of root colonization by *P. indica* is highly specific for each host (Figure 1) and cannot be explained by changes in the endogenous JA and GA levels in the roots which are induced by *P. indica* (Figures 1–3). However, for an individual host, we often observed a correlation between the expression of the investigated phytohormone-related genes and the root colonization strategy (Figures 3 and 4), but these correlations were found for specific gene/host combinations and cannot be generalized.
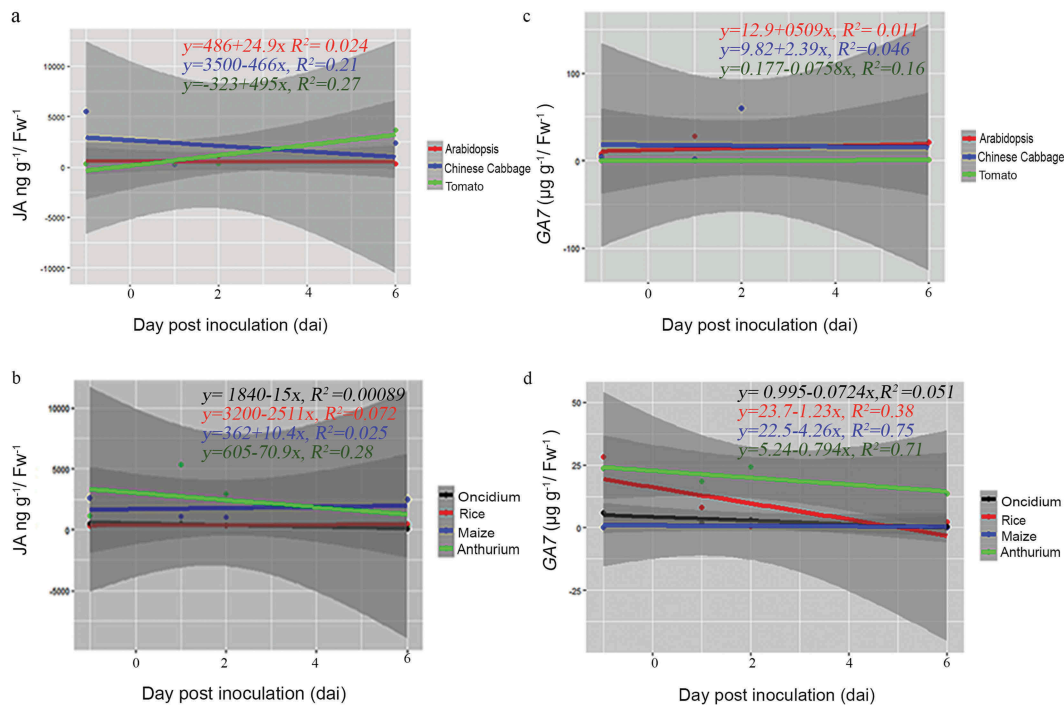
Figure 4. Scatter plots showing linear relationship between *P. indica* colonization and phytohormones (JA-, GA-) levels across plant species. Correlation of each dicot and monocot plants, *P indica* colonization period in days and JA (a and b) and GA (c and d) levels by rejecting 'null' hypothesis (equals to zero) are shown by linear regression analysis. Summary of regression coefficient values and R^2 values are represented. Testing the significance of the differences by the comparison of dependent (y) and independent variables (x) ($p \leq 0.05$ or ≥ 0.05) was performed using R-packages. The graphs are for statistical purpose only.

It appears that enormous differences in the endogenous phytohormone levels in the roots before the presence of the fungus might also be important for colonization, since the hormone balance is critical for overcoming the plant innate immunity. In some species, such as Chinese cabbage, the innate immunity appears to be immediately activated after exposure of the roots to *P. indica*, as reflected by the stimulation of JA and GA accumulation to orchestrate a complicated and interactive network of defense pathways. In other cases, these phytohormone levels do not respond strongly to the presence of the fungus (orchid and tomato) or occur only after colonization, such as in rice (Figures 1–3). The different responses of the JA levels in the host´s roots investigated in this study indicate that the fungus induces different threats either because the hosts recognize them differently, endogenous hormone levels restrict the fungal entry into the host or the fungus has different access to the root cells, e.g. due to different physical barriers. As long as the plant does not know whether it is approached by a beneficial or pathogenic fungus, it activates a mild JA response to restrict fungal growth in the roots and its rapid distribution. JA signaling affects other phytohormone functions including GA and ET signaling to prevent overcolonization and consequently loss of the benefits for the plants, since the plant resources have to be redirected from growth to defense when the roots become dominated by the overgrowing fungal hyphae. It appears that different species have established different mechanisms to cope with *P. indica*. It would be interesting to know whether the same or different fungal compound(s) induce the different responses in the host plants, and whether the fungus utilizes different strategies for a successful root colonization in different host species.

GA has been investigated in innate immune responses and early flowering induced by *P. indica* in various plant species.[20,42] Cosme et al.[43] showed that *P. indica* helps rice plants to tolerate root herbivory, and GA functions as a signal component of inducible plant tolerance against the biotic stress. Barley mutants impaired in GA synthesis, as well as perception, showed reduced colonization by *P. indica* which implicates that GA functions as a modulator of the root's basal defense.[34] A quintuple-DELLA mutant displaying constitutive gibberellin (GA) responses and the GA-biosynthesis mutant *ga1-6* (for *GA requiring 1*) showed higher and lower degrees of colonization in Arabidopsis roots, respectively, suggesting that *P. indica* recruits GA signaling to help establish root cell colonization.[26] Ent-kaurene synthases and ent-kaurene-like synthases are involved in the biosynthesis of phytoalexins and/or GAs. Li et al.[44] demonstrated that kaurene synthase activity is required for successful root colonization of *P. indica* in barley. Finally, *P. indica*-induced growth promotion of Chinese cabbage and barley seedlings correlated to an increased GA level in the colonized roots,[4,34] and the *GA2ox* gene involved in the inactivation of GA was downregulated in *P. indica*-colonized barley roots.[34,45] How these two phytohormones interact in order to control root colonization and entry of fungal hyphae into the root cell is a matter of intensive studies. However, our study clearly demonstrates that generalization over plant species is not possible.

The morphological observation also revealed that the growth pattern of root tissue was varied among the seven plant species.
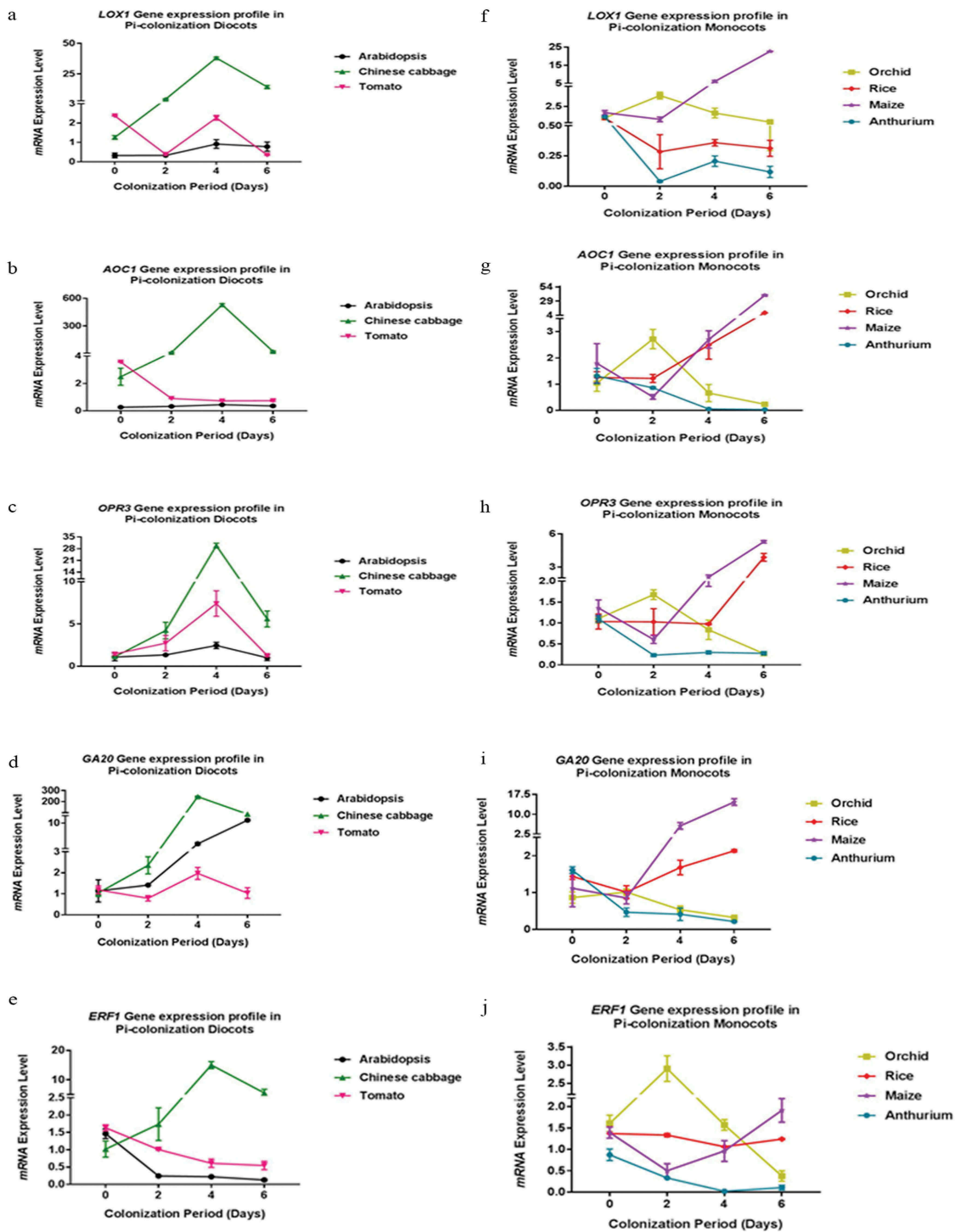
Figure 5. Relative mRNA levels for JA-, GA-, and ET-related genes in the roots of seven plant species after infection with P. indica for 0, 2, 4, and 6 dai. (a–j) The RNA levels of the JA synthesis-related *LOX1*, *AOC1*, and *OPR3*, the GA-responsive GA20-oxidase and the ET-related *ERF1* genes were analyzed in the roots of *A. thaliana*, *Oneidium* orchid, *Brassica campestris*, *Lycopersicon esculentum*, *Oryza sativa* cv. Japonica, *Zea mays* and *A. andraeanum* at 0, 2, 4, and 6 dai by qRT-PCT. For the latter species, additional data are also presented for later time points. The mRNA levels were normalized to those of the ACTIN mRNA level. The values are means ± SDs of three independent biological replications.

Especially, Chinese cabbage showed an obvious bushy root phenotype and Arabidopsis produces more branches. These observations are consistent with previous reports.[4] However, no changes were observed in *Oncidium* and *Anthurium*. The results demonstrated that plant has established a different mechanism to reprogram the host responses after *P. indica* colonization.

In this study, four monocots (orchid, rice, maize, and *Anthurium*) and three eudicots (Arabidopsis, cabbage and tomato) were cultivated with *P. indica*. Previous studies showed that *P. indica* preferentially colonizes the maturation zone of roots, and that fungal hyphae appeared later in the meristematic and elongation zones.[26,41,46] Therefore, microscopic analyses of colonization were performed with samples
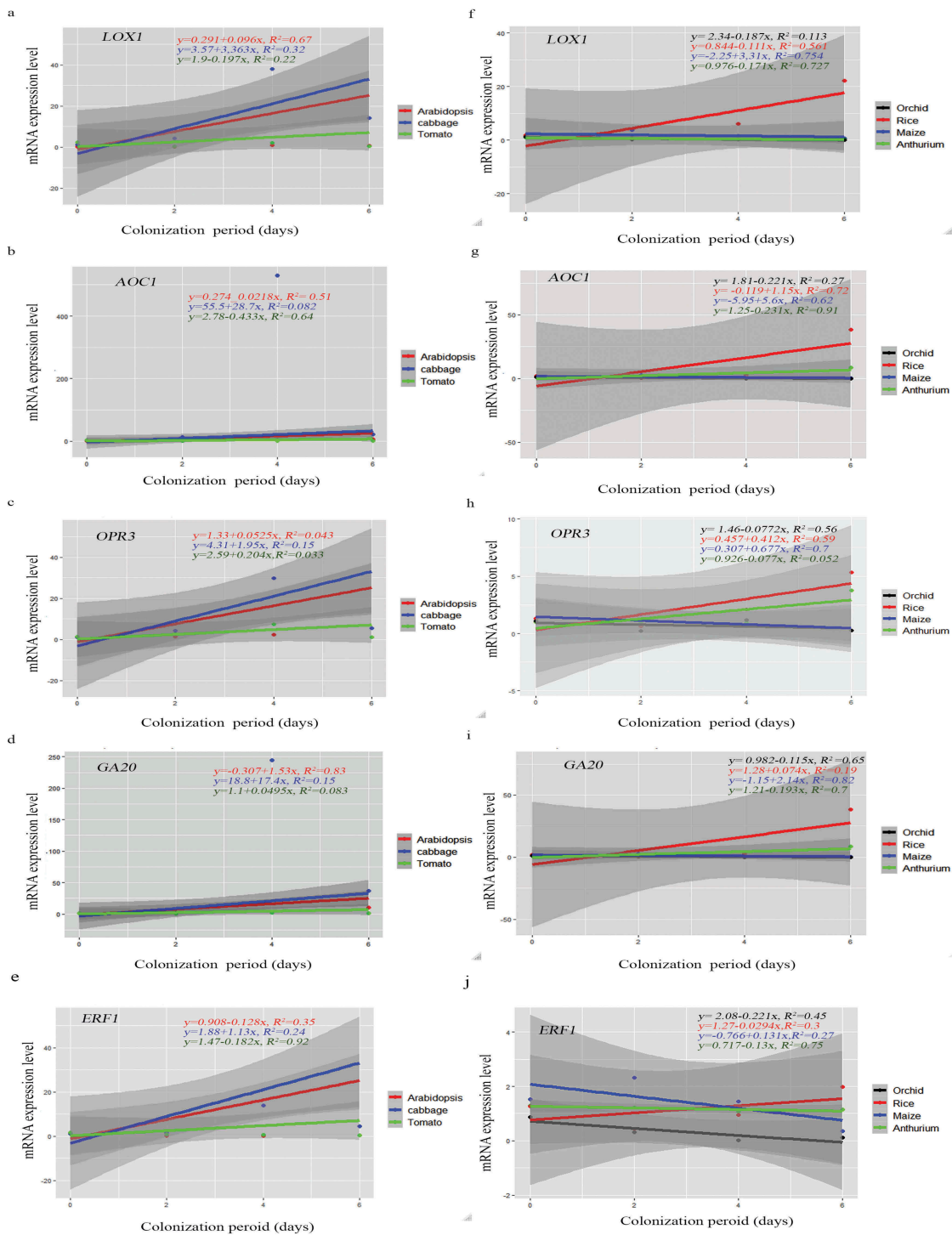
Figure 6. Scatter plots showing linear relationship between *P. indica* colonization and gene expression across plant species. (a–J) Correlation of each dicot and monocot plants, P. *indica* colonization period in days and gene expression levels by rejecting 'null' hypothesis (equals to zero) are shown by linear regression analysis. Summary of regression coefficient values and R^2 values are represented. Testing the significance of the differences by the comparison of dependent (y) and independent variables (x) (p<=0.5 or >=0,05) was performed using R-packages. The graphs are for statistical purpose only.

taken from the maturation zones of the roots, i.e. 3 cm away from the root tip. We show that the time period that is required for the appearance of *P. indica* in the maturation zone varied substantially among the species (Table 1). However, from the investigated plant species, rice, maize, and *Anthurium* were domesticated for the longest period of time.[47–49] Therefore, one might speculate that longer domestication of a species results in the retardation of root

colonization because these plants were bred to develop an optimal intrinsic adaptability to harm and resistance against pathogen attack. A stronger innate immunity in these species does not only prevent the invasion of pathogens but also of beneficial microbes which have to overcome the plant defense responses. A testable hypothesis might be that reprogramming of the phytohormone balance and signaling network to the demands of the beneficial endophyte *P. indica* might thus be

more difficult for domesticated plants compared to those not exposed to a selection pressure for optimizing the immune system.

Multiple reports have shown that effective innate immune systems of the hosts suppress successful root colonization by *P. indica* in Arabidopsis and barley.[26,34] The JA and GA levels in Arabidopsis increased after co-cultivation with *P. indica*, which is consistent with recent observations.[34,50,51,52] However, the stimulatory effect on JA accumulation is much stronger in rice and reverse in *Oncidium* (Figure 3). This suggests that *P. indica* might use JA to stimulate the host´s innate immunity in rice and Arabidopsis, whereas in *Oncidium*, the fungus already represses JA accumulation to promote root colonization. Tomato has been reported as a pathogenically vulnerable species due to its low endogenous JA level.[53,56] The strong increase during the later phases of *P. indica* colonization might indicate that the root utilizes GA to restrict root colonization.

The regulation of JA- and GA-related genes in response to *P. indica* colonization is also species-specific, which is not surprising considering the numerous processes which are regulated by these phytohormones. LOX, AOC, OPR3, and GA20-oxidase activate JA and GA biosynthesis and play important roles in senescence, wound and stress responses, pathogen attack and growth promotion.[39,40,57–61] Stimulation of *LOX1, AOC1* and *OPR3* expression by *P. indica* might be a crucial regulatory step for JA biosynthesis to balance root colonization and to stimulate growth.[50,57,58,62] GA20-oxidase produce active GA and the enzyme might be recruited by *P. indica* for correct colonization[26,34] Interestingly, the observed strong changes in the mRNA levels of these four genes during co-cultivation with *P. indica* occur during early phases of root colonization (summarized in Table 1). The only exception is the tomato *AOC1* mRNA level. A possible explanation could be that *AOC1* is expressed mainly in vascular bundles[61,63] which are not reached by the fungal hyphae. Furthermore, constitutive overexpression of *AOC1* in tomato resulted in elevated JA levels in flower organs but not in leaves.[61]

Ethylene (ET) is another important hormone that participates in the activation and regulation of plant innate immunity. It is a negative regulator in mutualistic root symbioses, but a positive modulator of mutualistic root – *P. indica* symbiosis.[33,64,65] *ERF1* is a target of ET and participates in defense-related gene expression, but also to *P. indica*.[33,66] Khatabi et al.[64] showed that overexpressors of ERF1 in Arabidopsis plants displayed constitutive ET-associated defense which also improved root colonization by *P. indica*. Similar to JA- and GA-biosynthesis genes, the *ERF1* mRNA levels were elevated in orchid, Chinese cabbage and maize roots 2, 4, and 6 dai with *P. indica*, while the mRNA level is downregulated in the other four hosts following the interaction with the fungus. Thus, *ERF1* represents another example of a phytohormone-related gene that is quite differently responding to *P. indica* in different host species.

In conclusion, the huge differences observed in root colonization and phytohormone actions in the symbioses between *P. indica* and different host species suggest a high degree of species-specificity and warn of generalizations during early phases of the establishment of the symbiosis.

The onset of root colonization and reprogramming of root development by the fungus are quite different among the seven plant species. This might be the result of a combination of different factors: The hyphal growth towards the roots may occur with different velocities because chemical mediators either promote or inhibit the contact between the two symbionts. Once a physical contact is established, propagation of the hyphae on the root surface and between the epidermal and subepidermal cell layers might be controlled by different plant-specific factors, such as the features of the cell walls. Finally, the plant machinery controlling the invasion of hyphae into the plant cell as well as the signaling events leading to the reprogramming of the root cells appears to be highly species-specific.

In conclusion, based on these results, it is difficult to come to a general conclusion by analyzing the symbiotic interaction of *P. indica* with just one host plant.

Materials and methods

Plant growth and fungal culture conditions

Rice (*Oryza sativa* L.) and *A. thaliana* seeds were surface-sterilized for 30 and 10 min, respectively, in a NaClO solution (1%), and maize (*Zea mays* L.), tomato (*Lycopersicon esculentum*) and Chinese cabbage (*Brassica campestris*) seeds for 10, 5 and 3 min, respectively, in a NaClO solution (5%). All seeds were then washed five times with sterile water and finally placed on a petri dish containing $^1/_2$ MS nutrient medium.[67] Plates with rice, tomato, maize, and Chinese cabbage were incubated at 22°C under continuous illumination (100 mol m^{-2} s^{-1}) for seed germination. *A. thaliana* seeds were incubated at 4°C in the dark for 48 h before transfer to 22°C and continuous illumination (100 µmol m^{-2} s^{-1}) for 10 days. *Anthurium andraeanum* and *Oncidium orchid* plants were cultured for two weeks until rooting, and then transferred to 1/2 MS medium for five days. After seed germination, the growing seedlings with healthy roots were transferred to fresh plates containing 1/2 MS medium. *P. indica* was cultured on Kaefer medium for 6 days.[68] One to six seedlings were used per petri dish (depending on the size of the seedlings) and one fungal plague of 5 mm in diameter per seedling was placed at a distance of 1 cm from the roots.[4] After exposure to the fungus, all co-cultures were grown under identical conditions to allow a comparative analysis. Mock-treatments were simultaneously performed with media without the fungus.

Microscopic detection of *P. indica* in roots

Seedlings of the seven species listed above were co-cultivated with *P. indica* for 0, 2, 4 or 6 days to detect the establishment of the symbiotic interaction and root colonization. For microscopical observations, the plant material was washed in running tap water to remove the planting medium, and the primary roots, 3 cm away from the root tip, were cut off and dissected into 2 cm sections. For the thin and soft root tissue of *A. thaliana*, tomato, and Chinese cabbage, cut roots were dissected into pieces of 1 cm each and boiled in 2% KOH for 2 min, then washed in sterile water and stained with 0.5% lactophenol blue solution (Fluka, USA) for 10 min. Subsequently, it was squeezed on a glass slide for microscopical observation. For the thick and soft root tissue of maize, orchid, and *Anthurium*, each root section was sliced into thin

pieces before staining with the same method. For the harder root tissue of rice, the roots were first fixed in formalin-acetic acid solution (formalin: acetic acid: 50% ethanol; 1:1:18; v/w) for 6 h and immersed in 2.5% sucrose solution overnight, and finally embedded in a frozen gel, followed by longitudinal microdissection into 25–30 μm slices. The frozen gel-embedded slices were stained with lactophenol solution as described above, before analyses with a bright field microscope. In pilot experiments with different staining procedures, we found that this analysis allows the best comparison of the roots from the different plant species.

Phytohormone analyses

Sample preparation

The seedlings were co-cultured with *P. indica* for 0, 2, 4 and 6 days, then their roots were washed under running tap water for 5 min and dried gently. Roots and cotyledons (excised from the hypocotyls) were harvested separately and quick-frozen in liquid N_2. Frozen samples were then ground under liquid N_2 with mortar and pestle. Samples (250 mg) were extracted with 750 μl MeOH–H_2O–HOAc (90:9:1, v/v/v) for 2 h in 4°C and centrifuged for 1 min at 10,000 rpm. The supernatant was collected, and the extraction was repeated. Pooled supernatants were dried under N_2, resuspended in 200 μl of 0.05% HOAc in H_2O–MeCN (85:15, v/v), and finally filtered through a Millex-HV 0.45 μm filter from Millipore (Bedford, USA). Samples were dried, resuspended in 80% HPLC-grade methanol (MeOH) and filtrated before use. In parallel, HPLC-grade SA and JA (100 ng) was treated in the same way and used as internal standards.

JA analysis

Analyses were carried out using a Dionex Ultimate 3000 RSLC at ambient temperature, and the injected volume was 10 μl. The elution gradient was carried out with binary solvent system consisting of 0.05% HOAc in H_2O (solvent A) and MeCN (solvent B) at a constant flow rate of 600 μl min^{-1} and a split 1/3. A linear gradient profile with the following proportions (v/v) of solvent B was applied (t (min), %B): (0, 15), (3, 15), (5, 100), (6, 100), (7, 15), (8, 15) with 5 min for re-equilibration. MS and MS/MS experiments were performed on a Bruker maXis UHR-QTOF mass spectrometer. All analyses were performed using the Turbo Ionspray source in negative ion mode with the following settings: capillary voltage −3500 V, nebulizer gas (N_2) 10 (arbitrary units), curtain gas (N_2) 12 (arbitrary units), collision gas (N_2) 4 (arbitrary units).

GA analysis

Analyses were carried out using a Dionex Ultimate 3000 RSLC at ambient temperature, and the injected volume was 10 μl. The elution gradient was a binary solvent system consisting of 0.05% HOAc in H_2O (solvent A) and MeCN (solvent B) at a constant flow rate of 600 μl min^{-1} and a split 1/3. A linear gradient profile with the following proportions (v/v)

Table 2. The following primer sequences were used in this study.

Primer names	Primer sequences (5′-3′)
Actin-F	TTTGCTGGTGATGATGC
Actin-R	GCAACATACATGGCAGG
LOX$_1$-F	TTGACCCAACCAAGCGGATA
LOX$_1$-R	TTACCGGACGAGCAAGTTCA
AOC$_1$-F	CGCATTCAGCTTTAGGTTTGG
AOC$_1$-R	GTGATTCCCACGCGTTTCTT
OPR$_3$-F	AAGATTCGATCTCTCTCATCGAGT
OPR$_3$-R	GGAGTGGTCCGTTGAGCA
GA$_{20}$-oxidase-F	CTCACCCAAACTCGTTCAACA
GA$_{20}$-oxidase-R	TCTGCTTACTCCAAGGCTCAT
ERF$_1$-F	GGCCATGGGGGAAATTCGC
ERF$_1$-R	GCTTGATCGTAGGCTAAAGC
PiTef-F	ACCGTCTTGGGGTTGTATCC
PiTef-R	TCGTCGCTGTCAACAAGATG

of solvent B was applied (t (min), %B): (0, 15), (3, 15), (5, 100), (6, 100), (7, 15), (8, 15) with 5 min for re-equilibration. MS and MS/MS experiments were performed on a Pegasus 4D GC×GC-TOFMS mass spectrometer. Column flow was set to 1 ml/min helium, injector temperature was set to 250°C, GC temperature program was set to 40°C at 1 min, 10°C/min to 300°C and held constant for 8 min, solvent delay was set to 470 s, transfer line temperature was set at 280°C, mass range saved was set to m/z 50–600, and ion source temperature was set to 200°C.

RNA preparation and analysis of gene expression by qRT-PCR

Roots of the seven species exposed to *P. indica* for 0, 2, 4 or 6 days were harvested. RNA was isolated from roots with an RNA isolation kit (RNA Plus Mini Kit, LabPrep^{TM}). Total RNA was isolated from three independent replicates of the roots. After DNAse treatment, the cDNA was synthesized with the RevertAidTM first strand cDNA synthesis kit (Fermentas, Waltham, MA, USA) using 2 μg of RNA. The quantitative reverse transcription-PCR (qRT-PCR) was performed with synthesized cDNA using the KAPA SYBR FAST qPCR kit (KAPA, Wilmington, MA, USA) and gene-specific primers listed in the following Table 2 in ABI 7500 (Applied Biosystems, USA) according to the manufacturer's instructions. To normalize the gene expression, the plant *ACTIN* gene was co-amplified as an internal control. The PCR cycle condition for qRT-PCR was set as default (40 cycles): 95.0°C for 3 min, 95.0°C for 3 s, 60.0°C for 30 s (instruction manual, ABI Biosystems 7500 RT-PCR machine). The mRNA levels for each cDNA probe were normalized with respect to the plant *ACTIN* mRNA level. The relative gene expression was analyzed by the 2$^{-\Delta\Delta Ct}$ method.[69] For optimizing the annealing temperatures, the PCR products were first analyzed on agarose gels to confirm that a single band with the expected length is generated.

Statistical analyses

Data display means with standard errors of three independent biological repeats. Statistical significance of the treatments was determined using Student's t-test in EXCEL 2010 at a significance level of $P < .05$. GraphPad Prism 5 software was used for statistical analysis. In all graphs, the error bars indicate the standard deviation. Linear regression analysis was

performed using R-packages (http://www.R-project.org/). The coefficient of determination (R^2) was analyzed, and test for the significance of the difference was performed.

Acknowledgments

Author contribution

HL, GA, QZ, XS, DT, FS, and MSH performed the experiments and analyses of the data, RSK performed statistical analysis. RO and KWY developed and supervised the experiments and wrote the manuscript.

Disclosure of potential conflicts of interest

There is no conflict of interests.

ORCID

Huichun Liu ⓘD http://orcid.org/0000-0003-4714-2359

References

1. Varma A, Verma S, Sahay N, Büttehorn B, Franken P. *Piriformospora indica*, a cultivable plant-growth-promoting root endophyte. Appl Environ Microbiol. 1999;65:2741–2744.
2. Verma S, Varma A, Rexer K, Hassel A, Kost G, Sarbhoy A, Bisen P, Bütehorn B, Franken P. *Piriformospora indica*, gen. et sp. nov., a new root-colonizing fungus. Mycologia. 1998;90:896–903. doi:10.1080/00275514.1998.12026983.
3. Fakhro A, Andrade-Linares DA, von Bargen S, Bandte M, Büttner C, Grosch R, Schwarz D, Franken P. Impact of *Piriformospora indica* on tomato growth and on interaction with fungal and viral pathogens. Mycorrhiza. 2010;20:191–200. doi:10.1007/s00572-009-0279-5.
4. Lee YC, Johnson JM, Chien CT, Sun C, Cai D, Lou B, Oelmüller R, Yeh K-W. Growth promotion of Chinese cabbage and Arabidopsis by *Piriformospora indica* is not stimulated by mycelium-synthesized auxin. Mol Plant Microbe Interact. 2011;24:421–431. doi:10.1094/MPMI-05-10-0110.
5. Molitor A, Zajic D, Voll L, Pons-Kuehnemann J, Samans B, Kogel KH, Waller F. Barley leaf transcriptome and metabolite analysis reveals new aspects of compatibility and *Piriformospora indica*-mediated systemic induced resistance to powdery mildew. Mol Plant Microbe Interact. 2011;24:1427–1439. doi:10.1094/MPMI-06-11-0177.
6. Oelmüller R, Sherameti I, Tripathi S, Varma A. *Piriformospora indica*, a cultivable root endophyte with multiple biotechnological applications. Symbiosis. 2009;49:1–17. doi:10.1007/s13199-009-0009-y.
7. Peškan-Berghöfer T, Shahollari B, Giong PH, Hehl S, Markert C, Blanked V, Kost G, Varma A, Oelmüller R. Association of *Piriformospora indica* with *Arabidopsis thaliana* roots represents a novel system to study beneficial plant–microbe interactions and involves early plant protein modifications in the endoplasmic reticulum and at the plasma membrane. Physiol Plant. 2004;122:465–477. doi:10.1111/j.1399-3054.2004.00424.x.
8. Pham GH, Kumari R, Singh A, Sachdev M, Prasad R, Kaldorf M, Buscot F, Oelmüller R, Peskan T, Weiss M, et al. Axenic cultures of *Piriformospora indica*. In: Varma A, Abbott L, Werner D,

9. Hampp R, editors. Plant surface microbiology. Berlin Heidelberg New York: Springer; 2004b. p. 593–616.
9. Prasad RP. Studies on interaction between symbiotic fungus (*Piriformospora indica*), rhizobacteria and selected plants [PhD Thesis]. Merrut, India: Merrut University; 2008.
10. Qiang X, Weiss M, Kogel KH, Schäfer P. *Piriformospora indica*-a mutualistic basidiomucete with an exceptionally large plant host range. Mol Plant Pathol. 2012;13(5):508–518. doi:10.1111/j.1364-3703.2011.00764.x.
11. Shahollari B, Varma A, Oelmüller R. 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. Plant Physiol. 2005;162:945–958. doi:10.1016/j.jplph.2004.08.012.
12. Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M, Heier T, Hückelhoven R, Neumann C, von Wettstein D, et al. The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. Proc Natl Acad Sci U S A. 2005 Sep 20;102(38):13386–13391. doi:10.1073/pnas.0504423102.
13. Weiß M, Waller F, Zuccaro A, Selosse MA. S*ebacinales* - one thousand and one interactions with land plants. New Phytol. 2016;211:20–40. doi:10.1111/nph.13977.
14. Kumar M, Yadav V, Tuteja N, Johri AK. Antioxidant enzyme activities in maize plants colonized with *Piriformospora indica*. Supporting Inf Microbiol. 2009;155:780–790.
15. Johri AK, Oelmüller R, Dua M, Yadav V, Kumar M, Tuteja N, Varma A, Bonfante P, Persson BL, Stroud R. Fungal association and utilization of phosphate by plants: success, limitations, and future prospects. Front Microbiol. 2015;6:984.
16. Sherameti I, Tripathi S, Varma A, Oelmüller R. The root-colonizing endophyte Pirifomospora indica confers drought tolerance in Arabidopsis by stimulating the expression of drought stress-related genes in leaves. Mol Plant Microbe Interact. 2008;21:799–807. doi:10.1094/MPMI-21-6-0799.
17. Yadav V, Kumar M, Deep DK, Kumar H, Sharma R, Tripathi T, Tuteja N, Saxena AK, Johri AK. A phosphate transporter from the root endophytic fungus *Piriformospora indica* plays a role in phosphate transport to the host plant. J Biol Chem. 2010;285:26532–26544. doi:10.1074/jbc.M110.111021.
18. Camehl I, Drzewiecki C, Vadassery J, Shahollari B, Sherameti I, Forzani C, Munnik T, Hirt H, Oelmüller R. The OXI1 kinase pathway mediates *Piriformospora indica*-induced growth promotion in Arabidopsis. PLoS Pathog. 2011;7:e1002051. doi:10.1371/journal.ppat.1002051.
19. Das A, Kamal S, Shakil Najam A, Sherameti I, Oelmüller R, Dua M, Tuteja N, Johri Atul K, Varma A. The root endophyte fungus *Piriformospora indica* leads to early flowering, higher biomass and altered secondary metabolites of the medicinal plant, *Coleus forskohlii*. Plant Sign Behav. 2012;7:1–10. doi:10.4161/psb.7.1.18472.
20. Pan R, Xu L, Wei Q, Wu C, Tang W, Oelmüller R, Zhang W. *Piriformospora indica* promotes early flowering in Arabidopsis through regulation of the photoperiod and gibberellin pathways. PLoS One. 2017;12:e0189791. doi:10.1371/journal.pone.0189791.
21. Hua MS, SenthilKumar R, Shyur LF, Cheng YB, Tian ZH, Oelmuller R, Yeh KW. Metabolomic compounds identified in *Piriformospora indica*-colonized Chinese cabbage roots delineate symbiotic functions of the interaction. Sci Rep. 2017;7(1). doi:10.1038/s41598-017-08715-2.
22. Baltruschat H, Fodor J, Harrach BD, Niemczyk E, Barna B, Gullner G, Janeczko A, Kogel K-H, Schäfer P, Schwarczinger I, et al. Salt tolerance of barley induced by the root endophyte *Piriformospora indica* is associated with a strong increase in antioxidants. New Phytol. 2008;180:501–510. doi:10.1111/j.1469-8137.2008.02583.x.
23. Daneshkhah R, Cabello S, Rozanska E, Sobczak M, Grundler FM, Wieczorek K, Hofmann J. *Piriformospora indica* antagonizes cyst nematode infection and development in *Arabidopsis* roots. J Exp Bot. 2013;64(12):3763–3774. doi:10.1093/jxb/ert213.

24. Knecht K, Seyffarth M, Desel C, Thurau T, Sherameti I, Lou B, Oelmüller R, Cai D. Expression of BvGLP-1 encoding a germin-like protein from sugar beet in Arabidopsis thaliana leads to resistance against phytopathogenic fungi. Mol Plant Microbe Interact. 2010;23(4):446–457. doi:10.1094/MPMI-23-4-0446.

25. Franken P. The plant strengthening root endophyte Piriformospora indica: potential application and the biology behind. Appl Microbiol Biotechnol. 2012;96:1455–1464. doi:10.1007/s00253-012-4506-1.

26. Jacobs S, Zechmann B, Molitor A, Trujillo M, Petutschnig E, Lipka V, Kogel K-H, Schafer P. Broad-spectrum suppression of innate immunity is required for colonization of Arabidopsis roots by the fungus Piriformospora indica. Plant Physiol. 2011;156:726–740. doi:10.1104/pp.111.176446.

27. Lahrmann U, Ding Y, Banhara A, Rath M, Hajirezaei MR, Döhlemann S, von Wirén N, Parniske M, Zuccaro A. Host-related metabolic cues affect colonization strategies of a root endophyte. Proc Natl Acad Sci U S A. 2013;110:13965–13970. doi:10.1073/pnas.1301653110.

28. Lahrmann U, Zuccaro A. Opprimo ergo sum–evasion and suppression in the root endophytic fungus Piriformospora indica. Mol Plant Microbe Interact. 2012;25:727–737. doi:10.1094/MPMI-11-11-0291.

29. Nongbri PL, Johnson JM, Sherameti I, Glawischnig E, Halkier BA, Oelmüller R. Indole-3-acetaldoxime-derived compounds restrict root colonization in the beneficial interaction between Arabidopsis roots and the endophyte Piriformospora indica. Mol Plant Microbe Interact. 2012;25:1186–1197. doi:10.1094/MPMI-03-12-0071-R.

30. Vadassery J, Oelmüller R. Calcium signaling in pathogenic and beneficial plant microbe interactions: what can we learn from the interaction between Piriformospora indica and Arabidopsis thaliana. Plant Signal Behav. 2009;4:1024–1027. doi:10.4161/psb.4.11.9800.

31. Ye W, Shen C-H, Lin Y, Chen P-C, Xu X, Oelmüller R, Yeh K-W LZ. Growth promotion-related miRNAs in Oncidium orchid roots colonized by the endophytic fungus Piriformospora indica. PLoS One. 2014;9:1–12.

32. Achatz B, Kogel KH, Franken P, Waller F. Piriformospora indica mycorrhization increases grain yield by accelerating early development of barley plants. Plant Signal Behav. 2010;5 (12):1685–1687. doi:10.4161/psb.5.12.14112.

33. Camehl I, Sherameti I, Venus Y, Bethke G, Varma A, Lee J, Oelmüller R. Ethylene signalling and ethylene-targeted transcription factors are required to balance beneficial and nonbeneficial traits in the symbiosis between the endophytic fungus Piriformospora indica and Arabidopsis thaliana. New Phytol. 2010;185:1062–1073. doi:10.1111/j.1469-8137.2009.03149.x.

34. Schäfer P, Pfiffi S, Voll LM, Zajic D, Chandler PM, Waller F, Scholz U, Pons-Kühnemann J, Sonnewald S, Sonnewald U, et al. Manipulation of plant innate immunity and gibberellin as factor of compatibility in the mutualistic association of barley roots with Piriformospora indica. Plant J. 2009;59:461–474.

35. Hilbert M, Voll LM, Ding Y, Hofmann J, Sharma M, Zuccaro A. Indole derivative production by the root endophyte Piriformospora indica is not required for growth promotion but for biotrophic colonization of barley roots. New Phytol. 2012;196 (2):520–534. doi:10.1111/j.1469-8137.2012.04275.x.

36. Vadassery J, Ritter C, Venus Y, Camehl I, Varma A, Shahollari B, Novák O, Strnad M, Ludwig-Müller J, Oelmüller R. The role of auxins and cytokinins in the mutualistic interaction between Arabidopsis and Piriformospora indica. Mol Plant Microbe Interact. 2008;21:1371–1383. doi:10.1094/MPMI-21-10-1371.

37. Stein E, Molitor A, Kogel KH, Waller F. Systemic resistance in Arabidopsis conferred by the mycorrhizal fungus Piriformospora indica requires jasmonic acid signaling and the cytoplasmic function of NPR1. Plant Cell Physiol. 2008;49:1747–1751. doi:10.1093/pcp/pcn147.

38. Zarea MJ, Hajinia S, Karimi N, Goltapeh EM, Rejali F, Varma A. Effect of Piriformospora indica and Azospirillum strains from saline

or non-saline soil on mitigation of the effects of NaCl. Soil Biol Biochem. 2012;45:139–146. doi:10.1016/j.soilbio.2011.11.006.

39. Chandler PM, Marion-Poll A, Ellis M, Gubler F. Mutants at the Slender1 locus of barley cv. Himalaya. Molecular and physiological characterization. Plant Physiol. 2002;129:181–190. doi:10.1104/pp.010917.

40. Sun TP. Gibberellin metabolism, perception and signaling pathways in Arabidopsis. Arabidopsis Book. 6:e0103.

41. Dong SQ, Tian ZH, Chen PJ, Senthilkumar R, Shen CH, Cai D, Oelmüller R, Yeh KW. The maturation zone is an important target of Piriformospora indica in Chinese cabbage roots. J Exp Bot. 2013;64:4529–4540. doi:10.1093/jxb/ert265.

42. Kim D, Abdelaziz ME, Ntui VO, Guo X, Al-Babili S. Colonization by the endophyte Piriformospora indica leads to early flowering in Arabidopsis thaliana likely by triggering gibberellin biosynthesis. Biochem Biophys Res Commun. 2017;490:1162–1167. doi:10.1016/j.bbrc.2017.06.169.

43. Cosme M, Lu J, Erb M, Stout MJ, Franken P, Wurst S. A fungal endophyte helps plants to tolerate root herbivory through changes in gibberellin and jasmonate signaling. New Phytol. 2016;211:1065–1076. doi:10.1111/nph.13957.

44. Li L, Chen X, Ma C, Wu H, Qi S. Piriformospora indica requires kaurene synthase activity for successful plant colonization. Plant Physiol Biochem. 2016;102:151–160. doi:10.1016/j.plaphy.2016.02.017.

45. Hedden P. Gibberellin metabolism and its regulation. Annu Rev Plant Biol. 2008;20:317–318.

46. Deshmukh S, Hückelhoven R, Schäfer P, Imani J, Sharma M, Weiss M, Waller F, Kogel KH. The root endophytic fungus Piriformospora indica requires host cell death for proliferation during mutualistic symbiosis with barley. Proc Natl Acad Sci USA. 2006;103:18450–18457. doi:10.1073/pnas.0605697103.

47. Jin J, Huang W, Gao JP, Yang J, Shi M, Zhu MZ, Luo D, Lin HX. Genetic control of rice plant architecture under domestication. Nat Gen. 2008;40:1365–1369. doi:10.1038/ng.247.

48. Kovach MJ, Sweeney MT, McCouch SR. New insights into the history of rice domestication. Trends Genet. 2007;23:578–587. doi:10.1016/j.tig.2007.08.012.

49. Li CB, Zhou AL, Sang T. Rice domestication by reducing shattering. Science. 2006;311:1936–1939. doi:10.1126/science.1123604.

50. Vahabi K, Sherameti I, Bakshi M, Mrozinska A, Ludwig A, Reichelt M, Oelmüller R. The interaction of Arabidopsis with Piriformospora indica shifts from initial transient stress induced by fungus-released chemical mediators to a mutualistic interaction after physical contact of the two symbionts. BMC Plant Biol. 2015;15:58. doi:10.1186/s12870-015-0533-2.

51. Vahabi K, Camehl I, Sherameti I, Oelmüller R. Growth of Arabidopsis seedlings on high fungal doses of Piriformospora indica has little effect on plant performance, stress, and defense gene expression in spite of elevated jasmonic acid and jasmonic acid-isoleucine levels in the roots. Plant Signal Behav. 2013;8: e26301. doi:10.4161/psb.26301.

52. Lahrmann U, Strehmel N, Langen G, Frerigmann H, Leson L, Ding Y, Scheel D, Herklotz S, Hilbert M, Zuccaro A. Mutualistic root endophytism is not associated with the reduction of saprotrophic traits and requires a noncompromised plant innate immunity. New Phytol. 2015;207:841–857. doi:10.1111/nph.13411.

53. Vahabi K, Dorcheh SK, Monajembashi S, Westermann M, Reichelt M, Falkenberg D, Hemmerich P, Sherameti I, Oelmüller R. Stress promotes Arabidopsis - Piriformospora indica interaction. Plant Signal Behav. 2016;11:e1136763. doi:10.1080/15592324.2015.1136763.

54. Bostock RM. Signal crosstalk and induced resistance: straddling the line between cost and benefit. Annu Rev Phytopathol. 2005;43:545–580. doi:10.1146/annurev.phyto.41.052002.095505.

55. Chen H, Daniel JA, Howe GA. Constitutive activation of the jasmonate signaling pathway enhances the production of secondary metabolites in tomato. FEBS Lett. 2006;580:2540–2546. doi:10.1016/j.febslet.2006.03.070.

56. Pedranzani H, Racagni G, Alemano S, Miersch O, Ram´ırez I, Pena-Cortes H, Taleisnik E, Machado-Domenech E, Abdala G. Salt tolerant tomato plants show increased levels of jasmonic acid. Plant Growth Regul. 2003;41:149–158. doi:10.1023/A:1027311319940.

57. Wasternack C, Hause B. Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. Ann Bot. 2013;111:1021–1058. doi:10.1093/aob/mct067.

58. Stintzi A, Browse J. The *Arabidopsis* male-sterile mutant, *opr3*, lacks the 12-oxophytodienoic acid reductase required for jasmonate synthesis. Proc Natl Acad Sci USA. 2000;97:10625–10630. doi:10.1073/pnas.190264497.

59. Vicentea J, Cascona T, Vicedob B, Garcı´a-Agustı´nb P, Hambergc M, Castresanaa C. Role of 9-lipoxygenase and a-dioxygenase oxylipin pathways as modulators of local and systemic defense. Mol Plant. 2012;5(4):914–928. doi:10.1093/mp/ssr105.

60. Stenzel I, Hause B, Maucher H, Pitzschke A, Miersch O, Ziegler J, Ryan CA, Wasternack C. Allene oxide cyclase dependence of the wound response and vascular bundle-specific generation of jasmonates in tomato – amplification in wound signaling. Plant J. 2003;33:577–589.

61. Stenzel I, Hause B, Miersch O, Kurz T, Maucher H, Weichert H, Ziegler J, Feussner I, Wasternack C. Jasmonate biosynthesis and the allene oxide cyclase family of *Arabidopsis thaliana*. Plant Mol Biol. 2003;51:895–911.

62. Melan MA, Enriquez AD, Peterman TK. The LOX1 gene of *Arabidopsis* is temporally and spatially regulated in germinating seedlings. Plant Physiol. 1993;105:385–393. doi:10.1104/pp.105.1.385.

63. Mierscha O, Weichert H, Stenzel I, Hause B, Maucher H, Feussner I, Wasternack C. Constitutive overexpression of allene oxide cyclase in tomato (*Lycopersicon esculentum* cv. Lukullus) elevates levels of some jasmonates and octadecanoids in flower organs but not in leaves. Phytochemistry. 2004;65:847–856. doi:10.1016/j.phytochem.2004.01.016.

64. Khatabi B, Molitor A, Lindermayr C, Pfiffi S, Durner J, von Wettstein D, Kogel KH, Schäfer P. Ethylene supports colonization of plant roots by the mutualistic fungus *Piriformospora indica*. PLoS One. 2012;7:1–8. doi:10.1371/journal.pone.0035502.

65. Khatabi B, Schäfer P. Ethylene in mutualistic symbioses. Plant Sign Behav. 2012;7:1634–1638. doi:10.4161/psb.22471.

66. Berrocal-Lobo M, Molina A, Solano R. Constitutive expression of ETHYLENE RESPONSE FACTOR1 in *Arabidopsis* confers resistance to several necrotrophic fungi. Plant J. 2002;29:23–32.

67. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol. 1962;15:473–497. doi:10.1111/j.1399-3054.1962.tb08052.x.

68. Hill TW, Kaefer E. Improved protocols for *Aspergillus* medium: trace elements and minimum medium salt stock solutions. Fungal Genet Newsl. 2001;48:20–21.

69. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 2001;25:402–408. doi:10.1006/meth.2001.1262.