The symbiotic effect of *Piriformospora indica* on induced resistance against bakanae disease in rice (*Oryza sativa* L.)

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ABSTRACT: The root endophytic fungus, *Piriformospora indica*, colonizes roots of a large number of plant species including Cereals and Brasicaceae. There are several reports indicating that *P. indica* protects roots from different pathogens. In the present study, rice plants were pre-inoculated with *P. indica* and were subsequently infected with *Fusarium proliferatum,* as the causal agent of root rot and crown rot (Bakanae) disease. The effects of *P. indica* on the protection of rice plants against *F. proliferatum* were evaluated. Transcription levels of some pathogenesis-related genes such as *NPR1*, *PR1*, *PR4* and *PR5*, as well as two transcriptional factor genes namely *WRKY62* and *WRKY85* were quantified via quantitative real time PCR in rice roots. Pre-inoculated and challenged rice plants showed mild disease symptoms with two weeks delay compared to non *P. indica* colonized plants. Quantification of *Fusarium* in roots via q-PCR confirmed the higher quantity of the fungi in non pre-inoculated plants. Expression analysis revealed a faster induction of pathogenesis related genes following *F. proliferatum* inoculation of pre-inoculated plants i.e. priming of these genes is a part of mechanism of *P. indica* induced systemic resistance.

KEYWORDS: Priming, q-PCR, Timing, Symbiosis

INTRODUCTION

The fungal genus *Fusarium* is composed of a large number of species that can be pathogenic on plants. *Fusarium* species are causal agents of various diseases affecting many economically important cereals, such as rice (*Oryza sativa* L.). *Fusarium fujikuroi* Nirenberg (teleomorph *Gibberella fujikuoi* Sawada) is an important rice pathogen, causing the bakanae disease or disease of foolish seedlings (6). Some of the *Fusarium* species produce a wide range of biologically active secondary metabolites, including mycotoxins, which are harmful to humans and animals (10).

The conventional technique to control the disease is chemical method, which is expensive and has destructive effects on the environment. Therefore, the development of strategies that promote sustainable resistance will be needed (24). One of the methods to achieve such an aim is induced resistance by using beneficial microorganisms (12).

Root endophytic fungus *Piriformospora indica* colonizes roots of a large number of plant species including Cereals and Brasicaceae (29; 19). The fungus is known to reproduce asexually by generating thick-walled chlamydospores, and in clear contrast to arbuscular mycorrhiza, can be cultured on synthetic media (29). Plants colonized with *P. indica* exhibit enhanced tolerance against abiotic stress and resistance to pathogens (32). In barley, it had been shown that *P. indica* infested plants were more resistant to *Blumeria graminis* compared to non-inoculated control

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plants (32). Another study indicated that *P. indica* also protects barley roots from root rot caused by *Fusarium graminearum* (9). Transcription level of several pathogenesis related (PR) genes such as *PR1b*, *PR2* and *PR5* in *P. indica* infested plants up-regulated *P. indica* protected *Arabidopsis* plants against the causal agent of powdery mildew, *Golovinomyces orontii*, through the Induced Systemic Resistance (ISR) pathway by accumulation of jasmonic acid and elevation of *NPR1* gene expression (25).

In the present study, plants were pre-inoculated with *P. indica* and were subsequently infected with *Fusarium proliferatum,* as the causal agent of Bakanae disease. The severity of the disease, transcription levels of some pathogenesis-related and transcriptional factor genes were evaluated using quantitative real time PCR technique. The aim of the study was to find the role of *P. indica* on the protection of rice plants against a major biotic stress in paddy fields of Northern provinces. It was also intended to analyze the expression pattern and type of induction of pathogenesis related genes during colonization of rice plants with *P. indica*.

MATERIALS AND METHODS

Plant material and growth conditions

Seeds of rice (*Oryza sativa* L. cv Tarom) were surface disinfected in 0.25% sodium hypochlorite for 10 minutes and then rinsed thoroughly with sterilized distilled water three times. Germination of seeds was performed at 25°C between filter papers inside a seed germinator (Weiss - Germany) at dark condition for 4 days.

P. indica inoculation procedures and bioassays

P. indica (a gift from Prof. Kogel, institute of phytopathology and applied zoology, university of Giessen, Germany) was propagated on solid modified KM or Complex Medium (CM; Modified Aspergillus minimal medium) containing: glucose 20 gr, pepton 2 gr, salt solution (12 gr $NaNo_3$, 10.4 gr KCl, 10.4 gr $MgSO_4.7H_2O$, 30.4 gr KH_2PO_4 per liter) 50 ml, casamino-acid 1 gr , agar 15 gr. Micro elements were prepared per liter as follow: (6 gr $MnCl_2.4H_2O$, 1.5 gr H_3BO_3, 2.65 gr $ZnSO_4.7H_2O$, 2.4 gr $Na_2MoO_4.2H_2O$, 750 mg KI, 130 mg $CuSO_4$. $5H_2O$). The fungi were incubated at 25°C for 4 weeks. Chlamydospores were collected by flooding the plate with 10 ml sterile water containing 0.05% (v/v) Tween 20, followed by gentle scraping. The inoculum was spined down in order to separate spores from mycelium (19).

To inoculate rice seedlings with fungus, 4-day-old seedlings were immersed in *P. indica* inoculum/chlamydospore suspension (5×10^5 ml^{-1}) containing 0.05% (v/v) Tween 20, for 5 hours. Mock plants were prepared by treatment of seedlings with sterile water (29). Finally, seedlings (colonized with *P. indica* and non-colonized) were planted into Yoshida medium (33) in a completely randomized design with three replications.

Staining and microscopic detection of *P. indica* in rice roots

To monitor the presence of *P. indica* inside the infected tissues, rice roots were stained using Vierheiling *et al.* (1998) method. The staining of the infected tissues was made at 15 and 30 days after inoculation as well as flowering period. The structure of fungus was visualized via mounting in 50% glycerol and was examined with Nikon imaging microscopy.

Fusarium proliferatum bioassays

Fusarium proliferatum (provided by Rice Research institute, Amol, Iran) was grown on potato dextrose agar (PDA) at 22 °C for 10 days. Fungal cultures were flooded with sterile distilled 0.05% (v/v) Tween 20. The resulting suspensions were filtered through two layers of sterile cotton lint and brought to a final concentration of 10^6 spores ml^{-1} with sterile distilled water (3).

In order to inoculate with *F. proliferatum*, root and crown of two-week-old *P. indica* colonized and non-colonized seedlings were immersed in *F. proliferatum* spore suspension (5×10^4 ml^{-1}). Mock inoculation was performed with water containing 0.05% (v/v) Tween 20. Finally, leaves from 4 treatments including the combinations of inoculated (P_1) and non-inoculated (P_0) with *P. indica*, challenge inoculated (F_1) and non-inoculated (F_0) with *F. proliferatum* were rinsed with water and harvested at 0, 24, 48, 72, and 96 hours post inoculation (hpi) with *F. proliferatum* into liquid nitrogen.

Quantification of *F. proliferatum* biomass in inoculated rice roots

The ratio of *F. proliferatum* DNA to plant DNA was used to monitor the fungal rate infection in rice roots. A pair of *FUM*-specific primer from *F. proliferatum* and *18S*-specific primer from rice genome was selected for quantification of fungal DNA based on respective Ct values. For

determination of *F. proliferatum* progression, Ct curves were generated by using the $2^{-\Delta Ct}$ method where ΔCt is the difference between raw Ct values of the fungus-specific gene and the raw Ct values of the plant specific gene. The levels of *F. proliferatum FUM* and *P. indica ITS* genes are relative to the level of rice *18S* gene, which was set at 1 (14).

Roots of rice plants pre-inoculated with *P. indica* were harvested one and 14dpi with *F. proliferatum*. Roots were washed with sterile water and DNA was isolated from whole roots using Murray and Thompson (1980) method. Furthermore, genomic DNA was extracted from roots of non-inoculation plants as well as *F. proliferatum* mycelia.

RNA extraction, cDNA synthesis and real-time quantitative PCR

Total RNA was extracted from crushed plant samples using TRIZOL reagent (Invitrogen) as described by Triant and Whitehead (2009). The extracted RNA was treated with RQ1 RNase-free DNase (Promega, Madison, WI) before cDNA synthesis using Quantitect cDNA synthesis kit (Qiagen). Two µl diluted cDNA (50 ng) of each sample was used as template. PCR reactions were performed in a 20 µl total reaction with SYBR Green plus 0.7 µl of 10 µM oligonucleotide primers (Table 1). Real-time quantitative PCR was carried out in an Applied 7000Sequence Detestation System. After 7 min activation / denaturation step at 94°C, 39 cycles (94°C for 30 s, 60°C for 30 s and 72°C for 30 s) were carried out and a single step fluorescent reading was taken place after each cycle. The expression rate of gene was calculated using set at 1 (14). The cycle threshold (C_t) values were determined by the 7700 Sequence Detection Software (Ver. 1.6.3). The equal amplification rate and the real-time efficiency of target and reference genes were calculated on the basis of a linear regression slope of a dilution row (E = 10 [–1/slope]) (21). The 18S ribosomal RNA (18S) gene was applied as an internal control.

A serial dilution of 20 ng to 10 pg of *P. indica* and rice genomic DNA were used to test the primers. The efficiency varied in a range of E = 1.60 to values over 2 (24). Heat dissociation (PCR product melting curve) analysis was performed at the end of the PCR reactions.

In vitro antagonistic activity of *P. indica* on *F. proliferatum*

To test for direct antagonistic effects, an agar disc of 0.5 cm diameter covered by *F. proliferatum* mycelium was placed in the middle of a PDA plate and four *P. indica* agar discs were placed around at an equal distance. In other sets, *P. indica* surrounded by *F. proliferatum* fungal cultures was propagated on SNA plates at 18°C.

Table 1. Primer pairs used in the present study and their nucleotide sequences

Gene	sequence	Accession number	Reference(s)
18 S Rice	F: 5'- ATGATAACTCGACGGATCGC -3' R: 5'- CTTGGATGTGGTAGCCGTTT -3'	AF069218	Hosseini *et al*, 2014
ITS *P. indica*	F: 5'-CAACACATGTGCACGTCGAT-3' R: 5'-CCAATGTGCATTCAGAACCA-3'	NR119580	Jacobs *et al*, 2011
FUM *F. prolifratum*	F: 5'-AGGATTGGCTGGATCTTCAC-3' R: 5'-TAATACGGTTGGAAATGGCA-3'	AY577454	Matic *et al*, 2013
PR1b	F: 5' - TCGTATGCTATGCTACGTGT -3' R: 5' - CACTAAGCAAATACGGCTGA -3'	EF061247	Zhang *et al*, 2009
PR4	F: 5'-CATTATTACAACCCACAACAGAACAA-3 R: 5'-GCATCCCATGTGGCACAAT-3'	AY050642	Zhang *et al*, 2014
PR5	F: 5'- ACCTCTTCCGCTGTCCTC -3' R: 5'- GAAGACGACTTGGTAGTTGC -3	X68196	Sayari *et al*, 2014
NPR1	F: 5'- GAACCCGGGATGGACACCACCATTG -3' R: 5'- AAGGATCCTCAAGGTACCTCCAAACCAAG -3'	DQ450948	Sayari *et al*, 2014
WRKY62	F: 5'- AGGATGGGTACCAATGGA-3' R: 5'- ACGAGTTGATGGAGATGGA -3	DQ298182	Ryu *et al*, 2006
WRKY85	F: 5'- CAGCAAGAAAAGGAATATACAAAT -3' R: 5'- CTCAATGTGTTTCCTAACATTACA -3	DQ298186	Ryu *et al*, 2006

RESULTS

Presence of *P. indica* inside the symbiotic plant roots

Microscopic inspection of rice plant tissues pre-inoculated with *P. indica* showed that the fungus colonized roots primarily via root hairs and later on grew intracellularly in the root cells (data not shown).

This observation was confirmed by performing PCR reactions in root materials using *P .indica ITS* gene specific primers (data not shown).

Fusarium symptoms in *P. indica* colonized rice plants

Plants challenged with *F. proliferatum* (P0F1) had less tillage, abnormal growth; chlorotic, thin and brownish leaves; shorter than normal seedlings; compared to non-inoculated control plants. Plants colonized by *P. indica* (P_1F_0) remained healthy during the course of the experiment with the optimum numbers of tillages. However, in rice plants pre-inoculated with *P. indica* and inoculated with *F. proliferatum* (P1F1) the disease symptoms were observed with two weeks delay compared to non *P. indica* colonized plant. In addition, the development of disease symptoms were significantly less than control (P0F1) plants.

Quantification of *F. proliferatum* DNA in rice roots by quantitative real-time PCR

The regression curve of serial dilutions of the respective templates including *ITS* gene from *P. indica* and *FUM* gene from Fusarium showed linearity of amplification over the dynamic range (data not shown).

F. proliferatum quantification by q-PCR indicated that 7 days post-inoculation (dpi) the relative amount of the fungal DNA in P_0F_1 plants reached the highest level while it decreased slightly 14 dpi time points. In contrast, the development of *F. proliferatum* in P_1F_1 plants was associated with mild symptoms and less fungal DNA compared to P_0F_1 samples, 7dpi. At 14 dpi, Fusarium DNA reduced to a quite low level in the same samples (Figure 1).

Expression patterns of *NPR1* (*NH1*) gene

The expression pattern of *NPR1* gene in rice plants challenge inoculated with *F. proliferatum* and pre-inoculated with *P. indica* indicated that the gene up-regulated significantly during the course of the experiment in a biphasic pattern with the highest at 24 and 96 hours post-inoculation (hpi). However, no significant changes of *NPR1* gene expression (< 2- fold) was detected in rice plants inoculated only with *Fusarium* (Figure 2).

Expression patterns of *PR1b* gene

The expression level of *PR1b* gene in rice plants pre-inoculated with endomycorrhizal fungus *P. indica* significantly up-regulated 24 (2.2- fold) and 72 (~2-fold) hours after inoculation (hai) with *F. proliferatum* in a biphasic

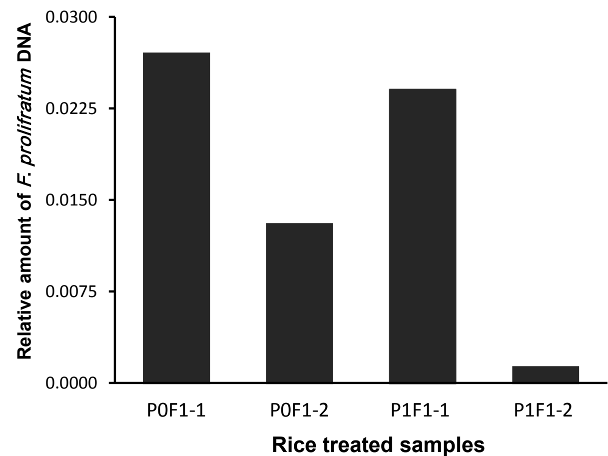

Figure 1. Quantification of *Fusarium prolifratum* in rice roots. The Relative amount of fungal DNA to plant DNA in *P. indica*-colonized (P_1F_1) and noncolonized (P0F1) rice plants 1 and 2 weeks after challenge with *F. prolifratum.*

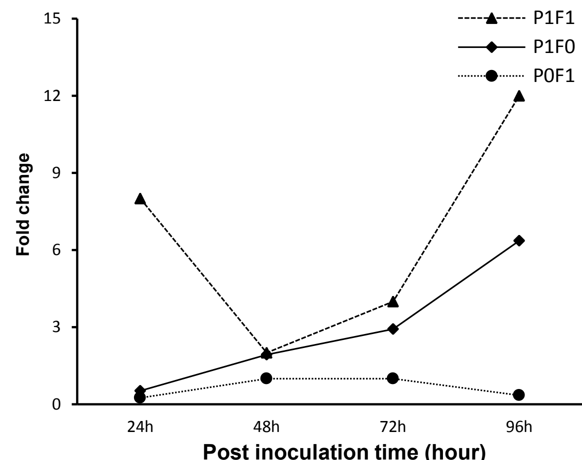

Figure 2. Expression patterns of *NPR1* gene in rice. Triangle (P1F1): pre-inoculated with *P. indica* and challenged with *F. prolifratum* 10 days later; Diamond (P1F0) *P. indica*-inoculated; Square (P0F1) *F. prolifratum*-inoculated plants. The Y-axis represents fold change induction of *NPR1* gene at 24, 48, 72 and 96 hours post inoculation.

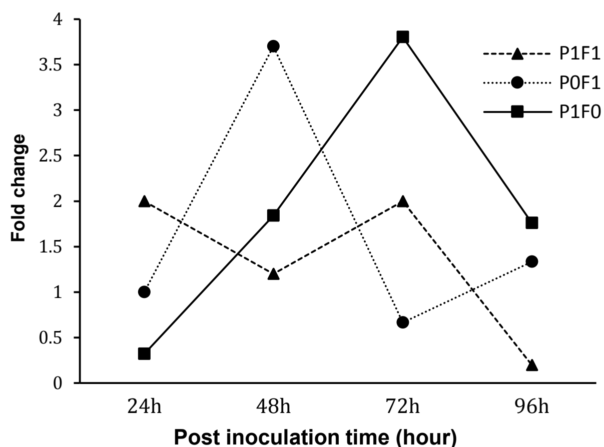

Figure 3. Expression patterns of *PR1b* gene in rice. Triangle (P1F1): pre-inoculated with *P. indica* and challenged with *F. prolifratum* 10 days later; Diamond (P1F0) *P. indica*-inoculated; Square (P0F1) *F. prolifratum*-inoculated plants. The Y-axis represents fold change induction of the gene at 24, 48, 72 and 96 hours post inoculation.

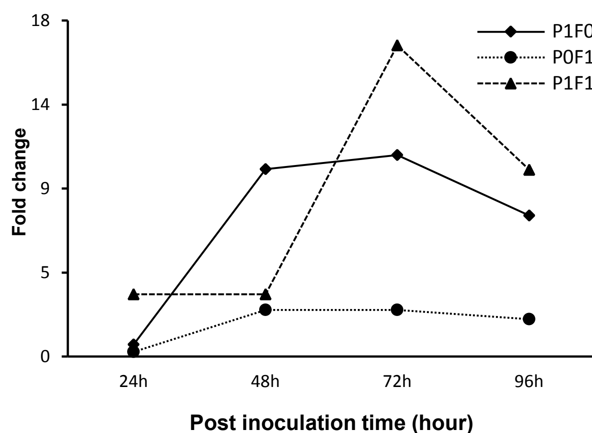

Figure 5. Expression patterns of *WRKY62* gene in rice. Triangle (P1F1): pre-inoculated with *P. indica* and challenged with *F. prolifratum* 10 days later; Diamond (P1F0) *P. indica*-inoculated; Square (P0F1) *F. prolifratum*-inoculated plants. The Y-axis represents fold change induction of *WRKY62* gene at 24, 48, 72 and 96 hours post inoculation with *F. prolifratum.*

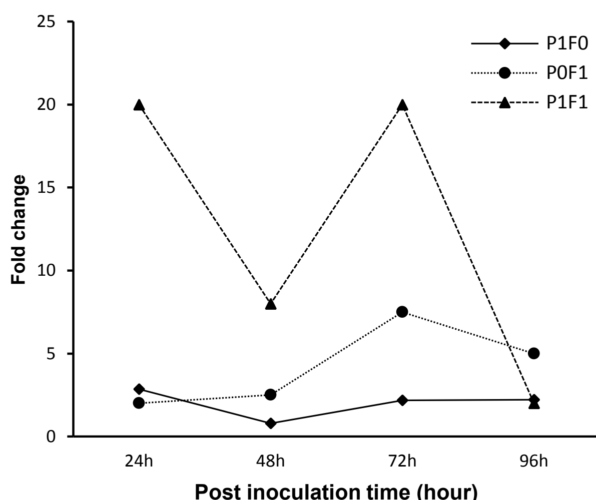

Figure 4. Expression patterns of *PR4* gene in rice. Triangle (P1F1): pre-inoculated with *P. indica* and challenged with *F. prolifratum* 10 days later; Diamond (P1F0) *P. indica*-inoculated; Square (P0F1) *F. prolifratum*-inoculated plants. The Y-axis represents fold change induction of the gene at 24, 48, 72 and 96 hours post inoculation with *F. prolifratum.*

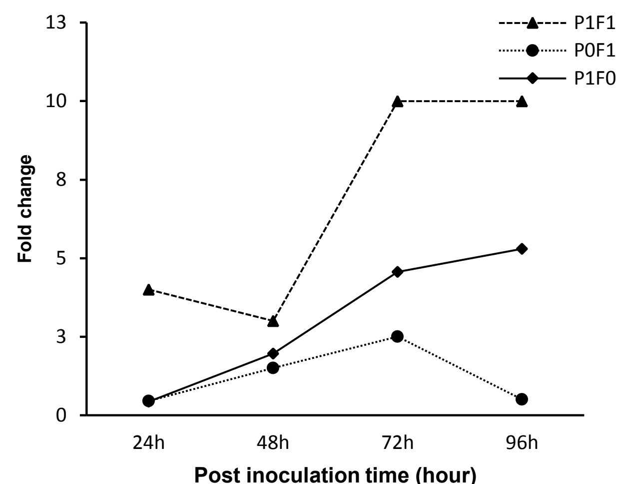

Figure 6. Expression patterns of *WRKY85* gene in rice. Triangle (P1F1): pre-inoculated with *P. indica* and challenged with *F. prolifratum* 10 days later; Diamond (P1F0) *P. indica*-inoculated; Square (P0F1) *F. prolifratum*-inoculated plants. The Y-axis represents fold change induction of *WRKY85* gene at 24, 48, 72 and 96 hours post inoculation with *F. prolifratum.*

manner, whereas the gene significantly up-regulated 48 hai (~7- fold) in plants, which merely inoculated with *Fusarium* in a monophasic pattern (Figure3).

Expression patterns of *PR4* gene

The expression level of *PR4* in rice plants pre-inoculated with *P. indica* and challenged with *F. proliferatum* demonstrated that the gene up-regulated significantly at 24 (~20- fold), 72 (~20- fold) and 96 hai (~2- fold) biphasic manner. The gene also up-regulated 48, 72 and 96 hai at 2.5, 6.4 and 5-fold, respectively, when rice plants only challenged with *Fusarium* (Figure 4).

Expression patterns of *WRKY62*

The expression level of *WRKY62* transcriptional factor gene in rice plants pre-inoculated with *P. indica* and challenged with *F. proliferatum* up-regulated significantly. The changes were at 24, 48, 72 and 96 hpi in 3.3; 3.3; 16.6

and 10- fold, respectively. The gene also up-regulated in Fusarium–inoculated plants at 48, 72 and 96 hpi in 2.5, 2.5 and 2 folds, respectively (Figure 5).

Expression patterns of WRKY85

The expression of *WRKY85* in rice plants pre-inoculated with *P. indica* and challenge inoculated with *F. proliferatum* revealed that the gene up-regulated significantly at 72 (2.5- fold) hpi in a monophasic manner. The gene also up-regulated at 24, 48, 72 and 96 hai by ~4, ~2.5, ~10 and ~10 - fold, respectively, when rice plants only challenged with *Fusarium* (Figure 6).

DISCUSSION

It is established in the current work that *P. indica* can protect rice plant from the damage caused by *F. proliferatum*. The chlorotic, thin and brownish leaves, decreasing plant length symptoms were visible in control plants (P0F1) 8 weeks after *F. proliferatum* inoculation; whereas *P. indica* pre-inoculated plants (P1F1) were look almost the same as non-infected plants with normal growth and slight yellowish-green. The same result was observed in *P. indica* wheat interaction (Mousavi et al 2015). However, there were no direct antagonistic effects between two fungi *in vitro*.

To characterize fungus plant interactions and to analyze the possible mechanism of protective effects of *P. indica*, a real-time PCR-based assay was developed. Rice plants with the established symbiotic interactions (12 dpi) were inoculated with *F. proliferatum*. The ratio of Fusarium DNA to plant DNA decreased ~13.5 fold from P0F1 to P1F1 in root samples 14 dpi. The figures highly correlate with the presence of mild symptoms in latter samples.

To test if endophytic fungus *P. indica* modulates PR and transcription factor (TF) genes, an experiment was conducted. It was observed that expression of *PR1b*, *NPR1*, *PR5*, *PR4*, *WRKY62* and *WRKY85* genes at 15 dpi with *P. indica* (P1F0) was at subtle change (< 2 fold) while the genes were up-regulated significantly for the next few days, which is an indication of induction of *PR* genes by *P. indica*. Comparing P1F1 with P0F1 treatments, the expression of *NPR1*, *PR4*, *WRKY62* and *WRKY85* genes in P1F1 samples were significantly higher than the expression in P0F1 samples (P1F1> P0F1) in almost all time points. Therefore, higher and stronger induction of the gene expression in P1F1 samples might be the consequence of presence of *P. indica* (Table 2).

Regarding expression of *NPR1*, *PR4*, *WRKY62* and *WRKY85* in P1F1 compared to P0F1 treatments, it can also figured out that *P. indica* caused a faster induction (24 hr) of the genes in P1F1 treatments (Table 3).

It is confirmed by several molecular studies that quantitative, rather than qualitative, differences in the defense responses regulate plant resistance or susceptibility to a pathogen (Nimchuk *et al.*, 2003). A rapid and strong activation of defence mechanisms is essential for success in controlling attackers.

Accordingly, preconditioning of plant tissues for a quick (earlier) and more effective (higher) activation of defence upon attack has important ecological fitness benefits and seems to be a common feature of the plant's immune system (Conrath *et al.*, 2006). This boost of basal defenses, known as priming, seems to be successfully triggered by certain beneficial micro-organisms such as *P. indica* (Balmer *et al.*, 015). Some pathogenesis related proteins are believed to have direct antifungal activities (Hejgaard *et al.*, 991). Faster induction results in higher concentration of antifungal products, which might slow down fungal penetration. The expression of *NPR1*, *PR1b*, *PR4*, *PR5*, *WRKY62* and *WRKY85* significantly reduced at 48hr post inoculation compared to 24h time point in P1F1 treatment. Significant up-regulations were observed in later time points. Constitutive expression of plant defence response is too costly. Thus, useful microorganisms have developed resistance through priming of the defence system, which is a physiologically less demanding approach in the challenging pathogens. (Van Wees *et al.*, 2008). Subtle elevated changes in defence-related transcripts might be the reason for the low metabolic cost of the higher resistance ability of *P. indica*-colonized plants, as it was observed in terms of enhanced growth and grain yield under field condition (Achatz *et al.*, 010).

Table 2. Gene expression in terms of fold induction 72 hours post inoculation.

	NPR1	PR4	WRKY62	WRKY85
P1F1	12	20	16.7	10
P0F1	-	5	2.5	2.5

Table 3. Gene expression in terms of fold induction 24 hours post inoculation.

	NPR1	PR4	WRKY62	WRKY85
P1F1	8	20	3.3	4
P1F0	-	2.85	-	-
P0F1	-	2	-	-

P. indica systematically induced transcripts related to transcription and suppressed photosynthetic transcripts in the absence of pathogen in barley, which was similar to the effect of the compatible interaction with *Blumeria graminis* (Molitor *et al.*, 2011). In barley roots following symbiosis by non-pathogenic rhizobacteria, leaf transcripts were not affected in the absence of pathogen (Verhagen *et al.*, 2004). Both cases considered as induced systemic resistance (ISR) triggered. ISR mechanism typically does not require additional host plant resources. ISR can provide resistance in distal tissues and requires jasmonic acid signaling and ethylene signaling (Van Loon, 1998).

In the present experiment, different transcripts which were involved in different signaling pathways were analyzed. SA and H_2O_2 strongly induced the mRNA level of both *OsPR1* genes in rice plants (Agrawal *et al.*, 2 001). *NPR1/NIM1* (no PR-1 expression; non-inducible immunity) gene is essential for regulation of SAR phenomenon downstream of salicylic acid and is needed for the expression of resistance and PR-protein gene expression (Cao *et al.*, 1997). Although the molecular basis for priming has not been established, a requirement for *NPR1/NIM1* has been shown (Kohler *et al.*, 2002). Mutation in *NPR1/NIM1* causes the failure of either ISR or SAR activation (Delaney *et al.*, 1995). Hevein-like protein or PR4 is a JA-induced (Schenk *et al.*, 2000) and ethylene responsive defence gene (Van Loon *et al.*, 2006). *PR5* is an SA signaling system-inducible gene (Zhang *et al.*, 2012). According to the results functional colonization had a similar effect in genes involved in both SA and JA pathways. However, some scientists believe that a functional colonization implies partial suppression of SA-dependent responses or up-regulation and priming of those that are JA-regulated (Pozo and Azcón-Aguilar, 2007).

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تاثیر همزیستی قارچ *Piriformospora indica* بر القاء مقاومت در مقابل بیماری پوسیدگی طوقه برنج (*Oryza sativa* L.)

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چکیده

بیماری پوسیدگی طوقه برنج یکی از بیماری‌های مهم برنج است که یکی از روش متداول کنترل آن استفاده از سموم شیمیایی است که علاوه بر پرهزینه بودن اثرات مخرب زیست محیطی را هم به دنبال دارد. قارچ درون هم‌زیست *Piriformospora indica* میکروارگانیسمی است که روی ریشه تعدادی از گیاهان زندگی می‌کند و موجب تحریک مقاومت سیستمیک علیه عوامل بیماری‌زای ریشه، ساقه و برگ در گیاه می‌شود. پژوهش حاضر به منظور مطالعه تاثیر هم‌زیستی قارچ مذکور بر ویژگی‌های مولکولی مقاومت القائی علیه بیماری پوسیدگی طوقه برنج انجام شده است. گیاهچه‌های برنج تلقیح شده با *P. indica* و نیز گیاهچه‌های فاقد *P. indica* با سوسپانسیون اسپور قارچ عامل بیماری (*Fusarium proliferatum*) مایه‌کوبی و در محیط آبی یوشیدا کشت و نمونه‌برداری از ریشه و برگ گیاهچه‌های کلیه تیمارها نمونه برداری انجام شد. بیان ژن‌های مقاومت *PR4, PR1, WRKY62, WRKY85, NPR1* ارزیابی شدند. بیان اغلب ژن های مطالعه شده در بافت‌های ریشه و برگ گیاهان پیش تلقیح شده با *P. Indica* و آلوده به قارچ فوزاریوم به طور معنی‌داری بالاتر از مقدار آنها در گیاهانی بود که فقط با قارچ فوزاریوم تلقیح شده بودند. علاوه بر این، سرعت بیان تعدادی از ژن ها نیز در گیاهان پیش تلقیح شده بیشتر بوده است که با پرایمینگ ژن‌های مذکور در ارتباط است. کمیت‌سنجی بیومس قارچ بیمارگر در ریشه گیاهانی که دارای قارچ هم‌زیست بودند نشان داد که میزان بیومس بیمارگر در هفته دوم پس از تلقیح کاهش قابل توجهی (۰/۰۵ برابر) نسبت به هفته اول داشت، بیومس قارچ بیمارگر در گیاهان فاقد *P. indica* در هفته اول و دوم اندازه‌گیری بسیار بیشتر از گیاهان دارای *P. indica* بود.

کلمات کلیدی: برنج، پوسیدگی طوقه، پرایمینگ، قارچ *P. indica*، مقاومت القائی