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Effects of a biocontrol bacterium on growth and defence of transgenic rice plants expressing a bacterial type-III effector

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Abstract – Expression of $HpaG_{Xoo}$, a bacterial type-III effector protein, in transgenic plants induces disease resistance. Resistance also can be elicited by biocontrol bacteria. We studied effects of the biocontrol *Bacillus subtilis* strain B-916 on the rice variety R109 and the the $hpaG_{Xoo}$- expressing rice line HER1. Colonisation of roots by B-916 caused 12.5 ± 1.3% and 0.5 ± 0.05% increases, in contrast to controls, in root growth of R109 and HER1. Growth of R109 leaves and stems was increased by 0.5 ± 0.05% but that of HER1 was inhibited. When B-916 colonisation was subsequent to plant inoculation with *Rhizoctonia solani*, a pathogen that causes sheath blight, the disease was less severe than controls in both R109 and HER1; HER1, nevertheless, was more resistant, suggesting that B-916 and $HpaG_{Xoo}$ cooperate in inducing disease resistance. In R109 roots, the *OsARF1* gene, which regulates plant growth, was expressed in consistence with growth promotion by B-916. Inversely, the depression of *OsARF1* expression was coincident with inhibition in growth of HER1 leaves and stems. In both plants, the expression of *OsEXP1*, which encodes an expansin protein involved in plant growth, was concomitant with growth promotion in leaves and roots responding to B-916. We also studied *OsMAPK5b* encoding a mitogen-activated protein kinase involved in multiple defence responses in rice. In response to B-916, early expression of *OsMAPK5b* was coincident with R109 resistance to the disease, while HER1 expressed the gene similarly whether B-916 was present or not. Evidently, B916 and HER1 interact differently in rice growth and resistance. The combinative effects should stimulate agricultural use and further studies on mechanisms that underlie the interaction.

Key words: rice, $hpaG_{Xoo}$-expressing rice line 1 (HER1), biocontrol bacteria, growth promotion, disease resistance.

INTRODUCTION

Due to public concerns on pesticide use in crops, exploration on alternative methods has been a global effort for secure crop protection. Biocontrol bacteria and bioactive natural products show a great potential of agricultural use in disease control and improvement of crop productivity (Dixon, 2001; Stuiver and Custers, 2001; Zasloff, 2001). *Bacillus* species is an important class of biocontrol bacteria; they protect plants by numbers of mechanisms. When applied to rice and other plants, *B. subtilis* produces iturin to inhibit *Botrytis cinerea* (Toure *et al*., 2004), *Fusarium graminearum*, *Rhizoctonia solani*, *Rhizoctonia cerealis*, *Pyricularia grisea* (Yao *et al*., 2003), *Xanthomonas oryzae* pv. *oryzae* (Lin *et al*., 2001), and *Aspergillus niger* (Podile and Prakash, 1996), which cause significant diseases in a variety of crops. *B. subtilis* also induces plants to produce defence compounds, like chitinase and peroxidase, which protect plants by action on the pathogens (Ongena *et al*., 2005). Moreover, biocontrol bacteria can promote seed germination and plant growth, indirectly increasing resistance (Romanenko and Alimov, 2000). Combinative use of these mechanisms may greatly increase effects in disease control and improvement of agronomic properties of crops.

Natural products that have a potential in crop improvement are various in nature and sources. Proteins of harpin group produced by many plant pathogenic bacteria belong to an important class of type-III effectors, which elicit multiple plant responses when secreted by pathogens during infection (Staskawicz *et al*., 2001; Alfano and Collmer, 2004) or applied to plants (Dong *et al*., 1999; Dong *et al*., 2004, 2005). $HpaG_{Xoo}$, a harpin protein produced by the rice leaf blight pathogen *X. oryzae* pv. *oryzae*, stimulates plant growth and induces defence toward pathogens and insects (Noel *et al*., 2002; Kim *et al*., 2003; Peng *et al*., 2003). In transgenic tobacco plants expressing $HpaG_{Xoo}$, systemic acquired resistance (SAR) is induced (Peng *et al*., 2004b). $HrpN_{Ea}$ from *Erwinia amylovora* not only stimulates abscisic acid signalling to induce drought tolerance (Dong *et al*., 2005) but also stimulates ethylene to promotes plant growth and induce insect defence (Dong *et al*., 2004). The defence responses often lead to production of various defence compounds, such as chitinase and peroxidase (Kim and Beer, 2000; Peng *et al*., 2003), which

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also can be induced by biocontrol bacteria (Nandakumar *et al*., 2001). Moreover, colonisation of plant roots by biocontrol bacteria activates induced systemic resistance (ISR), which antagonizes SAR and provides plants with a distinct battery of defence arsenal (Pieterse *et al*., 1996; Dong *et al*., 2001; Bostock, 2005). Therefore, a combinative use of biocontrol bacteria and the type-III effectors could be more effective than use of either of them in crop improvement.

This study was aimed at determining if a biocontrol bacterium and a type-III effector interact to affect plant growth and disease resistance. Previously, we used *B. subtilis* B-916 to control rice sheath blight with desired results (Chen and Mew, 1998). We have introduced $HpaG_{Xoo}$ into tobacco (variety Xanthi) and rice (variety R109), generating several transgenic lines. Transgenic tobacco plants have been determined to increase resistance to viral, bacterial, and fungal diseases (Peng *et al*., 2004b). Here we show that the $HpaG_{Xoo}$-expressing rice line 1 (HER1) increases growth and activates defences toward pathogens. We also present evidence that *B. subtilis* B-916 differentially affects growth and disease resistance in R109 and HER1.

MATERIALS AND METHODS

Microbes. The *Bacillus subtilis* strain B-916 has been transformed with the vector pRP22 containing a green florescent protein gene. The recombinant strain B-916::pRp22::GFP possesses chloromycetin resistance held by the vector and rifampicin resistance as screened previously (Chen and Mew, 1998). Bacterial cells were multiplied in YPGA medium (5 g of yeast extract, 5 g of Bacto peptone, 5 g of glucose, 1 g of NaCl and 17 g of agar, in a litre) (Pionnat *et al*., 1999) supplied with 20 µg/ml chloromycetin and 250 µg/ml rifampicin in a shaker at 28 °C for 48 h. The *Rhizoctonia solani* strain RH-2 was stored under -86 °C; before inoculation, it was incubated in Potato Dextrose agar medium (PDA) at 28 °C for 7 d.

Growth of rice. The rice (*Oryza sativa indica*) variety R109 was used. By engineering R109 with $HpaG_{Xoo}$ (Peng *et al*., 2003), HER1 was generated and maintained in our lab. Equivalent progenies from the empty vector-transformed rice (EVTR) line were a control compared to HER. Seeds were germinated by incubation for 2 d in Petri dishes at 28 °C in dark and transferred into plastic cups containing quartz sands saturated with Rice Nutrition Solution (The International Rice Research Institute, Makati City, Metro Manila, Philippines). Subsequently, germinal seedlings were grown in a chamber at 25 °C and a 16-h-light/8-h-dark cycle. Plants of 14-d old were used for experiments in lab.

Determination of effects of *Bacillus subtilis* B-916 on rice plants. To evaluate effects of B-916 on rice growth, seeds were soaked in water (control) or a B-916 suspension of 5×10^8 colony formation unit per millilitre (CFU/ml) for 2 h and incubated on a wet filter paper in a Petri dish. Root growth was monitored with a group of treated seeds. In another group, juvenile seedlings were transferred into nutrition solutions and incubated as described. At 14 d after seed treatment, plant weight was determined. B-916 population in root tissues was determined at intervals after treatment. Roots were excised, weighted, sterilised in 70% ethanol and 33% sodium hypochlorite. Sterilised roots were washed in sterile water, homogenized in 10 ml sterile water, and placed in a -20 °C refrigerator during operation to avoid bacterial multiplication in homogenates. Thawed homogenates were spun briefly and diluted properly; 5 µl of each dilution was placed on YPGA-Cm agar medium in a plate; one plate was used for one sample with different dilutions. Plates were incubated under 28 °C for 2 d; bacterial numbers were counted and given as CFU/g fresh root.

Sheath blight evaluation. In lab assays, HER1 in T3 generation were compared with EVTR or R109 for resistance to sheath blight resistance in the presence and absence of *R. solani* RH-2. The basal second sheaths of plants were inoculated with uniform RH-2 sclerotia placed between sheaths and stems. Inoculated sites were covered with wet absorbent cotton tied to stem by transparent pastern. Inoculated plants were incubated in the chamber. Sixteen h later, roots were soaked in water or a B-916 suspension (5×10^8 CFU/ml) for 2 h, followed by regular growth of plants. Sheath blight symptoms were monitored at 24, 48, and 72 h post treatment (hpt) with B-916. Disease severity was evaluated based on length of blight lesions.

Gene expression analysis. Total RNA was isolated from whole plants, roots or leaves at intervals using the Tripure Isolution Reagent (Roche Diagnostics, Indianapolis, IN, USA) as per the vender's instruction. Gene expression was analysed by RT-PCR done with primers and programs shown in Table 1. Reaction conditions were optimised and RT-PCR

TABLE 1 – PCR and RT-PCR primers and programs for genes tested in this study

Gene	Genbank accession No.	Primers (Product size in bp)	PCR programs
OsARF1	AJ306306	5′-gcagattactgcaggccatt-3′, 5′-tcatccgctgaacctcttct-3′ (899)	95 °C 5 min; 95 °C 30 s, 55 °C 50 s, 72 °C 50 s, 25 cycles; 72 °C 10 min
OsEF1α	AF030517	5′-ccgagcgtgagagaggtatc-3′, 5′-gccaataccaccgatcttgt-3′ (551)	95 °C 5 min; 95 °C 30 s, 55 °C 45 s, 72 °C 40 s, 25 cycles; 72 °C 10 min
OsEXP1	Y07782	5′-taccggatcatgtgcgacta-3′, 5′-gctgtgaggtcgagaaggtc-3′ (517)	95 °C 5 min; 95 °C 30 s, 50 °C 30 s, 72 °C 45 s, 25 cycles; 72 °C 5 min
hpaG$_{Xoo}$	AY139029	5′-gcggatttgttatcgattc-3′, 5′-tattactgcattgatgcgcttcc-3′ (420)	95 °C 5 min; 95 °C 30 s, 55 °C 30 s, 72 °C 30 s, 30 cycles; 72 °C 10 min
OsMAPK5b	AF479884	5′-cgacatgatgacggagtacg-3′, 5′-cagctgcttcatttggtcct-3′ (496)	95 °C 5 min; 95 °C 30 s, 50 °C 45 s, 72 °C 40 s, 25 cycles; 72 °C 10 min

products were confirmed by sequencing as described (Peng *et al*., 2003). The elongation translation factor gene *EF1α* was used as a control because it is expressed constitutively in eukaryotes (Berberich *et al*., 1995). RT-PCR products were resolved by electrophoresis in 1% agarose gel and visualized by staining with ethidium bromide. Relative levels of gene expression were determined with a gel documentation system (Molecular Imager Gel Doc XR System equipped with the Quantity One 1-D analysis software, Alfred Nobel Drive Hercules, California, USA).

Data analysis. Results were presented when they were similar in replicate experiments. Each experiment was done 3 times and involved 3 replicates each time; a replicate contained 15 plants. Exceptions were specified elsewhere in the text or in figure legends. T and ANOVA tests were used to determine significant differences among treatments.

RESULTS

Effects of *Bacillus subtilis* B-916 on growth of R109 and HER1

When applied to roots, the biocontrol bacterial strain B-916 differently affected growth of root parts, stems, and leaves of both plants. As depicted in Fig. 1A, when compared to control, colonisation of seeds by B-916 evidently increased root growth of R109. However, growth of HER1 stems and leaves apparently was inhibited. Growth of HER1 roots and R109 stems and leaves was promoted a little.

To relate growth variations with B-916 multiplication, we determined bacterial population in rice tissues. As shown in Fig. 1B, bacterial numbers in roots of both R109 and HER1 increased with time during the course of study; they were greater in HER1 relative to those in R109 at each time point after 6 h posttreatment (hpt). Difference in bacterial number between both genotypes was significant (ANOVA test, p = 0.05). Optimal population was found at 24 hpt; HER1 supported bacterial growth to a number that was 1.6 times bacterial population in R109.

The effects of B-916 on growth were quantified (Fig. 1C). Root growth increases for B-916 vs. control were determined as 12.5 ± 1.3% and 0.5 ± 0.05% in R109 and HER1, respectively. However, growth of stems and leaves was different between R109 and HER1. For R109, 0.5 ± 0.05% increase was detected; by contrary, HER1 showed an 8.1 ± 1.0% decrease in growth of stems and leaves. The differences were significant between control and B-916 treatment (T test, p = 0.05) and among treatments in R109 and HER1 (ANOVA test, p = 0.05), suggesting a significant effect of B-916 on rice growth. These results indicated that root, stem and leaf growth in R109 and HER1 was not coincident with B-916 population.

Effects of *Bacillus subtilis* B-916 on sheath blight in R109 and HER1

A lab assay for rice sheath blight revealed a cooperative effect of B-916 and HER1 in decreasing severity of the disease (Fig. 2). When plants were not treated with B-916, HER1 was less diseased than R109 (Fig. 2A). In R109, sheath blight symptoms appeared by 12 h after inoculation, when no symptom was seen in HER1. When observed at 48 h after inoculation, symptoms spread in R109 over the leaf and stem that contacted with sclerotia as inoculums, but symptoms occurred only on the stem in HER1 plants treated with water (Fig. 2A, CK). These results indicate that HER1 had acquired resistance. Following B-916 application to roots, on the other hand, sheath blight was alleviated markedly compared to control in both R109 and HER1. However, blight lesions were evidently shorter and less extensive on sheath of HER1 vs. R109 (Fig. 2A, B-916). These results were confirmed by quantifying disease severity evaluated based on length of blight lesions (Fig. 2B). When determined at 24, 48 and 72 hpt and compared to control, length of blight lesions of R109 were decreased by 100 ± 5%, 18.2 ± 1% and 12.1 ± 0.6%; the rate in HER1 was 100 ± 5%, 57.5 ± 2.9% and 36.4 ±

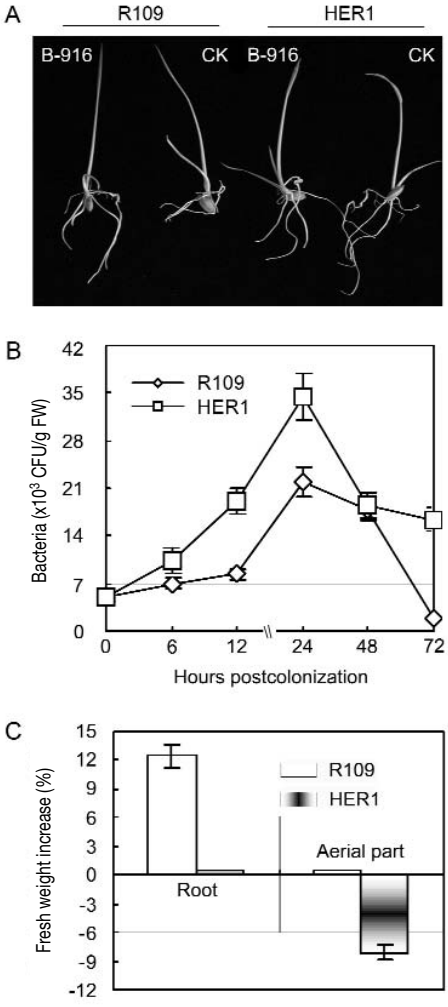

FIG. 1 – Effects of the biocontrol bacterial strain B-916 on growth of HER1 and R109. A - Seedlings of 14-d old. Seeds were germinated on wet filter paper in Petri-dish for 2 d. Juvenile roots were soaked in sterile water (CK) or a B-916 suspension (5 x 10^8 CFU/ml). Two h later, juvenile seedlings were transferred to nutrition solution in plastic cups, incubated as described. B - Bacterial numbers in root tissues of plants equivalent to those in A. Bacteria were recovered from plants immediately after soaking roots in a B-916 suspension and at the indicated intervals. Bacterial population was determined as CFU/g fresh weight (FW). Curves represent mean ± standard deviation (SD) of results from 3 replicates (n = 45 plants). C - Weight increase in B-916-colonised plants vs. CK plants. Plants from A were grown for 14 d. Increase in plant weight was determined for B-916-colonised plants in contrast to CK plants. SD bars (n = 45 plants) are shown cross tops of histograms.

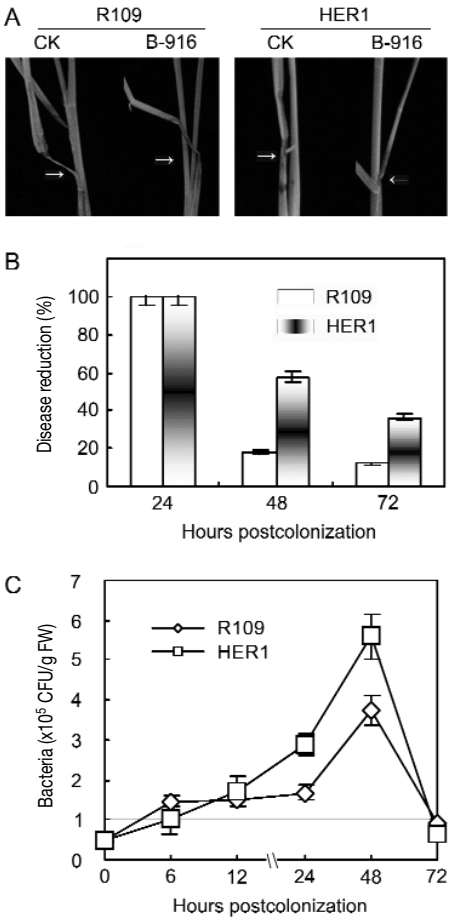

FIG. 2 – Effects of B-916 on sheath blight. A - Disease symptoms on R109 and HER1. Plants at 14-d old were inoculated with *R. solani* at the sites indicated by narrows. Sixteen h later, roots were soaked in sterile water (CK) or a B-916 suspension for 2 h. Plants were photographed at 24 h postinoculation (n = 15 plants). B - Disease severity reduction in B-916-colonised plants vs. CK plants. Length of sheath blight on plants equivalent to those in A was determined. Disease severity reduction was determined by comparing length of blight lesions on B-916-colonised plants with that on CK plants. SD bars (n = 45 plants) are shown cross tops of histograms. C - Bacterial numbers in tissues of plants equivalent to those in A. Assay was similar as in Fig. 1A. SD bars (n = 45 plants) are shown on curves.

1.8%. Clearly, B-916 and HER1 cooperated in alleviating sheath blight severity.

B-916 multiplication in *R. solani*-inoculated plants was consistent with the reduction of disease severity (Fig. 2C). During 6-48 hpt, bacterial population increased with time in R109 and HER1, but bacterial numbers were evidently greater in HER1 than R109 at 12-48 hpt. At 24 hpt, bacterial amounts in root tissues of HER1 were scored as ca. 2.9 ± 0.3 x 10^5 CFU/g, which was 1.7-fold of that in R109. These results represented significant differences between R109 and HER1, and between CK and B-916 treatment in both genotypes (ANOVA test, p = 0.05).

Growth-related gene expression in R109 and HER1

To seek a reason for the disparity between HER1 and R109 in growth promotion of roots, stems and leaves responding to B-916, as shown in Fig. 1, genes *OsARF1* (*Oryza sativa* auxin response factor 1) and *OsEXP1* (encoding a rice expansin) were studied by RT-PCR conducted with the *EF1α* gene used as a standard (Fig. 3A). OsARF1 regulates an early response to an auxin signal during plant growth and is most active in fast-growing parts of rice (Waller *et al*., 2002), whereas expansins are required for cell expansion and plant growth in regular growth process and in response to a harpin protein (Dong *et al*., 2004). When plants were not colonised by B-916, *OsARF1* was not expressed in R109 and HER1. *OsEXP1*, however, was expressed conspicuously in leaves but feebly in roots of both plants; expression level in HER1 leaves was higher than that in R109 leaves (Fig. 3A). When tested at 12 h after root colonisation by B-916, the two genes behaved differently in R109 and HER1. A great level of *OsARF1* transcript was detected in roots of R109 instead of HER1, while expression was not evident in R109 leaves and HER1 roots and leaves (Fig. 3B). Like *OsARF1*, *OsEXP1* was expressed at varied extents in roots and leaves of both plants (Fig. 3C). The expression level was close in R109 and HER1 roots and optimal in R109 roots, which accumulated the transcript to an extent that was ca. 10-fold higher than the level in HER1 leaves (Fig. 3C). Apparently, *OsARF1* and *OsEXP1* were quite related to growth of roots, leaves, and stems of R109 and HER1 in response to B-916. Induced expression of both genes was coincident with growth of roots, leaves and stems of R109 and HER1.

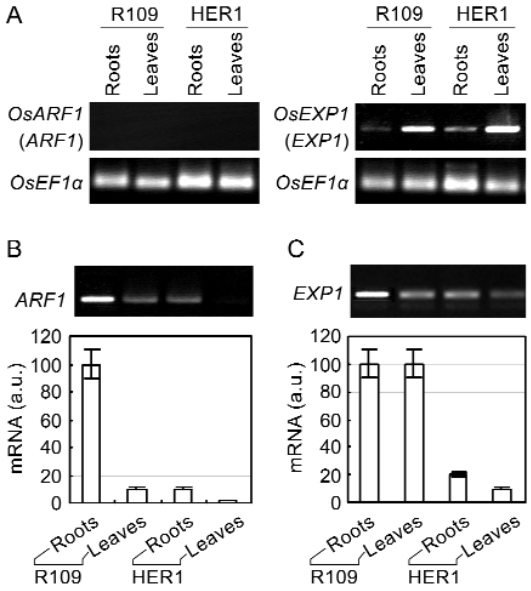

FIG. 3 – Differential expression of *OsARF1* and *OsEXP1* expression in roots and leaves. A - Assay with plants in the absence of B-916. Plants at 14-d old were studied. B and C - Assay with plants in the presence of B-916. Root colonisation by B-916 was executed 12 h prior to the assay. In A to C, RNA was isolated from roots and leaves as indicated. Gene expression was analysed by RT-PCR conducted with RNA isolated from whole plants. RT-PCR products were resolved in agarose gel by electrophoresis and visualized by staining with ethidium bromide. The constitutively expressed gene *OsEF1α* was as a standard to verify uniform amplification of genes (gel not shown). Relative amount of *OsARF1* and *OsEXP1* mRNA was quantified as arbitrary units (a.u.) with a gel documentation system.

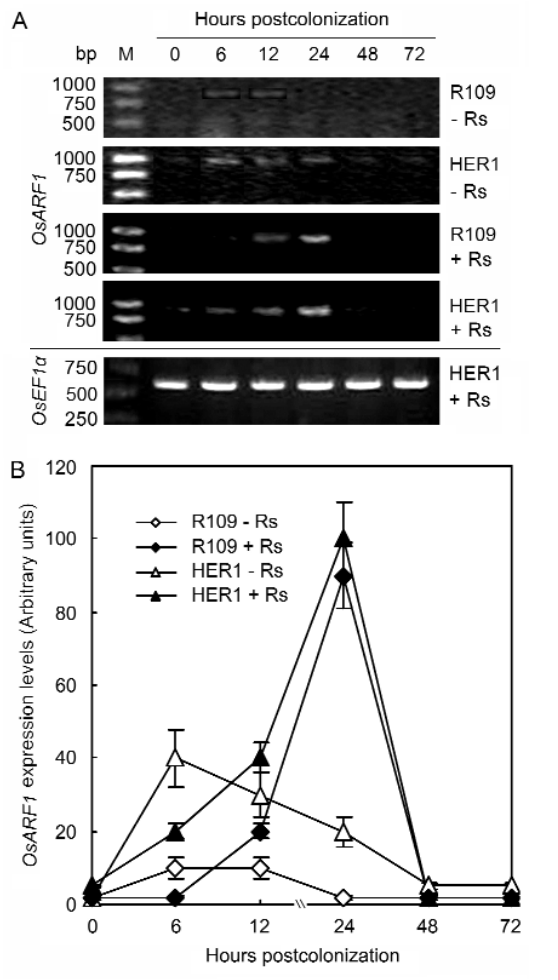

FIG. 4 – Time course (A) and level (B) of *OsARF1* expression in leaves of plants colonised by B-916 and inoculated with *R. solani* or without inoculation. Plants at 14-d old were inoculated; 16 h later, roots were soaked in a B-916 suspension for 2 h, followed by continuous incubation of plants. RNA was isolated at indicated times and analyzed as in Fig. 3. R109 - Rs meaned R09 plants colonised with B-916, but not inoculated with *R. solani* RH-2; R109 + Rs meaned R109 plants inoculated with RH-2 and subsequently colonised with B-916; HER1 - Rs meaned HER1 plants colonised with B-916, but not inoculated with RH-2; HER1 + Rs meaned HER1 plants inoculated with RH-2 and subsequently colonised with B-916.

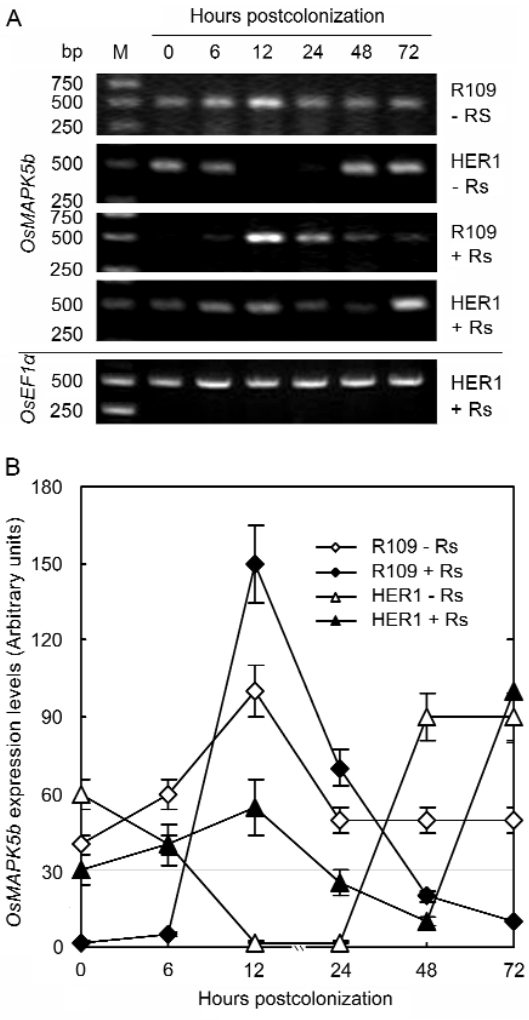

FIG. 5 – Time course (A) and level (B) of *OsMAPK5b* expression in leaves of plants colonised by B-916 and inoculated with *R. solani* or without inoculation. Plant treatments and assays were similar to those in Fig. 4.

OsARF1 expression in the interaction complex

We determined how *OsARF1* behaves in leaves when B-916, $HpaG_{Xoo}$ and *R. solani* are present together in rice plants. The gene expression was compared in leaves of plants after they were inoculated with *R. solani* RH-2 or not inoculated, followed by B-916 colonisation on roots or no colonisation. Figure 4A depicts gene expression pattern. When plants were not inoculated, *OsARF1* expression was detected feebly at 6 and 12 h but after that it was not detectable; when plants were colonised by B-916, *OsARF1* expression levels were similar in HER1 in contrast to R109. When plants were inoculated, *OsARF1* was expressed in both plants, but stronger in HER1 vs. R109; expression level also was increased with time. Quantifying gene expression level (Fig. 4B) and comparing it with growth (Fig. 1C) and disease (Fig. 2B) revealed that the earlier expression was coincident to plant growth promotion, whereas the decreased expression after 24 h of colonisation (40 h after inoculation) apparently was related to infection by the pathogen.

OsMAPK5b expression in the interaction complex

We also studied *OsMAPK5b*, a gene that encodes a mitogen-activated protein kinase in rice. OsMAPK5b is required for tolerance to drought, salinity and coldness but inhibits SAR (Xiong and Yang, 2003) and thus may be engaged in the role of biocontrol bacteria in R109 vs. HER1, which shows defence to pathogens and stresses (data not shown). *OsMAPK5b* had a constitutive expression but patterns (Fig. 5A) and levels (Fig. 5B) of expression varied with time and plant genotypes. In HER1, the gene expression was similar in the different combinations, indicating that it was not related with responses to B-916 or *R. solani*. In R109, however, *OsMAPK5b* expression was affected markedly by B-916 and *R. solani*. In R109 plants without inoculation, *OsMAPK5b* expression was evident during 0-72 h after B-916 colonisation on roots. When R109 plants were inoculated with *R. solani*, time of *OsMAPK5b* expression was promoted at 12 h and became feeble subsequently since 48 h, suggesting an effect by the pathogen.

DISCUSSION

We have engineered the rice variety R109 with *hpaG*$_{Xoo}$, a bacterial gene encoding a type-III effector protein, generated HER lines, and compared HER1 and R109 for growth and disease resistance in response to *B. subtilis* B-916, a biocontrol bacterial strain. Based on results detailed here, B-916 and HpaG$_{Xoo}$ interacted differently during rice growth and resistance development. Both cooperate in inducing resistance, promising a great potential in agricultural use. Complicated mechanisms underlie interactions involving B-916, HpaG$_{Xoo}$ and the sheath blight pathogen *R. solani*.

The increase of disease resistance in HER1 is consistence with our previous study showing that HpaG$_{Xoo}$ induces disease resistance in transgenic tobacco plants (Peng *et al*., 2004a, 2004b). Results described here show that B-916 is more effective in HER1 than it is in R109 to increase resistance. Evidence in supporting the conclusion was seen in the consistence of resistance with the *in planta* population of B-916 (Fig. 2). Clearly, B-916 and HpaG$_{Xoo}$ act together to affect disease resistance, and could be integrated into a practice to provide better effect than applying either of them. Moreover, B-916 experimentally antagonizes HpaG$_{Xoo}$ in rice stems and leaves growth. The negative interaction was seen in differences between R109 and HER1 in growth of roots and, particularly, stems and leaves (Fig. 1). The antagonism also was evident in the disparity between rice growth and B-916 population (Fig. 1). It is important to test whether the antagonism diminishes rice productivity.

Results of studying *OsARF1*, *OsEXP1* and *OsMAPK5b* disclose complicatedness in molecular basis of interactions involving B-916, HpaG$_{Xoo}$ and *R. solani*. Different signals may be recruited to affect rice growth in response to B-916 and HpaG$_{Xoo}$. Transcription of *OsARF1* is an early response to an auxin signal in rice and is most active in parts of facilitated growth (Waller *et al*., 2002). We found that *OsARF1* expression was induced in R109 roots but was nullified in HER1 stems and leaves (Fig. 3). Consistently, B-916 promoted growth of R109 roots but inhibited HER1 leaves and stems growth (Fig. 1). Growth inhibition in HER1 apparently matched B-916-compromised proportion of the growth increase relative to R109. Thus, *OsARF1* may at on aerial parts of the plant to affect B-916-HpaG$_{Xoo}$ antagonism. Moreover, plant growth stimulated by a harpin involves expansins, which promote cell growth by loosing the wall (Li *et al*., 2003). Actions by expansins and harpin both are subjected to ethylene signalling (Dong *et al*., 2004; Belfield *et al*., 2005; Vreeburg *et al*., 2005; Shi *et al.*, 2006). The behaviour of *OsEXP1* in R109 and HER1 (Fig. 3) suggests a role *OsEXP1* plays in the positive effect of B-916 on R109 and HER1, respectively. Further studies are required to determine how the involved signals and genes modulate the antagonism found in HER1.

The pattern and time course of *OsMAPK5b* expression (Fig. 5) indicate that B-916-HpaG$_{Xoo}$ interaction may affect distinct signalling pathways. In rice, OsMAPK5b regulates tolerance to drought, salinity and coldness but acts to suppress salicylic acid-mediated SAR (Xiong and Yang, 2003). SAR, however, serves as a pathway of type-III effectors in harpin group to induce disease resistance when the proteins are applied to plants (Strobel *et al*., 1996; Dong *et al*., 1999) or produced in transgenic plants (Peng *et al*., 2004b). Nevertheless, plant growth-promoting biocontrol bacteria are believed to induce ISR, which antagonizes SAR in signal transduction (Johri *et al*., 2003; Gielen *et al*., 2004; Tjamos, 2005). Studying an individual factor, like OsMAPK5b, in the proposed signalling network merely is the first step toward studies on components critical to the interaction.

In HER1 vs. R109, retarded infection is consistent with resistance (Fig. 2). After inoculated to rice plants, *R. solani* sclerotia produce tubes to infect the host usually in and causes disease in 24 h (Rohilla *et al*., 2002; Wang and Tan, 2005). The evident expression of *OsARF1* and *OsMAPK5b* within 12 h reasonably is attributed to B-916 colonisation on roots (Figs. 4 and 5). Plant basal defence (Dangl and Jones, 2001) can be induced by pathogens but occurs rather later during infection process than required for protecting plants from diseases (Vijayan *et al*., 1998; Maleck and Dietrich, 1999). This may be the case for *OsMAPK5b*. An inverse mode was seen for *OsARF1*. The pathogen seems to affect plant growth by affecting *OsARF1* expression, which is concomitant with plant growth in response to an auxin signal (Waller *et al*., 2002). The conspicuous expression of *OsARF1* after 40 h of inoculation (Fig. 4) indicates that the gene behaves depending on challenges that the plant encounters (Waller *et al*., 2002). The hypothesis remains to be tested.

Acknowledgements

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