Rice Defense Mechanisms Against *Cochliobolus miyabeanus* and *Magnaporthe grisea* Are Distinct

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ABSTRACT

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Responses of rice to *Magnaporthe grisea* and *Cochliobolus miyabeanus* were compared. In Tetep, a rice cultivar resistant to both fungi, pathogen inoculation rapidly triggered the hypersensitive reaction (HR), resulting in microscopic cell death. In rice cv. Nakdong, susceptible to both pathogens, *M. grisea* did not cause HR, whereas *C. miyabeanus* caused rapid cell death similar to that associated with HR, which appeared similar to that observed in cv. Tetep, yet failed to block fungal ramification. Treatment with conidial germination fluid (CGF) from *C. miyabeanus* induced rapid cell death in both cultivars, suggesting the presence of phytotoxins in CGF. Pretreatment of cv. Nakdong with CGF significantly increased resistance to *M. grisea*, while the same treatment was ineffective against *C. miyabeanus*. Similarly, in cv. Nakdong, benzo-

thiadiazole (BTH) significantly increased resistance to *M. grisea*, but was ineffective against *C. miyabeanus*. Methyl jasmonate (MeJA) treatment appeared to be ineffective against either fungus. Increased resistance of cv. Nakdong to *M. grisea* by BTH or CCF treatment was correlated with more rapid induction of three monitored *PR* genes. Application of MeJA resulted in the expression of *JAmyb* in cv. Nakdong being induced faster than in untreated plants in response to *M. grisea* infection. In contrast, the expression pattern of the *PR* and *JAmyb* genes in response to *C. miyabeanus* was nearly identical between cvs. Nakdong and Tetep, and neither BTH nor MeJA treatment significantly modified their expression patterns in response to *C. miyabeanus* infection. Our results suggest that rice employs distinct mechanisms for its defense against *M. grisea* versus *C. miyabeanus*.

Additional keywords: host selective toxins, rice blast, rice brown leaf spot.

Although a very large number of pathogens exist in nature, each plant species is susceptible only to a limited number of pathogens that have evolved to overcome the defense system of the plant species. In some plant-pathogen systems, cultivars of a host plant also exhibit differential resistance to individual races within a pathogen species, termed the gene-for-gene interaction (14). The gene-for-gene interaction, in which a pathogen isolate expressing an avirulence (*AVR*) gene triggers a rapid defense response in host cultivars expressing a matching resistance (*R*) gene, is a major determinant of plant susceptibility to diverse pathogens, including viruses, bacteria, fungi, and nematodes.

In contrast to the highly specific nature of the initial recognition event mediated by *AVR* and *R* gene interactions, subsequent defense responses typically involve an array of molecular and cellular changes that commonly occur in response to a diverse group of pathogens (7). Incompatible interactions lead to the onset of rapid local defense responses, including ion fluxes, activation of kinases, and accumulation of reactive oxygen intermediates. Hypersensitive response (HR)-associated cell death around the infection site limits the spread of the pathogen to surrounding cells, and also evokes a systemic defense response, which is mediated through the production of secondary signal molecules, such as salicylic acid (SA), jasmonic acid (JA), ethylene (ETH), and nitric oxide (NO). Plant defense responses are accompanied by the induced expression of pathogenesis-related (*PR*) genes locally at the point of infection, as well as systemically (35,37).

The rapid induction of these genes serves as an indicator of incompatible interactions (3,6,19).

Systemic acquired resistance (SAR), the most well characterized systemic defense response, confers resistance to a broad spectrum of pathogens and can be induced by treatment of SA or its analogs, such as benzothiadiazole (BTH) (15,16) and methyl-2,6-dichloroisonicotinic acid (INA) (9). Application of one of these SAR-inducing chemicals to a susceptible host prior to pathogen infection leads to very rapid induction of HR and *PR* gene expression upon pathogen infection, resulting in no or reduced disease (16,24,29). Although onset of SAR conferred increased resistance against a diverse group of pathogens, it appears ineffective against certain pathogens, such as *Botrytis cinerea* and *Alternaria alternata* (15), suggesting that different defense mechanisms, which are under the control of signals other than SA, are required. Indeed, analyses of *Arabidopsis thaliana* mutants that are altered in their defense responses against various pathogens/pests have revealed the involvement of multiple signaling pathways, some of which are under the control of JA and ETH (7,13). However, the SA-, ETH-, and/or JA-dependent signaling pathways do not function independently. Instead, these pathways are interconnected via both negative and positive regulatory mechanisms, permitting a plant to fine-tune and tailor its defense responses according to the nature of the invading pathogen (2).

Relative to the wealth of information on the molecular and cellular basis of defense mechanisms in *A. thaliana*, the corresponding information for rice is rather limited. The main objective of this study was to study and compare rice defense responses against two fungal pathogens of agricultural significance, *Magnaporthe grisea* (blast) and *Cochliobolus miyabeanus* (brown leaf spot). Early infection processes of these fungi share common

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features, including conidial attachment to the host surface, appressorium formation, and direct penetration of host cuticle using an infection hyphae differentiated from the appressorium. Both fungi also exhibit cultivar specificity (described below). However, the mechanism controlling their cultivar specificity and virulence may be distinct. Rice blast is a classical gene-for-gene system (5,22,45), and *M. grisea* is hemibiotrophic. The genetic basis of host cultivar specificity by *C. miyabeanus* is not well understood, but like other *Cochliobolus* species, *C. miyabeanus* appears to employ phytotoxins as a major virulence factor to cause host cell death (53). In this study, employing both resistant and susceptible rice cultivars, we compared how rice responds to *M. grisea* and *C. miyabeanus* at both cellular and molecular levels. We also investigated the effects of BTH, methyl jasmonate (MeJA), and conidial germination fluid (CGF) from *C. miyabeanus* on the disease severity and the expression patterns of four marker genes in rice infected with each fungus.

MATERIALS AND METHODS

Plant and fungal materials and infection assays. Rice seeds were sterilized by immersion in a 100 ppm solution of thiophanate-methylthiram for 16 h and sown in a commercial soil mixture at a density of three plants per pot (5 by 5 cm). Plants were propagated in a greenhouse as previously described (27) for 4 weeks until four to six leaves were fully expanded. *M. grisea* strain KJ401 and *C. miyabeanus* strain HIH-1 were obtained from the National Institute of Agricultural Science and Technology in the Rural Development Administration (Korea). Inoculation of rice with *M. grisea* was performed as previously described (27) using a conidial suspension (2×10^5 conidia per ml). Conidia of *C. miyabeanus* were collected from cultures grown on sucrose-proline agar (10) using 250 ppm of Tween 80, and the concentration was adjusted to 2×10^5 conidia per ml. Rice plants were inoculated by spraying with conidial suspension until all leaves were covered with fine droplets. Inoculated plants were kept in a dew chamber for 16 h at 25°C under 100% relative humidity to facilitate fungal penetration and then transferred to a greenhouse (25 to 30°C, 80% relative humidity) for disease development. Severity of disease was scored as previously described (20) using the following assessments: diseased areas per leaf, number of lesions, and lesion types. For both pathogens, evaluation of disease severity was stopped at 10 days postinoculation (dpi), and the infected plants were photographed. Infection assays were independently repeated at least three times.

Preparation of CGF from *C. miyabeanus*. Conidia of *C. miyabeanus* were harvested as described previously and washed twice with sterile distilled water. The final concentration of conidial suspension was adjusted to 1×10^6 conidia per ml. The conidial suspension was uniformly sprayed onto a sterile, prewetted paper towel placed in a sealed plastic container, which was subsequently placed at room temperature overnight. More than 90% of conidia germinated within 6 h. CGF was recovered from the paper towel by squeezing it, and large debris in CGF was removed by filtration through two layers of filter paper. After vacuum drying, CGF was dissolved in methanol to 50 mg/ml and kept at -70°C until use.

Chemical treatments. Seedlings of cv. Nakdong were treated with one of the following chemical or CGF preparations. BTH, supplied by Syngenta-Korea as a wettable powder (5.0% active ingredient), was dissolved in water containing 250 ppm of Tween 80 at a final concentration of 1 mM active ingredient. Insoluble components in the BTH solution were removed by filtration prior to application to rice. MeJA (Sigma Chemical, St. Louis) was prepared as a 10 mM methanol stock solution, which was diluted to 100 µM using water containing 250 ppm of Tween 80 prior to treatment. Seedlings of cv. Nakdong were misted with a fresh solution of BTH or MeJA (approximately 2.0 ml per pot). Three

days after the BTH and MeJA treatment, seedlings were inoculated with fungal spores. Six hours after spraying rice seedlings with 100 ppm of CGF, plants were inoculated with *M. grisea* and *C. miyabeanus*.

RNA extraction and northern analysis. Rice leaves were harvested for RNA isolation at 0, 1, 2, 3, and 4 days after fungal inoculation. Harvested plant materials were preserved at -70°C. Total RNA was extracted with a lithium chloride-precipitation method (8). For hybridization analysis, 15 µg of total RNA per lane was separated electrophoretically in denaturing formaldehyde-agarose gel (8% formaldehyde, 0.5× MOPS, and 1.5% agarose) and blotted to Hybond-N⁺ membrane (Amersham Pharmacia Biotech, UK) by capillary transfer. Uniform sample loading was confirmed by staining ribosomal RNA bands in the gel with ethidium bromide. DNA probes were labeled with [α -³²P] dCTP using a random primer labeling kit (Boehringer-Mannheim, Germany). The *JAmyb* gene probe was cloned by polymerase chain reaction (PCR) from genomic DNA of cv. Nakdong using a specific primer pair designed based on its reported sequence (30). The remaining probes were derived from previously characterized cDNA clones (26). Probe DNA was prepared from these clones by PCR using vector-specific primers. RNA gel blots were hybridized and washed as previously described (27) and exposed to X-ray film (AGFA, Germany).

RESULTS

Comparison of rice responses to *M. grisea* and *C. miyabeanus*. Twelve rice cultivars, including six *indica* and six *japonica* types, were screened with five isolates of *C. miyabeanus* (Table 1). With the exception of cv. Tetep (resistant to all isolates), all five isolates successfully infected these cultivars, causing brown leaf spot disease symptoms ranging from intermediate to severe. Strain HIH-1 and two cultivars, Nakdong (highly susceptible to HIH-1) and Tetep, were chosen for further analyses. To compare similarities and differences in host responses to *C. miyabeanus* and *M. grisea*, an *M. grisea* strain KJ401 that is virulent on cv. Nakdong but avirulent on cv. Tetep was included.

Cv. Nakdong infected with *M. grisea* strain KJ401 exhibited a disease progression pattern typical of rice blast (Fig. 1). At 3 dpi, water-soaked lesions began to form, with invasive mycelial growth apparent in these lesions. At 5 dpi, the leaf surface was covered with blast lesions, and massive sporulation began to

TABLE 1. Virulence of five *Cochliobolus miyabeanus* isolates on 12 rice cultivars

Rice cultivars ^a	Host response to individual <i>C. miyabeanus</i> isolates ^b				
	HIH-1	PSK-1	SW-1	SW-2	SW-3
<i>indica</i>					
Tetep	R	R	R	R	R
Tadukan	S	S	S	S	I
Milyang 23	S	S	S	I	S
Pungsan	S	I	S	S	S
Taebaek	S	S	S	S	I
IR36	S	I	S	S	I
<i>japonica</i>					
Nakdong	S	S	S	S	S
Hwacheong	S	S	S	S	S
Kanto51	S	S	S	S	S
Chucheong	S	S	S	S	S
Jinmi	S	S	S	S	S
Nongbaek	S	S	S	S	S

^a Six *indica* and six *japonica* types were screened.

^b A scale ranging from 0 to 10 was employed to evaluate disease severity (DS) according to the scale of the International Rice Research Institute. Cultivars exhibiting average DS of 3 or lower were recorded as resistant (R). Intermediate (I) corresponded to those exhibiting DS in the range of 3 to 6, and those exhibiting DS higher than 6 were recorded as susceptible (S).

appear in the center of lesions at 10 dpi. In contrast, in leaves of cv. Tetep inoculated with KJ401, abrupt host cell death around infection sites was observed by 36 h postinoculation (hpi). Microscopic lesions resulting from this localized host cell death failed to enlarge, representing a typical incompatible interaction mediated by HR.

When cv. Nakdong was inoculated with *C. miyabeanus* strain HIH-1, conidia germinated at the rate of >93% within 3 hpi. Visible host cell death was observed around 16 hpi. At 36 hpi, microscopic lesions started forming and became enlarged as time progressed. At 3 dpi, the average lesion area was approximately 0.5 by 3 mm (width by length), and chlorosis began to appear around the margin of lesions (Fig. 1). Although rapid cell death was also observed within 16 hpi in cv. Tetep inoculated with HIH-1, neither significant lesion enlargement nor chlorosis ensued thereafter. Lesions on cv. Tetep caused by HIH-1 were generally larger than those on Tetep infected with KJ401 (Fig. 1).

Effects of BTH and MeJA on the development of blast and brown leaf spot diseases. A number of previous studies suggested that successful defense of rice against *M. grisea* might be dependent on both the SA- and JA-dependent signaling pathways (1,30,32,41,55). We evaluated how the application of BTH, a functional analog of SA, and MeJA on cv. Nakdong prior to infection with KJ401 and HIH-1 affected disease development (Fig. 2). Although cv. Nakdong was highly susceptible to KJ401, treatment of Nakdong with 1 mM BTH 3 days prior to infection greatly increased its resistance against KJ401, almost to the level exhibited by cv. Tetep. Fungal growth was restricted within initial infection sites. Microscopic lesions around infection sites were observed at 2 dpi but failed to expand further than a diameter of 1 mm (Fig. 2A). No sporulation was observed on any of the lesions on the BTH-treated leaves (Fig. 2C). In contrast, BTH treatment did not noticeably increase resistance of cv. Nakdong to HIH-1 (Fig. 2B). Treatment of cv. Nakdong with MeJA prior to infection did not significantly modify the disease progression by either fungus (Fig. 2). Although at 3 dpi the rate of blast disease progression in cv. Nakdong pretreated with 100 μ M MeJA appeared somewhat retarded relative to that in untreated plants, the

difference disappeared within 24 h (Fig. 2A). MeJA did not appear to influence the interaction between cv. Nakdong and HIH-1 (Fig. 2D).

Comparative analyses of rice gene expression in response to *M. grisea*, *C. miyabeanus*, BTH, and MeJA. To compare rice responses to *M. grisea* and *C. miyabeanus* at the gene expression level, four marker genes, including *PR-1*, *PR-10* (=PBZ1), *PR-11* (=POX22.3), and *JAmyb*, were employed (Figs. 3 and 4). Previous studies showed that expression of the three *PR* genes was induced by pathogen infection, BTH, and/or probenazole (27,54). The *JAmyb* gene, encoding an MYB transcription factor, was highly induced at an early stage of *M. grisea* infection in both the compatible and incompatible interactions, and was also inducible by JA and wounding, but not by SA and BTH (30). In cv. Tetep infected with KJ401, the amount of transcript from all four genes peaked at 1 dpi and then gradually decreased (Fig. 3). The strong induction of these genes preceded the first visible signs of HR, which appeared around 36 hpi. In cv. Nakdong infected with KJ401, the induction of these genes was significantly delayed (by 1 to 2 days) relative to that in cv. Tetep, and their transcripts peaked at 3 dpi, which coincided with the formation of water-soaked lesions. Beyond 3 dpi, the level of transcripts remained unchanged (*PR-10* and *PR-11*) or decreased slightly (*PR-1* and *JAmyb*).

BTH treatment of cv. Nakdong significantly shortened the amount of time required for three *PR* genes to achieve the maximum transcript level after infection by KJ401 (1 dpi versus 3 dpi). Their expression patterns in cv. Nakdong pretreated with BTH resembled those in cv. Tetep infected by KJ401, suggesting that an earlier induction of these genes by BTH was associated with the increased resistance of BTH-treated Nakdong to KJ401 (Fig. 2). The expression pattern of *JAmyb* in cv. Nakdong was not significantly affected by BTH. MeJA treatment caused more rapid and stronger induction of *JAmyb* without affecting the *PR* genes.

In response to HIH-1, the expression patterns of all four genes were similar in cvs. Nakdong and Tetep (Fig. 3). In both cultivars, transcripts of all four genes started accumulating in infected

Fig. 1. Compatible and incompatible interactions of rice with *Magnaporthe grisea* and *Cochliobolus miyabeanus*. Left panel shows typical symptoms on cvs. Nakdong (compatible) and Tetep (incompatible) infected with *M. grisea* strain KJ401. Typical symptoms of the same cultivars infected with *C. miyabeanus* strain HIH-1 are shown in the right panel. Photographs were taken 10 days postinoculation.

leaves by 1 dpi. In cv. Nakdong infected with HIH-1, as the infection progressed, transcripts from *PR-10*, *PR-11*, and *JAmyb* decreased to varying degrees, whereas *PR-1* transcripts appeared to increase gradually. In cv. Tetep, however, the amount of transcript from all four genes appeared to decrease with the progression of time. Overall, the gene expression patterns in cvs. Nakdong and Tetep in response to HIH-1 infection appeared similar to those in cv. Tetep infected with KJ401. Application of BTH to cv. Nakdong prior to HIH-1 infection did not affect the expression patterns of these genes, with the exception of *PR-1*, which peaked at 1 dpi and then gradually decreased. In response to MeJA, the amount of *JAmyb* transcripts in cv. Nakdong infected with HIH-1 appeared higher than that in untreated Nakdong between 1 and 3 dpi, but the overall pattern did not change (peaked at 1 dpi and then gradually decreased in both cases).

CGF from *C. miyabeanus* caused host cell death and increased resistance to *M. grisea*. Xiao et al. (53) showed that CGF from *C. miyabeanus* contained a number of phytotoxic

metabolites. Spray application of CGF from HIH-1 caused HR-like lesions on the leaves of both cvs. Nakdong and Tetep within 12 h of application. These lesions, which formed only when CGF at a concentration higher than 100 ppm was applied, did not expand further (Fig. 4A). CGF treatment also significantly increased the level of *PR-1* transcripts in cvs. Tetep and Nakdong within 1 day after treatment (Fig. 4B). Considering the strong correlation between the rapid induction of *PR-1* transcripts and increased resistance to *M. grisea* (Figs. 2 and 3), we hypothesized that the application of CGF to cv. Nakdong prior to infection would increase resistance against *M. grisea*. Indeed, treatment with CGF 6 h prior to infection protected cv. Nakdong from KJ401 (Fig. 4C): no expanding invasive mycelial growth of KJ401 was observed in the CGF-treated leaves. Similar to BTH-treatment, CGF modified the expression patterns of the four marker genes in cv. Nakdong infected with KJ401. The modified expression patterns resembled those in cv. Tetep infected with KJ401 (Fig. 5). Transcripts from all four genes in CGF-treated cv.

Fig. 2. Effect of benzothiadiazole (BTH) and methyl jasmonic acid (MeJA) on the progression of blast and brown leaf spot diseases. **A**, Disease progression in cvs. Nakdong (compatible), Tetep (incompatible), and Nakdong pretreated with BTH (1 mM) or MeJA (100 μ M) followed subsequent to infection with *Magnaporthe grisea* strain KJ401. **B**, Disease progression in cvs. Nakdong, Tetep, and Nakdong pretreated with BTH (1 mM) or MeJA (100 μ M) followed subsequent to infection with *Cochliobolus miyabeanus* strain HIH-1. **C and D**, Photographs depicting representative symptoms taken 10 days after fungal inoculation.

Nakdong peaked at 1 dpi, whereas the transcripts of these genes in untreated Nakdong noticeably increased only at 2 to 3 dpi (Fig. 3).

DISCUSSION

During evolution, plant pathogens have adopted diverse strategies to successfully colonize their hosts. Against such diverse pathogenesis strategies, plants have also evolved elaborate regulatory mechanisms to tailor their defense responses according to the nature of invading pathogen. Because the innate defense mechanisms of plants have been, and continue to be, widely exploited for the management of many important plant diseases, a better understanding of the disease interaction and how the plant defense mechanisms operate is critical for the development of effective disease control measures via the use of breeding, genetic engineering, chemical/biological agents enhancing plant's disease resistance, or a combination of these means.

The main objective of this study was to enhance our understanding of rice defense mechanisms employed in response to two of its main pathogens, *M. grisea* and *C. miyabeanus*. Worldwide, rice blast is one of the most economically devastating crop diseases. Rice blast disease has been extensively studied as a plant-pathogen model system (23). In comparison, despite the destructive potential of *C. miyabeanus*, only limited information is currently available for the molecular and cellular basis of rice brown spot disease. We analyzed how *C. miyabeanus* interacts with both susceptible and resistant rice cultivars and compared the rice defense responses against *C. miyabeanus* with those against *M. grisea*.

Treatment of rice with BTH or CGF of *C. miyabeanus* was highly effective in controlling blast disease but ineffective against

brown leaf spot disease. Chemical and biological agents that systemically enhance plant's innate resistance against a broad spectrum of pathogens have been explored as a means for environmentally safe and economical disease control (33,50). Once such agent is BTH, which has been shown to be effective in enhancing resistance of tobacco, *Arabidopsis thaliana*, and wheat to a diverse array of pathogens (15,16,29). BTH appears to function via activation of the SAR signal transduction pathway (29). A BTH treatment prior to infection significantly increased the resistance of cv. Nakdong against KJ401 to a level of resistance comparable to that conferred by an incompatible gene-for-gene interaction (Fig. 2). There was no significant difference in the degree of resistance between BTH-treated cv. Nakdong and resistant cv. Tetep.

Fig. 3. Expression patterns of the rice *PR-1*, *PR-10* (=PBZ1), *PR-11* (=POX22.3), and *JAmyb* genes in response to infection by *Magnaporthe grisea*, *Cochliobolus miyabeanus*, and a treatment with benzothiadiazole (BTH) or methyl jasmonic acid (MeJA). Total RNA was extracted from the leaves of cvs. Nakdong, Tetep, and Nakdong pretreated with BTH or MeJA collected at 0, 1, 2, 3, and 4 days postinoculation (dpi) with *M. grisea* strain KJ401 or *C. miyabeanus* strain HH-1.

Fig. 4. Effect of conidial germination fluid (CGF) from *Cochliobolus miyabeanus* on rice. **A**, Symptoms on cvs. Nakdong and Tetep 24 h after the spray application of 100 ppm of CGF. **B**, Expression patterns of the *PR-1* gene in these cultivars in response to the CGF treatment. Total RNA was extracted from rice leaves at 0 to 4 days postapplication of CGF. dpt = days posttreatment. **C**, Severity of blast (white bar) and brown leaf spot (black bars) diseases in cv. Nakdong following a treatment with 100 ppm of CGF in 250 ppm of Tween 80 (CGF) or 250 ppm of Tween 80 (control) 6 h prior to pathogen inoculation. Disease severity was assessed 10 days after fungal inoculation.

Similar to BTH, CGF from *C. miyabeanus* successfully protected cv. Nakdong from *M. grisea* KJ401 (Fig. 4C). No invasive mycelial growth of *M. grisea* was observed in the CGF-treated leaves. Kim et al. (25) showed that pretreatment with a culture filtrate from *C. miyabeanus* also significantly reduced disease symptoms of tomato late blight, barley powdery mildew, and wheat leaf rust in these nonhost plants, suggesting that metabolites within CGF function as nonspecific defense elicitors. The effectiveness of BTH and CGF in successfully controlling blast disease seemed comparable to that of another SAR-inducing agent probenazole, which also successfully inhibited rice blast disease (36). Yoshioka et al. (57) reported that in *Arabidopsis thaliana*, the probenazole-mediated systemic resistance was dependent on the *NPRI* gene and SA, suggesting that both BTH and probenazole function via the same signal transduction pathway. Silverman et al. (46) showed that the innate level of SA in rice correlated with the degree of resistance against *M. grisea*. Although how the metabolites comprising CGF trigger rapid rice defense response remains to be determined, we hypothesize that host cell death caused by CGF leads to SAR (described below).

Expression patterns of three *PR* genes following the treatment with BTH supported the hypothesis that BTH protected rice from *M. grisea* by inducing SAR to the level comparable to that of incompatible gene-for-gene interactions (Fig. 3). The expression patterns of the three *PR* genes in BTH-treated cv. Nakdong infected with *M. grisea* strain KJ401 were similar to those observed in cv. Tetep infected with the same strain. Kim et al. (27) also showed that transcripts from these *PR* genes were increased rapidly in cv. Hwacheong in response to infection by an avirulent *M. grisea* strain, suggesting that these *PR* genes could serve as reliable molecular markers for monitoring rice defense response against *M. grisea*. Although BTH markedly increased the level of *PR* gene transcripts in response to fungal infection, BTH alone was not sufficient for inducing their accumulation. This finding is consistent with a previous report (27), suggesting that the induction of rice *PR* genes only became noticeable 8 days after BTH treatment. Thus, BTH appears to protect rice via its ability to shift the susceptible host into a "ready-to-respond" state. Katz et al. (24) reported that BTH brought about such a state in parsley cells. In contrast, CGF itself was sufficient for rapidly increasing the level of *PR-1* transcripts (Fig. 4), suggesting that although both BTH and CGF significantly increased rice resistance and rapidly increased the level of transcripts from three *PR* genes upon pathogen infection, they exert their effect via different mechanisms.

Whereas the application of BTH and CGF converted a compatible interaction to an incompatible interaction between rice and *M. grisea*, none of them appeared to be effective against brown leaf spot disease, suggesting that the defense system activated by BTH and CGF is only effective in controlling certain pathogens. Expression patterns of the three *PR* genes also suggested that the rice defense mechanisms against brown leaf spot disease are distinct from those against blast disease; their expression patterns in response to *C. miyabeanus* infection were nearly identical in cvs. Nakdong and Tetep and were not significantly altered by BTH treatment. Previously, Friedrich et al. (15) reported that the defense mechanisms activated by BTH effectively inhibited tobacco diseases caused by *Tobacco mosaic virus* and a number of biotrophic fungal pathogens. The same treatment, however, failed to protect tobacco plants from necrotrophic pathogens such as *Botrytis cinerea* and *Alternaria alternata*. Compatibility of tobacco with the biotrophic pathogens is under the control of gene-for-gene interaction, and thus the HR-mediated host cell death is sufficient for resistance. Resistance mechanisms against the necrotrophic pathogens have not been well characterized. It has been suggested that against necrotrophic pathogens, pathogen-specific defense mechanisms, such as the production of enzymes capable of detoxifying host-specific (or nonspecific) toxins secreted by a

pathogen (31,51) or counteracting the action of virulence factors (4,48), may be needed.

The plant hormone JA and its more volatile methyl ester derivative MeJA have been implicated in plant defense (43,48,49). The JA-dependent signal transduction pathway has been shown to be important for defense against insects (34,47) and a diverse array of pathogens, ranging from bacteria such as *Erwinia carotovora* (38) to fungal and oomycete pathogens, including *Alternaria brassicicola*, *B. cinerea*, and *Pythium* spp. (11,42). In our study, exogenous application of MeJA prior to infection did not appear to protect rice from either *M. grisea* or *C. miyabeanus*, suggesting that the JA-dependent signaling transduction pathway plays a minor role, if at all, in rice resistance against *M. grisea* and *C. miyabeanus*.

Rapid host cell death associated with pathogen infection: A double-edged sword? In incompatible gene-for-gene interactions, rapid host cell death in and around the initial infection sites prevents the ramification of the pathogen to surrounding cells, and evokes SAR effective against a broad spectrum of pathogens (28, 44). HR-associated host cell death appears to be a form of programmed cell death (PCD), which is a genetically controlled, cellular suicide mechanism that requires the active participation of the host. The resistance of rice to *M. grisea* strain KJ401 depended on HR-associated host cell death (Fig. 1); KJ401 triggered rapid HR, resulting in microscopic cell death in resistant cv. Tetep, but not in susceptible cv. Nakdong. BTH treatment, which induced rapid HR-associated host cell death in cv. Nakdong in response to KJ401 infection, or CGF, which caused host cell death even in the absence of pathogen, was sufficient for conferring resistance to cv. Nakdong against KJ401, further supporting the importance of the HR-associated cell death in resistance to *M. grisea*.

Activation of the HR-associated host cell death, however, appeared to be ineffective against *C. miyabeanus*; unlike *M. grisea*, *C. miyabeanus* caused rapid host cell death in both cvs. Tetep and Nakdong. Despite such rapid host cell death, fungal colonization in cv. Nakdong was not blocked, suggesting that a mechanism different from HR-associated host cell death may be needed for successful defense against *C. miyabeanus*. Considering that CGF from *C. miyabeanus* induced host cell death even in the absence of *C. miyabeanus*, host cell death by CGF seems to be a pathogen-initiated process that facilitates pathogenesis. Previous studies also supported the hypothesis that phytotoxins in CGF act as a major virulence factor (25,53). Phytotoxins secreted by other fungi,

Fig. 5. Effect of conidial germination fluid (CGF) from *Cochliobolus miyabeanus* on expression patterns of the rice *PR-1*, *PR-10* (=PBZ1), *PR-11* (=POX22.3), and *JAmyb* genes in response to infection by *Magnaporthe grisea*. Six hours after spraying seedlings of cvs. Nakdong and Tetep with 100 ppm of CGF, plants were inoculated with *M. grisea* strain KJ401. Total RNA was extracted from rice leaves at 0 to 4 days after fungal inoculation. dpi = days postinoculation.

notably those belonging to the genera *Cochliobolus* and *Alternaria*, also cause host cell death that exhibits characteristics of PCD (52). For some of these pathogens, the ability to induce host cell death via toxin production functions not only as a major virulence factor, but also as a host specificity determinant (52). One such example is the interaction between *Cochliobolus victoriae* and oats, in which the cyclic pentapeptide victorin produced by *C. victoriae* determines the pathogen's compatibility with its host (56). Another cyclic peptide HC-toxin also is an important virulence and specificity factor in *Cochliobolus car-bonum*-maize interactions (40).

Production of phytotoxins during germination, appressorium formation, and invasive growth of *C. miyabeanus* has been demonstrated in previous studies (17,39,53). In compatible rice cultivars, even prior to penetration, microscopic cell death was observed in plant cells that were in direct contact with fungal spores (17). The collapse of mesophyll cells not in direct contact with invading hyphae was also observed, suggesting the secretion and diffusion of phytotoxins in advance of the fungal growth. Following the application of CGF from *C. miyabeanus* or ophiobolin A (a major component in CGF), an isolate of *A. alternata* that is nonpathogenic to rice successfully infected and ramified in rice leaves (53), supporting the role of *C. miyabeanus* phytotoxins in virulence. However, causing host cell death through the secretion of such phytotoxins is not sufficient for colonizing cv. Tetep. This cultivar possesses the ability to block invasive mycelial growth even though it is sensitive to phytotoxins in CGF. Further analyses of the molecular and cellular basis of *C. miyabeanus*-rice interactions are needed to reveal the underlying resistance mechanisms in cv. Tetep. Rapidly accumulating genomic data and resources for rice and molecular/cytological tools for studying fungal pathogens should facilitate such analyses.

Host cell death in rice appears to play a dual role in both resistance and disease development depending on the nature of invading pathogen. This observation is supported by recent studies showing that for certain pathogens, chemical/biological treatments or genetic mutations that induce host cell death facilitate pathogen colonization and disease development (12,18,21). For instance, transgenic expression of animal genes that inhibit PCD conferred increased resistance to tobacco plants challenged by *B. cinerea*, *Cercospora nicotianae*, and *Sclerotinia sclerotiorum* (12). Similarly, an *A. thaliana dnd1* mutant, which exhibits a highly attenuated HR with no cell death upon pathogen infection (58), was more resistant to infection by *B. cinerea* and *S. sclerotiorum* than was the wild-type plant, and the treatment of *A. thaliana* with either reagents that produce reactive oxygen intermediates, or an avirulent strain of *Pseudomonas syringae*, prior to infection with *B. cinerea* or *S. sclerotiorum* increased disease severity (18), suggesting that HR-associated cell death facilitates pathogenesis by these pathogens.

In summary, rice appears to require distinct mechanisms for defense against *M. grisea* and *C. miyabeanus*. While rapid induction of HR-associated host cell death resulting from an incompatible gene-for-gene interaction, or a treatment of rice with BTH or CGF prior to pathogen infection, was highly effective against *M. grisea*, even converting a compatible interaction to an incompatible one (Figs. 2 and 4), it was ineffective against *C. miyabeanus*. Considering that the effect of host cell death on defense response depends on the type of pathogen, control measures effective against *M. grisea* may not work against *C. miyabeanus*. Our work underscores the importance of utilizing appropriate innate defense mechanisms so as to protect crops from pathogens employing diverse pathogenic strategies.

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