Acquired Resistance Functions in *mlo* Barley, Which Is Hypersusceptible to *Magnaporthe grisea*

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Barley plants carrying a mutation in the *Mlo* (barley [*Hordeum vulgare* L.] cultivar Ingrid) locus conferring a durable resistance against powdery mildew are hypersusceptible to the rice blast fungus *Magnaporthe grisea*. It has been speculated that a functional *Mlo* gene is required for the expression of basic pathogen resistance and that the loss of *Mlo* function mediating powdery mildew resistance is an exception for this particular disease. Here, we report that the onset of acquired resistance (AR) after chemical as well as biological treatments is sufficient to overcome the hypersusceptible phenotype of backcross line BCIngrid*mlo*₅ (*mlo*) barley plants against *M. grisea*. Moreover, even barley plants bearing a functional *Mlo* gene and thus showing a moderate infection phenotype against rice blast exhibit a further enhanced resistance after induction of AR. Cytological investigations reveal that acquired resistance in *mlo* genotypes is manifested by the restoration of the ability to form an effective papilla at sites of attempted penetration, similarly to wild-type *Mlo* plants. In addition, the rate of effective papillae formation in *Mlo* plants was further enhanced after the onset of AR. These results demonstrate that treatments leading to the AR state in barley function independently of the *Mlo/mlo* phenotype and suggest that the *Mlo* protein is not a component of the AR signaling network. Moreover, it seems that only concomitant action of *Mlo* together with AR permits high level resistance in barley against blast. Higher steady state levels of PR1 and barley chemically induced mRNA correlate with higher disease severity rather than with the degree of resistance observed in this particular interaction.

Plants have evolved a network of different defense strategies against pathogen attack, one of which is the so-called acquired resistance (AR) response. This phenomenon is well documented in model systems such as *Arabidopsis* and tobacco, and it describes the acquisition of resistance against potentially virulent pathogens after e.g., exposure to root colonizing microorganisms (so-called induced systemic resistance [ISR]) or necrotizing pathogens (so-called systemic acquired resistance [SAR]) as well as the application of certain chemicals (Kessmann et al. 1994; Ryals et al. 1994; Sticher et al. 1997). It has been demonstrated that distinct defense signaling pathways are involved in each of these processes (Ton et al. 2002). Simultaneous induction of ISR and SAR in *Arabidopsis* resulted in an additive level of resistance to *Pseudomonas syringae* pv. *tomato* (van Wees et al. 2000). However, salicylic acid, a key component of SAR, has

been found to inhibit jasmonic acid synthesis, which is involved in the ISR response (Doares et al. 1995). After receiving an initial stimulus, plants are primed for a faster and stronger defense reaction in response to a subsequent challenge inoculation (Conrath et al. 2001; Zimmerli et al. 2000). AR has been shown to function in important cereal species such as wheat, barley, and rice (Görlach et al. 1996; Schaffrath et al. 1997; Schweizer et al. 1989).

The barley backcross line BCIngrid*mlo*₅ (*mlo*) type of resistance against powdery mildew shares some analogies with AR, namely, that it is effective against a broader range of pathotypes compared with major gene resistance. In the case of *mlo*, resistance extends to all races of one fungal species, *Blumeria graminis* f. sp. *hordei* (Brown et al. 1991; Jørgensen 1977). Up until now, no breakdown of *mlo*-mediated resistance has been observed in the field, which is, in contrast, often the fate for major gene resistance (Jørgensen 1992). This outstanding performance against *B. graminis* f. sp. *hordei* in the field contrasts with our finding that *mlo* genotypes are extremely hypersusceptible to another plant pathogenic fungus, *Magnaporthe grisea* (Jarosch et al. 1999). Genotypes carrying the wild-type *Mlo* (barley [*Hordeum vulgare* L.] cultivar Ingrid) allele are only slightly susceptible. Thus, it seems that the functional *Mlo* allele is needed for expression of defense reactions against this pathogen and, in *mlo* genotypes, the penetration defense of the host seems to be compromised as many more papillae are successfully penetrated by the fungus. Similar observations were recently documented for infection of *mlo* barley genotypes with *Cochliobolus sativus*, a necrotrophic fungus (Kumar et al. 2001).

The present study was aimed at analyzing whether induction of the AR state can control rice blast in barley. Our results show that biotic as well as chemical pretreatments, known to induce AR in monocots, are effective in protecting *Mlo* as well as *mlo* genotypes of barley against rice blast. We identified the formation of effective papilla as the crucial resistance response in both genotypes. Thus, the *Mlo* resistance mechanism is phenocopied after the onset of AR in *mlo* genotypes. However, the greatest degree of resistance was found in *Mlo* genotypes after the onset of AR, whereas AR-induced *mlo* genotypes appeared only to be brought up to the level of resistance seen in the noninduced *Mlo* wild type. Thus, it seems that the AR resistance component is additive to the resistance component conditioned by the *Mlo* allele. It is tempting to speculate that two independent signal transduction pathways may exist and that only the concomitant activation of both leads to the highest degree of resistance.

RESULTS

Macroscopic evaluation of disease severity.

In order to test the capacity of AR to protect barley from blast disease, we first chose chemical inducers that have been previously reported to be active in monocots (Görlach et al. 1996;

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Fig. 1. Macroscopic symptoms on leaves infected with *Magnaporthe grisea*. Leaves from the barley (*Hordeum vulgare* L.) cultivar Ingrid (*Mlo*) genotype and the barley backcross line BCIngrid mlo_5 (*mlo*) genotype 6 days after inoculation with *M. grisea*. Plants were pretreated with **A**, Bion (benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester), **B**, INA (2,6-dichloroisonicotinic acid), and **C**, *Blumeria graminis* f. sp. *tritici* (*Bgt*), and the appropriate control. Pictures show three representative leaves for each treatment. All experiments were carried out in triplicate with similar results. WG = wettable granulate and WP = wettable powder.

Schaffrath et al. 1997). The application of Bion (benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester) prior to a challenge inoculation with *M. grisea* resulted in a strong reduction of macroscopically visible symptoms on both genotypes (Fig. 1A). This reduction affected the size as well as the number of blast lesions. The highest degree of protection was observed on Bion-treated *Mlo* plants, whereas *mlo* genotypes displayed a less pronounced effect. The arrangement of leaves in classes according to the diseased leaf area validated this observation (Fig. 2A). The classes with highly diseased leaf area were found on control *mlo* plants, whereas Bion treatment shifted the distribution of classes to a similar level of disease as that observed for untreated *Mlo* plants, and the lowest level of disease was found on Bion-treated *Mlo* plants. In our experiments, Bion treatment was unable to protect BCIngrid*mlo*₅ plants to the high level observed for treated *Mlo* plants. Next, we tested chemical inducer 2,6-dichloroisonicotinic acid (INA), which has been postulated to have a similar mode of action in inducing AR as Bion (Friedrich et al. 1996; Görlach et al. 1996; Lawton et al. 1996). In fact, macroscopic evaluation and quantitative assessment of resistance against *M. grisea* after INA treatment mirrored the results obtained for Bion (Fig. 1B and 2B).

In addition to chemical induction of AR, we tested a biological agent that might utilize different components of the host defense machinery during the onset of AR, as demonstrated previously (Schaffrath et al. 1997; Schweizer et al. 1999). The biological inducer of choice was the barley nonhost pathogen *Blumeria graminis* f. sp. *tritici*, which is the causal agent of powdery mildew on wheat. *B. graminis* f. sp. *tritici* is capable of inducing resistance in barley against the barley powdery mildew fungus *B. graminis* f. sp. *hordei* (Thordal-Christensen and Smedegaard-Petersen 1988). Similarly, a preinoculation with *B. graminis* f. sp. *hordei* is able to induce AR in wheat against a challenge inoculation with *B. graminis* f. sp. *tritici*, thus confirming a general principle of inducing abilities of nonhost powdery mildews against virulent *formae speciales* (Schweizer et al. 1989). In our experiments, a pretreatment with *B. graminis* f. sp. *tritici* protected barley from rice blast disease on *Mlo* as well as *mlo* plants, respectively (Fig. 1C). Again, pretreated *Mlo* genotypes were most efficiently protected. The distribution of infection classes confirmed this observation (Fig. 2C).

Microscopic analysis of infection sites.

Recently, we have reported that a defect in the formation of effective papillae is correlated with the hypersusceptible infection phenotype of BCIngrid*mlo*₅ genotypes against *M. grisea* (Jarosch et al. 1999). Here, we aimed to analyze the cytological determinants accompanying resistance after the onset of AR. To this end, we differentiated three classes of cytological events. The first of them consisted of the formation of an effective papilla that efficiently blocked fungal penetration (Jarosch et al. 1999). The second class is represented by the occurrence of a hypersensitive response (HR) in the first penetrated epidermal cell, which is thought to be, in analogy to the rice/*M. grisea* pathosystem, a resistance response that at least slows down pathogen invasion (Koga 1994). In the third class, we documented more extensive pathogen development accompanied by HR in the mesophyll. The autofluorescence occurring along with undisturbed pathogen colonization in the mesophyll is more likely a sign for susceptibility than for resistance.

Accordingly, the most susceptible *mlo* control plants showed the highest percentage of mesophyll HR and the lowest percentage of effective papillae (Fig. 3A). After treatment of the *mlo* genotype with Bion, we found a higher number of effective papillae than in control plants. The occurrence of HR in

epidermal cells remained unchanged, while the proportion of HR in the mesophyll was reduced. It seems that the onset of AR solely affected the early defense response, namely papilla formation, and did not influence triggering of HR in the epidermis. The lower amount of mesophyll HR reflected the inhibi-

Fig. 2. Distribution of infection classes according to diseased leaf area. Barley plants of the barley (*Hordeum vulgare* L.) cultivar Ingrid (*Mlo*) genotype and the barley backcross line BCIngrid*mlo*₅ (*mlo*) genotype were inoculated with *Magnaporthe grisea*. Macroscopic evaluation of disease severity was done 6 days after inoculation on 30 leaves per treatment, and leaves were classified according to the diseased leaf area, as indicated. Plants were pretreated with **A**, Bion (benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester), **B**, INA (2,6-dichloroisonicotinic acid), and **C**, *Blumeria graminis* f. sp. *tritici* (*Bgt*), and the appropriate control. All experiments were carried out in triplicate with similar results.

tion of fungal development before it invaded the mesophyll. Although less pronounced, the treatment of *Mlo* plants with Bion results in a higher percentage of effective papillae, while the proportion of HR in the mesophyll dropped.

Cytological investigation of plants treated with INA and *B. graminis* f. sp. *tritici* as inducers of AR confirmed these obser-

reaction types:

■ effective papillae ■ HR epidermis □ HR mesophyll

Fig. 3. Distribution of cellular interaction types. Frequency of interaction sites on barley (*Hordeum vulgare* L.) cultivar Ingrid (*Mlo*) and barley backcross line BCIngrid mlo_5 (*mlo*) genotypes infected with *Magnaporthe grisea*. Leaves were harvested 72 h after inoculation and were prepared for microscopy. Plants were pretreated with **A**, Bion (benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester), **B**, INA (2,6-dichloroisonicotinic acid), and **C**, *Blumeria graminis* f. sp. *tritici* (*Bgt*), and the appropriate control. Single-cell responses were discriminated as indicated. Each data point consists of the investigation of 3 to 4 leaves based on approximately 100 infection sites per leaf. The result of three independent experiments were calculated together, and the mean $\pm$ standard error is presented.

vations (Fig. 3B and C, respectively). The distribution of cellular reaction types in control treated plants was very similar within all of these experiments, and the pretreated *Mlo* plants showed the highest percentage of papilla formation and the lowest rate of mesophyll HR, both indicative for the most efficient resistance response. Due to the relative high percentage of mesophyll HR, it seemed that treatment of *mlo* genotypes with *B. graminis* f. sp. *tritici* is slightly less efficient at inducing AR than are INA and Bion treatments.

Gene expression profiling in the barley/*M. grisea* pathosystem.

Since it is desirable to have a molecular marker for disease susceptibility or resistance, or both, in the barley/*M. grisea* pathosystem, we performed Northern blot analyses of transcript accumulation for a series of cDNAs that had been shown to be associated with pathogen resistance in barley (Bryngelsson et al. 1994; Beßer et al. 2000).

First, we checked for accumulation of transcripts corresponding to a basic *PR1b*-type pathogenesis-related (PR) protein that accumulated in barley in response to pathogen inoculation in compatible as well as incompatible interactions with the powdery mildew fungus (Bryngelsson et al. 1994). While mock-inoculated genotypes showed only a weak accumulation of these transcripts, we found a strong induction after blast infection of *Mlo* genotypes and even the strongest hybridization signal in diseased *mlo* genotypes (Fig. 4). Thus, transcript accumulation related to *PR1b* showed a direct correlation with disease severity in the barley/*M. grisea* pathosystem. The accumulation of transcripts corresponding to a peroxidase (*PR9*) and a thaumatinlike protein (*PR5*) mirrored this result, although the differences between genotypes under investigation was not as high as in the case of *PR1b*. As a second class of cDNAs, we tested the so-called "barley chemically induced" genes (*BCI*), that are differentially up-regulated in barley after the onset of AR by chemicals but not in response to powdery mildew infections. In contrast, we found an accumulation of transcripts corresponding to these genes after infection with *M. grisea*. While *BCI3* transcripts also accumulate in mock-inoculated plants and the pattern did not differ between *Mlo* and *mlo* genotypes, we found a stronger, or at least an earlier, accumulation of transcripts corresponding to *BCI1* in the case of *mlo* plants. In summary, the accumulation of transcripts of the genes under investigation showed a negative correlation with disease resistance in the barley/*M. grisea* pathosystem.

Next, we tested whether the postulated role of *PR1b* as a molecular marker of disease severity also applies in plants showing AR-mediated resistance. After application of Bion, we found the strongest protection against *M. grisea* infections in *Mlo* genotypes (discussed above), and these plants showed the lowest *PR1b* transcript accumulation (Fig. 5). In contrast, *mlo* genotypes, which are hypersusceptible to blast, showed the highest expression. These findings were supported by experiments using INA and *B. graminis* f. sp. *tritici* as inducers for AR that gave very similar results (data not shown). Thus, a direct correlation between susceptibility against rice blast and high rates of *PR1b* transcript accumulation applied for *M. grisea*-infected barley plants showing AR.

DISCUSSION

The rice pathogen *M. grisea* can complete its life cycle on barley plants. Therefore, barley must be considered as a potential host for this pathogen. However, due to different ecological and agricultural requirements, the organisms seldom meet in cultivation, and blast infections on barley are reported only very infrequently. QTL analysis has revealed that some barley

genotypes have a quantitative type of resistance to one or more isolates of *M. grisea* (Sato et al. 2001). However, no entirely resistant genotype has been found. Recently, we reported that a functional Mlo protein is required for a moderate infection phenotype against *M. grisea* and that, in the absence of this protein, barley becomes hypersusceptible to the fungus (Jarosch et al. 1999). In the present study, we looked at the ability of known chemical and biological inducers of AR to protect *Mlo* and *mlo* genotypes of barley against infection with *M. grisea*. We show here that the concomitant action of a functional Mlo protein in combination with AR results in the highest degree of protection. Thus, it seems that the Mlo protein might have a more general role in resistance than was previously thought.

Here, we demonstrate for the first time that different pretreatments, including pathogen infection as well as the application of chemical substances known to induce AR in cereals, significantly reduce disease severity on barley attacked by *M. grisea*. As chemical inducers, we used Bion and INA, both of which have been reported previously to be effective in protecting barley against powdery mildew and rice against blast (Beßer et al. 2000; Kogel et al. 1994; Schweizer et al. 1997, 1999). Both chemicals prevented the hypersusceptibility of barley *mlo* plants to *M. grisea*. Inducers bring the level of protection in the *mlo* plants up to that of unprotected wild-type *Mlo* plants. This shows that the application of chemical inducers can phenotypically compensate for the lack of a functional Mlo protein. In addition, the *Mlo* genotype showed a further enhanced resistance towards blast after the onset of AR compared with control plants of the same genotype. This may be indicative of separate signaling pathways independently controlling blast resistance in barley, one of which needs Mlo activity and the other representing the AR machinery. Evidence for independent defense pathways were also documented for *Arabidopsis*, in which the concomitant activation of the salicy-

late- and jasmonate-dependent pathways resulted in enhanced disease resistance towards *Pseudomonas syringae* pv. *tomato* (van Wees et al. 2000). This demonstrates that the activation of AR does not necessarily lead to the expression of the highest possible resistance capacity of a plant. Obviously, in *Arabidopsis* showing AR, a margin of resistance capacity is left, which could be filled in by induction of jasmonate-dependent ISR. For barley, it seems that the scope of protection gained by the induction of AR could be further increased due to the activity of a functional Mlo protein. Experiments using double mutants of *mlo* plants (*mlo*, *ror1-2*) support the hypothesis that the onset of AR in barley does not require Mlo. This mutant lacks elements of the *mlo* resistance pathway and is, therefore, susceptible to *B. graminis* f. sp. *hordei*. Nevertheless, resistance against powdery mildew could be reestablished by inducers of AR (B. Jarosch, unpublished data).

We found no complete resistance in barley against blast after induction of AR. Therefore, this must be considered as a quantitative type of resistance, which in turn, seems to be a general feature of AR-mediated resistance (Friedrich et al. 1996; Görlach et al. 1996; Lawton et al. 1996). Studies using the barley nonhost pathogen *B. graminis* f. sp. *tritici* as a biological inducer of AR displayed a very similar pattern in reduction of disease severity compared with chemical inducers, although the magnitude of resistance seems to be less high. Nonhost resistance in barley against *B. graminis* f. sp. *tritici* is associated with a burst of hydrogen peroxide, which in turn, is a prerequisite for genetic as well as AR-mediated resistance against the barley powdery mildew fungus (Hückelhoven et al. 2000, 2001). Also in the barley/*M. grisea* pathosystem, resistance could be correlated with the accumulation of hydrogen peroxide at sites of attempted penetration (M. Jansen and U. Schaffrath, unpublished data). Thus, *B. graminis* f. sp. *tritici*-triggered hydrogen peroxide accumulation may contribute to the onset of biologically induced resistance.

Fig. 4. Accumulation of mRNA in barley in response to infection with *Magnaporthe grisea*. Barley plants of the barley (*Hordeum vulgare* L.) cultivar Ingrid (*Mlo*) and the barley backcross line BCIngridmlo₅ (*mlo*) genotypes were inoculated with *M. grisea* by spraying spores onto the leaves. Control plants of the same genotypes were mock-inoculated using the spray solution without spores. Total RNA was extracted after the timepoints indicated and was subjected to gel blot analysis. RNA loading was monitored by ethidium bromide staining (EtBr) of the gels before blotting. Blots were hybridized with digoxigenin-labeled probes generated either by polymerase chain reaction or in vitro transcription using plasmids containing the cDNAs of interest as indicated. The arrows mark the hybridization signal corresponding to the mRNAs of expected size. The experiment was carried out in duplicate with similar results.

To study the mode of action of AR against blast in more detail, we made microscopical investigations monitoring the early defense responses of the plants. The predominating microscopic interaction phenotype, correlating with the highest resistance towards blast, was identified as formation of effective papilla. The percentage of this resistance response peaks in *Mlo* plants showing AR and is lowest in control plants of *mlo* genotype showing the most disease. It was known previously that the moderate infection phenotype of *Mlo* plants depends on the formation of an effective penetration barrier and that hypersusceptibility of *mlo* plants is due to an insufficient expression of this response (Jarosch et al. 1999). Here, we demonstrate that the phenotypic complementation of resistance in *mlo* plants after the onset of AR is achieved at the cellular level by a phenocopy of the *Mlo*-dependent defense response. In barley, the formation of effective papilla was also demonstrated to be crucial for *Mlg*- and *mlo*-mediated resistance against powdery mildew (Görg et al. 1993; Jørgensen 1992; Schiffer et al. 1997). However, induction of resistance in barley can also be uncoupled from papilla formation, as shown after a heat treatment that reduces symptoms on young barley seedlings to subsequent infection with the barley powdery mildew fungus *B. graminis* f. sp. *tritici* (Schweizer et al. 1995). The fungus was stopped in the heat-treated leaves at the stage of appressorial penetration but independent of papilla formation and hypersensitive response. Also, *Mla*-specified resistance of barley against *B. graminis* f. sp. *hordei* does not rely on papilla formation (Schiffer et al. 1997). Thus, papilla formation in barley is not a component of the overall defense scenario rather than a response to specific stimuli. Obviously, the onset of AR meets the requirements for this activation in response to a *M. grisea* infection.

Gene expression studies were undertaken to monitor whether transcript levels of genes coding for PR proteins, *BCI* genes, or both were related to the infection phenotypes of *Mlo* and *mlo* plants in response to *M. grisea*. Transcripts of *PR1b* started to accumulate 24 h after inoculation in both genotypes, but by day 5, there was a considerably higher level in the hypersusceptible *mlo* plants than in the more resistant *Mlo* genotype. Although the biochemical function of the PR1 protein is still unknown, there are indications that this protein possesses antifungal activity

(Alexander et al. 1993). However, accumulation of *PR1b* transcripts seems to play no substantial role in the barley/*M. grisea* interaction. This might also apply for the barley/powdery mildew interaction, where *PR1b* transcripts accumulate to a higher degree in compatible, rather than in incompatible, interactions (Bryngelsson et al. 1994). Thus, elevated levels of *PR1b* transcripts are more likely a marker for enhanced pathogen development than for resistance. This hypothesis was tested using barley infected with *M. grisea* after the onset of AR. These findings are in contrast to results using dicotyledonous plants such as *Arabidopsis* and tobacco. In these systems, the induction of AR is accompanied by enhanced PR1 synthesis (Kessmann et al. 1994). Expression profiles of *PR5* and *PR9* confirm the observation found for *PR1b*. Monitoring transcript accumulation from the *BCII* and *BCI3* genes revealed no significant correlation to disease phenotypes. However, this report is the first showing that *BCII*, similar to *Lox2Hv1* (Vörös et al. 1998), is inducible by a pathogen attack.

On the molecular level, the *Mlo* locus is thought to be a key element controlling cell death in barley (Büschges et al. 1997). While the presence of a functional *Mlo* protein enables the development of biotrophic fungi like *B. graminis* f. sp. *hordei*, the absence of *Mlo* may support the growth of necrotrophic fungi (Kumar et al. 2001). However, in the case of the hemibiotrophic fungus *M. grisea*, hypersusceptibility in *mlo* plants clearly depends on the absence of an *Mlo*-dependent active defense response (Jarosch et al. 1999). Recent findings showing that the barley *Mlo* gene is pathogen- and stress-inducible are consistent with the hypothesis that *Mlo* is a pathogen-responsive protein (Piffanelli et al. 2002). Thus, it is tempting to speculate that the *Mlo* protein is a key element of the basic resistance response of barley, which is complemented by the AR defense machinery.

MATERIALS AND METHODS

Plant and fungal material.

Barley (*Hordeum vulgare* L.) cultivar Ingrid (designated as *Mlo*) and the near-isogenic backcross line BCIngrid*mlo*₅ (designated as *mlo*), were provided by P. Schulze-Lefert, Max Planck Institute for Plant Breeding Research, Cologne, Germany.

Fig. 5. Accumulation of *PR1b* transcripts. barley (*Hordeum vulgare* L.) cultivar Ingrid and barley backcross line BCIngrid*mlo*₅ plants were sprayed with a 250-ppm Bion (benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester) solution. Plants treated with wettable granulate (WG) served as controls. Inoculation with *Magnaporthe grisea* took place 3 days after the pretreatments, and mock-inoculated plants were sprayed without spores. Total RNA was extracted after the timepoints indicated and was subjected to gel blot analysis. RNA loading was monitored by ethidium bromide staining (EtBr) before blotting. Blots were hybridized with a digoxigenin-labeled probe generated either by polymerase chain reaction or in vitro transcription using a plasmid containing the cDNA for *PR1b*. Independent experiments resulted in similar gene expression profiles.

Seedlings were grown in a growth chamber at 16 to 18°C and 50 to 60% relative humidity with a 16 h photo period.

The *Magnaporthe grisea* race 031 (TH6772) was provided by the Institute of Biochemistry, Faculty of Agriculture, Tamagawa University, Machida-shi, Tokyo. The fungus was cultivated on potato dextrose agar and rice leaf agar (water extract of 50 g rice leaves, 2 g l⁻¹ Faex medicinales [yeast extract, Biolabor, Bremen, Germany], 10 g l⁻¹ watersoluble starch, 15 g l⁻¹ agar-agar) at 27°C under white and black light (310 to 360 nm) for 2 weeks. For inoculation, the concentration of conidia was adjusted to 2 × 10⁵ ml⁻¹ in (0.1% wt/vol gelatin, 0.05% wt/vol sodium oleate). Plants were inoculated by spraying the spore suspension onto the second leaf 3 days after treatment with chemical inducers and 2 days after *B. graminis* f. sp. *tritici* inoculation on the primary leaf. After incubation for 24 h in a moist chamber in the dark (26°C, up to 100% relative humidity), plants were kept at high humidity under the same temperature and light regime as described above.

Treatment with resistance inducing chemicals and *B. graminis* f. sp. *tritici* inoculation.

INA (CGA41396, Novartis, Basel, Switzerland), formulated as 25% (wt/wt) active ingredient with a wettable powder (WP) carrier, was dissolved in water to a final concentration of 5 ppm active ingredient for macroscopic and microscopic evaluation of resistance induction and Northern blot analyses. The solution was applied to 5- to 6-day-old barley seedlings as a soil drench. Bion (CGA245704, Novartis), formulated as 50% (wt/wt) active ingredient with a wettable granulate (WG) carrier, was sprayed onto 8- to 9-day-old seedlings until water runoff occurred. The final concentration of the spray solution was 250 ppm active ingredient. Control plants were treated with WP and WG, respectively. Primary leaves of 5- to 6-day-old barley plants were inoculated with 2 to 10 conidia mm⁻² from *B. graminis* f. sp. *tritici* field isolate (Aachen, Germany, 2001). *B. graminis* f. sp. *tritici* was cultivated on wheat cv. Feldkrone in a growth chamber under the conditions described above.

Macroscopic and microscopic analysis.

Disease symptoms on leaves were macroscopically evaluated 6 to 7 days after inoculation with *M. grisea*. For microscopy, leaves were harvested at the timepoints indicated by placing them in a clearing solution (0.15% [wt/vol] trichloroacetic acid in [4:1, {vol/vol}] ethanol/chloroform). The solution was exchanged once and subsequently replaced by 50% (vol/vol) glycerol. Fungal structures were observed by bright-field microscopy. Papillae and whole cell autofluorescence were observed by fluorescence microscopy (excitation filter 485 nm, dichoric mirror 510 nm, barrier filter 520 nm; TCS SP, Leica, Bensheim, Germany). Whole-cell autofluorescence was taken as a reliable measure of cell death (Görg et al. 1993; Koga et al. 1990). A total of 100 to 200 infection sites were inspected per 3-cm leaf segment and were scored independently. Seedlings (3 to 5) were analyzed for each data point. Because defense reactions of short and long epidermal cells against *M. grisea* are nearly identical, no distinction was made in their evaluation (Jarosch et al. 1999).

Northern blot analysis.

For extraction of total RNA, the PeqGold RNAPure kit (Peqlab, Erlangen, Germany) was used. For gel blot analyses, 10 µg of total RNA was separated on 1.5% (vol/vol) formaldehyde agarose gels, was transferred to GeneScreen membranes, and was cross-linked using a UV cross-linker (Amersham, Freiburg, Germany). Loading of the gel was monitored by ethidium bromide staining of the gel before transfer. Mem-

branes were hybridized according to Peterhänsel and associates (1998) in Dig Easy Hyb (Roche, Mannheim, Germany) at 60 or 68°C overnight with digoxigenin-labeled probes, generated by either polymerase chain reaction or in vitro transcription. After stringent washing at 60 or 68°C with 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 0.1% sodium dodecyl sulfate, immunological detection was performed by chemiluminescence, using antidigoxigenin Fab fragments and CSPD as a substitute (Roche, Mannheim, Germany). cDNAs used as probes are designated *PR1b* (Bryngelsson et al. 1994), *PR5* (thaumatinlike protein) (Hahn et al. 1993), *PR9* (*WIR-3*, wheat peroxidase, homologue to barley *Prx8*) (Rebmann et al. 1991), and *BCII*, *BCI3* (Beßer et al. 2000), respectively.

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