Genetically Engineered Resistance to Fusarium Head Blight in Wheat by Expression of *Arabidopsis NPR1*

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Fusarium head blight (FHB) is a devastating disease of wheat and barley which causes extensive losses worldwide. Monogenic, gene-for-gene resistance to FHB has not been reported. The best source of resistance to FHB is a complex, quantitative trait derived from the wheat cv. Sumai 3. Here, we show that the *Arabidopsis thaliana* *NPR1* gene (*AtNPR1*), which regulates the activation of systemic acquired resistance, when expressed in the FHB-susceptible wheat cv. Bobwhite, confers a heritable, type II resistance to FHB caused by *Fusarium graminearum*. The heightened FHB resistance in the transgenic *AtNPR1*-expressing wheat is associated with the faster activation of defense response when challenged by the fungus. *PR1* expression is induced rapidly to a high level in the fungus-challenged spikes of the *AtNPR1*-expressing wheat. Furthermore, benzothiadiazole, a functional analog of salicylic acid, induced *PR1* expression faster and to a higher level in the *AtNPR1*-expressing wheat than in the nontransgenic plants. We suggest that FHB resistance in the *AtNPR1*-expressing wheat is a result of these plants being more responsive to an endogenous activator of plant defense. Our results demonstrate that *NPR1* is an effective candidate for controlling FHB.

Additional keywords: *PR* gene, scab, transgenic wheat.

Fusarium head blight (FHB), generically known as scab disease, has reemerged as a devastating disease of wheat and barley, severely limiting crop productivity worldwide (Bai and Shaner 2004; McMullen et al. 1997a; Parry et al. 1995). *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* [Schwein.] Petch) is the principal causal agent of FHB (Bai and Shaner 2004). Losses due to FHB outbreaks in the United States during the period between 1991 and 1997 were estimated at \$4.8 billion (Johnson et al. 2003). Comparable losses due to FHB outbreaks have occurred in other wheat-growing regions of the world. Infection by *F. graminearum* ascospores is initiated in wheat florets at anthesis, from where the fungus spreads to other spikelets within the spike. Eventually, the fungus-infected spikelets become necrotic and bleached (McMullen et al. 1997a). Grain from *F. graminearum*-infected plants accumulate the mycotoxin deoxynivalenol (DON), a vomitoxin, which further limits grain quality (Bai and Shaner 2004). In addition, DON has a role in fungal virulence. Disruption of the DON biosynthetic pathway resulted in the reduction of disease progression by *G. zeae* (teleomorph) in wheat (Desjardins et al. 1996). In wheat and barley, monogenic, gene-for-gene re-

sistance to *F. graminearum* has not been identified (Bai and Shaner 2004). Resistance to FHB in some cultivated wheat cultivars is derived from the Chinese cv. Sumai 3 and its derivatives. Sumai 3-derived resistance to FHB is a complex, quantitative trait which confers type II resistance that limits fungal spread from the site of infection (Bai and Shaner 2004). The best control methods combine the planting of cultivars that are partially resistant to FHB with fungicide application and rotation with nonhost crops (Bai and Shaner 2004; McMullen et al. 1997a and b; Wilcoxson et al. 1993).

The genetic manipulation of components of defense signaling pathways offers an alternative strategy for controlling plant diseases. Regulatory genes that control the expression of multiple defense genes are excellent targets for developing broad-spectrum and durable resistance against pathogens (Stuiver and Custers 2001). Systemic acquired resistance (SAR) is an inducible defense mechanism that confers resistance to a broad spectrum of pathogens (Durrant and Dong 2004; Ryals et al. 1996; Sticher et al. 1997). SAR is associated with the accumulation of elevated levels of salicylic acid (SA) and expression of the pathogenesis-related (*PR*) group of genes, some of which encode antimicrobial proteins (Durrant and Dong 2004; Ryals et al. 1996; Sticher et al. 1997). The role of SA in SAR has been well characterized (Dempsey et al. 1999; Durrant and Dong 2004; Ryals et al. 1996; Shah 2003; Shah and Klessig 1999). The *Arabidopsis NPR1* (*AtNPR1*) gene is a key regulator of SAR. Loss-of-function mutations in the *AtNPR1* gene compromised the activation of SAR and enhanced susceptibility to a variety of pathogens (Dong 2004). In contrast, overexpression of *AtNPR1* in transgenic *Arabidopsis* enhanced resistance to bacterial and oomycete pathogens (Cao et al. 1998; Friedrich et al. 2001). The enhanced disease resistance in *Arabidopsis* conferred by the overexpression of *AtNPR1* was associated with the faster response of these plants to SA and its functional analog, benzothiadiazole (BTH) (Cao et al. 1998; Friedrich et al. 2001). *AtNPR1* expression also enhanced disease resistance in transgenic rice (Chern et al. 2001). Considerable progress has been made in understanding the role of the *NPR1* protein in the induction of SAR in *Arabidopsis* (Durrant and Dong 2004). SA application and exposure of the plant to the pathogen triggers a change in the molecular state of *NPR1*, resulting in its translocation into the nucleus where it interacts with the TGA family of DNA-binding proteins (Kinkema et al. 2000; Mou et al. 2003). The translocation of *NPR1* into the nucleus and its interaction with the TGA proteins is essential for the expression of the *PR1* gene and in governing disease resistance (Durrant and Dong 2004).

In wheat, a SAR-like mechanism has been documented (Schweizer et al. 1989) and BTH application shown to activate resistance to fungal pathogens (Görlach et al. 1996). Express-

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sion of the wheat *PR* genes is induced in response to *F. graminearum* inoculation and SA application (Anand et al. 2003a; Kruger et al. 2002; Pritsch et al. 2000), and the developmental resistance to FHB correlated with an increase in the endogenous level of SA (Anand et al. 2003a). Moreover, an NPR1 homolog is present in wheat (R. Makandar, J. Church and J. Shah, *unpublished*). In *Arabidopsis*, which is a host for *F. graminearum* (Skadsen et al. 2004; Urban et al. 2002), we have observed that overexpression of AtNPR1 markedly reduces growth of the pathogen in leaves (Fig. 1) and floral organs (R. Makandar and J. Shah, *unpublished*), suggesting that an NPR1-regulated mechanism is involved in defense against *F. graminearum*. Here, we show that the stable expression of the AtNPR1 transcript in wheat confers a heritable, type II resistance to FHB without any discernable grain yield penalty.

RESULTS

Ubi1:AtNPR1 expression in transgenic wheat.

The spring wheat cv. Bobwhite, which is susceptible to FHB, was used to generate the *Ubi1:AtNPR1* transgenic plants. The *Ubi1:AtNPR1* and *Ubi1:bar* constructs (Fig. 2), in which the AtNPR1 and *bar* expression in planta is driven from the maize *Ubiquitin1* gene promoter, were cobombarded into immature wheat embryos. The *bar* gene, which confers resistance to glufosinate, was used as a selectable marker to identify the stably transformed T₀ plants. Three glufosinate-resistant T₀ plants (117C, 125A and 192D) that expressed the *Ubi1:AtNPR1* chimera were identified. In the subsequent four generations, stable inheritance of the *Ubi1:AtNPR1* and *Ubi1:bar* chimeric genes were detected in progeny derived from lines 117C, 125A, and 192D (Fig. 3A). *Ubi1:bar* cosegregated with the *Ubi1:AtNPR1* chimeric gene in progeny derived from all three transgenic lines, suggesting integration of the two chimeric genes adjacent to each other. In the T₁ through T₄

Fig. 1. *Fusarium graminearum* growth in *Arabidopsis* plants overexpressing the *Arabidopsis thaliana* NPR1 gene (AtNPR1). Light microscopy of an *F. graminearum* inoculated leaf from wild-type (WT) and AtNPR1-overexpressing plant (OE [NPR1]) 48 h after fungal inoculation. Arrows point to fungal mycelia. Scale bar = 10 μm.

Fig. 2. *Ubi1:AtNPR1* and *Ubi1:bar* chimeric constructs. **A**, The *Arabidopsis thaliana* NPR1 gene (AtNPR1) cDNA was cloned behind the *Ubi1* promoter plus intron. The 3' nontranscribed region of the *Agrobacterium tumefaciens* nopaline synthase (*nos*) gene provides the transcription termination information. **B**, The *bar* gene transcribed region cloned behind the *Ubi1* promoter plus intron. The 3' nontranscribed region of the *A. tumefaciens nos* gene provides the transcription termination information.

generations, AtNPR1 expression was retained in the leaves of progeny of the transgenic lines 125A and 192D that contained the *Ubi1:AtNPR1* chimeric gene (Fig. 3B). However, expression of AtNPR1 was not detected in the *Ubi1:AtNPR1* containing progeny of 117C, suggesting that the *Ubi1:AtNPR1* chimera was silenced in the subsequent generations of this line. A different pattern of *Ubi1:AtNPR1* chimeric gene integration was observed in the transgenic lines 125A and 192D (Fig. 3C), confirming that these two lines were independently derived.

FHB resistance

in *Ubi1:AtNPR1*-expressing transgenic wheat.

T₄ progeny of the *Ubi1:AtNPR1*-expressing transgenic lines 125A and 192D and of the silenced line 117C were challenged with *F. graminearum* spores. Polymerase chain reaction (PCR)

Fig. 3. Integration and expression of the *Ubi1:AtNPR1* and *Ubi1:bar* chimeric genes in transgenic wheat. **A**, DNA isolated from leaves of the nontransgenic wheat cv. Bobwhite (Bob) and T₄ progeny derived from the *Ubi1:AtNPR1*-silenced transgenic line 117C and the *Ubi1:AtNPR1*-expressing transgenic lines 125A and 192D were used in a polymerase chain reaction (PCR) assay to detect the presence of the *Ubi1:AtNPR1* and *Ubi1:bar* chimeric genes. **B**, RNA extracted from leaves of the plants in **A** was used in a reverse-transcriptase (RT)-PCR assay to monitor expression of the *Ubi1:AtNPR1* chimeric gene. Expression of the Actin gene served as a control for RT-PCR. **C**, DNA extracted from leaves of nontransgenic Bobwhite (Bob) and the T₄ progeny derived from the transgenic lines 125A and 192D were digested with *EcoRI* (E), *BamHI* (B), and *BglIII* (Bg), resolved on a gel, transferred to a nylon membrane, and probed with an *Arabidopsis thaliana* NPR1 gene (AtNPR1) probe. The positions of molecular weight markers in kilobases are indicated on the right.

was used to confirm the presence of the *Ubi1:AtNPR1* transgene, and reverse-transcriptase (RT)-PCR to monitor expression of the *Ubi1:AtNPR1* transgene in the leaves of these T₄ progeny plants. The wheat cvs. Sumai 3 and Bobwhite served as the FHB-resistant and -susceptible controls, respectively. Similar to Sumai 3, the transgenic lines 125A and 192D exhibited a high level of resistance to scab (Fig. 4A and B). Fungal growth was restricted primarily to the pathogen-inoculated spikelets of 125A and 192D, suggesting a type II FHB resistance. Similar resistance to FHB also was observed in the *Ubi1:AtNPR1*-bearing T₂ and T₃ segregants derived from 125A and 192D, but not in the segregants that lacked the *Ubi1:AtNPR1* transgene (data not shown). In six of seven experiments, the presence of the *Ubi1:AtNPR1* chimera correlated with resistance to FHB in plants derived from 125A and 192D. In the one experiment where FHB resistance was not observed, expression of the *Ubi1:AtNPR1* chimeric gene was undetectable in the leaves of 125A and 192D plants. The reason behind the nonexpression of the transgene in this one experiment is not clear. Backcrosses to

the nontransgenic Bobwhite confirmed the dominance of the FHB-resistant trait in the *AtNPR1*-expressing transgenic plants. All the F₁ plants derived from crosses between Bobwhite and T₄ progeny of 125A and crosses between Bobwhite and T₄ progeny of 192D exhibited strong resistance to FHB (Table 1). In contrast to the FHB resistance observed in the *Ubi1:AtNPR1*-expressing 125A and 192D plants, in the nontransgenic Bobwhite and the silenced transgenic line 117C the fungus spread from the original site of inoculation throughout the spike (Fig. 4B; data not shown), confirming a strong correlation between expression of the *Ubi1:AtNPR1* transgene and FHB resistance.

Table 1. Dominant nature of the Fusarium head blight (FHB)-resistant trait in the *Ubi1:AtNPR1* transgenic wheat plants^a

Plant	No. of plants tested	R	S
Bobwhite	12	0	12
125A	12	12	0
192D	15	15	0
F ₁ (Bobwhite × 125A)	12	12	0
F ₁ (Bobwhite × 192D)	14	14	0

^a R = number of FHB-resistant plants and S = number of FHB-susceptible plants.

Fig. 4. Fusarium head blight (FHB) resistance in *Ubi1:AtNPR1* expressing transgenic wheat. **A**, FHB disease rating in the FHB-resistant cv. Sumai 3, the nontransgenic susceptible cv. Bobwhite (Bob), and T₄ progeny derived from the *Ubi1:AtNPR1*-silenced transgenic line 117C and the *Ubi1:AtNPR1*-expressing transgenic lines 125A and 192D 3, 9, 15, and 21 days postinoculation (DPI) of a single spikelet per spike with spores of *Fusarium graminearum*. Three spikes per plant were inoculated. The infected spikelets (%) are an average of 60 spikes from 20 plants per wheat line. The differences in severity of disease between Bob and the *Ubi1:AtNPR1*-expressing transgenic lines 125A and 192D were statistically significant ($P < 0.001$) at 9, 15, and 21 DPI with Student's *t* test. In addition, analysis of variance for single-factor variation was carried out using the PROC GLM procedure in SAS (v6.12). The variance for percentage of infected spikelets was observed to be significant among the lines at 9, 15, and 21 DPI ($P < 0.001$). Nonsignificant variation was observed between replications ($P = 0.48$ to 0.68). **B**, Photograph of a representative spike from the nontransgenic Bobwhite (Bob) and T₄ progeny derived from the transgenic lines 125A and 192D at 21 DPI of a single spikelet (indicated by an arrow) with spores of *F. graminearum*.

Fig. 5. Comparison of yield parameters between nontransgenic and *Ubi1:AtNPR1*-expressing transgenic wheat. Spikelet number per spike and seed yield were measured for the nontransgenic Bobwhite and T₄ progeny of the *Ubi1:AtNPR1*-expressing transgenic lines 125A and 192D. **A**, Spikelet #/spike is the average number of spikelets per spike $\pm$ standard error computed from 45 spikes derived from 15 individual plants per line. **B**, Seed yield from uninoculated (U) and pathogen-inoculated (I) spikes is the average seed yield (g/spike) $\pm$ standard error from 60 individual spikes per line. Different letters above the bars indicate values that are different from each other with a confidence of 95% or greater in a paired Student's *t* test.

The spikelet number per spike and seed yield are two agronomically important yield parameters. The spikelet number and seed yield among progeny of 125A and 192D were comparable with that in the nontransgenic Bobwhite plants (Fig. 5A and B), indicating that constitutive expression of *AtNPR1* does not confer any adverse effects on yield parameters. Moreover, compared with *F. graminearum*-inoculated Bobwhite plants, the seed yield in pathogen-inoculated 125A and 192D plants was considerably higher (Fig. 5B), suggesting a beneficial role of *AtNPR1* expression on grain productivity in the *F. graminearum*-challenged plants.

PR1 expression in *Ubi1:AtNPR1*-expressing plants.

The FHB resistance observed in the *AtNPR1*-expressing transgenic plants could result from the constitutive expression of defense responses. Alternatively, defense responses in the *AtNPR1*-expressing plants may be primed to respond faster to the pathogen. To distinguish between these two possibilities, we monitored expression of the wheat *PR1* gene in the transgenic lines 125A and 192D. In nontransgenic plants, the *F. graminearum*-induced expression of the *PR1* gene correlates with resistance to FHB; expression is induced faster and to higher levels in the *F. graminearum*-inoculated spikes of the FHB-resistant cv., Sumai 3 than in the susceptible cv. Bobwhite (Fig. 6A). A comparable low level of *PR1* transcript accumulated in the spikes and flag leaves of the unchallenged nontransgenic Bobwhite plant and the transgenic plants 125A and 192D (Fig. 6A and B), suggesting that *AtNPR1* expression does not result in a constitutively high level of expression of the *PR1*

gene. However, like Sumai 3, the *PR1* transcript accumulated faster and to higher levels in the pathogen-inoculated spikes of 125A and 192D than in the nontransgenic Bobwhite (Fig. 6A).

In *Arabidopsis*, the enhanced disease resistance conferred by overexpression of *AtNPR1* is due to the increased responsiveness of the transgenic plant to SA; both SA and its functional analog, BTH, were more effective in inducing *PR1* expression in the *AtNPR1*-overexpressing plant than in the wild-type plant (Cao et al. 1998; Friedrich et al. 2001). To determine whether the wheat lines 125A and 192D are similarly more responsive to inducers of SAR, we compared *PR1* expression among BTH-treated nontransgenic Bobwhite, the transgenic lines 125A and 192D, and Sumai 3. Similarly to the FHB-resistant Sumai 3, *PR1* expression in response to BTH treatment was faster and stronger in the transgenic wheat lines 125A and 192D than in the nontransgenic Bobwhite plant. *PR1* expression in the flag leaves of Sumai 3, 125A, and 192D was evident as early as 6 and 12 h post exposure to BTH at 100 and 50 μ M, respectively (Fig. 6B and C). In contrast, a more prolonged exposure to these concentrations of BTH was required for the induction of *PR1* expression in the nontransgenic Bobwhite plants (Fig. 6C).

DISCUSSION

We have successfully engineered the expression of *AtNPR1* from a maize *Ubiquitin* gene promoter to convert Bobwhite from an FHB-susceptible wheat cultivar into a highly resistant one. FHB resistance in the *AtNPR1*-expressing wheat plants

Fig. 6. *Fusarium graminearum*- and benzothiadiazole (BTH)-induced expression of pathogenesis-related (PR) gene *PR1* in transgenic and nontransgenic wheat plants. Northern blots were used to monitor *PR1* transcript accumulation with a 32 P-labeled wheat *PR1* gene-specific double-stranded cDNA probe. **A**, *PR1* expression in *F. graminearum*-inoculated spikes of the Fusarium head blight (FHB)-resistant cv. Sumai 3, the nontransgenic FHB-susceptible cv. Bobwhite (Bob), and the T_4 progeny derived from the transgenic lines 125A and 192D. RNA was extracted from entire spikes 24 h after mock (M) treatment or 24, 48, 72, and 96 h postinoculation with *F. graminearum* (F. g). **B**, *PR1* expression in flag leaves from Bobwhite (Bob) and T_4 progeny derived from the transgenic lines 125A and 192D treated with water (M) and BTH (50 and 100 μ M) 6 and 12 h posttreatment. **C**, Time course of *PR1* expression in flag leaves from Sumai 3, mock (M) treated with water and BTH (50 and 100 μ M), and mock- and BTH (50, 100, and 200 μ M)-treated flag leaves from Bobwhite (Bob). Flag leaves were harvested for RNA extraction at the indicated times after treatment. This experiment was done simultaneously with the experiment shown in B.

was associated with the faster and stronger activation of *PR1* expression in response to *F. graminearum*, presumably due to the enhanced responsiveness of these transgenic lines to an endogenous activator of plant defense. In contrast to the non-transgenic Bobwhite plants, BTH was more effective in activating *PR1* expression in the *AtNPR1*-expressing wheat plants (Fig. 6B). The similarity in the faster and stronger activation of *PR1* transcript accumulation in BTH-treated wheat and *Arabidopsis* plants that express elevated levels of *AtNPR1* suggests the evolutionary conservation of an NPR1-regulated defense mechanism between wheat and *Arabidopsis*. Indeed, wheat contains genes that exhibit homology to *AtNPR1* (R. Makandar, J. Church and J. Shah, *unpublished*). The enhanced resistance to *F. graminearum* in *Arabidopsis* overexpressing *AtNPR1* (Fig. 1) further supports this hypothesis. The FHB resistance and BTH sensitivity of *PR1* expression in the *AtNPR1*-expressing transgenic lines 125A and 192D were comparable with that of the FHB-resistant cv. Sumai 3, suggesting that faster and stronger response to an endogenous activator of plant defense may contribute to FHB resistance in Sumai 3.

In a previous study, the constitutive accumulation of high level of salicylates in a transgenic wheat plant, which was engineered to simultaneously express two *PR* genes, was shown to be accompanied by enhanced resistance to FHB (Anand et al. 2003a), suggesting that SA may function as an endogenous signaling molecule in wheat. Similarly, pathogen-induced SA accumulation may have a role in mediating FHB resistance in the *AtNPR1*-expressing transgenic wheat plants 125A and 192D. These *AtNPR1*-expressing plants may be primed to respond faster to SA and its analogs. Indeed, exogenously applied BTH was far more effective at activating *PR1* expression in the *AtNPR1*-expressing transgenic plants than in the non-transgenic Bobwhite. However, in *Arabidopsis*, *AtNPR1* also is associated with the activation of systemic defenses that are independent of SA (Pieterse et al. 1998). Moreover, although BTH induces resistance to powdery mildew in wheat, the BTH-induced wheat chemical induction (*WCI*) genes were not activated in response to powdery mildew and their expression was not associated with the activation of acquired resistance by the nonhost pathogen *Erysiphe graminis* f. sp. *hordei* (Schaffrath et al. 1997), thus suggesting that two different pathways may induce acquired resistance in wheat. Hence, additional experiments are required to determine if a SA-dependent or -independent mechanism is involved in mediating FHB resistance in the *AtNPR1*-expressing wheat plants.

Our findings are in accordance with earlier studies of overexpression of *AtNPR1* that resulted in enhanced resistance to bacterial and oomycete pathogens in *Arabidopsis* (Cao et al. 1998; Friedrich et al. 2001) and to bacterial blight in rice (Chern et al. 2001). Similarly, overexpression of a rice homolog of NPR1, *osNH1*, enhanced resistance to bacterial blight in rice (Chern et al. 2005). However, in rice, overexpression of *AtNPR1* and *osNH1* were associated with the spontaneous development of chlorotic lesions, cell death, accumulation of hydrogen peroxide, and the constitutive expression of rice defense genes (Chern et al. 2005; Fitzgerald et al. 2004). BTH application enhanced these phenotypes, suggesting that these detrimental phenotypes are a result of constitutive expression of plant defense response (Chern et al. 2005; Fitzgerald et al. 2004). Most cultivars of rice contain high basal level of salicylates (Silverman et al. 1995). These levels are a magnitude of order higher than that found in *Arabidopsis*, maize, and wheat (Anand et al. 2003a; Morris et al. 1998; Shah et al. 1997). This high level of salicylate may result in the constitutive expression of the NPR1-regulated pathways in rice plants overexpressing *AtNPR1* or *OsNH1* (Chern et al. 2005; Fitzgerald et al. 2004). The continuous diversion of energy for the constitu-

tive expression of defense responses is expected to lay constraints on plant productivity and could result in detrimental phenotypes; for example, chlorosis and lesions containing dead cells. Indeed, constitutive activation of SA-regulated defense responses in several *Arabidopsis*, rice, and maize mutants is associated with dwarfing and the spontaneous development of lesions (Campbell et al. 2002). Moreover, compared with control treatments, BTH treatment was shown to reduce plant productivity in the absence of a pathogen (Heil et al. 2000). Unlike the *AtNPR1*- and *OsNH1*-overexpressing rice plants, in the absence of pathogen, defense responses are not constitutively active and there is no detrimental effect associated with *AtNPR1* expression in wheat. This could be because wheat, unlike rice, does not contain high basal levels of SA and its conjugates and, hence, requires a pathogen-elicited signal to rapidly activate defense responses in the *AtNPR1*-expressing transgenic plants when challenged by pathogen. Alternatively, the detrimental phenotypes associated with *AtNPR1* expression in rice may be due to factors other than SA; as discussed above, the involvement of *AtNPR1* in some plant processes is independent of its association with SA signaling.

Previously, expression of the *FsTRI101* gene from *F. sporotrichioides*, which encodes a trichothecene acetyltransferase, was successfully utilized to limit DON levels and spread of *F. graminearum* in the spikes of a transgenic wheat plant (Okubara et al. 2002). This study and our work with *AtNPR1* indicate that genetic engineering provides an effective strategy for developing wheat with resistance to FHB, a devastating disease against which monogenic gene-for-gene resistance is not known. However, considering the detrimental phenotypes associated with *AtNPR1* expression in rice, the utilization of *AtNPR1* for enhancing disease resistance in crops should take into consideration the physiology of the individual plant species to be targeted.

MATERIALS AND METHODS

Plant and fungal material and growth conditions.

Wheat plants were grown in a greenhouse under a temperature regime of 20 and 18°C (day and night, respectively). Wheat seeds were germinated in autoclaved composed-peat-based planting mixture (Premier Pro Mix-BX, Procuier, Canada), three seeds per gallon-capacity pot. *Arabidopsis* plants were grown at 22°C in a growth chamber programmed for a 14-h light (100 $\mu\text{E m}^{-2} \text{s}^{-1}$) and 10-h dark cycle. A transgenic *Arabidopsis* line that expresses the *AtNPR1* cDNA from the cauliflower mosaic virus 35S gene promoter was described previously (Cao et al. 1998). Approximately 4-week-old *Arabidopsis* plants were used for fungal inoculation. A fungal spore suspension containing 30,000 spores/ml was infiltrated into the abaxial surface of leaves with a needleless syringe. The pathogen-inoculated plants were covered with a clear plastic dome. The disease spread was observed and recorded at 48 h postinoculation. *F. graminearum* isolate Z-3639 was cultured on half-strength potato dextrose agar (Difco Laboratories, Detroit, MI, U.S.A.) at 22°C. Sporulation was induced by culturing the fungus in mung bean broth for 2 days at 22°C. The fungal spore suspension was filtered through four layers of cheese cloth to remove fungal mycelia and the spore concentration quantified using a haemocytometer. To prepare the mung bean broth, 4 g of mung bean seed was boiled in 100 ml of water. The extract was filtered through four layers of cheesecloth and the filtrate autoclaved.

Fungal inoculation of wheat and disease rating.

One central spikelet of a spike at anthesis was clipped one-third from the top and inoculated with 1,200 spores of *F.*

graminearum isolate Z-3639 in a 20- μ l volume. The inoculated spike was covered with a moistened ziplock bag and the plant was kept at 22°C in the greenhouse. Three days postinoculation (dpi) the ziplock bag was removed. The fungus-inoculated plants were evaluated for type II disease reaction at 3, 9, 15, and 21 dpi. For each spike, the percentage of spikelets to which the infection had spread from the inoculated spikelet was determined. A spike was scored as resistant (0 to 25% spikelets infected), moderately resistant (25 to 50% spikelets infected), moderately susceptible (50 to 75% spikelets infected), and susceptible (75 to 100% spikelets infected). Three spikes per plant were inoculated.

Estimation of yield components.

The spikelet number per spike is one of the yield-contributing traits. For each plant, the spikelet number per spike was computed for three spikes. The average number of spikelets per spike in 15 individual plants represented the mean spikelet number for an individual wheat line. For calculating seed yield, the weight of seeds per spike was determined. The seed yield in grams per spike represented the average seed yield from 60 individual spikes per wheat line.

Plasmids and wheat transformation.

The *Ubi1:AtNPR1* chimeric gene construct (plasmid pJS406) contains a cDNA, which encompasses the translated plus the 5' and 3' transcribed but nontranslated regions of the *AtNPR1* gene from the *Arabidopsis* ecotype Dijon-17 (Zhou et al. 2000). In planta expression of *AtNPR1* is driven from the ubiquitously expressed maize *Ubiquitin1* (*Ubi1*) promoter, with the maize *Ubi1* intron added to enhance stability of transcript (Christensen and Quail 1996). The 3' nontranscribed region of the *Agrobacterium tumefaciens nopaline synthase (nos)* gene provides the transcription termination information. The gene construct *Ubi1:bar* in the plasmid pAHC20 (Christensen and Quail 1996) expresses the *bar* gene from the *Ubi1* promoter-intron. The *bar* gene confers resistance to the herbicide glufosinate (trade name Liberty; Bayer Crop Sciences, Research Triangle, NC, U.S.A.). Embryogenic calli were used for cotransforming wheat with the pJS406 plus pAHC20 plasmids using a previously described protocol (Altpeter et al. 1996) as modified by Anand and associates (2003b).

Leaf painting assay for resistance to glufosinate.

To detect expression of the *Ubi1:bar* chimeric gene in wheat transformants, an aqueous solution (0.2%, vol/vol) of the herbicide Liberty was applied on a portion of the leaf lamina (approximately 2.5 cm long) on the flag 1 leaf (one leaf below the flag leaf) with a paintbrush. The painted leaf area was marked with a marker and visual observations were recorded 3 to 4 days after leaf painting. Leaves with bleached symptoms on the Liberty-painted leaf portion were scored as sensitive to Liberty and leaves without bleaching were scored as insensitive to Liberty.

Molecular analysis of transgenic wheat plants.

DNA was extracted from plants as previously described (Das et al. 1990). RNA was extracted from plants using acid guanidinium thiocyanate-phenol-chloroform (Chomczynski and Sacchi 1987). ³²P-labeled *AtNPR1* cDNA was used as a probe to monitor the presence of the *Ubi1:AtNPR1* chimera and accumulation of *AtNPR1* transcript by Southern and Northern analysis, respectively, whereas a ³²P-labeled 950-bp fragment of an FHB-inducible wheat *PR1* gene (GenBank accession number DT045069) was used as a probe to monitor accumulation of the *PR1* transcript. Genomic PCR was used to monitor the presence of *Ubi1:AtNPR1* and *Ubi1:bar* chimeric

genes. The *AtNPR1*-specific oligonucleotide primers *AtNPR1-F1* (5'-GAGGACACATTGGTTATACTC-3') and *AtNPR1-R5* (5'-CAAGATCGAGCAGCGTCATCTT-3') were used to amplify a 750-bp fragment of the *Ubi1:AtNPR1* chimera, whereas a *Ubi1* primer (5'-CCTGCCTTCATACGCTATTATTATGTC-3') plus *bar* primer (5'-CTTCAGCAGCAGGTGGGTGTAGAGCGTG-3') were used to amplify a 460-bp fragment of the *Ubi1:bar* chimera. RT-PCR analysis was performed with the Superscript II One-step RT-PCR kit (Invitrogen, Carlsbad, CA, U.S.A.). For RT-PCR amplification analysis of Actin expression, the primers Actin 8-1 (5'-ATGAAGATTAAGGTCGTGGCA-3') and Actin 8-2 (5'-TCCGAGTTTGAAGAGGCTAC-3') were used.

BTH treatment.

Leaves were floated in individual solutions containing 50, 100, and 200 μ M BTH. Leaves floated in water (mock-treated) served as a negative control. Leaves were harvested at various time points after treatment, RNA extracted, and *PR1* expression monitored by Northern blot analysis.

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