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mlo5, a resistance gene effective against a biotrophic pathogen (*Blumeria graminis* fsp. *hordei*) confers enhanced susceptibility of barley to the necrotrophic fungus *Bipolaris sorokiniana* (teleomorph: *Cochliobolus sativus*)

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ABSTRACT The barley resistance gene *mlo5* determines race non-specific resistance to the biotrophic powdery mildew pathogen *Blumeria graminis* f.sp. *hordei*. On the other hand, we have shown that barley lines that contain the *mlo5* gene display enhanced susceptibility to the necrotrophic fungus *Bipolaris sorokiniana* (teleomorph: *Cochliobolus sativus*) and its toxic culture filtrate (Kumar et al. 2001). Enhanced susceptibility to necrotic disease symptoms was linked to increased accumulation of hydrogen peroxide (H_2O_2), a reactive oxygen intermediate. In addition, increased accumulation of transcripts of a barley pathogenesis-related gene (*PR1-b*) and slight increases in expression of two antioxidant genes, a glutathione S transferase and an ascorbate peroxidase occurred in association with enhanced cell/tissue death and H_2O_2 accumulation. These results might reflect an unsuccessful attempt by infected *mlo5*-barley to suppress necrotic disease symptoms and support the hypothesis that the barley *Mlo* gene product functions as a negative regulator of cell death. Therefore, a compromised *Mlo* pathway confers effective control of the biotrophic powdery mildew pathogen but not of the necrotroph *B. sorokiniana*, demonstrating the necessity of different host defense strategies in response to pathogens with different lifestyles (biotroph vs. necrotroph).
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KEY WORDS

barley
Blumeria graminis
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In barley, the recessive *mlo5* allele confers a broad spectrum, non-race specific resistance to the biotrophic powdery mildew pathogen (*Blumeria graminis* fsp. *hordei*) (Schulze-Lefert and Vogel 2000) that is not accompanied by cell death. However, pleiotropic effects associated with the *mlo5* disease resistance trait include spontaneous formation of macroscopically visible necrotic leaf lesions in late developmental stages (Wolter et al. 1993; Peterhänsel et al. 1997). Therefore, the *mlo5* mutation can be considered similar to lesion mimic mutations identified in several plant species, all of which seem to be altered in the induction of cell death (Dangl et al. 1996). The *Mlo* gene of barley has been cloned and the deduced protein was predicted to be membrane associated (Büschges et al. 1997). Based on these findings the wild type *Mlo* allele is hypothesized to be a negative regulator of not only broad spectrum powdery mildew resistance but also of spontaneous cell death in barley leaves. While a compromised *Mlo* pathway (*e.g.* in *mlo5* plants) is sufficient to confer resistance to the biotrophic powdery mildew pathogen, we have shown that it enhances susceptibility to necrotrophic fungal pathogens like *Magnaporthe grisea* and *Bipolaris sorokiniana* (Jarosch et al. 1999; Kumar et al. 2001). The aim of the present study was to characterize the biochemical and molecular background of cell death elicited in *mlo5* barley by the necrotroph *Bipolaris sorokiniana* (teleomorph: *Cochliobolus sativus*) and its toxic culture filtrate in order to gain further insight in the pleiotropic effects of an important plant resistance gene.

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Materials and Methods

Sources of host and pathogen genotypes used, production of toxin-containing culture filtrate of *B. sorokiniana* and detection of hydrogen peroxide (H_2O_2) by 3,3-diaminobenzidine (DAB) staining were as described previously (Kumar et al. 2001). For gene expression assays, isolation of total plant RNA and Northern blot analysis was according to standard methods. Non-radioactive, digoxygenin or fluorescein labeled RNA probes were used (Roche). Reverse transcription-polymerase chain reaction (RT-PCR) analysis was done by a one-step method (Qiagen).

Results and Discussion

Powdery mildew-resistant barley genotypes bearing the *mlo5* locus displayed enhanced susceptibility to normosensitive necrosis caused by *Bipolaris sorokiniana* or its toxin-containing culture filtrate (CF). Increased sensitivity of a barley cv. Ingrid backcross line (I-*mlo5*) was evident even at a CF dilution of 1:500. Furthermore, the extent and spatial distribution of necrosis in inoculated and CF-treated plants correlated well with high H_2O_2 levels as measured by diaminobenzidine (DAB) staining of leaves. Infiltration with increasing concentrations of ascorbate (1, 10, and 50 mM) suppressed both H_2O_2 accumulation and necrotic symptoms

which suggests a role for H_2O_2 in the necrotization process as previously shown for other plant-pathogen interactions (Levine et al. 1994; Tenhaken et al. 1995; Govrin and Levine 2000). Interestingly, H_2O_2 accumulation appears to be a general feature of *mlo5* barley in response to pathogen attack. Our previous work demonstrated that in powdery mildew-infected *mlo5* plants nonnecrotic resistance (papilla formation) is also closely associated with accumulation of H_2O_2 (Hückelhoven et al. 1999; Hückelhoven and Kogel 2000). Thus, in *mlo5* barley, enhanced H_2O_2 production accompanies both disease induced by the necrotroph *B. sorokiniana* and resistance to the biotrophic powdery mildew pathogen (*B. graminis* fsp. *hordei*). Understanding the role of H_2O_2 in these processes could be a key for clarification of the pleiotropic effects of *mlo* alleles in barley.

Besides H_2O_2 production, another reliable molecular marker of normosensitive necrosis induced by *B. sorokiniana* in *mlo5* barley appeared to be the expression of a pathogenesis-related (PR) gene, *PR-1b*. Enhanced *PR-1b* expression in powdery mildew-infected *mlo5* barley (non-necrotic resistance reaction) has been detected previously (Peterhänsel et al. 1997). However, it is important to point out that in plants exposed to *B. sorokiniana* or its culture filtrate, *PR-1b* expression correlated with disease susceptibility, rather than with resistance. This result is supported by earlier work showing enhanced accumulation of *PR-1b* in barley and rice infected with the necrotrophic pathogens *Drechslera teres* and *Magnaporthe grisea* or *B. sorokiniana*, respectively (Reiss and Bryngelsson 1996; Manandhar et al. 1999). The enhanced induction of *PR-1b* along with slight increases in expession of two antioxidant genes, a glutathione S transferase and an ascorbate peroxidase might reflect an unsuccessful attempt by infected *mlo5*-barley to suppress necrotic disease symptoms and support the hypothesis that the barley *Mlo* gene product functions as a negative regulator of cell death (Büschges et al. 1997). Therefore, a compromised *Mlo* pathway confers effective control of the biotrophic powdery mildew pathogen but not of the necrotroph *B. sorokiniana*, demonstrating the necessity of different host defense strategies in response to pathogens with different lifestyles (biotroph vs. necrotroph).

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