Brachypodium distachyon–*Cochliobolus sativus* Pathosystem is a New Model for Studying Plant–Fungal Interactions in Cereal Crops

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ABSTRACT

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Cochliobolus sativus (anamorph: *Bipolaris sorokiniana*) causes spot blotch, common root rot, and kernel blight or black point in barley and wheat. However, little is known about the molecular mechanisms underlying the pathogenicity of *C. sativus* or the molecular basis of resistance and susceptibility in the hosts. This study aims to establish the model grass *Brachypodium distachyon* as a new model for studying plant–fungus interactions in cereal crops. Six *B. distachyon* lines were inoculated with five *C. sativus* isolates. The results indicated that all six

B. distachyon lines were infected by the *C. sativus* isolates, with their levels of resistance varying depending on the fungal isolates used. Responses ranging from hypersensitive response-mediated resistance to complete susceptibility were observed in a large collection of *B. distachyon* ($2n = 2x = 10$) and *B. hybridum* ($2n = 4x = 30$) accessions inoculated with four of the *C. sativus* isolates. Evaluation of an F_2 population derived from the cross between two of the *B. distachyon* lines, Bd1-1 and Bd3-1, with isolate Cs07-47-1 showed quantitative and transgressive segregation for resistance to *C. sativus*, suggesting that the resistance may be governed by quantitative trait loci from both parents. The availability of whole-genome sequences of both the host (*B. distachyon*) and the pathogen (*C. sativus*) makes this pathosystem an attractive model for studying this important disease of cereal crops.

Brachypodium distachyon is a member of the Pooideae subfamily, which contains many important temperate cereal crops, including wheat, barley, oat, turf, and forage grasses. Due to its small stature, rapid generation time, self fertility, and a small diploid genome, *B. distachyon* has been developed as a model system for functional genomic studies of temperate cereals and grasses (11,17). Research areas of interest to plant pathologists include deciphering the molecular mechanisms dictating the outcomes of host–pathogen interactions, which is essential for designing novel plant-based approaches for disease management. For cereal crops such as barley and wheat with large genomes and inefficient transformation systems, research on host–pathogen interactions is challenging; thus, establishing *B. distachyon* as a model system for cereal crop diseases is attractive. In the past several years, *B. distachyon* has been reported to be a host for a range of commercially important fungal pathogens of cereals and grasses (17). *Magnaporthe oryzae*, the causal agent of rice blast disease, was among the first plant fungal pathogens that have been evaluated on *B. distachyon* and found to be capable of infecting it (11,32). A set of *B. distachyon* accessions was screened with *M. oryzae* isolates, and they had differing degrees of susceptibility (11,32). *B. distachyon* accessions were also challenged with cereal rust species. Some of them showed brown flecking symptoms when inoculated with isolates of *Puccinia hordei* G. H. Oth and *P. triticea* Erikss., which are the barley and wheat leaf rust pathogens, respectively, but no uredinia were observed (11).

However, when challenged with isolates of the wheat stripe rust pathogen *P. striiformis* Westend., the *B. distachyon* accessions displayed a range of responses from brown flecking to uredinial formation in necrotic areas (2,11). Recently, Peraldi et al. (30) showed susceptibility of *B. distachyon* to *Fusarium head blight*, a major wheat disease caused by *Fusarium graminearum* and *F. culmorum*. More recently, Figueroa et al. (15) demonstrated that *B. distachyon* exhibits natural variation for resistance to *P. graminis*, the causal agent of stem rust. Peraldi et al. (31) also showed that *B. distachyon* is compatible with the eyespot (*Oculimacula* spp.) and Ramularia leaf spot (*Ramularia collo-cygni*) diseases of barley.

Cochliobolus sativus is a fungal pathogen that causes several important diseases in cereal crops, including spot blotch and common root rot. Spot blotch occurs in both barley and wheat and is a serious problem in many regions of the world (26). Significant losses to spot blotch in barley commonly occur in areas with temperate climates such as the upper Midwest region of the United States and the central provinces of Canada (18,26). The spot blotch disease attacks six-rowed and two-rowed barley cultivars, resulting in marked reductions in yield and quality of the crops (26,28). Yield losses from 16 to 33% have been reported in susceptible barley cultivars under conditions favorable for disease development (8,41). Spot blotch of wheat is typically observed in warm and humid environments such as subtropical regions; however, recent observations indicate that the disease is dramatically expanding into more temperate regions of the world (37). Yield losses of 20 to 80% have been reported in wheat due to spot blotch (12,33). *C. sativus* also causes common root rot (CRR), which is the most widespread and persistent root disease of barley and wheat in the Great Plains of North America (27) and western Canada (13,20). The disease can cause thin crop stands, reduced numbers of fruiting tillers, fewer kernels, and reduced kernel weights (40). Yield loss due to CRR varies from year to year; for most of North America, yield losses of 3 to 6% are attributed to CRR in wheat (40), and yield reductions of

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approximately 10% were reported in barley in Canada (13) and North Dakota (34).

Despite of the importance of the diseases caused by *C. sativus*, little is known about the mechanisms regarding its pathogenicity. Our understanding of plant defenses against *C. sativus*, especially at the molecular level, is lacking as well. In part, this is due to the fact that barley and wheat have large and complex genomes and lack efficient transformation systems. In this study, we investigated the host status of *B. distachyon* to *C. sativus* and determined that the *B. distachyon*–*C. sativus* interaction is a new model pathosystem for studying this disease in cereal crops.

MATERIALS AND METHODS

Fungal isolates. Five *C. sativus* isolates (ND93-1, ND85F, ND90Pr, ND4008, and Cs07-47-1) were used in this study. These isolates exhibit differential virulence patterns on barley and wheat differential lines. ND93-1, ND85F, and ND90Pr belong to one of the three pathotypes (pathotypes 0, 1, and 2) previously identified in *C. sativus* based on their virulence pattern in three barley differential genotypes (Bowman, ND 5883, and ND B112) (36,45). Pathotype 1, represented by isolate ND85F, exhibits high virulence on ND 5883 and low virulence on Bowman and ND B112; pathotype 2, represented by isolate ND90Pr, exhibits high virulence on Bowman and low virulence on ND 5883 and ND B112; and pathotype 0, represented by isolate ND93-1, exhibits low virulence on all three of the barley differentials (36). ND4008 exhibits high virulence on all three of these barley lines (19) and, thus, it is considered a new pathotype (pathotype 3). Cs07-47-1 was isolated from a wheat leaf disease sample and exhibits a relatively higher level of virulence on wheat but low virulence on Bowman, ND 5883, and ND B112.

Brachypodium lines. Six inbred *B. distachyon* lines (Bd1-1, Bd21, Bd21-3, Bd2-3, Bd3-1, and Bd18-1) (Table 1) were initially used for inoculation with the *C. sativus* isolates mentioned above. These *B. distachyon* lines were selected because of their wide use by the scientific community (17). An additional 190 *Brachypodium* spp. accessions (Supplementary Table S1) were obtained from Dr. Jinguo Hu of the United States Department of Agriculture–Agricultural Research Service (USDA-ARS) Western Regional Plant Introduction and Research Station, Pullman, WA. Most of these accessions have been described by Filiz et al. (16) and Vogel et al. (38). In all, 153 accessions were *B. distachyon*, while the other 37 were the tetraploid *B. hybridum*, according to the taxonomic classification of Catalan et al. (7).

A population of 74 F₂ plants derived from a cross between Bd3-1 and Bd1-1 was used to study the genetics of spot blotch resistance in *B. distachyon*.

Plant growth and fungal inoculation. Seed of *B. distachyon* and *B. hybridum* were planted in plastic cone trainers (Stuewe and Sons, Inc., Corvallis, OR) with Sunshine mix SB100 (Sun Gro Horticulture, Bellevue, WA) and fertilized with Osmocote Plus 15-19-12 (Scotts Sierra Horticultural Product Company, Marysville, OH). Each line or accession had three replicate cones, each containing three seeds. Cones were randomly placed in RL98 trays and, 3 days after planting, the trays were moved into a cold room

(4 to 8°C) for 4 to 5 weeks of vernalization before being returned to a 20°C (±2°C) greenhouse. When the plants reached particular developmental stages (from two-leaf stage to heading), they were spray inoculated with a conidial suspension (2 × 10³ conidia ml⁻¹) of the *C. sativus* isolates, using the method described by Fetch and Steffenson (14). The inoculated plants were incubated in a humidity chamber for 24 h and then placed in a 20°C (±2°C) greenhouse for 7 to 10 days before disease ratings. A disease rating scale of 1 to 5 was used to determine the disease severity of each plant where 1 = resistant (lesions consisting of flecking or small dark spots); 2 = moderately resistant (lesions consisting of dark spots [1 to 3 mm], with little surrounding necrosis or chlorosis); 3 = moderately susceptible (dark lesions surrounded by necrosis or chlorosis, 3 to 5 mm long); 4 = susceptible (large necrotic or chlorotic lesions, 5 mm or greater, with some coalescence); and 5 = highly susceptible (larger necrotic or chlorotic lesions, with brown discoloration spreading to the whole leaf). For each *Brachypodium* spp. line or accession, an average disease rating from all inoculated plants was used as the infection response to the particular fungal isolate.

Examination of fungal infection in planta. To examine the infection process of *C. sativus* in planta, we inoculated the *B. distachyon* lines Bd21-3 and Bd18-1 with a spore suspension (5 × 10³ conidia ml⁻¹) prepared from each of the three isolates (Cs07-47-1, ND85F, and ND4008) separately. Infected leaf samples were harvested at different time points and stained according to the method described by Koch and Slusarenko (23). Briefly, the leaf samples were cut into small fragments and placed in a lactophenol-trypan blue stain solution containing 10 ml of lactic acid, 10 ml of glycerol, 10 g of phenol, and 10 ml of distilled water containing 10 mg of trypan blue. After boiling for approximately 1 min in the staining solution, the leaf fragments were cleared in chloral hydrate (2.5 g of chloral hydrate dissolved in 1 ml of distilled water) for at least 30 min. They were then mounted in chloral hydrate and viewed under an Olympus BX51 microscope (Olympus, Center Valley, PA) and photographed by a CCD camera (Diagnostic Instruments, Inc., Sterling Heights, MI).

RESULTS

***C. sativus* infects leaves, stems, and spikes of *B. distachyon*.** We first spray inoculated whole plants of Bd21-3 at the heading stage with the spores of isolate Cs07-47-1. At 2 days postinoculation (DPI), pin-sized, dark-brown, necrotic lesions were observed (Fig. 1A). At 3 DPI, the lesions expanded and extended primarily along the vascular bundles (Fig. 1B). At 5 DPI, the lesions enlarged and some joined with each other to form larger lesions. At 7 DPI, chlorosis and ink-like symptoms surrounding the dark-brown lesions had developed on some leaves (Fig. 1D). The lesion development on *B. distachyon* was similar to that observed on barley inoculated with isolate ND4008 (Fig. 1F). The fungus caused lesions not only on leaves but also on infected spikes (Fig. 1E).

To study the infection responses of *B. distachyon* plants at different growth stages, Bd21-3 plants at 15 (Fig. 2A), 25 (Fig. 2B), 32 (Fig. 2C), and 37 (Fig. 2D) days after planting, respectively, were spray inoculated with Cs07-47-1. The results indicated that

TABLE 1. Infection responses of *Brachypodium distachyon* (Bd) inbred lines to five isolates of *Cochliobolus sativus*

Bd line	Ploidy	ACP	ACNO	Country	<i>C. sativus</i> isolate				
					ND93-1	ND90Pr	ND85F	Cs07-47-1	ND4008
Bd1-1	Diploid	PI	170218	Turkey	1.8	2.0	2.0	2.5	2.0
Bd18-1	Diploid	PI	245730	Turkey	3.5	3.0	1.5	3.7	4.5
Bd21	Diploid	PI	254867	Iraq	3.0	2.5	2.0	3.7	3.8
Bd21-3	Diploid	W6	39233	Iraq	3.0	2.0	2.0	4.0	4.0
Bd2-3	Diploid	PI	185133	Iraq	2.5	2.0	1.5	3.0	3.5
Bd3-1	Diploid	PI	185134	Iraq	3.5	3.5	2.0	4.7	4.5

infections occurred on all expanded leaves regardless of the plant growth stage. However, the disease was more severe on old leaves than on young leaves (Fig. 2C and D). The youngest leaves that were infected were those next to the newly emerged leaves at the time of inoculation. Thus, disease severity on the youngest leaves was used to represent the infection response of inoculated *B. distachyon* plants.

Interactions of *B. distachyon* lines with five *C. sativus* isolates. To determine whether the six *B. distachyon* lines (Bd1-1, Bd21, Bd21-3, Bd2-3, Bd3-1, and Bd18-1) exhibited differential infection responses to different fungal isolates, they were inoculated with individual fungal isolates (ND93-1, ND85F, ND90Pr, ND4008, and Cs07-47-1). The result indicated that the responses varied depending on the host and isolate involved (Table 1). For a particular fungal isolate, infection responses of the six *B. distachyon* lines varied. For example, when inoculated with Cs07-47-1, Bd1-1 was resistant and Bd21 was moderately resistant whereas the other four lines (Bd21-3, Bd3-1, Bd2-3, and Bd18-1) were susceptible (Fig. 3). The five *C. sativus* isolates also showed

differences in virulence on the same *B. distachyon* lines. For example, Bd18-1 was susceptible to ND4008 but resistant to ND85F (Fig. 4). Among the six *B. distachyon* lines evaluated, Bd1-1 was, overall, the most resistant line, and Bd3-1 was the most susceptible line against the five fungal isolates (Table 1). Among the fungal isolates, ND4008 was the most virulent and ND85F was the least virulent on the *B. distachyon* lines evaluated (Table 1).

Infection process of *C. sativus* on *B. distachyon*. To examine the infection process of *C. sativus* on *B. distachyon*, leaf samples of Bd21-3 plants inoculated with isolate Cs07-47-1 at the heading stage were collected at 6, 12, 24, 48, 72, and 120 h postinoculation (HPI), stained with lactophenol-trypan blue, and examined under a microscope. The results showed that appressoria formed by 6 HPI and penetration occurred by 12 HPI. At 24 and 48 HPI, both intercellular and intracellular fungal hyphae were observed around the infection points. At 72 and 120 HPI, the fungus produced more proliferated mycelia, and macroscopic symptoms expanded to plant tissues beyond the limits of the area infected with fungal hyphae (Fig. 5).

Fig. 1. Infection responses of *Brachypodium distachyon* inbred line Bd21-3 and barley line ND B112 after inoculation with *Cochliobolus sativus* isolates Cs07-47-1 and ND 4008, respectively. The *B. distachyon* plants were spray inoculated at the heading stage. The disease symptoms on leaves were examined at **A**, 2; **B**, 3; **C**, 5; and **D**, 7 days postinoculation (DPI). **E**, Dark-brown lesions were also observed on spikes. **F**, Spot blotch symptoms of ND B112 induced by isolate ND4008 at 7 DPI are also shown for comparison.

Fig. 2. Infection responses of *Brachypodium distachyon* inbred line Bd21-3 inoculated with *Cochliobolus sativus* isolate Cs07-47-1 at different growth stages. Seedlings of Bd21-3 were spray inoculated with Cs07-47-1 at **A**, 15; **B**, 25; **C**, 32; and **D**, 37 days old after plant emergence. Disease symptoms were recorded and photographed at 7 days postinoculation.

To further examine the infection process of the two fungal isolates (ND85F and ND4008) to which Bd18-1 exhibited differential infection responses, we examined leaf samples of Bd18-1 at three time points after inoculation with each isolate. By 24 HPI, both ND85F and ND4008 formed appressoria and their infection hyphae penetrated into the plant cells (Fig. 6). However, at 72 HPI, the lesions caused by ND85F were much smaller than those caused by ND4008. At 168 HPI, the lesions caused by ND85F were still restricted to the infected sites whereas those lesions induced by ND4008 had expanded, leading to the collapse of leaf cells (Fig. 6).

Screening additional accessions of *Brachypodium* spp. for resistance to four *C. sativus* isolates. To examine variation in *Brachypodium* spp. for infection responses to *C. sativus*, we screened another 153 *B. distachyon* accessions and 37 *B. hybridum* accessions collected from different regions of the world using four *C. sativus* isolates (Cs07-47-1, ND90Pr, ND85F, and ND4008). In general, *B. hybridum* was more resistant to *C. sativus* than *B. distachyon* because all but one *B. hybridum* accession (PI 254868) had a disease reading below 2.0, regardless of the isolate. Among the 153 *B. distachyon* accessions, the degree of susceptibility varied depending on the isolate used. Representative differences in infection responses of *B. distachyon* accessions after inoculation with Cs07-47-1 are presented in Figure 7. In total, 42 and 24 *B. distachyon* accessions were rated as susceptible to ND4008 and Cs07-47-1, respectively, with a disease reading >3.5 . However, only four and one *B. distachyon* accessions were susceptible to ND90Pr and ND85F, respectively, with a disease reading >3.5 .

Inheritance of resistance to *C. sativus* in *B. distachyon*. To investigate the genetics of resistance to *C. sativus* in *B. distachyon*, a population consisting of 74 F_2 individual plants derived from the cross between Bd3-1 and Bd1-1 was evaluated with the isolate Cs07-47-1. These two parental lines (Bd1-1 and Bd3-1) exhibited significant differences in infection response to Cs07-47-1 (Fig. 3). Lesions on infected leaves of Bd1-1 were small and did not expand much as the disease progressed. In contrast, large necrotic lesions with chlorosis developed on infected leaves of Bd3-1 (Fig. 3). Among the Bd3-1 $\times$ Bd1-1 F_2 progeny, disease levels ranged from highly resistant, with pin-sized lesions without chlorosis, to highly susceptible, with large lesions covering the whole leaves. A visual score of the observed necrosis (from 1 to 5 according to the size of necrotic lesions, with 5 = largest lesion) was

recorded on the parents and F_2 seedlings. Bd1-1 and Bd3-1 had a disease score of 2 and 4, respectively, while the disease scores of the F_2 population ranged from 1 to 5 (Fig. 8), suggesting both quantitative and transgressive segregation for resistance to *C. sativus* in this population.

DISCUSSION

Since *B. distachyon* was proposed as a model system for facilitating biological studies of grasses and cereals (11), many areas of research have been explored with this unique grass species (6,17). One such area is the development of *B. distachyon* as a potential model for understanding plant–pathogen interactions. Interactions of *B. distachyon* with several fungal and viral pathogens of cereal crops were previously investigated (1,2,10,11,15,30–32). However, no reports have been published about the interaction between *B. distachyon* and *C. sativus*, the causal agent of spot blotch disease in barley and wheat. Our results in this study clearly demonstrated that *C. sativus*-infected *B. distachyon*, and *B. distachyon* accessions varied in their level of resistance to the pathogen, thus establishing the potential of using this model species to study the interaction of this pathogen with grasses.

C. sativus is a hemibiotrophic fungal pathogen with a biotrophic phase early in the infection process and a necrotrophic phase in the late-infection stage (25) and it infects a wide range of hosts, including barley, wheat, and many grasses. We observed spore germination and appressorium formation on *B. distachyon* leaves between 6 and 24 HPI. This result is in agreement with those observed on barley and wheat (24,43). Yadav (43) showed that spores of *C. sativus* germinated between 8 and 16 HPI and appressoria formed between 12 and 22 HPI onto barley or wheat plants. Kumar et al. (24) completed a microscopic analysis of *C. sativus* development in leaves of the susceptible barley ‘Ingrid’, and found that the biotrophic phase of the fungus occurs within 24 HPI and is characterized by the development of hyphae within the living epidermal host cell invaded by the pathogen, while the

Fig. 3. Infection responses of six *Brachypodium distachyon* lines (Bd1-1, Bd21, Bd21-3, Bd2-3, Bd3-1, and Bd18-1) to *Cochliobolus sativus* isolate Cs07-47-1. The *B. distachyon* plants were spray inoculated at the heading stage, and the disease symptoms were recorded and photographed at 7 days postinoculation.

Fig. 4. Infection responses of *Brachypodium distachyon* line Bd18-1 inoculated with five *Cochliobolus sativus* isolates (ND85F, ND90Pr, ND93-1, Cs07-47-1, and ND4008). The *B. distachyon* plants were spray inoculated at the heading stage and the disease symptoms were recorded and photographed at 7 days postinoculation.

necrotrophic phase happens thereafter, with hyphae invading the mesophyll layer, accompanied by epidermal and mesophyll cell death. We also observed the biotrophic and necrotrophic phases of *C. sativus* on *B. distachyon*, further demonstrating that the fungus is a hemibiotrophic pathogen, which is different from other *Cochliobolus* pathogens such as *C. heterostrophus*, *C. carbonum*, and *C. victoriae*, all of which are necrotrophic pathogens producing host-selective toxins as virulence factors (39,42).

Peraldi et al. (30) showed that the two major *Fusarium* head blight pathogens (*F. graminearum* and *F. culmorum*) preferentially penetrated the cuticle, and grew and branched at the base of the macrohairs of *B. distachyon* leaves. However, in our study, *C. sativus* hyphae were not observed to specifically target hairs in

B. distachyon leaf tissues. Instead, the fungus developed appressoria, which directly penetrated into the epidermal cells of the *B. distachyon* plants just as they do in barley plants (24,25). For other ascomycete fungal pathogens such as *M. oryzae* (the rice blast fungus) and *Oculimacula* spp. and *R. collo-cygni* (the eyespot and Ramularia leaf spot pathogens), infection of *B. distachyon* plants is also not restricted around the macrohairs (31,32). This indicates that not all fungi employ the same mechanisms of penetrating a host plant.

Of the *B. distachyon* lines used for interactions with plant fungal pathogens, variation for susceptibility to different pathotypes or races of a fungus has been reported. For example, Routledge et al. (32) showed that two *B. distachyon* lines, ABR1 and ABR5, reacted

Fig. 5. *Brachypodium distachyon* line Bd21-3 inoculated with *Cochliobolus sativus* isolate Cs07-47-1 at the heading stage, with leaf sections sampled and examined at 6, 12, 24, 48, 72, and 120 h after inoculation (HAI), respectively.

Fig. 6. *Brachypodium distachyon* line Bd18-1 inoculated with *Cochliobolus sativus* isolates ND85F and ND4008 at the heading stage, with representative stained leaf samples examined at 24, 72, and 168 h after inoculation (HAI), respectively. Lesions caused by ND4008 were much larger than those induced by ND85F.

differentially to two different isolates of *M. oryzae*. Peraldi et al. (30) showed that Bd3-1 was more susceptible than Bd21 to foliar and floral development by *F. graminearum* and to the fungal mycotoxin deoxynivalenol. A similar difference was reported between these two *B. distachyon* lines to both *Oculimacula* spp. and *R. collo-cygni* infection (31). We observed differences among *B. distachyon* accessions in their responses to different isolates of *C. sativus* and found that the interactions depend on both the *B. distachyon* genotype and *C. sativus* isolate involved. All of these studies suggest that *B. distachyon* accessions may differ in specific and basal or broad-spectrum resistance to fungal pathogens, and merit further characterization.

We evaluated F₂ progeny from a cross between Bd3-1 and Bd1-1 for responses to inoculation with the isolate Cs07-47-1 and found quantitative and transgressive segregation of the resistance in the population. This result suggests that multiple genes from both parents may contribute to resistance to Cs07-47-1. Barbieri et al. (3) also found that Bd3-1 and Bd1-1 differed in their level of resistance to the rust fungus *P. brachypodii*. Using a new amplified fragment length polymorphism-based *Brachypodium* linkage map consisting of 203 loci and spanning 812 centimorgans, they detected three false brome rust resistance quantitative trait loci (QTL) located on

chromosomes 2, 3, and 4, respectively (3). Similar quantitative genetic analysis can be undertaken for molecular mapping of QTL for spot blotch resistance in *B. distachyon* using the mapping population derived from the Bd3-1/Bd1-1 cross. The results will offer opportunities to begin elucidating the molecular basis of resistance to *C. sativus*.

The genetics of resistance to spot blotch caused by *C. sativus* in barley has been studied by several research groups over many years. Wilcoxson et al. (41) first postulated that one or two genes controlled the adult plant resistance in M33 (a resistant barley line derived from ND B112), after analyzing a cross between M33 and Larker (susceptible to spot blotch). Using a doubled haploid (DH) population from the cross between Steptoe and Morex (a resistant cultivar also derived from NDB112), Steffenson et al. (35) identified a seedling resistance gene (*Rcs5*) on the short arm of chromosome 7H and a major effect QTL conferring adult plant resistance on the long arm of chromosome 1H of Morex. *Rcs5* and the major effect adult resistance QTL (now designated Rcs-qt1-1H-6-7) have been consistently identified using the same or other crosses involving Morex, although additional minor QTL have also been detected (4). In a DH population derived from the cross between Bowman and Calicuchima-sib, a single gene (designated as *Rcs6*) on the short arm of chromosome 1H was found to control the segregation for resistance to ND90Pr, with the resistance allele contributed by Calicuchima-sib and the corresponding susceptibility allele (designated as *SVHv1*) contributed by Bowman (5,44). Resistance to the pathotype 1 isolate ND85F in Bowman is controlled by two major QTL, which are located on chromosomes 3H and 4H (5). However, none of the resistance genes mentioned above have been isolated and functionally characterized at the molecular level. The genetic analysis of spot blotch resistance and the isolation of genes conferring resistance to *C. sativus* in *B. distachyon* will provide valuable insights into potential mechanisms underlying barley-*C. sativus* interactions.

In conclusion, this work provides the first report demonstrating both resistant and susceptible responses of *B. distachyon* to the spot blotch pathogen *C. sativus*. Our results indicated that *C. sativus* infected *B. distachyon* in a manner similar to infection of barley and wheat, the natural hosts, further suggesting that this grass model plant is a powerful tool for studies of plant-pathogen interactions relevant to small-grain cereals. The inbred *B. distachyon* line Bd21

Fig. 7. Different infection responses of representative *Brachypodium distachyon* accessions inoculated with *Cochliobolus sativus* isolate Cs07-47-1 at the heading stage. The disease was evaluated at days postinoculation using a 1-to-5 rating scale, where 1 indicates the most resistant and 5 indicates the most susceptible.

Fig. 8. Frequency distribution of disease ratings in F₂ population from a cross between *Brachypodium distachyon* inbred lines Bd1-1 and Bd3-1. In total, 74 F₂ plants were inoculated with the *Cochliobolus sativus* isolate Cs07-47-1. Disease ratings of the parents are indicated by arrows.

has been sequenced using whole-genome shotgun sequencing (21). The genome assembly consists of 10 large scaffolds containing 99.6% of all sequenced nucleotides (272 Mb). The genome of *B. distachyon* is the simplest grass genome described to date, and contains 31,694 protein-coding gene loci according to the most recent annotation (v2.1) (http://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Bdistachyon) (21), similar to the gene numbers estimated in rice ($n = 28,236$) (22) and sorghum ($n = 27,640$) (29). The genome of *C. sativus* has also been sequenced (9). With these combined resources, it should be possible to develop a deeper understanding of molecular interactions between *B. distachyon* and *C. sativus*, and translate the information gained into improved disease management in barley, wheat, and other cereal crops.

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