Sources and Genetics of Spot Blotch Resistance to a New Pathotype of *Cochliobolus sativus* in the USDA National Small Grains Collection

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Abstract

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Spot blotch, caused by *Cochliobolus sativus*, is one of the important barley diseases in the northern Great Plains of the United States and the Prairie Provinces of Canada. The disease has been under control for almost five decades due to the use of durable spot blotch resistance derived from the barley line ND B112. However, the emergence of isolate ND4008 with virulence on ND B112 prompted us to identify new sources of resistance to this new pathotype. In this study, we screened 2,062 barley accessions from the United States Department of Agriculture National Small Grains Collection for spot blotch resistance, and identified 40 barley accessions exhibiting

a high level of resistance to isolate ND4008 at the seedling stage. In all, 24 of the barley accessions with seedling resistance also exhibited moderate to high adult plant resistance to ND4008 in greenhouse tests. Seven of the ND4008-resistant barley accessions showed seedling resistance to two other pathotypes (1 and 2) of the pathogen. Genetic study of resistant barley accessions PI 235186, PI 592275, and PI 643242 indicated that a single major dominant gene controls spot blotch resistance to ND4008 in each of these three accessions. These resistant sources are useful for developing barley cultivars with spot blotch resistance to all pathotypes of *C. sativus*.

Spot blotch is caused by the ascomycetous fungus *Cochliobolus sativus* (S. Ito & Kurib.) Drechsler ex Dastur with the asexual stage name of *Bipolaris sorokiniana* (Sacc.) Shoemaker. It is one of the major diseases of barley (*Hordeum vulgare* L.) in the Upper Midwest region of the United States and the Canadian Prairie Provinces, where significant losses in grain yield and quality can occur due to epidemics of the disease. In North Dakota, spot blotch causes yield losses ranging from 4 to 20% in six-rowed barley genotypes (Nutter et al. 1985). Field and greenhouse evaluations indicated that the disease caused significantly reduced grain weight in both resistant and susceptible barley cultivars (Anderson and Bantari 1976). In Manitoba, an annual grain yield loss of 5 to 10% is estimated for barley affected by the leaf spot disease complex of net blotch (caused by *Pyrenophora teres*; anamorph: *Drechslera teres*) and spot blotch (Ghazvini and Tekauz 2007). Under conducive environmental conditions, spot blotch can cause as high as 40% yield losses in susceptible barley cultivars (Clark 1979; Mathre 1982).

Deployment of resistant cultivars is the most economical and environmentally sound approach for management of spot blotch (Mathre 1982; Wilcoxson et al. 1990). In the Upper Midwest region of the United States, nearly all six-rowed malting barley cultivars carry spot blotch resistance derived from 'ND B112' ('CIho 11531'), which was selected from the cross 'CIho 7117-77'/'Kindred' (Wilcoxson et al. 1990). The ND B112 resistance has remained effective for almost 50 years (Steffenson et al. 1996; Zhou and Steffenson

2013). However, the same source of resistance has not been reported as effective in two-rowed barley cultivars as in the six-rowed barley cultivars (Bilgic et al. 2005). Furthermore, 'Bowman', a two-rowed barley cultivar with resistance to pathotype 1 (isolate ND85F) of *C. sativus*, is highly susceptible to pathotype 2 (isolate ND90Pr) of the pathogen (Fetch and Steffenson 1994). Recently, new *C. sativus* pathotypes with virulence on ND B112 were identified in Manitoba (Ghazvini and Tekauz 2007) and North Dakota (Gyawali 2010). Isolate ND4008, isolated from a barley root sample in 2008 at Langdon, ND, is one of the new pathotypes with high virulence on ND B112 (Gyawali 2010). These new *C. sativus* pathotypes may pose a potential threat to the barley industry because most six-rowed malting cultivars share spot blotch resistance derived from ND B112. Identification and deployment of new sources of spot blotch resistance are essential to avoid any potential future disease epidemics in this region.

Over 5,000 spring barley accessions from the United States Department of Agriculture–Agricultural Research Service (USDA-ARS) National Small Grains Collection were evaluated for spot blotch resistance to pathotype 1 (ND85F) of *C. sativus* in the field, and 5.8% (373 accessions) were identified as resistant (Fetch et al. 2008). Most of these resistant lines were from North America and possibly carry the resistance from ND B112 due to its widespread use in barley breeding programs in the Upper Midwest region (Bonman et al. 2005; Fetch et al. 2008). Using pathotype 1 isolate ND85F, Fetch et al. (2003) evaluated 116 accessions of the wild barley species *H. vulgare* subsp. *spontaneum* (C. Koch) Thell for reactions to six diseases and found that 53 showed resistance to spot blotch at the seedling stage. However, information on spot blotch resistance to the new pathotype isolate ND4008 has not been reported. The objectives of this study were to identify sources of resistance to isolate ND4008 of *C. sativus* in a USDA barley core collection and to investigate the genetics of resistance in selected barley accessions.

Materials and Methods

Plant materials. A barley core collection consisting of 2,062 accessions was obtained from the USDA National Small Grains Collection in Aberdeen, ID. These barley accessions represent the global genetic diversity of barley cultivars and landraces, and were selected by the Triticeae Coordinated Agricultural Project (T-CAP) for genotyping and multiple trait phenotyping (Muñoz-Amatriáin et al. 2014). The information on geographical origin and row type is listed in Supplementary Table S1. Five barley genotypes (Bowman, 'ND 5883',

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ND B112, 'ND 23329', and 'ND 23345') were also included as controls in the phenotyping experiments. ND 5883, Bowman, and ND B112 are three barley differential lines used for identification of pathotypes of *C. sativus* (Valjavec-Gratian and Steffenson 1997a). ND 23329 and ND 23345 are two barley lines with a moderate level of resistance to isolate ND4008 (Gyawali 2010).

Three barley accessions (PI 235186, PI 592275, and PI 643242) exhibiting a high level of seedling resistance to isolate ND4008 were selected from the barley core collection and used as resistant parents to cross with a susceptible barley accession (PI 356746). The parents, F₁ plants, and three F₂ populations consisting of 124, 151, and 162 progeny, respectively, from the three barley crosses (PI 356746 × PI 235186, PI 356746 × PI 592275, and PI 356746 × PI 643242) were evaluated for resistance or susceptibility to isolate ND4008 of *C. sativus*. The 124 F_{2,3} families (15 to 20 seeds/family) derived from the PI 356746 × PI 235186 cross were further tested for spot blotch reaction to isolate ND4008 at the seedling stage to verify the results obtained in the F₂ generation.

Spot blotch phenotyping at the seedling stage. For spot blotch evaluation at the seedling stage, three to four seeds per experiment were planted for each barley genotype in D40 Deepots (6.4 cm in diameter and 25.4 cm in depth; (Stuewe and Sons, Tangent, OR) filled with potting mix (Pro-Mix LP15; Premier Tech Horticulture, Quakertown, PA). A slow-release fertilizer (Osmocote Plus 15-9-12 N-P-K plus minors; Everris Inc., Dublin, OH) was added after planting at the rate of 1 g/cone. Plants were grown in the greenhouse at 23 ± 2°C with supplemental lighting provided by 600-W high-pressure sodium lamps (P. L. Light Systems Inc., Beamsville, ON Canada) for 14 h/day. After 10 to 12 days, when the second leaves of seedling plants were fully expanded, conidial suspensions were spray inoculated onto the plants using the procedure described by Fetch and Steffenson (1999).

Three *C. sativus* isolates (ND85F, ND90Pr, and ND4008) were utilized in this study. ND85F (pathotype 1) and ND90Pr (pathotype 2) (Valjavec-Gratian and Steffenson 1997a; Zhong and Steffenson 2001) are routinely used in spot blotch resistance screening at North Dakota State University. Isolate ND4008 was isolated from a barley root sample collected in 2008 at Langdon, ND, and exhibited high virulence on ND 5883, Bowman, and ND B112 (Gyawali 2010). This isolate was previously described as pathotype 3 (Zhong et al. 2015) but should be designated as pathotype 7 according to the triple-coded naming system (Limpert and Müller 1994; Valjavec-Gratian and Steffenson 1997a). To produce as many conidia as possible, each isolate was grown on minimal medium (Tinline et al. 1960) or V8 potato dextrose agar (150 ml of V8 juice, 10 g of BD Difco potato dextrose agar, 3 g of CaCO₃, 10 g of agar, and 850 ml of distilled water), and incubated under 14 h of fluorescent light each day at approximately 23°C for 7 days. Preparation of conidial suspension, measurement of spore concentration, spray inoculation, and assessment of infection responses (IR) were conducted by following the methods described by Fetch and Steffenson (1999). At least three inoculation experiments were conducted for all barley accessions. Range of IR (minimum and maximum) and the most common IR (mode of IR) were recorded for each barley accession. Based on the 1-to-9 rating scale of Fetch and Steffenson (1999), each genotype was classified as a low (with mode IR 1 to 3), intermediate (with mode IR 4 to 5), or high (with mode IR 6 to 9) IR.

Spot blotch phenotyping at the adult plant stage. For spot blotch phenotyping at the adult plant stage, selected barley accessions and controls (Bowman, ND 5883, and ND B112) were evaluated in the greenhouse using a completely randomized design with three replicates. Three to four seeds per replicate were planted for each barley line in clay pots (12 cm in diameter and 12 cm in depth) containing potting mix (Pro-Mix LP15; Premier Tech Horticulture). A slow-release fertilizer (Osmocote Plus 15-9-12 N-P-K plus minors; Everris Inc.) was added after planting at the rate of 3 g/pot. When the flag leaves of the plants were fully expanded, the plants were spray inoculated with a conidial suspension (1,500 conidia/ml) of isolate ND4008 at the rate of 3 ml/plant using the inoculation procedure described by Fetch and Steffenson (1999). Each barley accession was evaluated by performing

experiments twice and classified as resistant (R), moderately resistant (MR), moderately susceptible (MS), or susceptible (S) IR based on symptoms of the upper three leaves of plants 6 to 8 days after inoculation according to the descriptions of Fetch and Steffenson (1999).

Single-nucleotide polymorphism genotyping data. Single-nucleotide polymorphism (SNP) genotyping data for the barley accessions were previously described in Muñoz-Amatriáin et al. (2014). The final datasets are deposited in the Triticeae Toolbox database (<https://triticeaetoolbox.org/>). In total, 6,915 SNP markers were extracted from the database for the selected barley accessions but only 4,968 of the markers were used in our statistical analysis because they are located on the iSelect consensus genetic map developed by Muñoz-Amatriáin et al. (2014).

Statistical analysis. Using the SNP genotyping data, the familial relatedness among barley accessions with seedling resistance to isolate ND4008 were calculated using the method of identity by state in JMP Genomics statistical suite (v6.0; SAS Institute, Cary, NC). Based on the relatedness information, cluster analyses were conducted in JMP Genomics using the Fast Ward method. The cubic clustering criteria were used to determine the number of clusters. The χ^2 goodness-of-fit test was conducted in RStudio (v0.98.1091; RStudio, Inc., Boston) to compare the observed and expected frequencies of S and R F₂ individuals and F₃ families from the three barley crosses.

Results

IR to isolate ND4008 at the seedling stage. Isolate ND4008 caused typical infections (necrotic lesions with or without chlorosis) on barley accessions across the experiments, allowing for the clear and unambiguous classification of IR. The two intermediate IR controls, ND 23329 and ND 23345, exhibited IR modes of 4, with ranges of 4 to 5 and 3 to 6, respectively, whereas the S controls, ND 5883, Bowman, and ND B112, exhibited high IR modes of 6, with ranges of 6 to 8, 5 to 7, and 5 to 8, respectively. Of the 2,062 barley accessions evaluated, 40 (1.9%) were rated as R, with IR ranging from 2 to 3 (Table 1). In total, 407 (19.7%) accessions showed intermediate reactions, with IR ranging from 4 to 5, while the remaining 1,615 accessions (78.3%) were S, with IR ranging from 6 to 8 (Fig. 1).

IR to isolate ND4008 at the adult plant stage. To compare disease reactions at the seedling and adult plant stages, 40, 4, and 19 barley accessions, which showed R (IR of 2 to 3), intermediate (IR of 4), and S (IR of 6 to 8) reactions to isolate ND4008, respectively, at the seedling stage, were selected for evaluation at the adult plant stage in the greenhouse. Of the 44 accessions with intermediate to R reactions to isolate ND4008, 24 showed IR of MR to R, and the remainder were rated as MS or S at the adult plant stage. In contrast, the 19 barley accessions with susceptibility at the seedling stage showed IR of S to MS at the adult plant stage (Table 1; Fig. 2). Most of the 63 evaluated barley accessions exhibited normal spot blotch symptoms with regular lesion types but two of them (PI 157890 and PI 372084) had large, irregular, and diffuse brown necrotic lesions (Fig. 2), as reported by Fetch and Steffenson (1999).

IR to pathotypes 1 and 2 of *C. sativus* at the seedling stage. To determine the resistance spectrum of the ND4008-resistant accessions, *C. sativus* pathotypes 1 and 2, represented by isolates ND85F and ND90Pr, respectively, were also used for inoculation at the seedling stage. Of the 76 barley accessions selected for evaluation, 14 (18.4%) exhibited a high level of resistance (mode IR < 4) and 40 (52.6%) were S (mode IR > 5) to isolate ND85F at the seedling stage (Table 1). Of the 76 tested accessions, 22 (28.9%) exhibited intermediate reactions (mode IR of 4 to 5) to this isolate. For isolate ND90Pr, only 13 accessions were S, and the remainder showed R reactions. Seven barley accessions consisting of two two-rowed barley lines and five six-rowed barley lines exhibited seedling resistance to all three isolates (Table 1).

Genetic diversity analysis of barley accessions with seedling resistance to isolate ND4008. Of the 40 barley accessions with seedling resistance to isolate ND4008, 36 were genotyped with SNP markers by T-CAP and, thus, the genotype data were used for phylogenetic analysis. The result showed that the 36 barley accessions formed five clusters (Fig. 3). Most of the clusters corresponded with the barley row types or geographical origins (Supplementary Table

S2). For example, all five two-rowed barley accessions from North Dakota were classified into one cluster (C2) and the one six-rowed barley accession (PI 643212) from North Dakota was classified into another cluster (C4) containing nine six-rowed barley accessions from North America and East Asia. Furthermore, all six barley accessions from Mediterranean countries with six-rowed type were classified into the same cluster (C5) (Fig. 3).

Genetics of seedling resistance to isolate ND4008 in three barley accessions. F₁ plants and F₂ populations from the three barley crosses (PI 235186 × PI 356746, PI 592275 × PI 356746, and PI 643242 × PI 356746) were evaluated with isolate ND4008 at the seedling stage. The mode IR (range) observed on four F₁ plants from PI 235186 × PI 356746, PI 592275 × PI 356746, and PI 643242 × PI 356746 were 3 (2 to 4), 3 (2 to 5), and 3 (2 to 4), respectively, which were similar to those of their respective R parents (PI 235186, PI 592275, and PI 643242) but significantly lower than that of the S parent PI 356746 (Table 1). This result suggests that dominant genes control the resistance. A significant fit to a 3:1 ratio of R/S plants was observed in all three F₂ populations (Table 2), indicating the segregation of a single dominant resistance gene in each cross. In all,

132 F_{2,3} families from the PI 356746 × PI 235186 cross were further evaluated for spot blotch reaction at the seedling stage. The number of homozygous resistant, segregating, and homozygous susceptible F_{2,3} families fit a 1:2:1 ratio (Table 2), thereby confirming the results of the F₂ generation that a single dominant gene controls spot blotch resistance in PI 235186.

Discussion

The genetic uniformity of spot blotch resistance in two- and six-rowed barley cultivars (Zhou and Steffenson 2013) and the recent reports of *C. sativus* isolates carrying virulence on ND B112 (Ghazvini and Tekauz 2007; Gyawali 2010) make barley vulnerable to spot blotch epidemics. Identification of new sources of spot blotch resistance are essential to combat the disease with host resistance. In this study, we evaluated 2,062 barley accessions for their reactions to the new virulent isolate ND4008 of *C. sativus* at the seedling stage in the greenhouse and identified 40 with resistance. Although the frequency of R barley accessions was low (1.9%) among the large collection, they were from diverse geographic origins—North Africa (Ethiopia), northern Europe (Belgium), southern Europe (Italy), East Asia

Table 1. Infection responses (IR) of selected barley accessions to different *Cochliobolus sativus* isolates at the seedling and adult plant stages in the greenhouse studies

Lines	Origin	Row ^c	Accession	IR at the seedling stage ^a			IR adult ^b
				ND4008	ND85F	ND90Pr	ND4008
PI 190790	North Korea	6	Landrace	2 (2–3)	5 (5–6)	1 (1–2)	MR
PI 294743	Romania	2	Cultivar	2 (2–3)	6 (6–7)	2 (2–2)	R
PI 532035	Egypt	6	Cultivar	2 (2–3)	6 (6–7)	2 (2–2)	R
PI 640006	United States	6	Breeding	2 (2–3)	7 (7–7)	1 (1–2)	MR
PI 235181	Cyprus	6	Cultivar	2 (2–4)	5 (5–6)	1 (1–2)	R
PI 328619	Romania	2	Core line	2 (2–4)	6 (6–7)	2 (2–2)	R
PI 592172	United Kingdom	2	Cultivar	2 (2–4)	7 (7–8)	3 (3–4)	MR
PI 643271	United States	2	Breeding	2 (2–4)	6 (6–7)	2 (2–2)	MS
CIho 1457	Iraq	6	Core line	3 (2–4)	5 (4–5)	3 (2–4)	...
CIho 4188	Afghanistan	6	Landrace	3 (2–4)	3 (2–4)	2 (2–3)	S
PI 452324	United Kingdom	6	Breeding	3 (2–4)	6 (6–7)	3 (2–4)	R
PI 643294	United States	2	Breeding	3 (2–4)	2 (2–3)	2 (2–3)	MS
CIho 15270	Canada	6	Breeding	3 (2–5)	3 (3–4)	2 (2–2)	R
CIho 6091	Canada	6	Breeding	3 (2–5)	6 (6–7)	...	MS to S
CIho 6880	United States	2	Genetic	3 (2–5)	...	...	...
CIho 7153	United States	6	Breeding	3 (2–5)	4 (4–5)	2 (2–3)	MS
PI 157890	Italy	6	Cultivar	3 (2–5)	5 (5–6)	1 (1–2)	Irregular
PI 190786	North Korea	6	Landrace	3 (2–5)	6 (6–6)	1 (1–2)	S
PI 357314	Denmark	2	Breeding	3 (2–5)	7 (7–8)	3 (3–3)	R
PI 531890	Egypt	6	Cultivar	3 (2–5)	7 (7–8)	1 (1–2)	R
PI 592275	United Kingdom	2	Cultivar	3 (2–5)	3 (3–5)	8 (8–8)	MR to MS
PI 643212	United States	6	Breeding	3 (2–5)	1 (1–3)	2 (2–2)	MS
CIho 5068	Canada	6	Breeding	3 (2–6)	8 (8–8)	3 (3–3)	MS
PI 235186	Libya	6	Landrace	3 (2–6)	3 (3–5)	2 (2–2)	R
PI 371245	Switzerland	2	Landrace	3 (2–6)	6 (6–6)	2 (2–3)	MS
PI 53239	Libya	6	Core line	3 (2–6)	4 (4–5)	1 (1–2)	MS
PI 531984	Egypt	6	Cultivar	3 (3–3)	4 (4–6)	2 (2–2)	R
PI 532000	Egypt	6	Cultivar	3 (3–4)	6 (5–7)	...	R
PI 643242	United States	2	Breeding	3 (3–4)	2 (2–4)	8 (8–8)	MR
CIho 2480	Venezuela	6	Core line	3 (3–5)	4 (4–5)	3 (3–4)	MR
PI 268241	Iran	2	Landrace	3 (3–5)	7 (7–8)	8 (8–8)	R
PI 328808	Czechoslovakia	2	Cultivar	3 (3–5)	7 (6–8)	2 (2–3)	MR to MS
PI 392469	South Africa	2	Breeding	3 (3–5)	8 (8–9)	2 (2–3)	R
PI 434795	Canada	6	Breeding	3 (3–5)	1 (1–2)	1 (1–1)	MS
PI 592282	United Kingdom	2	Cultivar	3 (3–5)	4 (4–5)	2 (2–3)	MR

(continued on next page)

^a IR at the seedling stage were rated based on the symptoms of the second leaves at 7 days after inoculation using the 1-to-9 rating scale of Fetch and Steffenson (1999). IR 1 represents the most resistant reaction while IR 9 represents the most susceptible reaction. Mode (the most common infection response) and range (the lowest and highest infection responses) are shown for each barley accession; ... indicates data are not available.

^b IR at the adult plant stage. IR at the adult plant stage were assessed on the upper three leaves of plants 6 to 8 days after inoculation following the rating scale of Fetch and Steffenson (1999). R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible, and Irregular = IR with large, dark-brown necrotic lesions of diffusive pattern.

^c Row type: six- or two-rowed barley.

(Japan), South Asia (India), Central Asia (Afghanistan), West Asia (Turkey), and North America (Canada and United States)—and belong to five different clusters based on phylogenetic analysis. These R barley accessions may represent distinct sources of spot blotch resistance but further molecular mapping studies and allelism tests are required to determine the chromosomal locations of the resistance genes and the relationship between them.

In this study, we evaluated the IR of selected barley accessions to *C. sativus* isolate ND4008 at the adult plant stages and found that most of the barley accessions with seedling resistance also exhibited adult plant resistance. This result indicates that the resistance alleles effective at the seedling stage may also function at the adult plant stage or the accessions may carry additional alleles that confer resistance in adult plants. Previous studies also showed that some genes confer resistance to spot blotch at both seedling and adult plant stages while others function only at a particular plant stage (seedling or adult plant stage) (Bilgic et al. 2005, 2006; Bovill et al. 2010; Steffenson et al. 1996). A similar situation has been observed in resistance of barley to the net blotch pathogen (Lehmannsiek et al. 2007). Adult plant resistance is essential for barley production in North America because the risk of spot blotch epidemics is high after anthesis, and yield losses increase significantly when the top three leaves are seriously damaged

by the disease (Nutter et al. 1985). Therefore, barley accessions with adult plant resistance are particularly useful sources for barley breeding programs.

Of the 40 barley accessions with seedling resistance to isolate ND4008, 7 (CIho 4188, CIho 15270, PI 235186, PI 434795, PI 643212, PI 643283, and PI 643294) also showed seedling resistance to pathotype 1 (isolate ND85F) and pathotype 2 (isolate ND90Pr) of *C. sativus*. Two of them (CIho 15270 and PI 235186) were R to isolate ND4008 at the adult plant stage. These accessions originated from four countries, including Afghanistan (CIho 4188), Canada (CIho 15270 and PI 434795), Libya (PI 235186), and the United States (PI 643212, PI 643283, and PI 643294). Cluster analysis with SNP markers indicated that these seven accessions were in four different clusters, with CIho 4188 and CIho 15270 in C3, PI 434795 and PI 643212 in C4, PI 643283 and PI 643294 in C2, and PI 235186 in C5. The barley accessions in the same cluster are genetically more closely related to each other and may share the same source of resistance, whereas those in different clusters may represent different resistance sources. These R barley lines should be useful for developing new cultivars with diverse resistance to spot blotch.

C. sativus is a fungal pathogen that can infect both leaves and roots of barley and cause spot blotch and common root rot, respectively

Table 1. (continued from preceding page)

Lines	Origin	Row ^c	Accession	IR at the seedling stage ^a			IR adult ^b
				ND4008	ND85F	ND90Pr	ND4008
PI 643283	United States	2	Breeding	3 (3–5)	1 (1–2)	1 (1–2)	MS
PI 643370	United States	2	Breeding	3 (3–5)	7 (7–8)	3 (3–4)	S
PI 70839	China	6	Landrace	3 (3–5)	6 (6–7)	1 (1–2)	MS
PI 87836	North Korea	6	Landrace	3 (3–5)	7 (7–8)	2 (2–3)	R
CIho 1388	Denmark	2	Cultivar	3 (3–6)	7 (7–8)	4 (4–4)	S
PI 531908	Egypt	6	Cultivar	4 (2–6)	7 (7–8)	2 (2–3)	MR
CIho 2543	Italy	6	Breeding	4 (3–5)	7 (7–8)	2 (2–3)	MR
PI 429995	India	6	Landrace	4 (3–5)	4 (4–5)	8 (8–8)	...
PI 599649	Czech Republic	2	Cultivar	4 (3–5)	2 (2–3)	2 (2–3)	MR
CIho 7247	United States	6	Breeding	6 (4–8)	3 (3–4)	3 (3–3)	MS
CIho 1688	Sudan	6	Core line	6 (5–9)	4 (4–5)	4 (4–5)	MS
PI 372084	Estonia	2	Cultivar	6 (6–8)	6 (6–7)	3 (3–4)	Irregular
PI 168904	Argentina	2	Cultivar	7 (6–8)	7 (6–7)	7 (6–8)	S
PI 356741	Morocco	6	Landrace	7 (6–8)	6 (6–8)	1 (1–2)	S
CIho 15282	Tunisia	6	Landrace	7 (7–9)	8 (7–8)	4 (4–4)	S
CIho 11829	Germany	6	Cultivar	8 (7–9)	7 (6–9)	8 (8–8)	S
CIho 13797	Canada	2	Cultivar	8 (7–9)	4 (3–5)	3 (3–4)	S
CIho 14055	Algeria	6	Landrace	8 (7–9)	7 (7–9)	3 (2–4)	...
CIho 14216	Mongolia	6	Landrace	8 (7–9)	3 (3–4)	8 (8–8)	MS
CIho 14255	Afghanistan	6	Landrace	8 (7–9)	4 (3–5)	2 (2–3)	...
CIho 5023	India	6	Landrace	8 (7–9)	4 (3–5)	3 (3–4)	...
CIho 5024	India	6	Landrace	8 (7–9)	6 (6–8)	6 (6–6)	...
PI 165883	India	6	Landrace	8 (7–9)	4 (4–5)	2 (2–2)	...
PI 172506	Turkey	...	Core line	8 (7–9)	3 (3–5)	3 (3–3)	S
PI 173518	Turkey	6	Landrace	8 (7–9)	5 (4–7)	2 (2–3)	...
PI 223509	Afghanistan	6	Landrace	8 (7–9)	6 (6–7)	3 (3–5)	...
PI 250775	Afghanistan	6	Landrace	8 (7–9)	8 (8–9)	5 (4–6)	S
PI 283413	Czechoslovakia	2	Cultivar	8 (7–9)	5 (4–7)	3 (3–4)	MS to S
PI 290306	Hungary	6	Cultivar	8 (7–9)	3 (2–5)	2 (2–2)	...
PI 429594	Nepal	6	Landrace	8 (7–9)	7 (5–8)	7 (7–7)	...
PI 95175	Unknown	2	Landrace	8 (7–9)	7 (6–8)	3 (3–4)	S
PI 356742	Morocco	6	Landrace	8 (8–8)	6 (5–6)	2 (2–2)	S
PI 356746	Morocco	6	Landrace	8 (8–8)	7 (7–9)	1 (1–2)	S
PI 392458	South Africa	2	Breeding	8 (8–8)	7 (6–8)	7 (7–7)	S
PI 405792	Macedonia	6	Landrace	8 (8–8)	6 (6–7)	3 (3–4)	...
PI 573985	Nepal	6	Landrace	8 (8–8)	7 (6–8)	8 (8–8)	S
PI 640028	United States	6	Breeding	8 (8–8)	5 (5–7)	6 (6–7)	...
PI 640069	United States	6	Breeding	8 (8–8)	5 (4–6)	2 (2–3)	...
PI 164385	India	6	Landrace	8 (8–9)	4 (4–5)	3 (3–4)	S
PI 290316	Hungary	6	Cultivar	8 (8–9)	7 (7–8)	2 (2–2)	...
PI 403984	Nepal	6	Breeding	8 (8–9)	4 (4–5)	6 (6–7)	...
PI 429533	Nepal	6	Landrace	8 (8–9)	4 (3–6)	3 (3–3)	...

Fig. 1. Percentages of barley accessions with different infection responses (IR) to *Cochliobolus sativus* isolate ND4008. In total, 2,062 barley accessions were evaluated at the seedling stage. IR was assessed by using the 1-to-9 rating scale of Fetch and Steffenson (1999). IR 1 represents the most resistant and IR 9 represents the most susceptible.

Fig. 2. Infection responses of representative barley accessions to *Cochliobolus sativus* isolate ND4008 at the adult plant stage.

Fig. 3. Heat map of 36 barley accessions with seedling resistance to *Cochliobolus sativus* isolate ND4008. Symmetric off-diagonal elements represent relationship measures for pairs of barley accessions. Different colors indicate different identity by state (IBS) values, as shown at the upper right corner. The dark red diagonals (IBS = 1) stand for the perfect relationship of each barley accession with itself. Clusters (C1 to C5) are shown at the left of the heat map.

(Mathre 1982). Previous studies indicate that *C. sativus* isolates originating from leaves are generally more virulent in causing spot blotch on leaves than those originating from roots, whereas isolates originating from roots were generally more virulent in causing common root rot than those originating from leaves (Gyawali 2010; Gyawali et al. 2012; Knight et al. 2010). However, some *C. sativus* isolates recovered from either leaves or roots were able to cause serious diseases on both plant tissues (Gyawali 2010). These studies indicate that both specialists (isolates with high virulence on either leaves or roots) and generalists (isolates with high virulence on both plant tissues) exist in this fungal population. Isolate ND4008 is one of the generalists with high virulence in causing both spot blotch and common root rot (Gyawali 2010; Gyawali et al. 2012; this study). It would be interesting to know whether those barley accessions with spot blotch resistance to isolate ND4008 also exhibit resistance to common root rot; however, further investigation is needed.

Our study with the three crosses (PI 235186 $\times$ PI 356746, PI 592275 $\times$ PI 356746, and PI 643242 $\times$ PI 356746) showed that all F_1 plants were R, indicating that the genes controlling the resistance are dominant. This result was confirmed by the genetic analysis of segregation in F_2 or F_3 progenies from the crosses. However, in several previous genetic studies of spot blotch resistance, the resistance alleles were found to be recessive or partially recessive (Valjavec-Gratian 1996; Valjavec-Gratian and Steffenson 1997b). For example, Valjavec-Gratian and Steffenson (1997b) evaluated eight F_1 seedlings from the Bowman $\times$ ND 5883 cross for their IR to the pathotype 2 isolate ND90Pr and showed that the IR of F_1 progeny were similar to those exhibited by the S parent Bowman. Analysis of F_2 progenies and F_3 families further indicated that a single recessive gene governs resistance to isolate ND90Pr in ND 5883. Using the same cross of Bowman and ND 5883, Valjavec-Gratian (1996) showed that F_1 plants were also S to the pathotype 1 isolate ND85F, like the S parent ND 5883, indicating that resistance to ND85F is controlled by a recessive gene allele in Bowman. These results suggest that the genetic mechanism for resistance to ND4008 is probably different from those for resistance to ND90Pr and ND85F. Further studies are needed to map and clone the susceptibility and resistance genes with the ultimate goal of deciphering their functions at a molecular level.

Table 2. Segregation for seedling reactions to *Cochliobolus sativus* isolate ND4008 in F₂ and F₃ populations from barley crosses between the resistant lines (PI 235186, PI 592275, and PI 643242) and the susceptible accession (PI 356746)

Cross	Segregation ratio ^a	Expected ratio	χ^2	Probability value
F ₂ plants				
PI 356746 × PI 235186	94:30 (R:S)	3:1	0.043	0.84
PI 356746 × PI 592275	124:38 (R:S)	3:1	0.206	0.65
PI 356746 × PI 643242	114:37 (R:S)	3:1	0.020	0.89
F _{2,3} families				
PI 356746 × PI 235186	32:62:30 (HR:SEG:HS)	1:2:1	0.065	0.97

^a R = resistant plants exhibiting low infection responses similar to those of their respective resistant parents, S = susceptible plants exhibiting high infection responses similar to the susceptible parent PI 356746, HR = homozygous resistant, SEG = segregating, and HS = homozygous susceptible.

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