# Development of Barley Resistant to Spot Blotch and Genetics of Resistance

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### ABSTRACT

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Cree, Manker, Morex, and Robust are barley (Hordeum vulgare) cultivars resistant to spot blotch (caused by Bipolaris sorokiniana) that have been released by the University of Minnesota. The latter two cultivars are planted in about 95% of the barley-producing area in Minnesota and are widely grown in other areas. The resistance of these four cultivars and of many advanced breeding lines was derived from CI 7117-77 through ND B-112 and Dickson, which were developed at North Dakota State University. This resistance has been stable since its discovery in the mid-1950s. Other germ plasm resistant to B. sorokiniana has been developed from Wisc 691-1, CI 1227, CI 6311, CI 9584, and H. agriocrithon, sources that differ from ND B-112. In an inheritance study, resistance from ND B-112 and that from the other sources appeared to be conditioned by one or two genes. Some progenies of Minn 33 (derived from ND B-112) crossed with resistant lines that trace to CI 1227 and CI 6311 were moderately susceptible, indicating that different resistance genes were involved.

Spot blotch of barley (Hordeum vulgare L.), caused by Bipolaris sorokiniana (Sacc.) Shoemaker, has been an important disease in the Upper Midwest of the United States for many years (9). However, during the past decade the disease has been controlled by the use of resistant cultivars such as Glenn (5) from North Dakota State University and Morex (19) and Robust (20) from the University of Minnesota.

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The University of Minnesota program of breeding barley cultivars that are resistant to spot blotch has been ongoing since the 1950s. During this time many lines and cultivars have been examined in naturally occurring spot blotch epidemics at Crookston and St. Paul, MN, but some epidemics in St. Paul were supplemented by spraying plants with water suspensions of conidia of *B. soro-kiniana* or by scattering autoclaved cereal grains colonized by the pathogen among plants growing in the field (21).

In about 1958 the Minnesota project selected the line ND B-112 as a superior source of resistance to spot blotch. About 10 yr later, the cultivar Dickson was also designated as a resistance source because it has the ND B-112 resistance and is

adapted to conditions in Minnesota (16). These sources were crossed with agronomically superior cultivars and advanced breeding lines. The resulting breeding populations were developed by a modified single seed descent procedure with selection for resistance to spot blotch in the  $F_2$  and  $F_5$  generations as well as in the  $F_6$ - $F_9$  generations when lines were in replicated yield nurseries.

Additional sources of resistance were identified in Wisc 691-1 (4); CI 1227, CI 6311, and CI 9584 (2); and H. agriocrithon Aberg (13). These additional sources probably are not genetically related to ND B-112, and with the exception of Wisc 691-1, they are poor agronomic types. Wisc 691-1 was crossed with Swan, a Canadian barley well adapted to Minnesota, and the other sources were crossed with Larker or Trophy, the principal malting cultivars of the time. The resulting populations were screened for resistance to spot blotch at Crookston and St. Paul, and in each case one resistant line was identified as a germ plasm source.

The objectives of this study were to compare the resistance to spot blotch of barley cultivars and lines that have been developed at the University of Minnesota and to report on the genetics of resistance conditioned by genes from ND B-112 and from other sources.

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#### MATERIALS AND METHODS

Evaluation of resistance in cultivars and lines. Barley cultivars and lines developed at the University of Minnesota (Table 1) were reevaluated between 1980 and 1986 for resistance to spot blotch in artificial epidemics in St. Paul. Each year, the barleys were planted as single rows in a randomized complete block with four replications.

Genetics of resistance. The inheritance of resistance to spot blotch was studied in lines Minn 33, Minn 65-241, Minn 65-243, Minn 65-244, and Minn 7. The latter four lines were derived from CI 6311, CI 1227, CI 9584, and Wisc 691-1, respectively (Table 1). Minn 33 possesses the ND B-112 resistance and is an agronomically elite breeding line with a pedigree similar to that of Robust (Fig. 1).

Each of these five lines was crossed with spot blotch-susceptible cultivar Larker to estimate the number of genes controlling resistance.  $F_3$  families (50-75 plants per family) of each cross, derived from single  $F_2$  plants without selection, were evaluated for spot blotch resistance

in the field in 1985. F<sub>3</sub> families of each cross were classified as resistant, segregating, or susceptible, and goodness-of-fit to genetic ratios was tested using the chi-squared statistic modified by the Yates correction for continuity (10).

The reactions of some  $F_3$  families of each cross in each resistance category were reevaluated in the  $F_4$  generation in the field in 1986. Head rows were planted from five single heads randomly chosen from each  $F_3$  family. The disease reaction of these randomly chosen  $F_3$  plants was not noted. The  $F_4$  plants were inoculated with *B. sorokiniana*, irrigated, and evaluated for spot blotch.

To develop lines that combined resistance from different sources and to test whether resistances were conferred by different genes, three resistant  $\times$  resistant crosses were made: Minn 65-241  $\times$  Minn 33, Minn 65-243  $\times$  Minn 33, and Minn 65-244  $\times$  Minn 33. In 1979, about 250 plants of  $F_2$  populations of each cross were evaluated for resistance to spot blotch in a natural epidemic at Crookston. In 1980, 40  $F_5$  lines of each cross

Table 1. Spot blotch reactions of barley cultivars that derive their resistance from line ND B-112 and of germ plasm lines that derive their resistance from other sources

Cultivar	Resistance source	Spot blotch reaction <sup>a</sup>				
		1980	1981	1983	1985	1986
Larker <sup>b</sup>	None	S	S	S	S	S
Minnesota cultiva	ars					
Cree	ND B-112	MR	MR	MR	MR	MR
Manker	ND B-112	R	R	R	R	R
Morex	ND B-112	MR	MR	MR	MR	MR
Robust	ND B-112	R-MR	R-MR	R-MR	R-MR	R-MR
Minn 33	ND B-112	R	R	R	MR	R
Minnesota germ p	plasm <sup>c</sup>					
Minn 65-244	CI 9584	R	R	R-MR	R-MR	R-MR
Minn 65-243	CI 1227	MR	MR	MR	R-MR	R-MR
Minn 65-241	CI 6311	MR	MS	MS	MR-MS	R-MR
Minn 7	Wisc 691-1	R	R	R	R-MR	R-MR
Minn 61-16	Hordeum agriocrithon	R	R	MR	R	R-MR
Minn 65-195	H. agriocrithon	R	R	MR	R-MR	R-MR

 $<sup>^{</sup>a}$  S = susceptible, MS = moderately susceptible, MR = moderately resistant, R = resistant.

<sup>&</sup>lt;sup>c</sup> Pedigrees given in text.

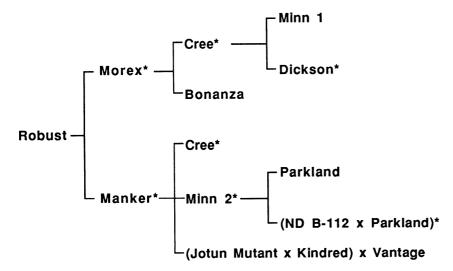


Fig. 1. Pedigree of Robust barley; the resistant parents are indicated by asterisks.

were inoculated and evaluated for spot blotch resistance in the spot blotch nursery at St. Paul. The F<sub>5</sub> lines were developed by single seed descent from randomly selected F<sub>2</sub> plants. In 1981, 20 F<sub>6</sub> lines from each cross that represented the spot blotch reactions observed in the F<sub>5</sub> populations were evaluated in the nursery at St. Paul.

**Spot blotch evaluations.** Plants were inoculated with *B. sorokiniana* on alternate evenings for 2 wk beginning when about 50% were headed. Each morning after inoculation, plants were watered with overhead sprinklers for 1–2 hr. Irrigation was continued every evening for 2 wk following the inoculation period.

To produce inoculum of B. sorokiniana, a mixture of 40 single-spore cultures collected at various locations in Minnesota was grown on a medium of perlite, cornmeal, and potato-dextrose agar (12). When the pathogen was sporulating profusely, cultures were airdried and stored at 4 C until used. Inoculum was applied as a conidial suspension in tap water (5  $\times$  10<sup>5</sup> to 1  $\times$  10<sup>6</sup> conidia per milliliter) at 0.0058 MPa (40 psi) from a 60-L tank mounted on a garden tractor. Tween 20 was used as a wetting agent at 0.4 ml per liter of conidial suspension. Inoculum was applied at the rate of approximately 400 ml per meter of plant row.

Spot blotch reactions were evaluated when plants were in the hard-dough stage of development just before senescence: R = resistant (lesions absent or small and without chlorosis); MR = moderately resistant (lesions small but with some chlorosis around most lesions); MS = moderately susceptible (lesions large with extensive chlorosis but little or no coalescence); and S = susceptible (lesions large and coalescing, with severe chlorosis). Spot blotch reactions were evaluated in the field because disease reactions of cultivars in glasshouses have not corresponded to reactions observed in the field (14).

### RESULTS

Evaluation of cultivars and lines. Cultivars Cree, Manker, Morex, and Robust were consistently more resistant to spot blotch than Larker, the susceptible cultivar that was widely grown until 1980 (Table 1). Cree, Manker, Morex, and Robust were resistant or moderately resistant. Minn 33, an agronomically superior breeding line with a pedigree similar to that of Robust (Fig. 1), was also resistant and is representative of many breeding lines that derive their resistance from ND B-112. Resistance sources Minn 7, Minn 65-244, Minn 61-16, and Minn 65-195 were resistant to moderately resistant and were comparable in resistance to Minn 33 (Table 1). Line Minn 65-243 was usually rated moderately resistant, but it appeared to

<sup>&</sup>lt;sup>b</sup> Highly susceptible to spot blotch and widely grown in Minnesota until replaced by Morex.

be somewhat less resistant than Minn 33. Line Minn 65-241 possessed a lower level of resistance than the other lines. It was moderately susceptible in two trials, moderately resistant to moderately susceptible in one trial, and moderately resistant in two trials (Table 1).

Genetics of resistance. F<sub>3</sub> families from crosses between resistant and susceptible parents were classified as resistant (class obtained by combining resistant and moderately resistant classes), segregating, or susceptible (class obtained by combining moderately susceptible and susceptible classes) to spot blotch. The segregating class was a mixture of all the classes.

The frequency of F<sub>3</sub> families of each cross deviated significantly from the 1:2:1 ratio expected for the segregation of a single gene, apparently because an excess number of families were classified as segregating. This difficulty arose because of overlapping of the disease reaction classes, especially with the intermediate classes and to some extent with the resistant and moderately resistant classes.

Because of the difficulty in distinguishing between resistant and segregating families, these classes were combined (Table 2). A satisfactory fit to a 3:1 ratio was observed in three crosses. However, in Minn 7 × Larker and Larker X Minn 65-243, the fit to a one-gene model was not good. Although the data in these two crosses are consistent with the 13:3 ratio of resistant to susceptible, the difficulties in classifying disease reactions make the two-gene model unlikely. Accordingly, the number of genes controlling resistance in these two crosses could not be specified. Nevertheless, the recovery of several resistant and susceptible families indicated that resistance is probably controlled by no more than one or two major genes. The presence of modifying genes with minor effects could neither be ruled out nor confirmed in any of the five crosses.

When families of crosses between the resistant parents and Larker were classified in the F<sub>3</sub> generation one year and in the F<sub>4</sub> generation the next year, there was, in general, good agreement between generations (Table 3). In a few cases, families of the two generations were not classified alike. This lack of agreement may have been the result of the limited sample of each F<sub>3</sub> family as well as the difficulty in classifying spot blotch reactions on segregating families.

In crosses between resistant and resistant parents (Minn 65-241  $\times$  Minn 33, Minn 65-243  $\times$  Minn 33, and Minn 65-244  $\times$  Minn 33), all  $F_2$  plants were classified as resistant or moderately resistant. However, in two of the three crosses, a few  $F_5$  and  $F_6$  lines were classified as moderately susceptible (Table 4). The absence of susceptible lines precludes a definite statement, but we hypothesize that Minn 65-241 and

Minn 65-243 (derived from CI 6311 and CI 1227, respectively) possess genes for resistance that are different from those of Minn 33 (derived from ND B-112).

## **DISCUSSION**

A major objective of barley breeding in Minnesota has been to transfer genes for resistance to spot blotch from ND B-112 and Dickson into cultivars useful for growers and the malting and brewing industries. The success of this resistance breeding program is demonstrated by the wide adoption of cultivars Morex and Robust. These two cultivars account for the bulk of barley acreage in Michigan, Wisconsin, Minnesota, North Dakota, and South Dakota, and Morex is widely grown in Montana, Idaho, Washington, and Oregon (1). Cree and Manker are also products of the program but are no longer grown (17-20). In addition, many resistant breeding lines like Minn 33 have been produced.

Two sources of resistance to spot blotch (ND B-112 and Dickson) were used to develop the resistant barley cultivars released by the University of Minnesota. Both of these sources trace to CI 7117-77, which was selected by A. J. Le Jeune from composite cross CC-XIII at North Dakota State University. Line ND B-112 was selected by A. J. Le Jeune and W. W. Sisler from the cross CI 7117-77 × Kindred (A. E. Foster, North Dakota State University, personal communication).

Although resistance to spot blotch in barley cultivars developed at the University of Minnesota may be traced to a single source, there have been no reports that resistant cultivars or advanced breeding lines derived from ND B-112 have become susceptible to spot blotch. However, when environmental conditions favor the development of spot blotch, some cultivars and breeding lines that are usually rated as resistant or moderately resistant may be classified as moderately susceptible. This apparently happened in our evaluation of F<sub>3</sub> families and resulted in an excess number of

Table 2. Number of resistant (R) and susceptible (S) F<sub>3</sub> families of five barley crosses<sup>a</sup> infected with *Bipolaris sorokiniana* in the field

	No. of families		F <sub>3</sub>		P
Cross	R <sup>b</sup> S	ratio	$\chi^2$		
Minn 65-244 × Larker	106	31	3:1	0.41	0.70-0.50
Larker × Minn 33	94	26	3:1	0.70	0.50 - 0.30
Larker × Minn 65-241	74	28	3:1	0.33	0.70 - 0.50
Minn 7 × Larker	85	15	3:1	5.33	0.05 - 0.02
Larker × Minn 65-243	57	9	3:1	4.55	0.05 - 0.02

<sup>&</sup>lt;sup>a</sup> Between spot blotch-resistant lines and the susceptible cultivar Larker.

**Table 3.** Number of  $F_3$  and  $F_4$  families of five barley crosses<sup>a</sup> classified as resistant, segregating, and susceptible to spot blotch

Cross	Generation <sup>b</sup>	Resistant	Segregating	Susceptible
Minn 65-244 × Larker	F <sub>3</sub>	19	11	10
	$\mathbf{F}_{4}^{J}$	18	12	10
Larker × Minn 33	$\mathbf{F}_{3}$	20	10	10
	$\mathbf{F}_{4}^{J}$	18	12	10
Larker × Minn 65-241	$\mathbf{F}_{3}$	8	11	9
	$\mathbf{F}_{4}$	8	11	9
Minn 7 × Larker	$\mathbf{F}_{3}^{7}$	15	6	6
	$\mathbf{F}_{4}^{J}$	15	7	5
Larker × Minn 65-243	$\mathbf{F}_{3}^{T}$	6	3	4
	$\mathbf{F}_{4}$	4	6	3

<sup>&</sup>lt;sup>a</sup> Between spot blotch-resistant lines and the susceptible cultivar Larker.

Table 4. Spot blotch reaction classification of  $F_5$  and  $F_6$  lines from three crosses of spot blotch-resistant barleys

		Class <sup>a</sup>			
Cross	Generation	Resistant	Moderately resistant	Moderately susceptible	
Minn 65-243 × Minn 33	F <sub>5</sub>	35	5	0	
	$F_6$	11	6	3	
Minn 65-241 × Minn 33	$F_5$	34	4	2	
	$\mathbf{F}_{6}$	12	7	1	
Minn 65-244 × Minn 33	F <sub>5</sub>	39	1	0	
	$\mathbf{F_6}$	18	2	0	

<sup>&</sup>lt;sup>a</sup> Data show the number of lines in each class. In each cross, 40 F<sub>5</sub> lines were tested in 1980 and 20 F<sub>6</sub> lines in 1981.

<sup>&</sup>lt;sup>b</sup> Numbers obtained by combining resistant and segregating classes.

<sup>&</sup>lt;sup>b</sup> The F<sub>4</sub> families were derived from the F<sub>3</sub> families. The F<sub>4</sub> classification was based on five single-head lines derived from each F<sub>3</sub> family. F<sub>3</sub> was tested in 1985 and F<sub>4</sub> in 1986.

families being placed in the segregating class rather than the resistant class. We have also classified Morex as moderately susceptible when weather has favored spot blotch development.

Net blotch, caused by *Pyrenophora* teres Drechs., can be confused with spot blotch unless the identity of the pathogen is checked by examination of conidia. Confusion of the two diseases is even more likely when symptoms of net blotch are not typical. Thus, Morex and Robust have been reported as susceptible to spot blotch when in fact they were severely infected with net blotch.

Although the resistance provided by ND B-112 continues to be effective, other sources of resistance to spot blotch have been prepared for use should the pathogen become virulent on the resistant cultivars now in use. The resistance genes of Wisc 691-1 probably differ from those of ND B-112. Wisc 691-1 was crossed with Swan to produce Minn 7, which is being held for future use. The resistance genes of CI 1227, CI 6311, and H. agriocrithon (2,13) also are probably different from those of ND B-112 and Dickson. These sources were crossed with Larker or Trophy, and the lines are being held for future use. In addition, Minn 65-241 and Minn 65-243, which derive resistance from CI 6311 and CI 1227, respectively, were crossed with Minn 33 to provide lines that combine different resistance genes.

The resistance genes of Minn 65-244, derived from CI 9584, may be similar to those of Minn 33, derived from ND B-112. We tentatively offer this suggestion because a cross of these sources produced only resistant to moderately resistant F<sub>2</sub> plants and F<sub>5</sub> and F<sub>6</sub> lines.

Experience with the development of barley lines and cultivars resistant to spot blotch indicates that resistance from ND B-112 and Dickson is readily transferred from parents to progenies. Our inheritance data suggest that resistance is probably conditioned by one or two genes. Other investigators (3,6–8,11) have also concluded that resistance to spot blotch is due to a small number of genes.

The value of resistance to spot blotch conferred by genes from ND B-112 may be estimated from yield data from fungicide trials involving resistant and susceptible barley cultivars and from comparisons of the yields of related resistant and susceptible lines. Musick (14) reported a test done in 1977 at Crookston in which mancozeb increased grain yields of susceptible Larker 20% and of resistant Manker 1%. In further tests done at St. Paul and Rosemount, MN, in 1978 and 1979, the fungicide raised yields of Larker 15% and of moderately resistant Morex 4% (14). Nutter et al (15) reported that mancozeb increased yields 20% in susceptible Larker but only 4-10% in resistant Bumper, Dickson, Glenn, Hazen, and Robust.

In 1977 we studied the value of resistance to spot blotch in enhancing grain yields of 13 resistant and nine susceptible  $F_6$  lines from the cross Manker  $\times$  Primus. The lines were tested in replicated plots at Crookston in a severe natural epidemic and at St. Paul in the absence of spot blotch and other diseases. When spot blotch was absent, mean yields of the resistant and susceptible lines were approximately equal. When spot blotch was severe, the mean yield of resistant lines was 1,129 kg more per hectare than that of the susceptible lines, an increase of 33%.

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