The Use of Molecular Markers to Study *Setosphaeria turcica* Resistance in Maize

Kevin D. Simcox and Jeffrey L. Bennetzen

United States Department of Agriculture, Agricultural Research Service (USDA-ARS), Plant Genetics Research Unit, Columbia, MO 65211; and Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, respectively.

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ABSTRACT

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To facilitate understanding of the molecular basis of *Htn1* resistance in maize to northern corn leaf blight, we mapped the *Htn1* locus by restriction fragment length polymorphism (RFLP) analysis. Linkage with the *Htn1* locus, using a backcross population, was initially detected with the RFLP probe UMC16, which hybridized to two loci: UMC16A on the long arm of chromosome three and the unmapped locus UMC16B. Independent assortment of *Htn1* with UMC16A and linkage to UMC16B was verified with RFLP markers closely flanking UMC16A on the long arm of chromosome three. Information obtained from previous studies, detailing duplicate loci in maize, indicated that RFLP loci in the region around UMC16A are duplicated on the long arm of chromosome eight in the region of *Idh1*. RFLP loci mapping within this region on chromosome eight detected linkage with both *Htn1* and UMC16B. Analysis of additional backcross progeny mapped the *Htn1* locus 0.8 cM (centiMorgans) distal to UMC117, near the recently reported location of the *Ht2* locus. Segregation analysis of F_2 progeny from crosses of the inbred W22*Htn1* with A619*Ht2* confirmed the linkage of *Htn1* with *Ht2*. RFLP analysis of susceptible F_2 progeny indicates that *Htn1* and *Ht2* are not allelic, and *Ht2* maps are approximately 10 cM proximal to *Htn1*.

Additional keywords: disease resistance, gene mapping.

The *Htn1* gene of maize (*Zea mays* L.) confers resistance to *Setosphaeria turcica* (Luttrell) K.J. Leonard & E.G. Suggs (anamorph: *Exserohilum turcicum* (Pass.) K.J. Leonard & E.G. Suggs = *Helminthosporium turcicum* Pass.), both by extending the period between infection and disease symptom expression and by delaying the onset of sporulation (8,22). The delayed onset of sporulation effectively reduces the extent of disease present during critical developmental periods in maize. Resistance specified by the *Htn1* gene is distinct from the typical chlorotic-necrotic lesion resistance genes *Ht1*, *Ht2*, and *Ht3* associated with resistance to *S. turcica* (12,14,18). Originally derived from the Mexican land race Pepitilla (8), no information is available concerning the map position of *Htn1* or possible allelism to other *S. turcica*-resistance genes. In the maize inbred B37, *Htn1* resistance effectively inhibited lesion formation throughout the growing season. In a more susceptible genetic background, such as the maize inbred Hy, *Htn1* resistance is expressed as small, susceptible lesions that are delayed from forming for up to 26 days and that rarely sporulate (22). Variation in *Htn1* expression also is observed under low light intensities (27). The reported variation in the expression of *Htn1* suggests the presence of modifiers that may or may not be linked to *Htn1*.

The *Htn1* gene is effective against the most common *S. turcica* virulence-gene combinations present in the United States (18). However, isolates of *S. turcica* virulent on *Htn1* genotypes have been identified in nurseries located in Florida, Texas, and Hawaii (21,27,29). The identification of virulent biotypes of the pathogen suggests that incorporating *Htn1* into breeding lines could eventually result in an increase in the frequency of biotypes virulent on *Htn1* and an accompanying loss of resistance. When *Ht1* and *Htn1* are in combination, an intermediate resistance phenotype

is seen when inoculated with an avirulent isolate of *S. turcica* (22). This intermediate lesion type is a smaller chlorotic-necrotic lesion than what is seen in *Ht1* genotypes. It remains to be seen if the same interaction occurs when *Ht2* or *Ht3* are combined with *Htn1* and how these various combinations interact with the virulence genotypes of *S. turcica*.

Identification of restriction fragment length polymorphism (RFLP) markers flanking each *S. turcica*-resistance gene would facilitate genotype determinations when pyramiding resistance genes and when studying interactions among *S. turcica*-resistance genes (20). The *Ht1* gene has been mapped to the long arm of chromosome two, and RFLP markers closely flanking the *Ht1* gene have been identified (3,10). Recently, the *Ht2* locus was mapped to the long arm of chromosome eight by RFLP analysis (D. Zaitlin, *personal communication*; 30). The chromosome locations of the *Htn1* and *Ht3* genes are currently unreported. This study describes the use of RFLP analysis to map the *Htn1* resistance gene.

MATERIALS AND METHODS

Plant and fungal materials. Maize inbred lines A619, A619*Ht1*, A619*Ht2*, A619*Ht3*, and W22*Htn1* were obtained from W. Pedersen, Department of Plant Pathology, University of Illinois, Urbana-Champaign. The maize chromosome eight tester line, Coop 802A, homozygous recessive for *glossy18* (*gl18*), *virescent16* (*v16*), and *japonica1* (*j1*) was obtained from the Maize Genetics Cooperative Stock Center, Department of Agronomy, University of Illinois, Urbana-Champaign. Isolates of *S. turcica* avirulent on *Ht1*, *Ht2*, *Ht3*, and *Htn1* genotypes (race 0) and avirulent on *Ht2*, *Ht3*, and *Htn1* genotypes (race 1) (18) were obtained from W. Pedersen. The authenticity of the isolates was confirmed by inoculating A619, A619*Ht1*, A619*Ht2*, A619*Ht3*, and W22*Htn1*, using inoculation procedures described below.

Linkage relationships between *Htn1* and the *Ht1*, *Ht2*, and *Ht3* genes were determined with F_2 progeny from crosses of W22*Htn1* × A619*Ht1*, A619*Ht2*, or A619*Ht3*. Resistant and susceptible F_2 progeny were identified by inoculating with *S. turcica* race 0. The *Htn1* locus was mapped by RFLP analysis with a BC_1 population consisting of W22*Htn1* crossed by the recurrent parent, A619*Ht1*. Resistant and susceptible BC_1 progeny were identified with *S. turcica* race 1. Analysis of the linkage of the *Htn1* locus to morphological markers on the long arm of chromosome eight was accomplished with a BC_1 population consisting of W22*Htn1* crossed to the homozygous *gl18 Htn1 v16 j1* tester, Coop 802A, the recurrent parent. Resistant and susceptible BC_1 progeny were identified with *S. turcica* race 0.

Screening procedures. Resistant and susceptible individuals were identified by inoculating 3-wk-old seedlings (approximately five-leaf stage) with either *S. turcica* race 0 or 1. Inoculum production consisted of adding 5 ml of sterile water containing 0.1% Tween-20 (Sigma Chemical Co., St. Louis, MO) to the surface of 2-wk-old lactose-casein hydrolysate agar (28) cultures and dislodging the conidia with a rubber policeman. After adjusting the conidia concentration to 5,000 conidia per milliliter, 100 μl of the conidial suspension was inoculated into the seedling whorl with a P-200 Pipetmann (Rainin Instrument Co., Woburn, MA). Inoculated seedlings were incubated overnight in high humidity at 15 C and maintained in the greenhouse under 16 h of light/8 h of dark. Greenhouse temperatures ranged from 15 to 25 C throughout the experiment. Seedlings were scored for resistance and susceptibility after 2 wk. All experiments were repeated at least twice with the exception of the BC_1 cross used to map the *Htn1* locus by RFLP analysis. For RFLP analysis, inoculations of individual BC_1 plants were repeated after 2 wk to verify seedling scores, and the seedlings were collected for DNA isolation.

DNA isolation. DNA was prepared from seedlings at the 10-leaf stage by a procedure similar to that used by Saghai-Maroof et al (23). Approximately 5 g of frozen leaf material was ground to a fine powder with dry ice in an electric coffee grinder. Samples were resuspended in 2× CTAB buffer (1.4 M NaCl, 2% [w/v] hexadecyl-trimethylammonium bromide [Sigma], 1% [v/v] β-mercaptoethanol, 100 mM Tris-HCl, pH 8.0), preheated to 65 C, and incubated at 65 C in a water bath for 30 min. After two extractions with chloroform/isoamyl alcohol (24:1), the DNA was precipitated by the addition of 0.6 volumes of isopropanol. The precipitated DNA was transferred to a 1.5-ml tube with a wide-bore pipet tip and washed twice with 70% ethanol at room temperature. The DNA was resuspended in TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) with RNase A at 10 μg/ml and stored at 5 C until use.

DNA digestion and membrane hybridization. Restriction enzymes were purchased from Gibco BRL (Grand Island, NY), New England Biolabs (Beverly, MA), and Boehringer Mannheim (Indianapolis, IN), and used in threefold excess under conditions specified by the supplier. Genomic DNA (5–8 μg per lane) was digested with the appropriate restriction enzymes and electrophoresed through 0.8% agarose gels. The DNA was transferred to MSI Hybond-N blotting membrane (Amersham Corp., Arlington Heights, IL), as previously described (1), or to GeneScreen Plus (DuPont NEN Research Products, Boston, MA) transfer membrane, using our modification (24) of the "dry-blot" procedure developed by Kempter et al (16). Labeled DNA probes were prepared by random priming and hybridized to membranes as previously described (1). The membranes were washed four times with 2× SSC/0.1% (w/v) SDS (1× SSC is 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0; SDS is sodium dodecyl sulfate) for 5 min each at 65 C and four times with either 0.1× SSC/0.1% (w/v) SDS or 0.2× SSC/0.1% (w/v) SDS for an additional 5 min each at 65 C. Autoradiography was performed as previously described (19).

Detection of polymorphisms and segregation analysis. Cloned random low copy-number *Pst*I genomic DNA fragments previously mapped on the maize linkage map were obtained from D. Hoisington, University of Missouri, Columbia (11). These clones were designated either UMC (University of Missouri, Columbia) or BNL (Brookhaven National Laboratory, Upton, NY). Isolated inserts of the RFLP clones were hybridized to membranes containing DNA from the two parental maize inbred lines, W22*Htn1* and A619*Ht1*, independently digested with each of nine restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Kpn*I, *Pst*I, *Sst*I, and *Xba*I). Probes that detected polymorphisms were employed as hybridization probes in segregation analysis of backcross progeny derived from the two parental inbred lines. Linkage analysis was performed and map distances determined with MAPMAKER (version 3.0 for DOS; 17). χ^2 goodness-of-fit values for segregation and independent assortment were calculated with the Linkage-1 program (26).

RESULTS

Linkage of *Htn1* to RFLP loci on the long arm of chromosome three. Because no previous information on the map location of *Htn1* was available, we initially sought to assign *Htn1* to a specific linkage group. A series of RFLP probes mapping to each of the 10 homologous chromosomes in maize, separated by approximately 50 cM (centiMorgans), was selected to provide a maximum distance of 25 cM from a RFLP locus. In the initial screen, RFLP probes were hybridized to filters containing genomic DNA from nine resistant and nine susceptible W22*Htn1* × A619*Ht1* BC_1 progeny. Departure from random segregation determined by the χ^2 test for independence ($P = 0.05$, df = 1) was used as the criteria to determine if linkage existed between *Htn1* and a RFLP locus. If evidence of linkage was obtained in the initial screen, additional BC_1 progeny were screened to confirm linkage to *Htn1*.

Linkage to *Htn1* was first detected with UMC16, located at map position 136 on the long arm of chromosome three (7). Probe UMC16 hybridized to two duplicate loci: UMC16A, which was unlinked to *Htn1*, and UMC16B, which cosegregated with *Htn1* (Table 1). Because UMC16A gave the strongest signal, this indicated that UMC16A was probably the locus originally mapped to chromosome three. Hybridizations with probes detecting loci flanking UMC16, BNL5.33A, BNL15.20, and BNL10.24A confirmed the location of UMC16A on chromosome three, whereas

no linkage was detected with either UMC16B or *Htn1* (Table 1). Hence, UMC16B and *Htn1* did not map to the long arm of chromosome three in the region of UMC16A.

Use of duplicated regions of the genome to map the *Htn1* gene. Information supplied by Helentjaris et al (9) was used as a guide for the most likely chromosomal location of the duplicated locus (UMC16B) detected by UMC16. RFLP loci mapping to the region around UMC16A are highly duplicated, sharing homology with all of the other nine chromosomes. However, the most extensive duplications in the region around UMC16A were found on the long arm of chromosome eight near *Isocitrate Dehydrogenase1* (*Idh1*) (7). RFLP loci distal to *Idh1* include UMC48, UMC30, UMC117, and BNL10.24B, and loci proximal to *Idh1* include UMC93, UMC89, and UMC12. Hybridization of the RFLP probe UMC30 to DNA of BC_1 progeny detected two duplicate loci, UMC30A and UMC30B, with UMC30A exhibiting the greater signal strength. The *Htn1* gene segregated independently of UMC30B but was linked to UMC30A (Table 1). The location of UMC30A on the long arm of chromosome eight was confirmed by the flanking locus, UMC117, which also was linked to *Htn1*. Linkage to the *Htn1* gene also was detected with UMC12, UMC48, UMC89, UMC93, and BNL10.24B (Table 1). Table 2 lists the appropriate restriction enzymes and molecular weight of the RFLP alleles used to map *Htn1* to the long arm of chromosome eight.

The position of *Htn1* on the long arm of chromosome eight. To determine the map order of *Htn1* with respect to RFLP markers on chromosome eight, 94 additional W22*Htn1* × A619*Ht1* BC_1 progeny were analyzed with RFLP loci that map near *Idh1*. Analysis of the backcross progeny revealed a single crossover between *Htn1* and both UMC30A and UMC117, which themselves were not separated by recombination (Table 3). The exchange of markers between UMC48 and *Htn1* indicated that *Htn1* is distal to UMC30A and UMC117. RFLP loci proximal to *Htn1* included UMC30A and UMC117 (0.8 cM) and UMC48 (11.8 cM). The 0.8 cM distance between *Htn1* and UMC117 places the *Htn1* locus near BNL10.24B in the interval between UMC117 and NPI268 (7). As reported by Helentjaris et al (9), the duplicated region on chromosome three between BNL12.30B and BNL10.24A appears to be inverted with respect to their orientation on chromosome eight.

Analysis of the W22*Htn1* × *gl18 Htn1 v16 j1* BC_1 data indicated that both *v16* and *j1* mapped distal to *Htn1* on the long arm of chromosome eight (Table 3). The *v16* locus was mapped 15.4 cM distal to *Htn1*, whereas *j1* was 46.0 cM distal to *v16*. Linkage of *Htn1* with *gl18* was not observed. Recombinants recovered from the W22*Htn1* × *gl18 Htn1 v16 j1* BC_1 progeny were analyzed by RFLP analysis to confirm the map order of the *Htn1* and *v16* loci. All resistant virescent backcross progeny (*Htn1 v16/Htn1 v16*) were heterozygous at UMC93 and UMC30, whereas susceptible normal backcross progeny (*Htn1 v16/Htn1 v16*) were homozygous for the *gl18 Htn1 v16 j1* tester alleles at UMC93 and UMC30. Thus, all recovered recombinants had an exchange distal to the *Htn1* locus between *Htn1* and *v16*.

Interrelationships and interactions between *Htn1* and the *Ht1*, *Ht2*, and *Ht3* resistance loci. Observations of *S. turcica* race 0 inoculated A619*Ht1* × W22*Htn1* F_1 progeny confirmed earlier observations of Raymundo et al (22) that an intermediate resistant lesion type is seen when *Ht1* and *Htn1* are both heterozygous. This intermediate lesion type is similar in pigmentation to the

TABLE 1. Localization of the *Htn1* locus to the long arm of chromosome eight

Loci	Chromosome	Progeny	χ^{2a}	cM[b]
UMC16A	3	33	0.0	...
UMC30B	3	18	3.6	...
BNL5.33A	3	18	2.1	...
BNL10.24A	3	18	2.0	...
BNL15.20A	3	25	1.2	...
UMC12	8	18	10.9*	...
UMC89	8	18	11.5*	...
UMC93	8	18	10.4*	...
UMC48	8	36	32.2*	2.9
UMC16B	8	45	37.0*	5.6
UMC30A	8	60	49.0*	3.3
UMC117A	8	27	19.0*	6.8
BNL10.24B	8	18	5.5*	...

[a] χ^2 values greater than 3.84 (df = 1), indicated by an asterisk (*), reject the hypothesis that *Htn1* segregates independently from the restriction fragment length polymorphism tested.
[b] Map distances determined by MAPMAKER 3.0 (17).

TABLE 2. Molecular weight of DNA polymorphisms used to locate *Htn1* to the long arm of chromosome eight

Loci	Enzyme[a]	W22*Htn1*[b]	A619*Ht1*[b]
UMC16A	*Xba*I	7.4	9.8
UMC16B	*Xba*I	3.8	5.4
UMC30A	*Eco*RV	8.3	12.8
UMC30B	*Eco*RV	3.8	8.5
UMC48	*Hin*dIII	8.1	23.1
UMC93	*Hin*dIII	7.5	13.1
UMC117	*Hin*dIII	13.7	10.7
BNL10.24A	*Hin*dIII	4.5	3.9
BNL10.24B	*Hin*dIII	5.5	5.2
BNL15.20	*Hin*dIII	8.8	8.0

[a] Restriction enzyme used to map restriction fragment length polymorphism (RFLP) allele variation in the maize inbred lines W22*Htn1* and A619*Ht1*.
[b] Approximate molecular weight, in kilobases, of polymorphic RFLP alleles.

TABLE 3. Linkage between *Htn1* and chromosome eight RFLP and morphological markers

Backcross and loci	Number of progeny	Number of recombinants	cM[a]
(W22*Htn1* × A619*Ht1*) × A619*Ht1*[b]			
Htn1-UMC117	91	2	0.8
Htn1-UMC30A	92	2	0.8
UMC117-UMC30	90	0	0.0
Htn1-UMC48	90	9	12.6
UMC117-UMC48	89	8	11.8
(W22*Htn1* × *gl18 Htn1 v16 j1*) × *gl18 Htn1 v16 j1*[c]			
Htn1-v16	113	15	15.4
Htn1-j1	112	41	>50.0
v16-j1	113	34	46.0

[a] Map distances determiend with MAPMAKER 3.0 (17).
[b] Gene order = UMC48-UMC30/117-*Htn1*; log likelihood = −131.86.
[c] Gene order = *Htn1-v16-j1*; log likelihood = −83.55.

TABLE 4. F_2 segregation data between *Htn1* and the *Ht1*, *Ht2*, and *Ht3* loci

Crosses	Total progeny	Symptom response: Resistant	Symptom response: Susceptible	Expected ratio	χ^{2a}
W22*Htn1*	20	20[b]	0		
A619*Ht1*	20	20[c]	0		
Htn1 × *Ht1* F_1	50	50[d]	0		
Htn1 × *Ht2* F_1	50	50[d]	0		
F_2					
Exp. 1	405	390	15	15:1	4.5*
Exp. 2	180	176	4	15:1	5.0*
A619*Ht3*	20	20[c]	0		
Htn1 × *Ht3* F_1	50	50[b]	0		
F_2					
Exp. 1	162	151	11	15:1	0.1
Exp. 2	155	152	3	15:1	4.9*
Exp. 3	188	175	13	15:1	0.1

[a] χ^2 values greater than 3.84 (df = 1), indicated by an asterisk (*), reject the hypothesis that *Htn1* segregates independently from the locus tested.
[b] Delayed symptom response to inoculation by *Setosphaeria turcica* race 0.
[c] Chlorotic-necrotic resistance response to inoculation by *S. turcica* race 0.
[d] Intermediate chlorotic response to inoculation by *S. turcica* race 0.

chlorotic-necrotic lesion *Ht1* phenotype except that the lesion is oval and slightly smaller. Inoculated F_1 progeny from crosses of W22*Htn1* with both A619*Ht2* and A619*Ht3* displayed a similar intermediate phenotype except that the chlorotic-necrotic lesions were limited to the region between adjacent intermediate vascular bundles and were seldom greater than 10 mm long. In all three cases, the expression of the chlorotic-necrotic lesion phenotype did not appear to be delayed by the presence of *Htn1*.

Analysis of segregation of resistant and susceptible classes in F_2 progeny from crosses of W22*Htn1* with A619*Ht1*, A619*Ht2*, and A619*Ht3* indicated that *Htn1* was tightly linked to *Ht2* but not *Ht1* or *Ht3* (Table 4). The absence of linkage between *Ht1* and *Htn1* was expected because *Ht1* has been mapped previously to chromosome two (3,10). In the case of *Ht3*, only two of the three experiments suggested that *Ht3* was not linked to *Htn1* (Table 4). The susceptible F_2 progeny from these crosses were further examined by RFLP analysis. In all progeny examined, RFLP loci linked to *Htn1* were homozygous for the A619*Ht3* allele, indicating that *Ht3* was not linked to *Htn1* (K. D. Simcox and J. L. Bennetzen, *unpublished data*).

As in the case of *Ht1*, linkage between *Htn1* and *Ht2* was anticipated because Zaitlin et al (D. Zaitlin, *personal communication*; 30) mapped *Ht2* proximal to UMC48 on the long arm of chromosome eight. The occurrence of susceptible progeny, presumably recombinants between *Htn1* and *Ht2*, suggests that these two loci are nonallelic. RFLP analysis indicated that susceptible F_2 progeny arose through recombination between *Htn1* and *Ht2*. All susceptible individuals were heterozygous for the W22 and A619 alleles at the proximal locus UMC93 whereas loci distal to *Ht2*, UMC48 and UMC30, were homozygous for the A619 allele. Thus, an exchange occurred between the A619 *Ht2* and W22 *Htn1* alleles in the *Ht2*-UMC48 interval in all eight of the susceptible F_2 progeny tested. Mapping *Ht2* within the UMC93-UMC48 interval supports the data of Zaitlin et al (D. Zaitlin, *personal communication*; 30). Using distances obtained from the maize core RFLP map, *Ht2* would map approximately 10 cM proximal to *Htn1* (7).

DISCUSSION

Understanding the genetics of host-pathogen interactions is greatly facilitated by the identification of molecular markers linked to disease-resistance genes in the host (20). Variability in disease symptom expression can be rapidly correlated with host genotype without the need of additional pollinations, and the extent of variation not attributed to a specific locus can be determined. In this study, the *Htn1* locus was mapped to the long arm of chromosome eight, 0.8 cM distal to UMC117 and 15.4 cM proximal to *v16*. We are currently developing a mapping population consisting of F_2:F_3 families to further analyze additional RFLP loci in the region of *Ht2* and *Htn1* and to identify loci that might modify the expression of *Htn1*. Although the mapping data indicates that the *v16* locus will not be useful as a tightly linked marker in marker-assisted selections, *v16* can be used in enhanced mapping procedures to recover recombination events in and around the *Ht2* and *Htn1* loci (6).

The use of RFLP clones that detect duplicate loci played a major role in the rapid localization of the *Htn1* locus. A single RFLP clone that detected two unlinked loci effectively allowed a "skip" from one chromosome to a region of known homology. Only 20 of the estimated 80 RFLP clones required to cover the maize genome were used to localize the *Htn1* locus to chromosome eight. This was due to the homology present between the long arm of chromosome three in the region of UMC16A and the long arm of chromosome eight near UMC30A (9). This suggests a method of using duplicate loci to facilitate the mapping of dominant genes such as disease- and herbicide-resistance genes, cytoplasmic restorer genes, and dominant developmental mutations not normally present in recombinant inbred lines and other specialized mapping populations (5,25). Use of a set of characterized RFLP clones that detect duplicate loci of known location would reduce the time and expense required to map dominant factors because a single clone facilitates multichromosomal linkage analysis.

Analysis of F_2 data confirms the preliminary results of Hooker (13) that *Ht3* is not linked to *Ht1*, *Ht2*, or *Htn1*. Similarities in lesion phenotype and race specificity between *Ht2* and *Ht3* presents a problem in determining whether a particular genotype contains either *Ht2* or *Ht3*. The clarification of the inconsistent W22*Htn1* × A619*Ht3* F_2 segregation data in experiment 2 (Table 4) demonstrates the usefulness of molecular probes in confirming genotypes. Localization of *Ht2* near UMC48 provides a useful marker to confirm whether *Ht2* or *Ht3* is present. The similarity in phenotype between *Ht2* and *Ht3* expression also suggests that *Ht2* and *Ht3* may be homologous loci. The *Ht3* gene was introgressed into maize from *Tripsacum floridanum* (13). It is possible that *Ht3* is the *Tripsacum* homolog of *Ht2* and that the absence of linkage between these genes indicates that the *Tripsacum* chromosome segment containing *Ht3* integrated at a nonhomologous site in the maize genome.

Another interesting aspect of resistance to *S. turcica* is the presence of a dominant inhibitor, *Sht1*, of *Ht2*, *Ht3*, and *Htn1* resistance in inbred lines related to inbred B14 (4; W. L. Pedersen, *personal communication*). In the presence of the *Sht1* allele, expression of *Ht2*, *Ht3*, or *Htn1* is inhibited and results in complete susceptibility. However, *Sht1* does not inhibit expression of *Ht1*. Whether inhibition of *Ht2*, *Ht3*, and *Htn1* implies some functional similarity among these genes is not certain. It is unusual that *Sht1* inhibits expression of *Ht2* and *Ht3* while *Ht1* is not effected, despite the similar chlorotic-necrotic lesion phenotype.

The identification of molecular markers tightly linked to the *Htn1* locus will allow marker-assisted selection to investigate the effects of pyramiding resistance genes on resistance to *S. turcica* (20), facilitate marker-assisted incorporation of *Htn1* into breeding lines (20), and provide useful markers for molecular analysis of *Htn1* with map-based cloning (6) and transposon-tagging techniques (2,15). Three of the four monogenic resistance genes to *S. turcica* have been mapped by RFLP analysis, and this will enhance our ability to dissect the genetic basis of this interesting pathosystem.

LITERATURE CITED

1. Bennetzen, J. L. 1984. Transposable element *Mu1* is found in multiple copies only in Robertson's *Mutator* maize lines. J. Mol. Appl. Genet. 2:519-524.
2. Bennetzen, J. L., Creese, A., Brown, W. E., and Lee, L. 1987. Molecular cloning of maize genes by transposon tagging with *Mutator*. Pages 183-204 in: Plant Gene Systems and Their Biology. J. L. Key and L. McIntosh, eds. Alan R. Liss, Inc., New York.
3. Bentolila, S., Guitton, C., Bouvet, N., Sailland, A., Nykaza, S., and Freyssient, G. 1991. Identification of an RFLP marker tightly linked to the *Ht1* gene in maize. Theor. Appl. Genet. 82:393-398.
4. Ceballos, H., and Gracen, V. A. 1989. A dominant inhibitor gene inhibits the expression of *Ht2* against *Exserohilum turcicum* race 2 in corn inbred lines related to B14. Plant Breeding 102:35-44.
5. Evola, S. V., Burr, F. A., and Burr, B. 1986. The suitability of restriction fragment polymorphisms as genetic markers in maize. Theor. Appl. Genet. 71:765-771.
6. Ganal, M. W., Young, N. D., and Tanksley, S. D. 1989. Pulsed field gel electrophoresis and physical mapping of large DNA fragments in the *Tm-2a* region of chromosome 9 in tomato. Mol. Gen. Genet. 215:395-400.
7. Gardiner, J. M., Coe, E. H., Melia-Hancock, S., Hoisington, D. A., and Chao, S. 1993. Development of a core RFLP map in maize using an immortalized F_2 population. Genetics 134:917-930.
8. Gevers, H. O. 1975. A new major gene for resistance to *Helminthosporium turcicum* leaf blight of maize. Plant Dis. Rep. 59:296-299.
9. Helentjaris, T., Weber, D., and Wright, S. 1988. Identification of the genomic locations of duplicate nucleotide sequences in maize by analysis of restriction fragment length polymorphisms. Genetics 118:353-363.
10. Hoisington, D. A., and Coe, E. H., Jr. 1989. Methods for correlating RFLP maps with conventional genetic and physical maps in maize. Pages 19-24 in: Development and Application of Molecular Markers to Problems in Plant Genetics. T. Helentjaris and B. Burr, eds. Cold

Spring Harbor Laboratory, Cold Spring Harbor, NY.
11. Hoisington, D. A., and Coe, E. H., Jr. 1990. Mapping in maize using RFLPs. Pages 331-352 in: Stadler Genetics Symposium—Gene manipulation in Plant Improvement II. J. P. Gustafson, ed. Plenum Press, New York.
12. Hooker, A. L. 1977. A second major gene locus in corn for chlorotic-lesion resistance to *Helminthosporium turcicum*. Crop Sci. 17:132-135.
13. Hooker, A. L. 1981. Resistance to *Helminthosporium turcicum* from *Tripsacum floridanum* incorporated into corn. Maize Genet. Coop. Newsl. 55:87-88.
14. Hooker, A. L., and Perkins, J. M. 1980. Helminthosporium leaf blights of corn: The state of the art. Annu. Corn Sorghum Res. Conf. 35:68-87.
15. Johal, G. S., and Brigg, S. P. 1992. Reductase activity encoded by the *Hm1* disease resistance gene in maize. Science 258:985-987.
16. Kempter, B., Luppa, P., and Neumeier, D. 1991. A short procedure for southern blotting on neutral and anionic membranes. Trends Genet. 7:109-110.
17. Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, M. J., Lincoln, S. E., and Newburg, L. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181.
18. Leonard, K. J., Levy, Y., and Smith, D. R. 1989. Proposed nomenclature for pathogenic races of *Exserohilum turcicum* on corn. Plant Dis. 73:776-777.
19. Maniatis, T. A., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 545 pp.
20. Melchinger, A. E. 1990. Use of molecular markers in breeding for oligogenic disease resistance. Plant Breeding 104:1-9.
21. Pataky, J. K., Carson, M. L., and Mosely, P. R. 1991. Race 23N of *Exserohilum turcicum* in Florida. Plant Dis. 75:863.
22. Raymundo, A. D., Hooker, A. L., and Perkins, J. M. 1981. Effect of gene *Htn* on the development of northern corn leaf blight epidemics. Plant Dis. 65:327-330.
23. Saghai-Maroof, M., Soliman, K., Jorgensen, R., and Allard, R. 1984. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. USA 91:8014-8018.
24. Simcox, K. D., and McMullen, M. D. 1993. The use of the "modified dry blot" procedure for RFLP analysis. Maize Genet. Coop. Newsl. 67:116-117.
25. Sisco, P. H. 1991. Duplications complicate genetic mapping of *Rf4*, a restorer gene for cms-C cytoplasmic male sterility in corn. Crop Sci. 31:1263-1266.
26. Suiter, K. A., Wendel, J. F., and Case, J. S. 1983. Linkage-1: A PASCAL computer program for the detection and analysis of genetic linkage. J. Hered. 74:203-204.
27. Thakur, R. P., Leonard, K. J., and Jones, R. K. 1989. Characterization of a new race of *Exserohilum turcicum* virulent on corn with resistance gene *Htn*. Plant Dis. 73:151-155.
28. Tuite, J. 1969. Plant Pathological Methods. Burgess Publishing Company, Minneapolis, MN.
29. Windes, J. M., and Pedersen, W. L. 1991. An isolate of *Exserohilum turcicum* virulent on maize inbreds with resistance gene *Htn*. Plant Dis. 75:430.
30. Zaitlin, D., DeMars, S. J., and Gupta, M. 1992. Linkage of a second gene for NCLB resistance to molecular markers in maize. Maize Genet. Coop. Newsl. 66:69-70.