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Identification of an RFLP marker tightly linked to the *Ht1* gene in maize

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Summary. We have identified tight linkage of an RFLP marker to the *Ht1* gene of maize that confers resistance to the fungal pathogen *Helminthosporium turcicum* race 1. This was accomplished by the use of four pairs of near isogenic lines (NILs; B73, A619, W153R, and CM105), each differing by the presence or the absence of the gene *Ht1*. Since *Ht1* maps to chromosome 2, 26 clones already mapped to this chromosome were labeled and probed against Southern blots of these NILs DNA digested with three restriction enzymes: *Eco*RI, *Bam*HI, and *Hin*dIII. Six markers exhibited an RFLP for at least one pair of NILs. Presumptive linkage was further tested by analyzing the segregation of five of the six markers (one was monomorphic in the cross studied) and resistance to *H. turcicum* race 1 on 95 F_2 individuals from the cross DF20 × LH146Ht. The results indicate a tight linkage between one of the DNA markers, *UMC150B*, and the *Ht1* gene.

Key words: Maize – *Helminthosporium turcicum* race1 – RFLP – NILs – Mapping

Introduction

Northern leaf blight caused by *Helminthosporium turcicum* Pass. is an important foliar disease of maize (*Zea mays*) in the northern U.S. corn belt and other temperate areas of the world. The disease is sporadic in occurrence, depending on the environmental conditions (it is favored by moderate temperature and high humidity) and the level of disease resistance of the cultivars. When it becomes severe, yield losses of 30% or more can occur when susceptible lines are grown (Perkins and Hooker 1981). Two types of resistance to the pathogen are known. They are expressed in maize in the form of lesion number (Jenkins and Robert 1952; Hughes and Hooker 1971) or lesion type (Hilu and Hooker 1963; Hooker 1963). Lesion number is quantitative in expression, not expressed in seedlings, usually polygenic in inheritance, and highly heritable. This type results in a reduction in the number and size of lesions (Jenkins and Robert 1952; Hughes and Hooker 1971). A major gene, *HtN*, isolated from the Mexican maize variety Pepitilla acts in the same way, by preventing the formation of lesions on adult plants (Gevers 1975). Lesion type is qualitatively expressed as chlorotic-necrotic lesions, with limited fungus sporulation in the necrotic centers. This resistance is inherited as a single dominant gene, *Ht1*, and was first observed in inbred GE 440 and Ladyfinger popcorn (Hooker 1963). Two other major genes conferring chlorotic lesion resistance, *Ht2* and *Ht3*, appear to be present at independent loci (Hooker 1977, 1981). Many inbred lines were converted to *Ht1*-resistant varieties by the backcross procedure (Hooker and Soon Kwon Kim 1973).

The size variation of homologous DNA fragments after cleavage with restriction endonucleases has been termed restriction fragment length polymorphism (RFLPs). This recent class of genetic polymorphism has numerous characteristics of particular usefulness in genetic improvement (reviewed in Beckmann and Soller 1986).

Despite a high level of polymorphism in maize (Helentjaris et al. 1986), little work has been published concerning its use in mapping genes of agronomic interest. As a first approach, we decided to identify DNA markers linked to the *Ht1* gene. The strategy followed involved two steps. In the first step, we used near isogenic lines

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(NILs). Young et al. (1988) analyzed in detail the advantages of such a strategy in tagging the *Tm2a* gene in tomato. Informative DNA markers were identified by the polymorphism that they exhibited between the NILs. In the second step, these DNA markers were used in a linkage analysis on an F_2 population segregating for the *Ht1* resistance gene.

Materials and methods

Plant materials

Four pairs of NILs involving the inbreds B73, A619, W153R, and CM105 were used for preliminary screening of markers putatively linked to the *Ht1* gene. Segregation studies were then made on 95 F_2 plants from a cross between a susceptible line (DF20) and a resistant line (LH146Ht). Experiments for the determination of the source of *Ht1* resistance in the converted lines were made using two varieties: inbred GE440 and Ladyfinger popcorn. For NILs, inbred GE440 and Ladyfinger popcorn plant material was grown from seeds in the greenhouse (Lyon, France) and F_2 material was grown in the field (Lebanon/IN, USA).

Plant DNA preparation, restriction digests, and blotting

DNA was prepared and digested with *Eco*RI, *Bam*HI, or *Hind*III as described by Helentjaris et al. (1986), except that DNA was extracted from fresh material. DNA fragments were separated on agarose gel, denatured, and blotted onto nylon membrane (Amersham Hybond N).

Probe preparation, hybridization, and autoradiography

Inserts of genomic or cDNA clones were isolated by electroelution from agarose gels and labeled with [α^{32}P]-dCTP (Amersham) using the random primer labeling method of Feinberg and Volgelstein (1983). The labeled probe was separated from unincorporated nucleotides by chromatography on G100 Sephadex. Hybridization and washes of nylon membranes were performed according to Amersham's instructions. Filters were exposed at −80 °C to Amersham MP films for 3–7 days with an intensifying screen. Blots were prepared for reuse (up to five times) by a wash in 0.2 *M* NaOH for 30 min at 42 °C, followed by a wash in 0.2 *M* TRIS-HCl (ph 7.5), 0.5% SDS, 0.1 × SSC for 30 min at 42 °C.

NILs screening

Twenty-five probes [kindly donated by Dr. D. Hoisington (University of Missouri, Columbia/MO, USA) and by Dr. B. Burr (Brookhaven National Lab, Upton/NY, USA)] and one probe from a Rhone Poulenc Agrochimie cDNA library (RPA9A-SSU), all of them mapping to chromosome 2, were radiolabeled and hybridized individually to screen the NILs filters.

Helminthosporium turcicum inoculation and phenotypic scoring

The F_2 seeds were planted in the field on May 24, 1989 in a Ragsdale silty clay loam soil. Ten-day-old cultures of *Helminthosporium turcicum [Exserohilum turcicum* (Pass.) Leonard & Suggs] race 1 were grown on lactose casein hydrolysate medium in 100-mm diameter petri plates. The cultures and medium were mixed with water in a Waring blender. The resulting suspension was filtered through two layers of cheese cloth, and ca. 20 ml of the inoculum was sprayed with an approximately 8-liter garden sprayer into the whorl of each plant at the eight- to ten-leaf stage.

Each plant was classified as resistant (chlorotic lesion with little or no necrosis) or susceptible (wilted and necrotic lesion without chlorosis). The ratings were first taken approximatively 3 weeks after pollination, and every following week the plants were observed for any changes in the original reading.

Mapping by F_2 segregation analysis

Linkage analysis was done using the MAPMAKER (Lander et al. 1987) version 2.0 computer program. Two-point linkage analysis was first performed to determine the maximum-likelihood recombination fraction and the associated lod score of the 15 pairs of the five informative loci and the *Ht1* locus. Loci were considered to be unlinked if the lod score was <3. The gene order was determined by three-point analysis. For each of the three possible orders of each triplet, MAPMAKER computes the likelihood that the data would have arisen given the maximum-likelihood three-point map of that order. We then inferred multipoint map orders by rejecting any order containing unlikely triplets. Finally, full multipoint analysis was used to confirm the order deduced from the three-point analysis and to compute the likelihood of the map.

Results and discussion

Analysis of DNA from NILs

Of the 26 loci tested, six (*UMC139, UMC125A, UMC4, UMC22, UMC150B,* and *BNL6.20*), all located on the long arm of chromosome 2, exhibited a variable pattern between the NILs (Fig. 1, Table 1). These results fit with the mapped position of *Ht1* (Coe et al. 1988).

The observed polymorphism spreads from *UMC139* to *UMC150B* for B73 and W153R, and from *UMC125A* to *UMC150B* for A619 and CM105. Two DNA markers, *UMC125A* and *UMC150B,* were highly informative, being polymorphic for all pairs of NILs. The minimum size of the introgressed segment in the converted lines varies from about 25 cM for A619Ht and CM105Ht to about

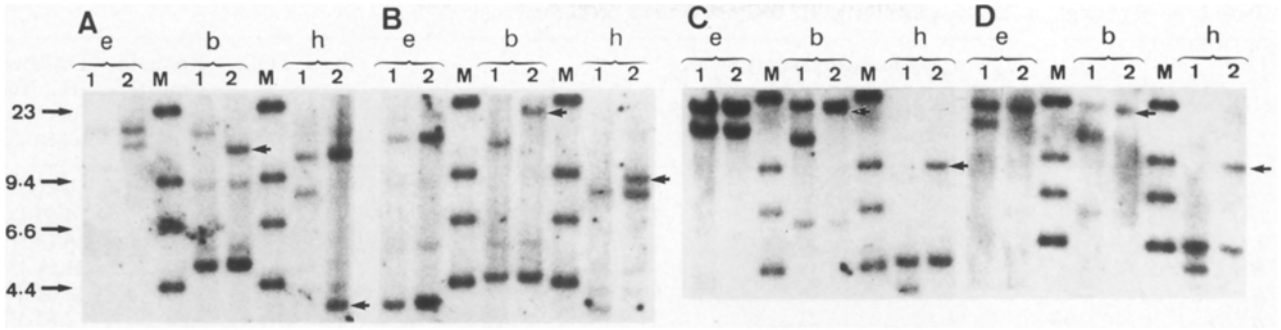

Fig. 1 A–D. Example of near isogenic line (NILs) screening. Filters contain pairs of NILs DNA without (*lane 1*) or with (*lane 2*) the *Ht1* gene. Genomic DNA was digested with *Eco*RI (*e*), *Bam*HI (*b*), or *Hin*dIII (*h*), electrophoresed in an agarose gel, blotted onto hybond N membrane, and hybridized to probe *UMC150*. **A** B73/B73Ht, **B** A619/A619Ht, **C** W153R/W153RHt, **D** CM105/CM105Ht. *Lane M* contains a molecular weight ladder of ^{32}P-labeled lambda *Hin*dIII-digested DNA, with the size of the fragments indicated in kilobase pairs on the upper left. Hybridizing bands of the converted lines corresponding to the locus *UMC150B* are indicated by *arrows*

Table 1. RFLPs exhibited between NILs by five of the six informative loci located on chromosome 2

	Loci	NILs											
		B73/B73Ht			A619/A619Ht			W153R/W153RHt			CM105/CM105Ht		
		E	B	H	E	B	H	E	B	H	E	B	H
UMC139	*UMC139*	*	*	0	0	0	0	*	0	0	0	0	0
6.6													
UMC98													
12.0													
UMC125A	*UMC125A*	*	0	*	*	*	0	*	*	*	*	0	*
4.4													
UMC122													
4.8													
2.5 *UMC137*	*UMC4*	0	0	0	0	0	0	0	*	0	0	0	0
1.8 *UMC4* *Ht1*													
UMC22=	*UMC22*	0	0	0	0	0	0	*	*	*	*	*	*
6.6 *UMC88*													
2.6 *NPI278A*													
NPI210													
2.9 *UMC150B*	*UMC150B*	*	*	*	0	*	*	0	*	*	*	*	*
9.3													
UMC171B-													
5.9 *OEC23*													
UMC49													
14.4													
UMC36													

E: *Eco*RI digests, B: *Bam*HI digests, H: *Hin*dIII digests; O: homomorphic patterns between NILs, *: polymorphic patterns between NILs; on the left of the table, the location of the markers on chromosome 2 (Hoisington and Coe 1989a). *BNL6.20* has not been located on this map

45 cM for B73Ht and W153RHt, according to a previously published map (Hoisington and Coe 1989a). Although these distances are approximations resulting from summations, they appear to be quite large, particularly for B73 Ht and W153RHt. According to Hanson (1959), the length of the introgressed segment for a chromosome of 1 Morgan is predicted to equal about 30 cM when the number of backcross generations is five (which is generally the minimum required in a breeding program). Young and Tanksley (1989), however, found a

Table 2. Joint segregation analysis involving *Ht1* and marker loci on chromosome 2

Loci	F_2 progeny in each phenotypic class[a]									n	LOD	Recombinant fraction MLE (cM)	Distance (Haldane function (cM)
	11,11	12,11	11,12	12,12	11,22	12,22	22,11	22,12	22,22				
Ht1[b], *UMC150B*	17		25		0		0	1	49	92	32.82	1	0.81
Ht1[b], *BNL6.20*[b]		39			2			3	40	84	22.2	4	4.69
Ht1[b], *UMC139*	17		26		1		0	9	38	91	19.12	8	9.13
Ht1[b], *UMC125A*[b]		39			5			15	29	88	8.19	19	23.58
Ht1[b], *UMC4*	7		6		6		2	4	13	38	2.38	27	38.61
BNL6.20[b], *UMC150B*	16		23		2		0	1	40	82	24.46	3	2.87
UMC125A[b], *UMC150B*	17		22		14		0	4	29	86	9.63	17	20.91
UMC139, *UMC150B*	14	3	3	22	0	8	0	1	38	89	24.7	9	9.49
UMC4, *UMC150B*	4	3	3	3	2	4	1	4	13	37	2.8	28	41.28
BNL6.20[b], *UMC125A*[b]		37			4			10	28	79	9.93	15	17.16
BNL6.20[b], *UMC139*	16		25		0		0	4	36	81	21.83	4	4
BNL6.20[b], *UMC4*	7		7		5		2	3	14	38	3.12	23	31.52
UMC125A[b], *UMC139*	17		31		6		0	4	30	88	14.62	10	10.59
UMC4, *UMC139*	6	3	2	5	1	2	0	6	13	38	5.11	21	26.49
UMC125A[b], *UMC4*	8		9		4		0	1	15	37	6.56	11	12.12

[a] Phenotypic designations: 1 = LH146Ht, 2 = DF20

[b] 11 and 12 cannot be differentiated due to dominance (*Ht1*) null allele (*UMC125A*) or similar hybridization patterns (*BNL6.20*)

wide variation in the size of introgressed segments, with a maximum of 51 cM for Craigella *Tm2a*, a tomato converted line produced through 11 backcross generations.

Analysis of DNA from F_2 plants

To further support the putative linkage between the six chromosome 2 DNA markers and the *Ht1* gene, a segregation analysis was carried out on an F_2 population from the cross DF20 × LH146Ht. *UMC22* was monomorphic in this cross with the restriction enzymes used, thus precluding the study of its segregation. Table 2 shows significant linkage between all pairs of characters surveyed, except for two of them: *UMC4-Ht1* and *UMC4-UMC150B*. Only one recombinant was found between *Ht1* and *UMC150B*. The recombination frequency between them is estimated to be 0.8 cM by the method of maximum likelihood (Allard 1956). It should be pointed out that Hoisington and Coe (1989a, b) indicated the relative position of the *Ht1* gene on the map of chromosome 2 by showing linkage between this gene and the RFLP markers *UMC125A, UMC122, UMC137, UMC4,* and *UMC22* (see the map presented in Table 1). Their results converge with those presented in this paper for the markers *UMC4* and *UMC125A* (we did not analyze *UMC137* because of the poor quality of the hybridization bands and *UMC122* because it was not available). In addition, our results reveal a tight linkage of the *Ht1* gene with the RFLP marker *UMC150B*.

This tight linkage is supported by the observation that the converted lines can be easily classified into two groups by their allelic form of *UMC150B*. In one group (A619Ht, W153RHt, CM105Ht, and LH146Ht) all lines show the same specific band attributed to the locus *UMC150B* (absent in the corresponding NILs without the *Ht1* gene) when their DNA are digested by *Bam*HI (20 kbp) or by *Hin*dIII (9.3 kbp) (Fig. 1, data not shown for LH146Ht). In the other grou we only find B73Ht, which shows a different specific band: 13.5 kbp or 3.8 kbp when its DNA is digested by *Bam*HI or *Hin*dIII, respectively (Fig. 1). These two allelic forms of *UMC150B* fit well with the presumptive origin of the *Ht1* resistance in the converted lines: either inbred GE440 or Ladyfinger popcorn. We have further examined the patterns of hybridization of probe *UMC150* with these two lines. GE440 shares the same hybridizing band that is present in A619Ht, W153RHt, CM105Ht, and LH146Ht, while Ladyfinger popcorn shares the same band with B73Ht (data not shown). This suggests that the *Ht1* resistance allele has been bred from GE440 to A619Ht, W153RHt, CM105Ht, and LH146Ht, and from Ladyfinger popcorn to B73Ht.

The map of chromosome 2 near gene *Ht1* is shown in Fig. 2. Two probes *UMC36* and *UMC98*, failed to disclose any polymorphism between the two parents, despite the use of 25 restriction enzymes, and hence could not be used to determine the orientation of the present map in relation to the previously published map (Hoisington and Coe 1989a). Therefore, we chose the orientation that gives the best fit (*UMC4* at the bottom) with the previous map. In addition, it should be noted that a new position for *UMC150B* (now proximal to *UMC139*) has been determined (compare the maps presented in Table 1 and Fig. 2). With this new order and the new calculated map distances, the minimum size of introgressed fragments are reduced to about 20 cM for B73Ht, A619Ht,

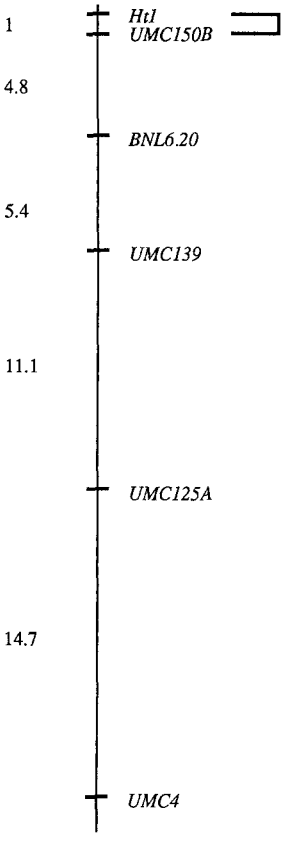

Fig. 2. Linkage map of chromosome 2 showing the region near the *Ht1* gene. All distances are given in centiMorgans using Haldane's mapping function (Haldane 1919) and were derived from the multipoint analysis, explaining the small discrepancies with the distances calculated from the two-point analysis (Table 2). This map (with a possible inversion of *Ht1* and *UMC150B* as indicated by the bracket) is at least 10^3 more probable than any other map which can be established with our data

and CM105Ht. This better fits the expected length for introgressed fragments (Hanson 1959). In contrast, the minimum size of the introgressed fragment is still ca. 40 cM for W153RHt. In fact, this converted line presents the weakest rate of isogenicity since it contains other donor fragments on chromosomes 3, 4, and 7 (data not shown).

Conclusion

The strategy followed allowed us to identify tight linkage of an RFLP marker, *UMC150B*, to the *Ht1* gene conferring resistance to *Helminthosporium turcicum* race 1. This linkage (≤ 1 cM) would be of great usefulness in breeding programs in facilitating the introgression of the *Ht1* gene into commercial varieties. Another interesting application of our results would be the use of *UMC150B* as a starting point for molecular approaches such as chromosomal walking to clone the *Ht1* gene (Ellis et al. 1988).

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