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Molecular-marker-facilitated investigation of host-plant response to *Exserohilum turcicum* in maize (*Zea mays* L.): components of resistance

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Abstract RFLPs were used to investigate components of host-plant response to *Exserohilum turcicum* in 150 unselected $F_{2:3}$ lines of a B52/Mo17 maize population. Following inoculation with spore suspensions of the pathogen (race 0), components of disease development were measured and then quantitative trait mapping was performed to identify the location and effects of quantitative trait loci (QTLs) determining host-plant response. Components of interest were the average number of lesions per leaf, the average percent leaf tissue diseased (severity) and the average size of lesions (cm^2). Based on a LOD threshold of 2.31 ($P < 0.05$), the number of lesions appears to be associated with QTLs on chromosomes 1S, 3L, 5S. Severity was associated with analogous regions and, in addition, QTLs on chromosomes 7L and 8L. Most QTLs, for either of these two components, involve additive gene action and partial dominance or overdominance. In contrast, lesion size was associated with QTLs on chromosomes 7L and 5L; recessive gene action may be involved at 7L.

Key words Breeding · *Helminthosporium turcicum* · RFLP · QTLs · Disease-resistance · Genetics

Introduction

Helminthosporium diseases are widespread throughout the world with the different species of causal fungi assigned to anamorphic genera of *Biopolaris*, *Drechslera*, or *Exserohilum*. They cause leaf blights, spots, or rots on many important crops (Agrios 1988). In maize, Northern Corn Leaf Blight (NCLB) is incited by *Exserohilum turcicum* Pass. (syn. *Helminthosporium turcicum* Pass.) and is a significant limitation to yield potential in humid and temperate regions. Plants affected by NCLB will also be predisposed to stalk- and root-rot infections (Perkins and Pedersen 1987; Dodd 1980).

Control of NCLB in maize is achieved largely by use of resistant germplasm (Ullstrup 1977). Several qualitative sources of resistance are known, namely *Ht1*, *Ht2*, *Ht3* and *HtN* – referring to their locus designations (Hooker 1963, 1975, 1977; Gevers 1975). *Ht1*, *Ht2* and *Ht3*, result in small chlorotic lesions and the amount of necrotic tissue, fungal sporulation, and inoculum for secondary infections are all reduced (Hooker and Kim 1973; Ullstrup 1977). The *HtN* gene results in a delay in lesion development until after flowering (Gevers 1975). The *Ht1* gene was formerly widely utilized in commercial maize hybrids. Estimates suggest 90% of the hybrids grown in the northern two-thirds of the U.S. maize belt during the mid 1970s carried the *Ht1* gene (Simone 1978).

Current breeding programs rely predominantly on resistance which exhibits a quantitative inheritance pattern. This type of resistance is effective against all presently known races of *E. turcicum*, in contrast to loci with qualitative effects (Smith and White 1988). With quantitative inheritance patterns, resistance seems to be manifested primarily by a reduction in lesion number and a broad range of levels of resistance has been observed (Hooker and Kim 1973). There are conflicting reports about the effect of quantitatively-inherited resistance on lesion size; some indicate that lesion size is reduced (Hilu and Hooker 1964; Smith and White 1988) while others suggest that there is no reduction in lesion size (Ullstrup 1977). There are many other components of resistance; for example, infection efficiency, incubation period, latent period, lesion expansion rate, and

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sporulation capacity. These can also be considered when assessing host-plant response to *E. turcicum* (Brewster et al. 1992). Precise measurements on a large number of components, however, is generally not feasible for replicated field evaluations of segregating populations.

Initial attempts to map quantitatively-inherited resistance to NCLB in maize involved a series of reciprocal translocations. Resistance, based on visual estimates of disease severity, appears associated with as many as 12 chromosome arms. Regions on the long arm of chromosomes 3 and 5 and the short arm of chromosome 7 appear to play a particularly important part in conditioning resistance in several inbred lines (Jenkins et al. 1957; Jenkins and Robert 1961). Generation-means analysis indicated that much of this resistance was highly heritable, involving between three to six genes with predominantly additive gene action (Hughes and Hooker 1971). A more recent study indicated that chromosome 3, the short arm of chromosome 4, and the long arm of chromosome 6 were of importance in reducing disease severity; the latter two regions were also associated with a reduction in lesion number and incubation period (Brewster et al. 1992).

The objectives of the present study were to use RFLP markers to obtain more precise estimates of the chromosomal location and genetic effects of QTLs conferring resistance to *E. turcicum* in maize; in particular to dissect host-plant response into separate components by using non-subjective disease assessments. The components chosen are number of lesions, size of lesions and their composite, percent leaf tissue diseased (severity).

Materials and methods

Population development, and disease and agronomic data analysis

Two inbred maize lines, Mo17 and B52, were used as parental material for the population and as check inbreds (per se). Mo17 shows partial resistance to NCLB, while B52 is very susceptible. From a single self-pollinated F_1 plant, 150 random F_2 plants were self-pollinated to create the 150 unselected F_3 lines used in field trials. Border rows consisted of bulked F_3 seed from the same population. Row length was 5.5 m with 0.76 m spacing between rows. Plots were thinned to 20 plants per row, approximately 48 000 plants ha^{-1}. Standard management practices regarding fertilization and cultivation were followed. Two replications of a sets-within-replications design were used. Twenty-seven entries, including both parents, were nested within each of six sets (12 sets total). Experiments were grown at Ames, Iowa, and at Urbana, Illinois, in the summer of 1991. The Illinois location was subsequently abandoned due to inadequate disease development. Disease inoculation (race 0) and disease assessment at Ames, Iowa, have been previously described (Freymark et al. 1993). For spore production, isolate HE62 of *E. turcicum* race 0 was cultured on agar under fluorescent lighting for 12 h daily. Plants were twice inoculated in the whorl with a spore suspension of race 0 (24 June and 9th July, 1991; 45 and 60 days after planting respectively). Disease development was rated twice commencing on the 7th August, and 4th September, 1991 (89 and 117 days after planting respectively). Data were collected from the 12 innermost plants per row on four leaves per plant for a total of 48 leaves per entry per replication. The F_3 lines were evaluated for host-plant response focusing on the average number of lesions per leaf (number), the average percent leaf tissue diseased (severity), and the average size of lesions in cm^2 (size). The first assessment took 1 day per replication, the second assessment took 4 days per replication to complete. Trait values in the second replication of the second assessment tended to be higher than those of the first replication. $F_{2:3}$ trait means for QTL mapping were produced by averaging over both replications as this represents the most precise estimate.

In addition, at the Ames location, the following agronomic data were recorded, grain weight (Gwt, weight in grams per plot of shelled grain dried to uniform moisture), plant height (Plth, average height in cm from the soil level to the tip of the central tassel spike for five randomly-chosen plants per plot), growing degree days to pollen shed (P_GDD, based on daily maximum, $\leq 30\,^{\circ}C$, and minimum, $\geq 10\,^{\circ}C$, temperatures; calculated as accumulated heat units from time of planting to time when 50% of the plants in a plot reached anthesis), growing degree days to silk emergence (S_GDD, based on daily maximum, $\leq 30\,^{\circ}C$, and minimum, $\geq 10\,^{\circ}C$, temperatures; calculated as accumulated heat units from time of planting to time when 50% of the plants in a plot have exposed silks), and number of ears per plot (Ears).

Phenotypic (r_p) correlations among traits were calculated according to Mode and Robinson (1959). Heritability estimates, on a progeny means basis, and variance components, were calculated according to the formula given by Hallauer and Miranda (1988). Exact 90% confidence intervals for heritabilities were calculated according to the formula given by Knapp et al. (1985). Data from the first assessment (day 89) were not normally distributed even with square root, arc sin, or $\log_{10}$ transformations; consequently these data were not used. Data from the second assessment (day 117) responded favorably to transformation, in particular using $\log_{10}$. With this transformation, normality was achieved for lesion number, percent severity, and lesion size in cm^2 ($W = 0.97$, 0.97 and 0.99 respectively, all non-significant at the 0.05 probability level) as described by the W statistic (Shapiro and Wilk 1965). Consequently the $\log_{10}$-transformed means of second assessment data were used for QTL mapping.

RFLP data analysis

RFLP assays and quantitative trait mapping for this population have been previously described (Freymark et al. 1993). RFLP analyses included 109 probes hybridized to single digests of genomic DNA samples. The samples were prepared from bulk leaf tissue samples of ten F_3 seedlings of each self-pollinated F_2 plant. The linkage map was constructed using MAPMAKER 1.9 (Lander et al. 1987). A lod score of 2.31 was calculated as the LOD significance threshold ($P < 0.05$) based on the number of intervals (Freymark et al. 1993). Disease data (unadjusted means) from the F_3 lines were used when scanning the genome for putative QTLs (Lincoln and Lander 1990). Once individual QTLs were identified, a multiple-QTL model was employed by considering all QTLs for a trait simultaneously in a single model.

Single-factor analysis of variance (SFAOV) (Edwards et al. 1987) was also employed to detect significant variation among marker class means at the 0.05, 0.01 and 0.001 probability levels.

Each individual F_3 line has one of three possible genotypes at any QTL, namely AA (homozygous for Mo17), AB (heterozygous) or BB (homozygous for B52). Trait values for each QTL, averaging over F_3 lines, can be represented as μAA, μAB and μBB respectively. These describe putative gene effects at each QTL and were calculated using the models as presented by Lincoln and Lander (1990):

$$\mu AA = \mu$$

$$\mu AB = \mu + A_B + D_B$$

$$\mu BB = \mu + 2\,(A_B),$$

where μ = residual value in the absence of B alleles, A_B = the additive component of the QTL B allele effect, D_B = the dominance component of the QTL B allele effect. Since a transformation had been used to normalize the F_3 line means, μAA, μAB and μBB were transformed back to their original scale only for comparative purposes.

The degree of dominance (d/a), which describes the predominant type of gene action, can be calculated as the estimated dominance effect divided by the estimated additive effect. The d/a ratio was reported on the effects as calculated by MAPMAKER/QTL without restoration to the original scale. The d/a ratio describes the type of gene action for each QTL and the guidelines presented by Stuber et al. (1987) were adopted, where additiive gene action = 0 to 0.20, partial dominance = 0.21 to 0.80, dominance = 0.81 to 1.20, over-dominance = > 1.20.

The contributing parent was determined by the sign of the additive component of the QTL B allele effect. This was then confirmed by comparing the average trait values. Estimates of dominance effects in this study are double those reported by MAPMAKER/QTL as F_3 lines were used for phenotyping rather than individual F_2 plants.

Genomic composition

The genomic composition of individual F_3 lines at each scoreable locus falls into one of three possible classes, namely homozygous for Mo17 (*AA*), homozygous for B52 (BB) and the heterozygote B52/Mo17 (*AB*). Frequency distributions for individual lines were calculated by summing the number of loci representing each class and dividing this by the total number of scoreable loci. In contrast to the method reported by Paterson et al. (1988), interval length was not taken into account; consequently the results reported in the present study may be more biased. Neither method accounts for double recombinations within an interval.

Leaf penetration by pathogen and early establishment

A preliminary study was conducted to see if there were observable differences in the early stages of pathogenesis of *E. turcicum* race 0 on leaves of inbreds Mo17 and B52. Fifty plants of each inbred were grown in the greenhouse, five plants per pot. Nineteen days after planting, leaf samples 5–10 cm in length were taken from the 2nd uppermost leaf; a total of 30 samples were collected for each inbred. Samples were washed in cold water, dried, and then placed in a moist crisper for inoculation with spores of race 0. The upper surface of the leaf was rubbed with the flat surface of a scalpel in order to break leaf hairs. The midrib of each sample was marked three times at approximately 1.5 cm intervals with a permanent marker. Inoculum (10 µl, 25–30 spores) was applied on either side of the upper surface of the leaf blade at a point equidistant from the margin and the midrib. Samples were kept moist and removed at 6, 12 and 24 h and examined for surface penetration phenomena using 0.1% acid fuchsin in lactophenol as a stain, and histological examination following leaf clearing (Mohan Ram and Nayyar 1978).

Results

Genome composition

Frequency distributions are shown in Fig. 1. Genomic composition ranged from 3.5 to 47.8% (mean 22.9%) homozygous from Mo17 (*AA*); from 0.0 to 51.4% (mean 22.8%) homozygous for B52 (*BB*); and from 25.7 to 88.9% (mean 54.3%) for the heterozygote B52/Mo17 (*AB*). Standard deviations were 9.3%, 10.0% and 11.6% respectively. These values are within the frequencies expected for F_3 lines.

Variance components

Genotypic variances of F_3 lines were significant for all attributes of disease development (Table 1). Broad-sense heritabilities, in percent, were 69.6% for number of lesions, 62.8% for severity, and 31.9% for size of lesions (Table 1). These estimates, however, are biased upwards as estimates of genotype-by-environment interactions were not available.

Disease development

Trait values, based upon each inbred check within all 12 sets, were not significantly different ($P < 0.05$) indicating that the inoculum procedures and subsequent disease development were relatively uniform across the experiment. Border plots that were not inoculated were virtually free of NCLB indicating that there was little

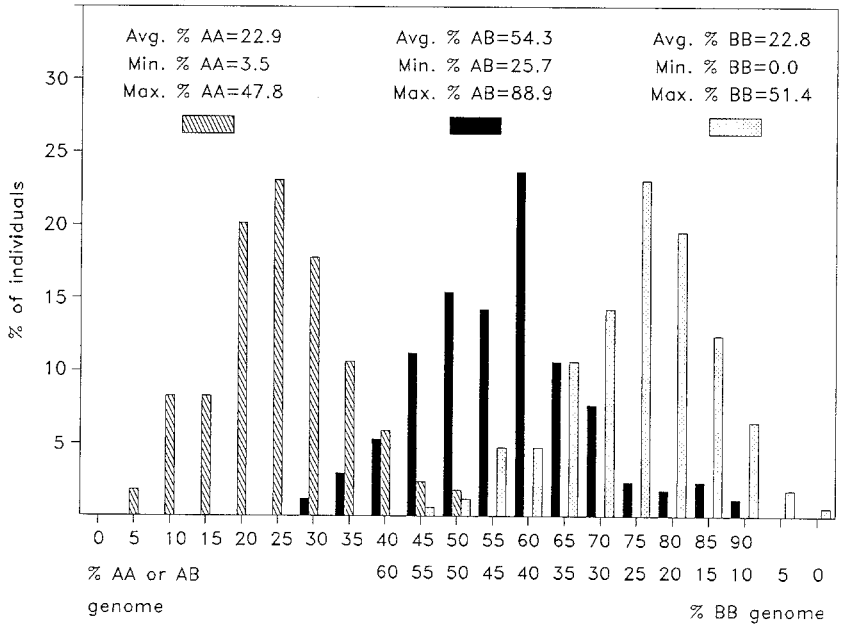

Fig. 1 Frequency distribution for percent heterozygosity (*AB*), percent homozygous Mo17 (*AA*) and percent homozygous B52 (*BB*) from 150 $F_{2:3}$ lines derived from the cross B52 × Mo17. For details on computation see Materials and methods

Table 1 Heritability estimates, variance components and associated standard errors for attributes of host-plant response to *E. turcicum* in F_3 lines of a B52/Mo17 population (h^2 percent heritability on a progeny mean basis, *UCL* and *LCL* 90% exact upper and lower confidence intervals for heritability estimates, σ_e^2 error variance, σ_{ph}^2 phenotypic variance, σ_g^2 genotypic variance)

Trait	h^2	UCL	LCL	$\sigma_e^2 \pm$ SE	$\sigma_{ph}^2 \pm$ SE	$\sigma_g^2 \pm$ SE
Number	69.6	76.9	60.0	0.86 ± 0.10	1.41 ± 0.17	$0.98^{**} \pm 0.17$
Severity	62.8	71.7	51.0	2.46 ± 0.29	3.30 ± 0.39	$2.07^{**} \pm 0.41$
Size	31.9	48.3	10.3	12.82 ± 1.50	9.41 ± 1.10	$3.00^{**} \pm 1.33$

** Significant at 0.01 level of probability

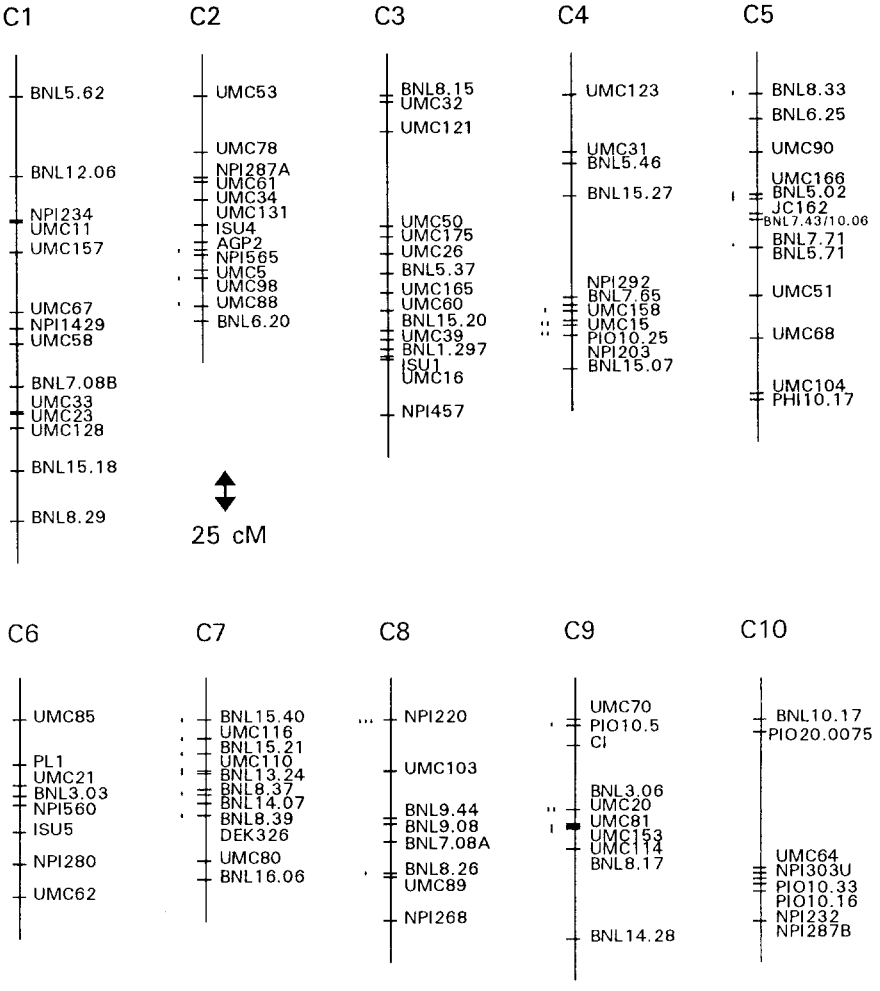

Fig. 2 Linkage map for the Mo17/B52 population created using 169 random $F_{2:3}$ lines and 112 probes. BNL5.02, BNL13.24, UMC153, UMC114, UMC81, UMC11, BNL10.06, UMC23 and ISU1 were included subsequent to performing quantitative trait mapping. All are closely linked (< 2 cM) to existing loci; their inclusion did not change total map distance or LOD scores appreciably. Probes with significant distortion from 1:2:1 goodness of fit ratio are marked with *, ** and *** indicating probability levels of 0.05, 0.01 and 0.001 respectively

natural inoculum. On average, B52 had twice as many lesions per leaf as Mo17 (2.09 and 1.08 respectively) and over four-times as much leaf tissue diseased (4.38% and 0.99% respectively). In addition, average lesion size on B52 was over twice that of those on Mo17 (10.83 cm^2 and 4.07 cm^2 respectively).

Quantitative trait mapping

The 111 loci included in quantitative trait mapping were used to produce the linkage map shown in Fig. 2. Twenty-six loci, involving seven linkage groups, deviated ($P < 0.05$) from a 1:2:1 ratio. This is not unusual and has been observed in other maize RFLP mapping studies (Doebley et al. 1990); consequently, loci that deviated from the expected ratio were not excluded. The distorted ratios and their frequencies were as follows: excess of heterozygotes (21 probes), excess B52 homozygotes (four probes), and excess Mo17 homozygotes (one probe).

The location and effects of QTLs affecting host-plant response are summarized in Table 2. Overall, five linkage groups are associated with host-plant response;

however, the number varies with each component analyzed. With the exception of QTLs on the short arm of chromosome 1, resistance is always contributed by Mo17 (Table 2). Three QTLs, on chromosomes 1S, 3L, and 5S are important in determining lesion number, explaining from 6.8% to 13.2% of the phenotypic variation for this trait. Severity is associated with corresponding regions and, in addition, chromosome 7L and 8L. Phenotypic variation explained ranges from 7.5% to 13.4%. These two traits involve primarily partial dominance or overdominance.

Chromosomes 5L and 7L are important in determining lesion size. Recessive gene action appears important on 7L; the *d/a* ratio suggests overdominance but closer inspection of gene effects, and the sign of the additive component of the B52 allele effect (Table 2), reveal that resistance is contributed by the Mo17 (A) allele and a reduction in lesion size is most evident when the two copies of this allele are present. The log-likelihood plots for chromosomes with QTLs are shown in Fig. 3. The number of lesions and the percent leaf tissue diseased in this population appear to be controlled by analogous regions of the genome and consequently these two traits have comparable log-likelihood plots (chromosomes 1S, 3L, 5S, 7L and 8L). This is not surprising considering the high phenotypic ($r_p = 0.95$) correlation between these two traits. For each trait the multiple QTL LOD scores effectively equaled the arithmetic summation of the individual QTL map LOD scores (Table 2) suggesting that, while QTLs may explain autonomous portions of the variation, their action is cumulative (Lincoln and Lander 1990).

Table 2 Location and effects of QTLs affecting host-plant response to *E. turcicum* in F_3 lines of a B52/Mo17 population. (*Interval* RFLP loci interval in which the LOD threshold was exceeded, neighboring peaks not separated by a log-likelihood reduction of 1.0 were considered local maxima of the same peak. *Chr.* linkage group which includes the QTL interval. Short (S) or long (L) arm designations are approximate only as centromere probes were not included. *Var.* percent phenotypic variation for a trait explained by an individual QTL. *a*, additive effects ($\log_{10}$ units) associated with the B (B52) parent allele. A negative value indicates that the B52 allele affects host-plant response favorably, a positive value indicates that the B52 allele affects host-plant response favorably. *d* dominance effects ($\log_{10}$ units). Estimates of *d* from MAPMAKER/QTL refer to the $F_{2:3}$ lines, consequently these values were doubled for inference to the F_2 plants. *Gene effects*, putative gene effects at each QTL where μAA represents the QTL trait value for all individuals homozygous Mo17; μBB, the QTL trait value for all individuals homozygous B52, and μAB the QTL trait value for all heterozygous individuals. For details of computations see Materials and methods. *Contrb. parent* parental inbred responsible for a reduction in trait values at a QTL. *Gene act.* gene action determined from the ratio of dominance to additive effects (*d/a*), *OD* overdominance, *DOM* dominance, *PD* partial dominance, and *ADD* additive gene action respectively, see Materials and methods for guidelines adopted. Multiple-QTL model, total percent variation explained and LOD score calculated for each trait in a model that included all individual QTLs

Average number of lesions per leaf (number)

Interval	Chr.	Var.	LOD[a]	*a*	*d*	Gene effects			Contrb. parent	*d/a*	Gene act.
						μAA	μAB	μBB			
UMC157-UMC67	1S	6.8%	2.31	−0.077	−0.250	1.69	0.80	1.19	B52	3.26	OD
UMC16-NPI457	3L	13.2%	4.60	0.171	0.067	0.82	1.43	1.81	Mo17	0.39	PD
BNL6.25-UMC90	5S	11.8%	3.85	0.161	−0.210	1.01	0.90	2.12	Mo17	−1.30	OD
Multiple-QTL model		29.5%	11.10								

Average percent leaf tissue diseased (severity)

Interval	Chr.	Var.	LOD[a]	*a*	*d*	Gene effects			Contrb. parent	*d/a*	Gene act.
						μAA	μAB	μBB			
UMC157-UMC67	1S	8.0%	2.71	−0.080	−0.300	2.42	1.01	1.67	B52	3.75	OD
UMC16-NPI457	3L	9.4%	3.22	0.156	−0.057	1.27	1.59	2.60	Mo17	−0.36	PD
UMC90-UMC166	5S	13.4%	4.14	0.184	−0.193	1.31	1.29	3.06	Mo17	−1.05	DOM
BNL15.21-UMC110	7L	13.2%	3.84	0.200	−0.011	1.08	1.67	2.70	Mo17	−0.06	ADD
BNL9.08-BNL7.08A	8L	7.5%	2.32	0.133	−0.054	1.31	1.57	2.41	Mo17	−0.41	PD
Multiple-QTL model		44.6%	17.30								

Average size of lesions in cm^2 (size)

Interval	Chr.	Var.	LOD[a]	*a*	*d*	Gene effects			Contrb. parent	*d/a*	Gene act.
						μAA	μAB	μBB			
BNL5.71-UMC51	5L	18.1%	4.32	0.093	−0.083	6.71	6.86	10.28	Mo17	−0.90	DOM
UMC116-BNL15.21	7L	12.3%	3.70	0.065	0.143	6.11	9.87	8.23	Mo17	2.21	OD
Multiple-QTL model		28.9%	7.98								

[a] LOD threshold 2.31

Phenotypic correlations in relation to disease development

Phenotypic correlations between attributes of disease development, agronomic traits, and genome composition are shown in Table 3. Maturity, expressed as grow-

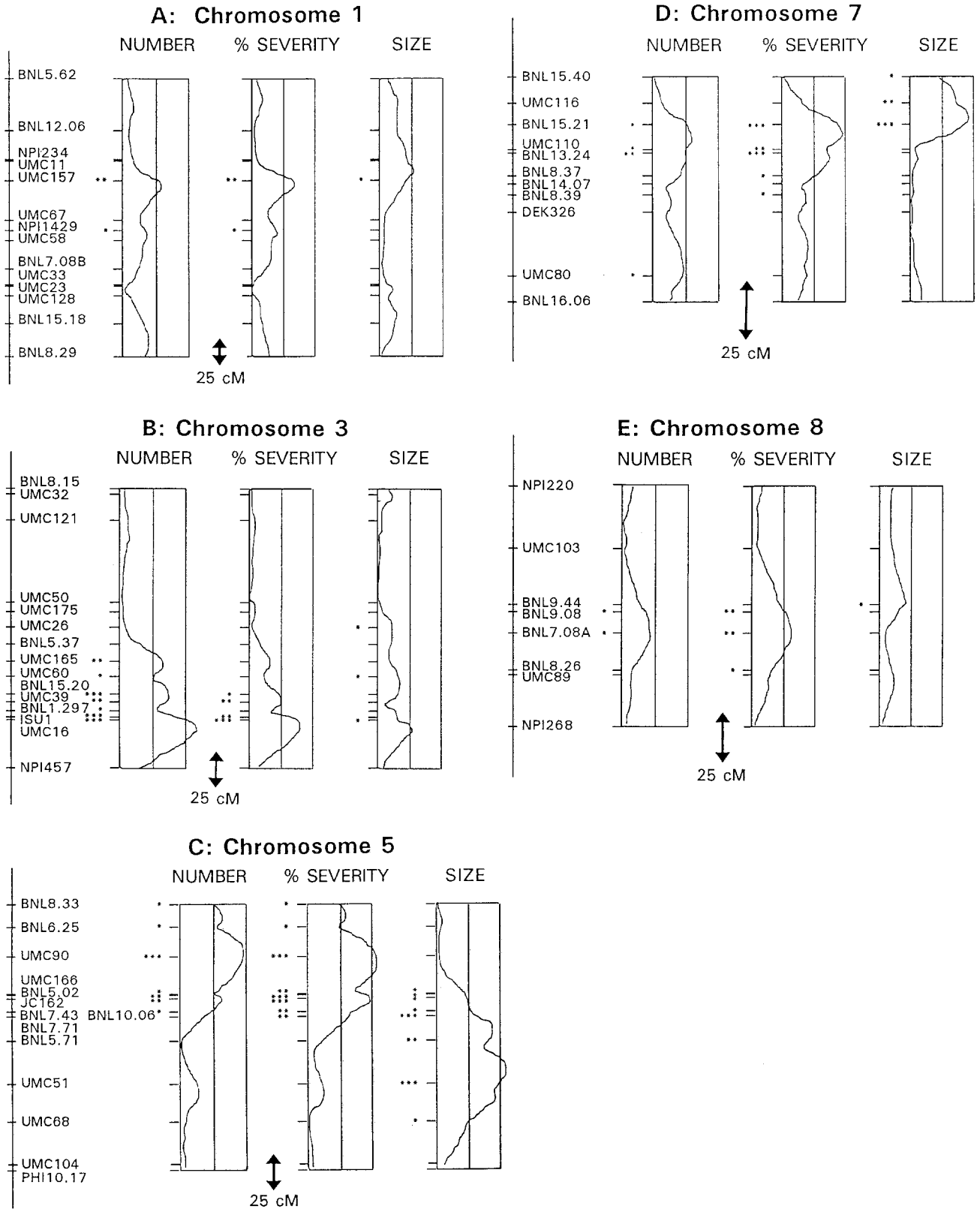

Fig. 3 A–E Linkage map and log-likelihood plots of chromosomes 1, 3, 5, 7 and 8 for average number of lesions per leaf (number), average percent leaf tissue diseased (severity) and average size of lesions in cm^2 (size). *Vertical lines* represent LOD scores of 0, 2 and 4 respectively. (LOD of 2.31 used as threshold for declaration of a QTL). SFAOV results: *, ** and *** indicate significance at 0.05, 0.01 and 0.001 levels respectively

Table 3 Phenotypic correlations between attributes of disease development, agronomic traits, and genome composition as measured in F_3 lines[a]

Trait	Disease development			Agronomic traits					Genome composition		
	Number	Severity	Size	Pltha	Gwt	Ears	P_GDD	S_GDD	%*AB*	%*AA*	%*BB*
Number		0.95**	−0.22**	0.04	−0.06	−0.13	−0.02	−0.12	0.00	−0.14	0.13
Severity			−0.08	0.06	−0.07	−0.14	0.05	−0.06	−0.03	−0.20*	0.22**
Size				0.12	−0.07	−0.08	0.38**	0.35**	−0.03	−0.24**	0.26**
Pltht					0.07	−0.02	0.37**	0.29**	0.07	0.04	−0.12
Gwt						0.80**	−0.30**	−0.46**	0.24*	−0.02	−0.26**
Ears							−0.24**	−0.41**	0.04	0.14	−0.17*
P_GDD								0.80**	−0.14	−0.15	0.29**
S_GDD									−0.20*	−0.07	0.29**
%*AB*										−0.56**	−0.64**
%*AA*											−0.30**

[a] For explanation of symbols, see Materials and methods
*,** Significant at 0.05 and 0.01 levels of probability, respectively

ing degree days to pollen shed or silk emergence, appears to be correlated with lesion size ($r_p = 0.38$ and 0.35, respectively); however, this is not true for the number of lesions or the percent leaf tissue diseased (Table 3). All other correlations between attributes of disease development and agronomic traits are of smaller magnitude. Plant height was not correlated with disease attributes. Phenotypic correlations between attributes of disease development and percent homozygous Mo17 (*AA*) are always positive. The percent heterozygosity is not correlated with attributes of disease development, unlike agronomic traits, for example grain weight, where a low correlation was detected.

Leaf penetration by pathogen and early establishment

Spore germination was good on both inbreds and exceeded 85% in all samples. With both Mo17 and B52 appressorium development was evident within 6 h after inoculation. Fungal penetration was direct and occasionally through stomata. Differences between Mo17 and B52 were not evident from the histological examinations following leaf clearing. Mycelium growth within leaf tissue on both inbreds was evident 24 h after inoculation; however, there was a high degree of spore disruption and quantitative comparisons were not possible.

Discussion

The use of RFLPs to study host-plant response in maize to plant pathogens is a relatively new application. Host-plant response to maize dwarf mosaic virus was investigated using individual backcross plants screened in the greenhouse. Three-point linkage analysis suggests tight linkage between an RFLP locus and a gene conditioning host-plant response on chromosome 6 (McMullen and Louie 1989). Bubeck et al. (1993) used RFLPs to study host-plant response to gray leaf spot in three populations using natural inoculum and visual disease assessments; the QTLs identified were not consistent over environments. Tight linkages between RFLPs and the *Ht1* and *Ht2* genes have been demonstrated in studies utilizing near-isogenic lines and individual F_2 plants (Bentolila et al. 1991; Zaitlin et al. 1992).

Using molecular-marker-facilitated quantitative trait mapping, the resolution of host-plant response into discernible components is evident in the present study. Different regions of the genome, as well as different modes of gene action, are implicated in controlling attributes of host-plant response. Estimates of overdominance in the F_2 populations may be pseudo-overdominance due to the combined action of linked genes rather than overdominance at individual loci (Hallauer and Miranda 1988). Imposing generations of random mating may break these linkages.

The number of lesions and disease severity show similar log-likelihood plots across five linkage groups. In contrast, lesion size in this population appears to be controlled by different regions of the genome (5L as opposed to 5S) or by somewhat different modes of gene action where QTLs coincide (7L). Regarding QTLs on chromosome 7L, QTL mapping gives indications of genomic regions rather than exact gene locations; consequently, it is impossible to say if the same gene or set of genes is involved in determining lesion size versus lesion number on chromosme 7L. One gene may have more than one distinct phenotype (pleiotropy) or alternatively, several closely-linked genes may be involved. The RFLP mapping results obtained in this study concur with the early translocation mapping experiments (Jenkins et al. 1957; Jenkins and Robert 1961) on the importance of chromosomes 3, 5 and 7,

despite the fact that these studies have utilized diverse germplasm.

The high phenotypic correlation for lesion number and percent severity, in addition to the comparable log-likelihood plots for the five linkage groups, strongly support earlier studies indicating that quantitatively-inherited resistance seems to be manifested primarily by a reduction in lesion number (Hooker and Kim 1973). The fact that the number of lesions is highly correlated with the percent leaf tissue diseased but not with the size of lesions may appear confusing at first. Essentially, in this population host-plant resistance appears to be achieved by a reduction of numbers of lesions, rather than their size. That is not to say that QTLs affecting size are not discernible, but rather that their contribution to determining the composite trait, severity, is small relative to that made by lesion number. If lesions are prevented from forming then their size becomes a moot point; consequently correlations between size and severity are low. In contrast, resistance genes characterized by chlorotic margins around lesions (e.g., *Ht1* and *Ht2*) have qualitative-inheritance patterns and a more marked effect on lesion size. This, in addition to observable differences in lesion morphology, suggests that different resistance mechanisms may be involved. QTLs that primarily control lesion number may be doing this by resisting the pathogen at an earlier stage of pathogenesis than the qualitatively-inherited loci. Different mechanisms are also implicated by the fact that the quantitatively-inherited resistance is effective against all presently known races of *E. turcicum*, in contrast to loci with known qualitative effects where race-specific interactions are evident (Smith and White 1988). An additional piece of evidence comes from the observation that qualitatively-inherited sources of resistance, in particular those conferring chlorotic lesion types, are clearly discernible in seedlings, whereas quantitatively-inherited sources of resistance are not (Hilu and Hooker 1964). The QTLs identified in this study do not coincide with the location of the *Ht1* or *Ht2* genes (Freymark et al. 1993); the location of *Ht3* is currently unknown. The *HtN* gene has recently been mapped to the long arm of chromosome 8 (J. Bennetzen, personal communication). While the *HtN* gene is inherited as a single dominant gene, it has been termed a 'lesion number type' of resistance (Hooker 1977) and may have similar mechanisms to those of quantitatively-inherited loci. The mapping information, however, suggests at the *HtN* gene is located on the long arm of chromosome 8 at a point distal to that of the QTLs identified in this study.

QTLs conferring resistance in the host were almost always contributed by Mo17. The sign of the additive component of the B52 allele effect for each QTL (data not shown), and trait correlations with genome composition, both support this statement. The host-plant response of F_3 lines to NCLB was not related to heterozygosity, but rather to the proportion of the parental genomes.

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