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Interval mapping of genes for quantitative resistance of maize to *Setosphaeria turcica*, cause of northern leaf blight, in a tropical environment

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Abstract

Quantitative trait loci (QTL) involved in the resistance of maize to *Setosphaeria turcica*, the causal agent of northern leaf blight, were located by interval mapping analysis of 121 $F_{2:3}$ lines derived from a cross between Mo17 (moderately resistant) and B52 (susceptible). A linkage map spanning 112 RFLP loci with 15 cM mean interval length was constructed, based on marker data recorded in a previous study. Field tests with artificial inoculation were conducted at three sites in tropical mid- to high-altitude regions of Kenya, East Africa. Host-plant response was measured in terms of incubation period, disease severity (five scoring dates), and the area under the disease progress curve (AUDPC). Heritability of all traits was high (around 0.75). QTL associated with the incubation period were located on chromosomes 2S and 8L. For disease severity and AUDPC, significant QTL were detected in the putative centromeric region of chromosome 1 and on 2S, 3L, 5S, 6L, 7L, 8L and 9S. On 2S the same marker interval which carried a gene enhancing latent period was also associated with reduced disease severity of juvenile plants. QTL on chromosomes 3L, 5S, 7L and 8L were significant across environments but all other QTL were affected by a large genotype × environment interaction. Partially dominant gene action for resistance as well as for susceptibility was prevailing. Single QTL explained 10 to 38% of the phenotypic variation of the traits. All but the QTL on chromosomes 1, 6 and 9 were contributed by the resistant parent Mo17. On chromosome 8L a QTL mapped to the same region as the major race-specific gene *Ht2*, supporting the hypothesis that some qualitative and quantitative resistance genes may be allelic.

Abbreviations: AUDPC, area under the disease progress curve; CIMMYT, International Maize and Wheat Improvement Center; KARI, Kenya Agricultural Research Institute; NCLB, northern corn leaf blight; QTL, quantitative trait locus/loci

Introduction

The ascomycete *Setosphaeria turcica* (Luttrell) Leonard & Suggs, in its anamorphic stage *Exserohilum turcicum* (Pass.) Leonard & Suggs [syn. *Helminthosporium turcicum* (Pass.)], is the causal agent of NCLB, a severe foliar disease of maize (*Zea mays* L.). In many maize growing areas of the world, particularly the midaltitude regions of the tropics, where temperatures are moderate and dew periods long, NCLB is a constant menace [1, 32, 36]. The symptoms of NCLB are wilting local lesions which turn necrotic at a later stage and coalesce in susceptible plant tissue, thus causing the destruction of large portions of the leaf area. Based on disease ratings three to four weeks after mid-silk, grain yield is reduced by an estimated 4.2% per 10% diseased leaf tissue [29 reevaluating 10] but infection before silking is considerably more damaging. In maize the disease is almost exclusively controlled by resistant cultivars. Two types of qualitative resistance genes are known: the chlorotic lesion type resistance conferred by genes *Ht1*, *Ht2* and *Ht3* [18–20], and the prolonged incubation period resistance caused by gene *HtN* [14], renamed *Htn1* [46]. All qualitative resistance genes were overcome by matching virulence genes in the fungal population. However, quantitative resistance (e.g. of Mo17) has been stable in diverse ecogeographic areas with their different *S. turcica* populations. Therefore, breeding for quantitative NCLB resistance is a major goal in maize breeding programs worldwide.

Sources of quantitative resistance have been reported [23, 25]. By means of translocation studies several chromosome arms harboring resistance genes were identified [5, 24, 26]. A more precise dissection of quantitative disease resistance determinants into Mendelian characters, the QTL, became manageable with the recently developed molecular and biometrical tools. QTL for NCLB resistance were first mapped in an $F_{2:3}$ population derived from the popular, partially resistant maize inbred line Mo17 [57] and the susceptible inbred line B52 by Freymark *et al.* [11, 12] using RFLP markers. The population was evaluated under field conditions with artificial infection at one location in Iowa, USA, with low disease severity relative to tropical latitudes. In the present study, the same population was tested at three locations in the southwestern maize-growing area of Kenya, at high disease severity. The Kenyan *S. turcica* population also contains pathotypes that have not been found in the USA [55]. In both experiments, interval mapping developed by Lander and Botstein [28] was used to locate QTL and to estimate their genetic effects.

The objectives of this study were (1) to determine whether the same QTL were detectable in the two vastly differing macro-environments of Iowa and Kenya, (2) whether QTL for reduced disease severity may also be detected by the trait incubation period, (3) to compare the mapping results with preceding translocation studies involving Mo17 and other inbreds, and (4) to compare genetic locations of QTL and qualitative factors.

Materials and methods

Germplasm

The mapping population was derived by Freymark *et al.* [11] from the two dent maize inbred lines, Mo17 and B52. Both lines descend from US corn belt materials. Mo17 is known to be quantitatively resistant against NCLB [2, 6, 41] while B52 reacts highly susceptible. The F_1 of the cross was self-pollinated and 169 F_2 plants were obtained. These were likewise self-pollinated to get the 150 $F_{2:3}$ lines which were previously evaluated [11, 12]. In Kenya twenty plants from each of the 150 $F_{2:3}$ lines were propagated by chain crossing thus maintaining the F_3 level of heterozygosity. Enough seed for evaluation at three locations was obtained from 121 $F_{2:3}$ lines. The US corn belt inbred lines B14A and B73, being highly susceptible, plus the resistent inbred lines CML202 (bred by CIMMYT-Zimbabwe) and E12-210 (KARI-Embu) were included as checks. Additionally, a set of differential lines with resistance genes *Ht1*, *Ht2*, *Ht3* and *Htn1* in the genetic background of the inbred lines B37 and

A619 was grown at each location to provide information about the virulence of the fungal population.

Field trials

The 121 $F_{2:3}$ lines, both parent lines, the F_1, and the four check inbreds were evaluated during the long rainy season (April to September) of 1994 at three locations, Embu, Kitale and Muguga, in the southwestern maize-growing area of Kenya. The locations differ in altitude, annual rainfall and mean temperature. Embu is situated southeast of Mount Kenya in a modestly warm and humid area (altitude 1494 m above sea level (m.a.s.l.), annual rainfall 1230 mm, mean temperature 19.5 °C). Kitale, located southeast of Mount Elgon near the Ugandan border, is the most conducive environment for NCLB with a tropical climate characterized by considerable amount of dew and daily rainfall during the growing season (1885 m.a.s.l., 1182 mm, 19.2 °C). Muguga, northwest of Nairobi, has lower temperatures which delay fungal development (2095 m.a.s.l., 954 mm, 15.8 °C).

A generalized 4 × 32 lattice [40] with two replications per location was chosen as experimental design. Plots consisted of four rows at Embu and Kitale. Limited field space allowed only three-row plots to be planted at Muguga. The rows were 3 m long with a spacing of 0.25 m between plants and 0.75 m between rows. The experiments were overplanted, then thinned to 12 plants per row to a final density of 53000 plants per ha. To protect the seedlings against cutworm (*Agrotis* sp.) damage, granules of the insecticide Furadan 5G were placed by the seeds. Also at planting time, 50 kg/ha nitrogen and 120 kg/ha phosphate were applied. During the growing season, experiments were irrigated and hand-weeded when necessary.

At the 4–6 leaf stage, the 12 plants of the inner rows of the plots at Muguga and of the two inner rows at Embu and Kitale, respectively, were artificially inoculated. At each location local inoculum was used. It was produced by collecting NCLB lesions of surrounding farmers' fields and incubating them for 48 h in polyethylene bags at 100% humidity and in total darkness. Spores were brushed from the leaves and suspended in water. The conidium concentration was determined with a microscope (× 100) and adjusted. About 1400 conidia per plant were inoculated at Embu, 600 at Kitale and 1500 at Muguga by pipetting the suspension into the leaf whorl.

Disease assessment

At each location, the incubation period was recorded as the number of days from inoculation to the day when 50% of the inoculated plants of a plot showed small, water-soaked lesions [47]. Disease severity was assessed as the percentage of diseased leaf tissue relative to the total leaf area. The assessment was done separately for each leaf blade and then averaged across all (except the decayed bottom) leaves of the plant. The resulting percentage of damaged leaf tissue of a plant was assigned to one of the eleven classes of an equidistant scale (class 0, 0% diseased tissue; class 1, 1–10%; class 2, 11–20%). Disease severity was recorded five times during the growing season at intervals of two weeks, beginning at about 4 weeks after inoculation. The first three scorings were before, the last two after mid-silk (the date when half of the plants have visible silks). On each scoring date, ten randomly selected plants, excluding the border plants, were evaluated. Plot means were calculated from these ten individual scorings. Lattice-adjusted entry means of the individual scoring dates were used to calculate the AUDPC according to Shaner and Finney [45]. Because individual lesions had rapidly coalesced in fairly susceptible genotypes in previous experiments in Kenya (A. Schechert, unpublished), the counting of lesions and the measurement of lesion size seemed unpractical and was not attempted.

RFLP assays and data analysis

RFLP analysis of the Mo17 × B52 mapping population was performed by Freymark *et al.* as

previously described [11, 12]. Mapmaker/Exp 3.0 [34] with improved error detection [33] was used to reconstruct the published [12] linkage map. Lattice-adjusted entry means and effective error mean squares from the individual experiments were used to compute the combined analyses across locations. Homogeneity of error mean squares of the locations was tested by Bartlett's test criterion [48]. Estimates of variance components for genotypic variation and genotype × environment variation were calculated according to Cochran and Cox [8]. In the biometrical model, effects of locations and genotypes were considered as random. Repeatability [i.e., variance component of entries/(variance component of entries + effective error mean square)] was calculated on a plot mean basis. Broad-sense heritability [16] with confidence intervals [27] was also estimated. Phenotypic and genotypic coefficients of correlation among incubation period, disease severity at five scoring dates, and AUDPC were calculated and tested for significance as suggested by Mode and Robinson [38]. For all statistical analyses and tests listed so far, the PLABSTAT and PLABCOV software [50, 51] was used. The normal distribution of phenotypic data, an inherent assumption on the interval mapping approach, was tested with SAS 'PROC UNIVARIATE' [43] by applying the Shapiro and Wilk statistic to adjusted entry means and means across locations. Some data subsets were not normally distributed. In none of these cases normal distribution was achieved by arcsinus, log or square-root transformation functions. If not mentioned otherwise, the data sets were normally distributed.

QTL analysis was performed on adjusted entry means for individual locations and on the average of adjusted entry means across locations in the combined analysis. Interval mapping was used to estimate genetic positions and effects of QTL with the computer program Mapmaker/QTL 1.1 [35]. LOD values (log-likelihood ratios) were computed every 2 cM of each linkage group and plotted accordingly. QTL were postulated when the LOD of the single-QTL model exceeded the threshold [28] of 2.36, corresponding to a probability of $P < 0.05$ that a false-positive occurred. The support interval of a QTL position was defined as the interval embracing the local maximum of the LOD curve by one LOD unit [49], meaning a relative likelihood of one tenth that a QTL is located outside vs. being located inside the interval. If the QTL profile suggested the presence of two linked QTL, the significance of the linked QTL was tested by keeping the primary QTL, having the higher LOD score, fixed and demanding that the LOD score at the second position exceed 2.36. After declaring putative QTL individually, the genetic effects of all putative QTL were calculated in a simultaneous fit as the effect of substituting the alleles of the resistant parent Mo17 by those of the susceptible parent B52. Additive and dominance effects at a locus were estimated as suggested for F_2 intercrosses in the tutorial [35, page 22]. To get comparable estimates of the dominance effects, estimates from Mapmaker/QTL were multiplied by 2 because they were based on data derived from a $F_{2:3}$ population having only one half of the dominance attributable to the F_2 [37]. The total genotypic variance was determined as the ratio of the total phenotypic variance, calculated by simultaneously fitting multiple QTL, and the heritability [44]. The gene action of a QTL was defined by the d/a ratio either as additive (0 to 0.2), partially dominant (0.2 to 0.8), partially recessive (-0.2 to -0.8), or recessive (-0.8 to -1.2).

Results

Incubation period and disease severity at the different locations

The length of the incubation period and even more so disease severity assessed at five scoring dates and the area under the disease progress curve (AUDPC) varied considerably among the three locations (Table 1). The average incubation period at Embu was about 1.5 days shorter than at Muguga and at Kitale. The blight became very severe at Kitale and was moderately damaging at Muguga. The ranking of the three sites for disease severity was the same at all scoring dates.

Table 1. Means across 128 entries (B52, Mo17, F_1, 121 $F_{2:3}$ lines, four check inbreds) and repeatability values of the incubation period (IP), disease severity assessed at five dates (DS1–5), and the area under the disease progress curve (AUDPC) after artificial inoculation with *Setosphaeria turcica* at three locations in Kenya 1994.

Trait	Embu		Kitale		Muguga	
	mean	repy[1]	mean	repy	mean	repy
IP [d]	9.19	0.47	10.85	0.54	10.68	0.57
DS1 [score 1–10]	1.95[2]	0.65	2.67	0.73	1.30	0.44
DS2	3.37	0.70	4.98	0.82	2.55	0.68
DS3	4.23	0.70	6.29	0.87	3.19	0.77
DS4	6.00	0.65	8.84	0.90	4.48	0.81
DS5	7.89	0.47	9.65	0.93	5.61	0.75
AUDPC	297.70	0.68	408.53	0.89	220.74	0.74

[1] Repeatability (range 0–1).
[2] A score of 1.95 relates to 19.5% disease severity.

Repeatability values were higher at Kitale than at the other two sites despite very high disease scores (Table 1). Moreover, repeatability was higher for intermediate than for early or late scoring dates and higher for ratings of disease severity than of incubation period. Standard errors of the means in Table 1 were generally small (0.02 to 0.07 for IP and DS, 0.66 to 1.66 for AUDPC) and in the ANOVA, the mean square for entries was highly significant ($P \leq 0.01$) for each trait at each site.

Among the differential lines, those with the qualitative resistance genes *Ht1*, *Ht2* and *Ht3* showed chlorotic lesions. Line B37*Htn1*, carrying the gene *Htn1*, showed a moderate proportion of necrotic leaf tissue at all locations and no chlorosis. This pattern indicates the presence of races 0 and N [30]. The occurrence of both races in Kenya has been verified in the laboratory [55].

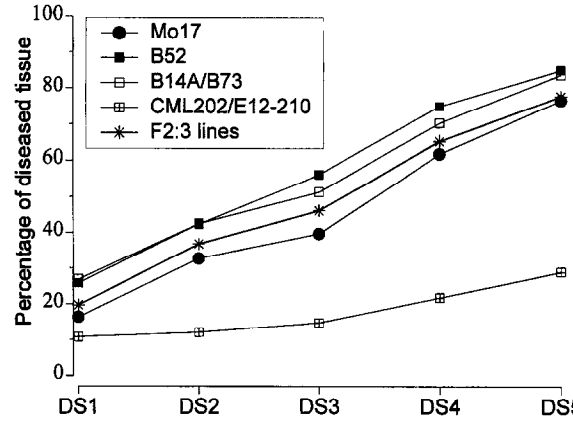

Fig. 1. Disease progress curves of different maize germplasm after artificial inoculation with *Setosphaeria turcica*. Disease severity was rated at five scoring dates (DS1–5) and is presented as means across three locations in Kenya. The germplasm includes the parent lines Mo17 and B52, susceptible check inbreds B14A and B73 (mean presented), resistant check inbreds CML202 and E12-210 (mean presented), and 121 $F_{2:3}$ lines of the cross Mo17 × B52.

Reaction of different plant genotypes

Disease development of the resistant parent Mo17 and the susceptible parent B52 was similar (Fig. 1) but inbred Mo17 was consistently less diseased than B52. Although the parental means were not significantly different across locations except for the third scoring date, the AUDPC of Mo17 was significantly ($P = 0.05$) smaller than that of B52 at each individual location and also across locations (282.2 vs. 365.7). The final disease severity of both parent lines was ca. 80%, indicating that Mo17 was quite susceptible under the environmental conditions of Kenya. The $F_{2:3}$ lines ranged intermediate between the two parents. Their AUDPC, averaged across sites,

ranged from 244.1 to 388.8 with a mean of 304.5. Disease development of the susceptible checks, B14A and B73, was comparable to that of the parent lines (mean AUDPC 329.3 and 379.6, respectively), while the resistant checks, CML202 and E12-210, became only moderately infected (mean AUDPC 105.0 and 132.3, respectively).

Resistance components and correlations among traits

For all traits, genotypic variances among $F_{2:3}$ lines and genotype × environment interaction variances were highly significant (Table 2). On average, variance component estimates of genotype × environment interaction were about half the size of those of the genetic effects. The importance of interaction increased at later scoring dates. Heritability estimates based on entry means [3] were generally high (around 0.75). Tight and highly significant ($P < 0.01$ throughout) phenotypic correlations, based on entry means, occurred among the disease severity scores at five dates (0.67 to 0.89) and between these scores and AUDPC (0.88 to 0.95) whereas incubation period was only moderately related to disease severity scores (-0.42 to -0.69) and AUDPC (-0.63). Estimates of genotypic correlations among the various resistance traits were all high ($r_g \geq 0.85$ among individual scores, $r_g \leq -0.75$ between individual scores and incubation period) and larger than twice their standard errors. Almost complete genotypic agreement ($r_g \geq 0.97$) existed between AUDPC and disease severity at each of the scoring dates.

Interval mapping

The linkage map of the cross Mo17 × B52 based on 169 F_2 individuals was well saturated, spanning 112 RFLP loci (genomic and cDNA probes) with an average spacing of 14.6 cM and a total length of 1492 cM (Fig. 4).

Incubation period was significantly associated with two genomic regions on the short arm of chromosome 2 and on the long arm of chromosome 8 (Fig. 2). LOD profiles of the QTL on chromosome 2S were similar for individual test sites with LOD scores ranging from 3.89 at Embu to 4.78 at Kitale, amounting to 7.36 across sites. The QTL on chromosome 8L was only significant at Kitale, the location with the most severe disease development, and across sites (LOD scores 3.65 and 2.47, respectively). There was evidence of genotype × environment interaction, as the peaks of the LOD profiles of chromosome 8 varied greatly among locations. Across locations the peak of the LOD curve mapped ca. 40 cM proximal to the QTL detected with the Kitale data (Fig. 2). At both QTL, the resistance gene was contributed by the more resistant parent Mo17. Fitted simultaneously, the two QTL explained

Table 2. Estimates of components of variance and heritability for the traits incubation period (IP), disease severity at five scoring dates (DS1–5), and AUDPC of 121 $F_{2:3}$ lines of the cross Mo17 × B52 after artificial inoculation with *Setosphaeria turcica* at three locations in Kenya, 1994.

Parameter	IP	DS1	DS2	DS3	DS4	DS5	AUDPC
Variance components							
Genotypes (G)	0.240**	0.056**	0.131**	0.163**	0.304**	0.178**	685.7**
G × environment	0.120**	0.039**	0.043**	0.063**	0.188**	0.167**	313.6**
Pooled error	0.380	0.068	0.094	0.110	0.139	0.236	299.8
Heritability	0.70	0.70	0.81	0.81	0.74	0.57	0.82
C.I.[1]	0.59, 0.78	0.58, 0.77	0.74, 0.86	0.74, 0.86	0.64, 0.81	0.41, 0.68	0.75, 0.86

** Significant at $P = 0.01$.

[1] Lower and upper boundary of the 95% confidence interval of the heritability estimate according to Knapp and Bridges [27].

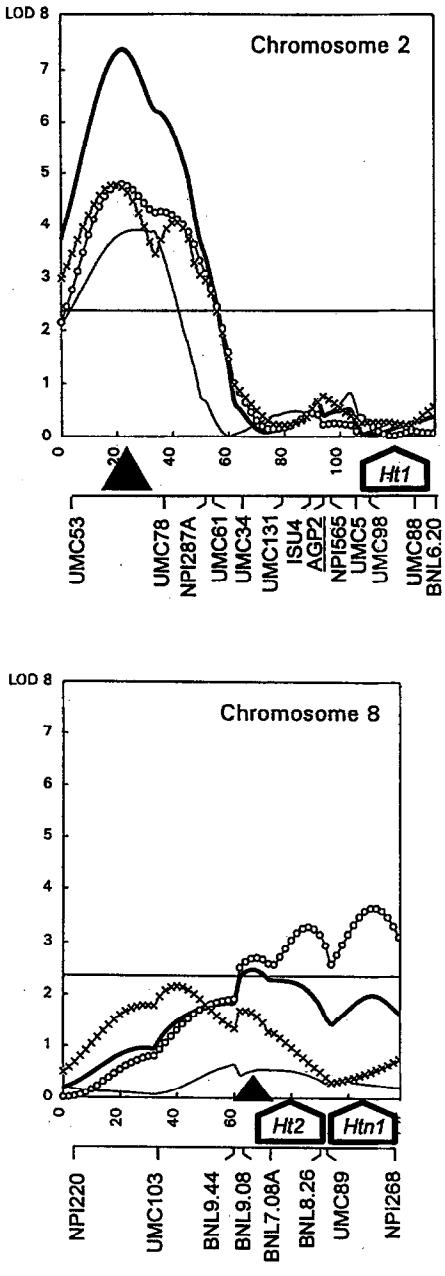

Fig. 2. QTL likelihood profiles of the incubation period of NCLB for chromosomes 2 and 8, as determined by interval mapping of 121 $F_{2:3}$ lines of the cross Mo17 × B52. LOD profiles for single locations (Embu: —; Kitale: –o–; Muguga: –×–) and across locations (—) were plotted with a spacing of 2 cM (see abscissa). The LOD significance threshold of 2.36 is marked. Positions of RFLP loci, QTL (triangle), and *Ht* gene loci (trapezium) are indicated on the abscissa.

40.9% of the phenotypic and 58.4% of the genotypic variation of the trait (Table 3). The QTL on 2S acted partially dominant whereas the QTL on 8L was recessive. Because the incubation period data of the combined analysis were not normally distributed, their interpretation should be cautious. However, the normally distributed disease severity data also revealed a QTL in the *BNL9.08-BNL7.08A* interval on 8L (Table 3).

Most QTL with a significant and large effect on the disease severity at individual scoring dates also had a large effect on AUDPC. This applies to four genomic regions located on 3L, 5S, 7L and 8L (Fig. 3). Their LOD profiles had the same shape for individual and across locations, although not all curves exceeded the LOD threshold. Fitted simultaneously, these four QTL explained about the same fraction of genotypic variation for AUDPC (59.0%) as the two QTL affecting the incubation period (58.4%) but a larger proportion of the phenotypic variation (Table 3). Gene action of the four QTL contributed by Mo17 ranged from additive to partially dominant to recessive.

The trimodal LOD curve for AUDPC on chromosome 3 (Fig. 3) was indicative of multiple, linked QTL but neither of the two peaks flanking the central peak in *UMC60-BNL15.20* surpassed the 2.36 LOD threshold when the postulated QTL (Fig. 3) was held fixed in runs of Mapmaker/QTL. A bimodal LOD curve was also observed for chromosome 5, regarding AUDPC (Fig. 3), but no statistical significance was obtained for the presence of a linked QTL in the *BNL5.02-JC162* interval. Chromosomes 3 and 5 suggest genotype × environment interaction: for chromosome 3, much higher LOD scores were obtained for AUDPC at Embu than at Kitale whereas the reverse was true for chromosome 5 (Fig. 3). At the same time, the shape of the curves was largely identical among the two sites.

Genomic regions near the suspected centromere position of chromosome 1 and on chromosome arms 2S, 6L, and 9S were also associated with significant delay in disease development (Fig. 4). However, these QTL were significant only for individual scoring dates, but not for the

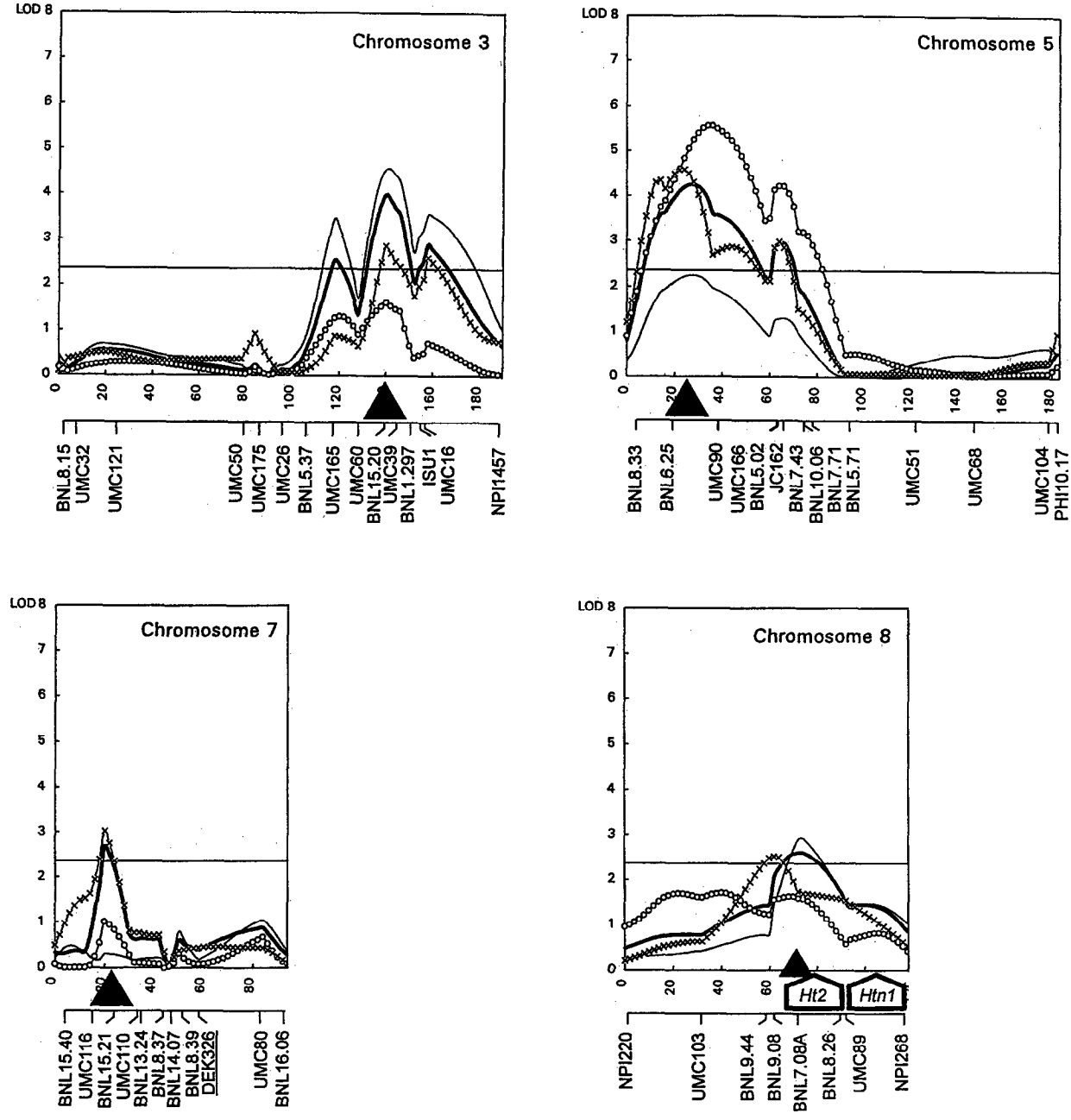

Fig. 3. QTL likelihood profiles of the area under the disease progress curve (AUDPC) of NCLB for chromosomes 3, 5, 7 and 8, as determined by interval mapping of 121 $F_{2:3}$ lines of the cross Mo17 × B52. LOD profiles for single locations (Embu: —; Kitale: -o-; Muguga: - × -) and across locations (▬) were plotted with a spacing of 2 cM (see abscissa). The LOD significance threshold of 2.36 is marked. Positions of RFLP loci, QTL (triangle), and *Ht* gene loci (trapezium) are indicated on the abscissa.

cumulative trait AUDPC, and subject to large genotype × environment interaction (for details, see [9]). Three of them were contributed by the susceptible parent, B52.

Discussion

Obviously the parent line Mo17 showed only a low level of resistance in this experiment in Kenya

Table 3. Mapmaker/QTL estimates of positions and genetic effects at QTL for resistance [component incubation period (IP) and the area under the disease progress curve (AUDPC)] of maize against *Setosphaeria turcica*, based on data from $F_{2:3}$ lines of the cross Mo17 × B52 at three test sites in Kenya. All QTL were contributed by the resistant parent, Mo17.

Trait	Chromosome	Marker interval	QTL position (cM)[1]	LOD[2]	Phenotypic variance[3] (%)	Genetic effects		
						additive[4]	dominant	dominant/additive
IP (d)	2S	*UMC53-UMC78*	14–**22**–32	7.36	38.0	−0.48	−0.22	0.45
	8L	*BNL9.08-BNL7.08A*	41–**67**–91	2.47	9.8	−0.21	0.27	−1.27
					40.9[5]			
AUDPC	3L	*UMC60-BNL15.20*	134–**140**–154	3.98	14.2	17.21	0.73	0.04
	5S	*BNL6.25-UMC90*	12–**25**–45	4.24	18.3	13.69	−6.61	−0.48
	7L	*BNL15.21-UMC110*	17–**20**–26	2.66	9.8	12.24	3.96	0.32
	8L	*BNL9.08-BNL7.08A*	61–**71**–107	2.58	9.8	15.54	−10.68	−0.69
					47.8			

[1] Peak of LOD curve (bold figure) and 1.0 LOD confidence interval.
[2] LOD scores exceeding the threshold of 2.36 ($P = 0.05$) in the combined analysis.
[3] Proportion of total phenotypic variance attributable to segregation at a QTL.
[4] Effect of substitution of Mo17 allele by B52 allele. Negative effects for IP are equivalent to positive effects for AUDPC.
[5] Proportion of total phenotypic variance explained by all segregating QTL simultaneously.

as the disease severity reached more than 70%. This was not totally unexpected because in an epidemiological study of NCLB in Uganda [2] the final disease severity of Mo17 was 20 to 40% whereas in Ohio merely 1.3% of the leaf area became infected. In Iowa, Freymark *et al.* [11] rated a disease severity of 1.0% on Mo17 vs. 4.4% on inbred B52. In each experiment, plant stands were artificially inoculated. The reason for the so much better epidemic development of NCLB in East Africa is unclear. It may be the latitude (longer nights) and altitude (heavier dew fall) of the tropical highlands plus frequent rains causing high humidity which support better spore germination and infection. Further reasons may be a higher amount of natural inoculum or higher aggressiveness of the East African *S. turcica* population.

There was a difference between the initial disease severity of B52 and Mo17 which remained constant over the whole epidemic (Fig. 1). Thus, the partial resistance of Mo17 in this experiment was dilatory and not rate-reducing, which would be expected for quantitatively inherited resistance [13]. Check line CML202 in contrast had a similar initial disease severity as Mo17 but thereafter became only slowly blighted, i.e. at a reduced rate.

The incubation or latent period is an important component of quantitative resistance in the maize/*Setosphaeria turcica* pathosystem [2, 6, 47] and may be a useful selection criterion. We approve this recommendation of Smith and Kinsey [47] because in our experiment the mean phenotypic correlation between incubation period and AUDPC ($r = -0.63$) was nearly as tight as in theirs ($r = -0.70$ between incubation period and disease severity 4 weeks after mid-silk). However, as the third and fourth scoring were even more tightly correlated with AUDPC ($r = 0.95$), a single scoring just before silking may be the best option for selection in large-scale breeding programs. This approach would still allow to discard unwanted genotypes before making crosses. We should continue to use the composite trait AUDPC for mapping resistance-QTL in this pathosystem because it is most informative of quantitative resistance [45]; many more QTL governing AUDPC than incubation period were detected in this study. Multiple scorings, which are needed

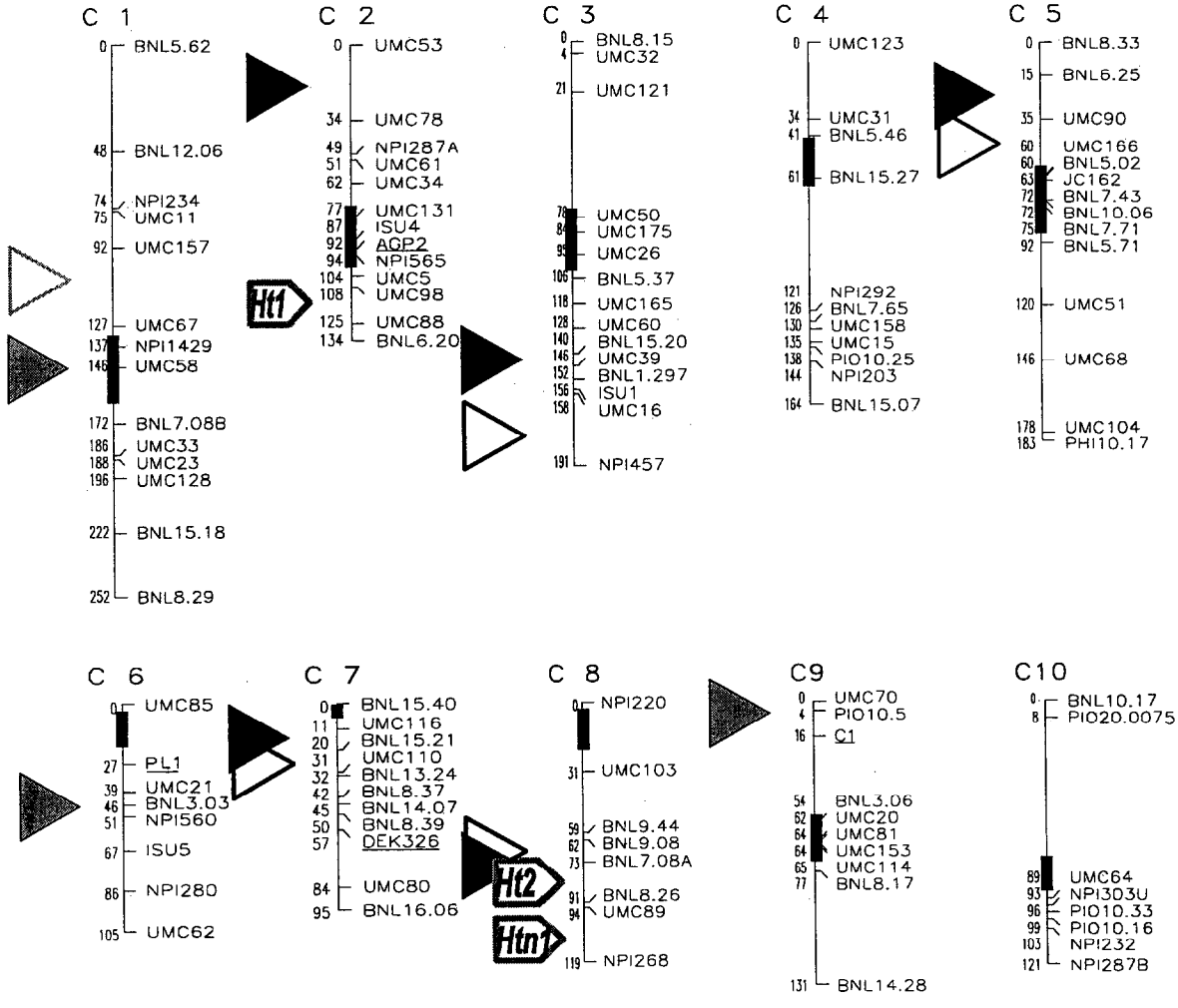

Fig. 4. Summarized illustration of QTL involved in the resistance reaction of an $F_{2:3}$ line population of the cross Mo17 x B52 against *Setosphaeria turcica* under field conditions in Kenya (this study) and Iowa [11, 12]. Marker distances for the ten maize chromosomes (C1 to C10) are given in cM. Approximate centrometric regions [17, 54] and positions of *Ht* genes [4, 46] are marked according to published data. For comparability of the two studies, only QTL affecting disease severity (in this study regardless of scoring date) are depicted. Symbols represent QTL donated by Mo17 as mapped in Kenya (filled arrowhead) and Iowa (open, solid line), QTL from B52 in Kenya (dotted) and Iowa (open, dotted line), and qualitative (*Ht*) resistance genes (*Ht1*, *Ht2*, *Htn1*).

to calculate the AUDPC, should also be useful in further studies to gain a better understanding of the developmental dynamics of resistance expression (seedling vs. adult-plant resistance) at individual QTL.

We reported important resistance QTL affecting AUDPC on chromosomes 3L, 5S, 7L and 8L. This is very consistent with Freymark *et al.* [11, 12] finding that single QTL on 1S, 3L, 5S, 7L and 8L accounted for 45% of the phenotypic variation of final disease severity. Exact QTL positions differed slightly between the two trials, especially with respect to chromosomes 3L and 5S (Fig. 4). It is unlikely that the smaller number of $F_{2:3}$ lines tested in our experiment (121 vs. 150) was responsible for these differences because a repeated analysis of Freymark's original data set, reduced to the 121 $F_{2:3}$ lines tested in Kenya, revealed exactly the same marker intervals as the complete set [12]. The linkage map distances be-

tween the LOD peaks in the two studies (Fig. 4) may not be statistically significant though. According to simulation studies by van Ooijen [39], the customary one-LOD support interval of the position of a QTL comprises the QTL with a 85% probability. A 99% probability level would equal a three-LOD confidence interval corresponding to a chromosomal distance of 40 to 60 cM. The good agreement between the results obtained with this mapping population in the two very different megaenvironments suggests that rather the high heritability of the parameters analyzed (Table 2) and the precision of the field trials (cf. repeatability values in Table 1) than the level of disease severity may be decisive for the power of a resistance-QTL mapping experiment.

The QTL we reported for chromosome 2S (Fig. 4) was not significant in Iowa [11]. The reason may be that the QTL mainly extended the incubation period and reduced early disease development (Fig. 2) but became less effective during later disease development. Freymark *et al.* [11] did not record the incubation period and thus may have missed this 'seedling resistance' QTL when scoring disease severity at 117 days after planting. The QTL we found on 6L and 9S (Fig. 4), contributed by the susceptible parent B52, were not detected at each location in Kenya [9]. In the same way they may have been ineffective in Iowa where the trial was conducted at only one location. We consider these QTL less important.

Genotype × environment (G × E) interactions are typically associated with quantitative resistance [13]. G × E interactions were statistically significant in a generation means analysis of Mo17 × A632 [6] but of limited importance because no change in ranking of generations was observed between a field and a greenhouse experiment, but a change in the magnitude of differences. Similarly, G × E interaction in this study was significant but the high heritability of NCLB resistance indicates that the effect was not large. It appears that some genes involved in quantitative resistance (QTL on 6L, 9S) are environmentally less stable than others (QTL on 3L, 5S, 7L, 8L). Those four chromosomal regions may harbor resistance genes in different germplasm because translocation studies in the 1950s with Mo21A and other unrelated inbred lines had already identified the importance of chromosomes 3, 5 and 7 [24, 26]. More recent mapping studies with translocations of Mo17 found that chromosomes 3, 4S and 6L were associated with the largest reductions of disease severity [5]. The reason for the discrepancy to our QTL mapping results may be the incomplete genome coverage of the translocations available to Brewster *et al.* [5], as was discussed previously [11], G × E interactions, or different genetic heterogeneity. Brewster *et al.* [5] used a different susceptible parent (W23) in their crosses. This inbred line may have shared resistance QTL with Mo17 which then were not segregating in their mapping population. Vice versa, Mo17 and B52 may share resistance alleles that would not segregate.

Gene action was mostly partially dominant or recessive. This is consistent with the mapping study in the US [12]. Generation means analyses with Mo17 [6] and other germplasm [21] suggested that additive gene action prevailed and that the size of non-additive effects varied among populations and between years. The stability of QTL effects across environments is a prerequisite for marker-assisted selection to be effective and warrants further study.

Robertson's [42] hypothesis that qualitative genes may be extreme (mutant) alleles at quantitative trait loci was recently supported by resistance gene mapping studies in the potato/*Phytophthora infestans* [31] and rice/*Pyricularia oryzae* [53] pathosystems. Freymark *et al.* [11] extended the list to maize/*S. turcica*, referring to minor effects (LOD < 2.0) of regions on chromosomes 2L, 4S and 8L to which the major genes *Ht1*, *bx1* and *Ht2* map in other inbred lines. The present results provide additional evidence: the major QTL of Mo17 on 8L mapped to the same region as the race-specific *Ht2* gene [46]. A chlorotic lesion type, which *Ht2* genotypes express with avirulent isolates, was never observed among the $F_{2:3}$ lines. It is open to further studies, though, whether the QTL we found on 8L is a true allele of the *Ht2* locus or a linked gene. The other major

gene on chromosome 8L is *Htn1* [46], prolonging the incubation and latent period of avirulent isolates until after anthesis [14]. Simcox and Bennetzen [46] placed the *Htn1* locus in the interval between *UMC117* and *NPI268*. We mapped a QTL affecting the incubation period to the interval *UMC89-NPI268*. However, the LOD score surpassed the significance threshold only for one location, Kitale (Fig. 2). One allele of this QTL reduced the incubation period of Mo17 by 0.43 days, i.e. by about 4% [9]. A recessive major resistance gene that confers a 'chlorotic halo' reaction to infection by *S. turcica* was described only recently in the inbred line 357 [7]. Based upon reciprocal translocation mapping studies, the gene appears to be located on the short arm of chromosome 1, near the centromere, i.e. in the region where we mapped a QTL contributed by B52 (Fig. 4). There is at least one more major gene for NCLB resistance in the Thai line Ki14 which is unliked to the known *Ht* genes (J.L. Brewbaker, pers. comm.). It will be interesting to see if it also maps to a region where we identified QTL.

Some questions remain unsolved. Are the putative QTL × environment interactions rather due to physical factors (weather, soil) or to different virulence characteristics among local pathogen populations? In the latter case this would mean that not all genes for quantitative resistance are necessarily durable. If LOD plots have a multimodal shape like the one obtained for chromosome 3 (Fig. 3) or that which Freymark *et al.* [11] found with chromosome 7, how can one distinguish ghost QTL from linked QTL? Improved biometrical tools using the multiple regression approach [15, 22, 52] will hopefully provide an answer in the near future.

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