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Dynamic gene action at QTLs for resistance to *Setosphaeria turcica* in maize

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Abstract Cultivars with quantitative resistance are widely used to control *Setosphaeria turcica* (Luttrell) Leonard & Suggs, the causal organism of northern corn leaf blight (NCLB). Here the effectiveness of quantitative trait loci (QTLs) for NCLB resistance was investigated over the course of host plant development in inoculated field trials. A population of 194–256 $F_{2:3}$ lines derived from a cross between a susceptible Italian (Lo951) and a highly resistant African inbred line (CML202) was tested in three environments in Kenya. The traits assessed were the incubation period (IP), the percentage disease severity (DS 1 to 5, taken biweekly), and the area under the disease progress curve (AUDPC). Considering all resistance traits and environments, a total of 19 putative QTLs were detected by composite interval mapping using a linkage map with 110 RFLP markers. In the combined analysis across environments, nine QTLs were significant (LOD >3.0) for DS 3, recorded around flowering time, explaining 71% of the genotypic variance. Four of these nine QTLs displayed significant ($P<0.05$) QTL $\times$ environment (QTL $\times$ E) interaction. Most QTLs were already significant in the juvenile stage (IP) and became less effective after flowering. Across environments, three QTLs conditioned adult-plant resistance, in the sense that they were only significant after flowering. Six QTL alleles on chromosomes 2, 4, 5, 8, and 9 of CML202 should be useful for marker-assisted backcrossing.

Key words *Exserohilum turcicum* · Maize · Northern corn leaf blight · Quantitative resistance · QTL mapping · *Setosphaeria turcica* · *Zea mays*

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Introduction

Setosphaeria turcica Leonard & Suggs, anamorph *Exserohilum turcicum* Leonard & Suggs, causes northern corn leaf blight (NCLB), a ubiquitous foliar disease of maize. Cultivars with effective quantitative resistance are widely grown to prevent yield losses by NCLB. Quantitative NCLB resistance is characterized by fewer, and sometimes smaller, lesions and a prolonged incubation period, but not by reduced sporulation (Ullstrup 1970; Brewster et al. 1992; Smith and Kinsey 1993; Carson 1995). The prolonged incubation period is already reliably expressed by younger plants and is tightly correlated with a lower blight severity of adult plants in the field (Smith and Kinsey 1993; Carson 1995; Schechert et al. 1997). Quantitative NCLB resistance is largely insensitive to environmental conditions (Carson and Van Dyke 1994), highly heritable (Carson 1995; Dingerdissen et al. 1996; Schechert et al. 1997, 1999), and has never succumbed to new pathogenic races of *S. turcica* (Leonard 1993), in contrast to the qualitative resistance conferred by *Ht* genes. Most maize breeders therefore prefer the use of quantitative NCLB resistance in their cultivar development programs.

The genomic basis of quantitative NCLB resistance was first investigated with backcross and F_2 progenies of crosses between resistant lines and susceptible chromosomal translocation stocks. Fourteen chromosome arms were found to be associated with quantitative leaf blight resistance (Jenkins et al. 1957; Jenkins and Robert 1961; Brewster et al. 1992). More recently, molecular-marker technology and genome-mapping software have facilitated the dissection of quantitative disease resistance into Mendelian factors,

the quantitative trait loci (QTLs, for review see Tanksley 1993; Young 1996). To identify QTLs for resistance to *S. turcica*, a population of $F_{2:3}$ lines, derived from the moderately resistant line Mo17 and the susceptible line B52, was genotyped with RFLP markers and field-tested in Iowa (Freymark et al. 1993, 1994) and Kenya (Dingerdissen et al. 1996). QTLs with partially dominant effects were detected on chromosomes 1, 3, 5, 7 and 8, in both mega-environments and at essentially the same genomic positions. On chromosome 8, the position of a QTL coincided with that of the major gene *Ht2* (Simcox and Bennetzen 1993). In another QTL mapping study conducted in Kenya, Schechert et al. (1999) used the tropical African inbred line CML202 from CIMMYT-Zimbabwe as a resistance donor. Looking at the means of IP and AUDPC across three environments, ten QTLs on seven chromosomes were discovered. Gene action was additive throughout. Some QTLs were closely linked to loci affecting the maturity of maize.

In the present companion paper of Schechert et al. (1999) we investigate how the effectiveness of resistance QTLs changed over time. By an analysis of the incubation period and five consecutive disease assessments we show that the QTLs were either effective during all stages of plant development, or else in the juvenile or the adult plant stage. The interaction of QTLs with the environment (QTL × E) is also examined.

Materials and methods

Field experiment

The field experiment was conducted in three Kenyan environments with a population of $F_{2:3}$ lines derived from a cross between the highly susceptible inbred line Lo951 and the highly resistant inbred line CML202. Line Lo951, developed from U.S. Corn Belt maize in Italy (Bertolini et al. 1991), has yellow dent-type kernels, and CML202, developed by CIMMYT-Zimbabwe (Pixley and Zambezi 1996), has white semi-dent-type kernels. Both inbreds belong to the same maturity group (Schechert et al. 1999) and presumably to the same heterotic pool (Welz 1998). In the Long Rainy Season of 1995, 280 $F_{2:3}$ lines and 20 check genotypes were tested at Kakamega (1585 m altitude, 18.6°C annual mean temperature, 1918 mm annual rainfall) and at Embu (1494 m, 19.5°C, 1230 mm; Embu-L). In the Short Rainy Season of the same year, a subset of 194 $F_{2:3}$ lines was again tested at Embu (Embu-S). Trials were laid out as a lattice with two replications and 2-row plots separated by one guard row. Conidial suspensions prepared from the local *S. turcica* populations were used to inoculate each plant at the 4–6-leaf stage (for further details see Schechert et al. 1999).

Disease assessment

Incubation period (IP, the number of days from inoculation to the appearance of wilting lesions) and disease severity were assessed on 15 randomly chosen plants per plot. Disease severity DS was estimated at five dates (Table 1) as the percentage of infected leaf area of the entire plant, disregarding decayed bottom leaves, in classes of 0%, 1 to 10%, 11 to 20%, … , and 91 to 100% blight severity. In Embu-S, only DS 2 to 4 were recorded. The area under the disease-progress curve (AUDPC) was calculated from DS 1 to 5 (Kakamega, Embu-L) or DS 2 to 4 (Embu-S, across environments) according to the standard equation of Campbell and Madden (1990).

Table 1 Timing of disease severity (DS) assessment in field trials

Weeks after	DS				
	1[a]	2	3	4	5[a]
Planting	7–8	9–10	11–12	13–14	15 –16
Inoculation	4	6	8	10	12
Anthesis	− 4	− 2	0	2	4

[a] Not recorded in Embu-S (Short Rainy Season)

Genotyping and data analysis

The RFLP analyses were performed with 105 single- or low-copy probes for 256 $F_{2:3}$ lines of the mapping population by Biofords Consultants, Evry, France, yielding a total of 113 marker loci. For constructing the linkage map, we used MAPMAKER/EXP 3.0 (Lincoln et al. 1992) with Haldane's mapping function. The threshold LOD ($\log_{10}$ of the likelihood odds ratio) value for declaring linkage in two-point analyses was 3.0. Three markers were left unassigned to linkage groups (for details see Schechert et al. 1999). The final map encompassed 110 RFLP loci and had a length of 1853 cM with an average interval length of 16.9 cM. QTL mapping was based on the adjusted entry means of 256, 255, and 194 $F_{2:3}$ lines tested at Kakamega, Embu-L, and Embu-S, respectively. The data of the individual environments and across the three environments ($n = 194$ lines) were analyzed with the software PLABQTL (Utz and Melchinger 1996). This program performs composite interval mapping using a multiple regression approach with selected markers as cofactors. The goodness-of-fit of the regression model was tested with Akaike's information criterion (AIC, Jansen 1993). Via stepwise regression, individual sets of cofactors were selected for each trait and data set by PLABQTL and, by maximizing the R^2 value, subsequently reduced or extended. A dominant-inheritance model was assumed but rejected when the AIC value of the respective additive-effects model was two or more units smaller (for details see Bohn et al. 1996). The likelihood-ratio test of presumed QTLs was given by comparison of the maximized likelihood of a model including a putative QTL and a model without the QTL. Both models included all selected cofactors except those flanking the QTL under consideration. The LOD threshold for declaring the presence of a QTL was set at 3.0, corresponding to an approximate nominal error rate of $\alpha = 0.001$ per test (i.e. marker interval), or 0.10 for the entire genome (Lander and Botstein 1989). The position of a QTL was given by the local maximum of a LOD-score curve. Adjacent QTLs on the same chromosome were considered different when the curve had a minimum between peaks that was at least 1 LOD unit below either peak. The proportions of the phenotypic variance explained by a single or by all QTLs were given by the partial and multiple coefficients of determination, respectively, fitting a model with all putative QTLs at once. The proportion of the genotypic variance explained by QTLs, Q, was obtained by division of the multiple coefficient of determination by the heritability, estimated on an entry-mean basis (Hallauer and Miranda 1988). For testing the hypothesis of no additive or dominance effect of the QTLs, F tests were carried out where F equalled the partial sum of squares of the single-QTL effect divided by the residual mean squares of the multiple-QTL model. The hypothesis of QTL × E interaction was tested as described by Bohn et al. (1996) including first a global F test of QTL × E interaction and, subsequently, F tests

of individual QTL × E interactions. These tests covered only QTLs that were significant in the combined analysis across environments.

Results

Disease progress

In both seasons, moderate levels of leaf blight were reached at Embu. At Kakamega, the epidemic became extremely severe (Fig. 1) and several lines were prematurely killed by *S. turcica*. Differentiation between the parent lines, Lo951 and CML202, and among the $F_{2:3}$ lines was clear in each environment, the mapping population showing a continuous distribution for each trait (Schechert et al. 1999). The resistance levels of Lo951 and CML202 were similar to those of the respective check inbreds A632, being highly susceptible, and CML204, being highly resistant (Fig. 2). The reaction of the $F_{2:3}$ lines was intermediate, relative to the parent lines, indicating additive gene action (Schechert et al. 1999). Repeatability values tended to increase from DS 1 to 5 in each of the trials (Table 2). Across environments, the heritability was highest for IP (86%).

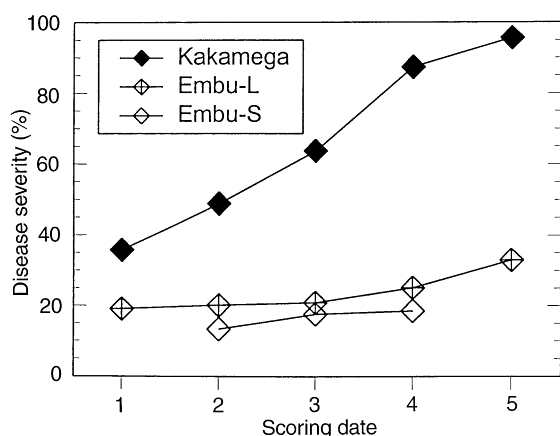

Fig. 1 Disease progress of northern corn leaf blight in three Kenyan environments, averaged across 194 $F_{2:3}$ lines of the cross Lo951 × CML202

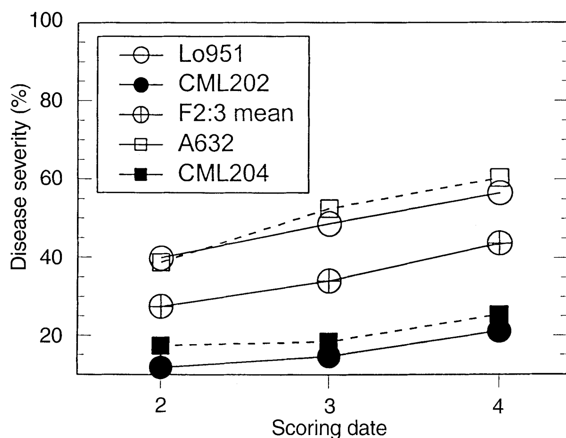

Fig. 2 Disease progress of northern corn leaf blight in 194 $F_{2:3}$ lines of the cross Lo951 CML202, the parental lines, and check inbreds (A632; CML204), averaged across three Kenyan environments

QTL mapping

For each trait and environment, significant QTLs for resistance were detected (Table 2). The number of QTLs ranged between one and ten for the different trait-environment combinations. About equal numbers of QTLs were found with the Kakamega and Embu-L data while Embu-S yielded the fewest QTLs (Table 2). Partial coefficients of determination (%) ranged from 5 to 25%. The smallest significant values were 5.4% for Kakamega, 5.3% for Embu-L, and 7.1% for Embu-S, indicating that the experimental power was smallest in the Embu-S trial. Multiple-R^2 values were highest in Kakamega for DS 2, in Embu-L and across environments for DS 3, and in Embu-S for IP (Table 2). The proportion of the genotypic variance explained by QTLs (Q) was highest for DS 2, in each individual and in the combined trials (Table 2). In Embu-L, the Q for DS 2 was 85%, and in the combined analysis, it reached 78%.

In total, PLABQTL identified 19 putative QTLs. Each chromosome, except number 7, carried QTLs (Table 3). Multiple QTLs resided on chromosomes 2, 3, 4, 5, 9 and 10. The genomic positions of the QTLs were mostly very stable across the component traits of resistance, and across environments. The chromosomal "window" containing the LOD curve peaks was quite narrow for all the trait-environment combinations of each QTL (Table 3, Figs. 3 and 4). One QTL was significant for AUDPC at a genomic position where there was no QTL for IP nor for any of the component scores (Table 3: Embu-S, chromosome 8, *bnl12.30* – *umc030*).

Except for two QTLs on chromosomes 3 (*umc010* – *umc389b*) and 5 (*umc068* – *bnl5.24*), all resistance alleles were contributed by the resistant parent, CML202 (Table 3). The sign of each QTL effect was consistent over the different resistance traits.

Additive gene effects were prevalent. Few QTLs exhibited significant, though not consistent, dominance effects. These were on chromosomes 4 (*bnl7.65* – *umc015*), 5 (*bnl5.40* – *npi461*), and 9 (*umc358* – *bnl5.09*).

QTL × environment interaction

Six QTLs were significant in each of the three environments for at least one resistance trait (Table 3). They were located on chromosomes 2 (*umc371* – *umc381*), 3 (*umc010* – *umc389b*), 4 (*umc119* – *bnl10.05*), 5 (*bnl5.40*

Table 2 QTLs for various traits of resistance to *S. turcica* in the maize population $F_{2:3}$ (Lo951 × CML202) tested in three environments in Kenya

Environment	Trait	No. of QTLs	Partial R^2 range (%)	Multiple R^{2a}(%)	Q^b(%)	h^{2c}	No. of cofactors[d]
Kakamega ($n = 256$)[f]	IP[e]	6	7.1–10.2	42.1	67.1	62.7	10
	DS 1	8	5.4–23.7	54.7	67.9	80.6	9
	DS 2	9	5.4–13.5	55.7	68.3	81.5	12
	DS 3	6	5.4–13.1	49.3	58.8	83.8	9
	DS 4	6	6.6–13.3	46.6	52.8	88.3	10
	DS 5	4	5.9–8.5	35.9	40.7	88.2	10
	AUDPC[g]	9	5.7–11.6	57.6	64.0	90.0	9
Embu-L ($n = 255$)	IP	6	5.3–16.3	41.4	67.3	61.5	10
	DS 1	8	5.4–13.2	49.4	80.3	61.5	11
	DS 2	8	6.1–9.5	50.2	84.7	59.3	16
	DS 3	9	5.3–10.6	52.3	80.0	65.4	15
	DS 4	9	5.4–14.3	47.1	67.5	69.8	14
	DS 5	8	5.4–11.7	46.1	65.1	70.8	13
	AUDPC	10	5.4–15.8	54.4	75.2	72.3	11
Embu-S ($n = 194$)	IP	3	9.5–12.6	29.7	50.5	58.8	10
	DS 2	5	7.1–13.2	27.1	60.6	44.7	13
	DS 3	1	10.5	5.8	10.9	53.1	10
	DS 4	3	7.6–11.8	22.0	32.7	67.3	7
	AUDPC	5	7.6–15.4	27.7	49.8	55.6	9
Across environments ($n = 194$)	IP	8	7.0–11.8	52.2	60.8	85.9	10
	DS 2	7	7.2–24.8	55.4	77.5	71.5	14
	DS 3	9	6.9–18.0	56.4	71.4	79.0	13
	DS 4	6	6.9–22.5	47.7	59.9	79.6	16
	AUDPC	8	6.9–18.3	55.8	69.3	80.5	17

[a] Proportion of phenotypic variance explained by QTLs
[b] Proportion of genotypic variance explained by QTLs
[c] Repeatability (for individual environments) or heritability (across environments)
[d] Number of markers used as cofactors in multiple regression analysis
[e] Incubation period (IP) and disease severity (DS)
[f] Number of $F_{2:3}$ lines tested
[g] Area under the disease progress curve, calculated over DS1 to DS5 (Kakamega; Embu-L) or over DS2 to DS4 (Embu-S; across environments)

– *npi461*), 8 (*umc323 – umc030*), and 9 (*umc380 – bnl7.57*). Another three QTLs, on chromosomes 1 (*umc372 – umc325*) and 5 (*umc001 – bnl5.40, umc068 – bnl5.24*), were significant in two environments. The remaining QTLs were only significant in a single environment (Table 3). All QTLs being significant in three or two environments, and even two of the QTLs detected in just one environment (on chromosomes 2: *umc044 – umc331*; 3: *umc361 – bnl15.20*), were also significant in the combined analysis. One QTL (chromosome 10: *umc157 – npi264*), possibly a false positive, was significant in the combined analysis, but not for any trait in any specific environment.

Meaningful statistical tests of QTL × E interaction could be conducted for the DS 2, DS 3, and AUDPC traits. DS 1 and DS 5 had only been assessed in two environments, and for IP and DS 4, too many missing values resulted in negative mean-square estimates for the global QTL × E variance component. In this case no F tests could be performed on individual QTL × E interaction. The global test of QTL × E interaction indicated significance ($P < 0.01$) for DS 2, DS 3, and AUDPC. Five out of seven QTLs for DS 2, and four out of seven QTLs for DS 3 and AUDPC were responsible for the effect (for both traits, no test was performed for the QTLs on chromosome 8 due to a critical proportion of missing values). Four QTLs were nearly consistently affected by interaction with the environment (Table 3), viz. the ones on chromosomes 2 (*umc371 – umc381*), 5 (*umc001 – bnl5.40*; *bnl5.40 – npi461*), and 9 (*umc380 – bnl7.57*). All of these QTLs were not significant in the Embu-S environment.

An appraisal of the importance of QTL × E interaction can also be gained by comparing the results from Kakamega and Embu-L, two trials with nearly the same sample size and thus power of QTL detection, and with an equal number of disease assessments (Table 2). Of the 15 QTLs discovered with both data sets for at least one resistance trait, eight were significant for both Kakamega and Embu-L. Three were significant only at Kakamega and four only at Embu-L (Table 3).

Dynamic gene action at QTLs

The QTL on chromosome 9 near *umc340* was consistently highly significant throughout the epidemic

Table 3 Magnitudes of additive gene effects at 19 putative QTLs for resistance to *S. turcica* in the maize population $F_{2:3}$ (Lo951 × CML202), in individual and across environments (ACROSS). Boxed segments indicate significant QTL × environment interaction (solid line: $P < 0.01$; thin line: $P < 0.05$)

QTL position		Environment	Trait						
Chromosome	Marker interval[a]		IP[b] (d)	DS 1[c] (%)	DS 2 (%)	DS 3 (%)	DS 4 (%)	DS 5 (%)	AUDPC[d] (area units)
1	*umc372 – umc325*	Kakamega	ns[e]	– 2.76	– 3.35	ns	ns	ns	ns
		Embu-L	0.38	ns	ns	ns	ns	ns	ns
		Embu-S	ns	–[f]	ns	ns	ns	–	ns
		ACROSS	0.47	–	ns	ns	ns	–	ns
2	*umc044 – umc331*	Kakamega	0.25	ns	ns	– 3.82	– 4.76	– 2.33[D]	– 199
		Embu-L	ns	ns	ns	ns	ns	ns	ns
		Embu-S	ns	–	ns	ns	ns	–	ns
		ACROSS	ns	–	ns	ns	– 1.95	–	ns
	umc371 – umc381	Kakamega	ns	– 3.61	– 4.62	– 3.26	ns	ns	– 190
		Embu-L	0.26	– 1.66	– 1.82	– 2.47	– 2.52	– 3.89	– 201
		Embu-S	ns	–	ns	ns	– 1.66	–	ns
		ACROSS	0.27	–	– 2.72	– 3.13	ns	–	– 127
3	*umc010 – umc 389b*	Kakamega	– 0.24	ns	2.35*	ns	ns	ns	ns
		Embu-L	ns	1.51	1.64	1.80	ns	ns	113
		Embu-S	ns	–	0.64[D]	ns	1.81	–	48*
		ACROSS	– 0.24	–	2.91	2.76	ns	–	111
	umc361 – bnl15.20	Kakamega	ns	ns	ns	ns	ns	ns	ns
		Embu-L	ns	ns	ns	ns	ns	ns	ns
		Embu-S	ns	–	– 1.50	– 2.08	– 2.37	–	– 119
		ACROSS	ns	–	– 1.59	– 2.11	ns	–	– 96
	umc096 – umc339b	Kakamega	ns	– 1.93[D]	– 2.79	– 3.89	– 5.22	– 2.41	– 195
		Embu-L	ns	ns	ns	ns	ns	ns	ns
		Embu-S	ns	–	ns	ns	ns	–	ns
		ACROSS	ns	–	ns	ns	ns	–	ns
4	*umc119 – bnl10.05*	Kakamega	0.33	– 1.60	ns	ns	ns	ns	– 156*
		Embu-L	0.36	– 0.97	ns	– 1.58	ns	ns	– 105
		Embu-S	0.34	–	ns	ns	ns	–	ns
		ACROSS	0.29	–	ns	ns	ns	–	ns
	bnl7.65 – umc015	Kakamega	ns	ns	ns	ns	ns	ns	ns
		Embu-L	ns	ns	– 2.11	ns	– 1.85	– 4.02[D]	– 14[D]
		Embu-S	ns	–	ns	ns	ns	–	ns
		ACROSS	ns	–	ns	ns	ns	–	ns
5	*umc001 – bnl5.40*	Kakamega	0.49	– 3.06	– 2.75	– 3.83	– 3.58	ns	– 216
		Embu-L	ns	– 1.91	– 2.92	ns	ns	ns	– 157
		Embu-S	ns	–	ns	ns	ns	–	ns
		ACROSS	0.33	–	– 1.75	– 1.84	– 1.88	–	– 91
	bnl5.40 – npi461	Kakamega	0.25[+]	– 1.93	– 2.50[D]	ns	ns	ns	– 210[D]
		Embu-L	0.37	– 1.67[D]	ns	– 2.56	– 3.05	– 5.33	– 175[D]
		Embu-S	0.29	–	ns	ns	ns	–	ns
		ACROSS	0.30	–	– 1.54	– 1.82	ns	–	– 76
	umc068 – bnl5.24	Kakamega	ns	ns	ns	ns	ns	ns	ns
		Embu-L	ns	ns	ns	2.11	2.56	4.49	148
		Embu-S	ns	–	0.83[D]	ns	ns	–	115*
		ACROSS	ns	–	ns	1.87	2.30	–	77
6	*umc313 – bnl5.47*	Kakamega	ns	ns	ns	ns	ns	ns	ns
		Embu-L	ns	ns	ns	ns	– 2.13	– 3.29	ns
		Embu-S	ns	–	ns	ns	ns	–	ns
		ACROSS	ns	–	ns	ns	ns	–	ns
8	*bnl12.30 – umc030*	Kakamega	ns	– 1.88	– 2.47	ns	– 2.33[+]	ns	– 132
		Embu-L	0.46	– 1.49	– 1.63	– 2.16	– 2.53	– 3.72	– 163
		Embu-S	ns	–	ns	ns	ns	–	– 59
		ACROSS	0.29	–	– 1.95	– 2.78	– 3.16	–	– 117

Table 3 Continued

QTL position: Chromosome	QTL position: Marker interval[a]	Environment	Trait: IP[b] (d)	DS 1[c] (%)	DS 2 (%)	DS 3 (%)	DS 4 (%)	DS 5 (%)	AUDPC[d] (area units)
9	*umc380 – bnl7.57*	Kakamega	0.26[D]	– 2.65	– 3.70	– 3.95	– 3.22[D]	– 2.10	– 205
		Embu-L	0.35[D]	– 1.93	– 1.84	– 2.45	– 3.46	– 5.24[D]	– 220
		Embu-S	0.37	–	ns	ns	ns	–	ns
		ACROSS	0.33	–	– 1.84	– 2.24	– 2.26	–	– 104
	umc358 – bnl5.09	Kakamega	ns	ns	ns	ns	ns	ns	ns
		Embu-L	ns	ns	– 0.99[D]	– 1.31[D]	– 0.86[D]	ns	– 44[D]
		Embu-S	ns	–	ns	ns	ns	–	ns
		ACROSS	ns	–	ns	ns	ns	–	ns
10	*umc373 – umc064*	Kakamega	ns	ns	ns	ns	ns	ns	ns
		Embu-L	ns	ns	ns	ns	ns	ns	ns
		Embu-S	ns	–	– 1.03	ns	ns	–	– 81
		ACROSS	ns	–	ns	ns	ns	–	ns
	umc157 – npi264	Kakamega	ns	ns	ns	ns	ns	ns	ns
		Embu-L	ns	ns	ns	ns	ns	ns	ns
		Embu-S	ns	–	ns	ns	ns	–	ns
		ACROSS	ns	–	ns	– 1.84	ns	–	ns
	npi264 – umc130	Kakamega	ns	ns	2.45	5.05	5.94	2.92	– 226
		Embu-L	ns	ns	ns	ns	ns	ns	ns
		Embu-S	ns	–	ns	ns	ns	–	ns
		ACROSS	ns	–	ns	ns	– 2.33	–	ns
Total of absolute gene effects		Kakamega	1.82	19.42	26.98	23.80	25.05	9.76	1729
		Embu-L	2.18	11.14	12.95	16.44	18.96	29.98	1340
		Embu-S	1.00	–	4.00	2.08	5.84	–	422
		ACROSS	2.52	–	14.30	20.39	13.88	–	799

+,* Additive effect significant at $P = 0.10$ or 0.05, respectively. Where nothing is indicated, effect significant at $P = 0.01$
[a] Cf. Figs. 3 and 4, or Schechert et al. (1999), for chromosomes 6 and 10
[b] Incubation period
[c] Disease severity. Negative effects for DS1 to DS5 indicate resistance allele from CML202, positive effects: resistance allele from Lo951. Reverse applies for incubation period
[d] Calculated across five (Kakamega; Embu-L) or three (Embu-S; ACROSS) disease readings
[e] Likelihood of QTL below significance threshold (LOD = 3.0)
[f] Not assessed
[D] QTL has significant dominance effect, too ($P = 0.05$)

(Fig. 3). But for most QTLs, the likelihood of their presence at a certain genomic position and the magnitude of their gene effects changed during host plant development. The QTL on chromosome 1, for example, had a significant effect on IP but was not significant for later disease scores (Fig. 3). The same is true for the QTL on the short arm of chromosome 4 (Fig. 3). Similarly, the resistance allele from Lo951 on chromosome 3, was ineffective at later stages, even at Embu-L where NCLB severity remained moderate (Table 3). In contrast, the QTL on chromosome 8 gained stepwise in significance from IP to DS 2, DS 3, and lastly DS 4 (Fig. 4).

Across environments, only three QTLs conditioned adult-plant resistance, in the sense that they were only effective after flowering (chromosomes 2: *umc044 – umc331*; 5: *umc068 – bnl5.24*; 10: *npi264 – umc130*; Table 3).

A unique pattern of gene action was associated with the three QTLs on chromosome 5 (Fig. 3). Two resistance alleles came from CML202 and the third was contributed by Lo951 (Table 3). The first QTL, close to *umc001*, was significant throughout the epidemic (Fig. 3). The second QTL, close to *umc051*, was highly significant at the start of the epidemic (IP) but its likelihood gradually decreased towards DS 4. The third QTL, close to *umc068*, was not significant for IP but its likelihood gradually increased towards DS 4.

At Kakamega, the strongest effects were detected for the intermediate disease readings, DS 2 to 4, whereas in Embu-L and Embu-S the last reading showed the strongest total effect. For example on chromosome 2, the QTL flanked by *umc371* and *umc381* (Table 3) was most effective for DS 2 at Kakamega, after which it declined, whereas in Embu-L the effect gradually increased toward DS 5. In Embu-S, only DS 4 gave a significant effect. The same pattern was expressed by the second QTL on chromosome 5 (*bnl5.40 – npi461*). This QTL was not significant beyond DS 2 at Kakamega, but had a rising effect in Embu-L (Table 3);

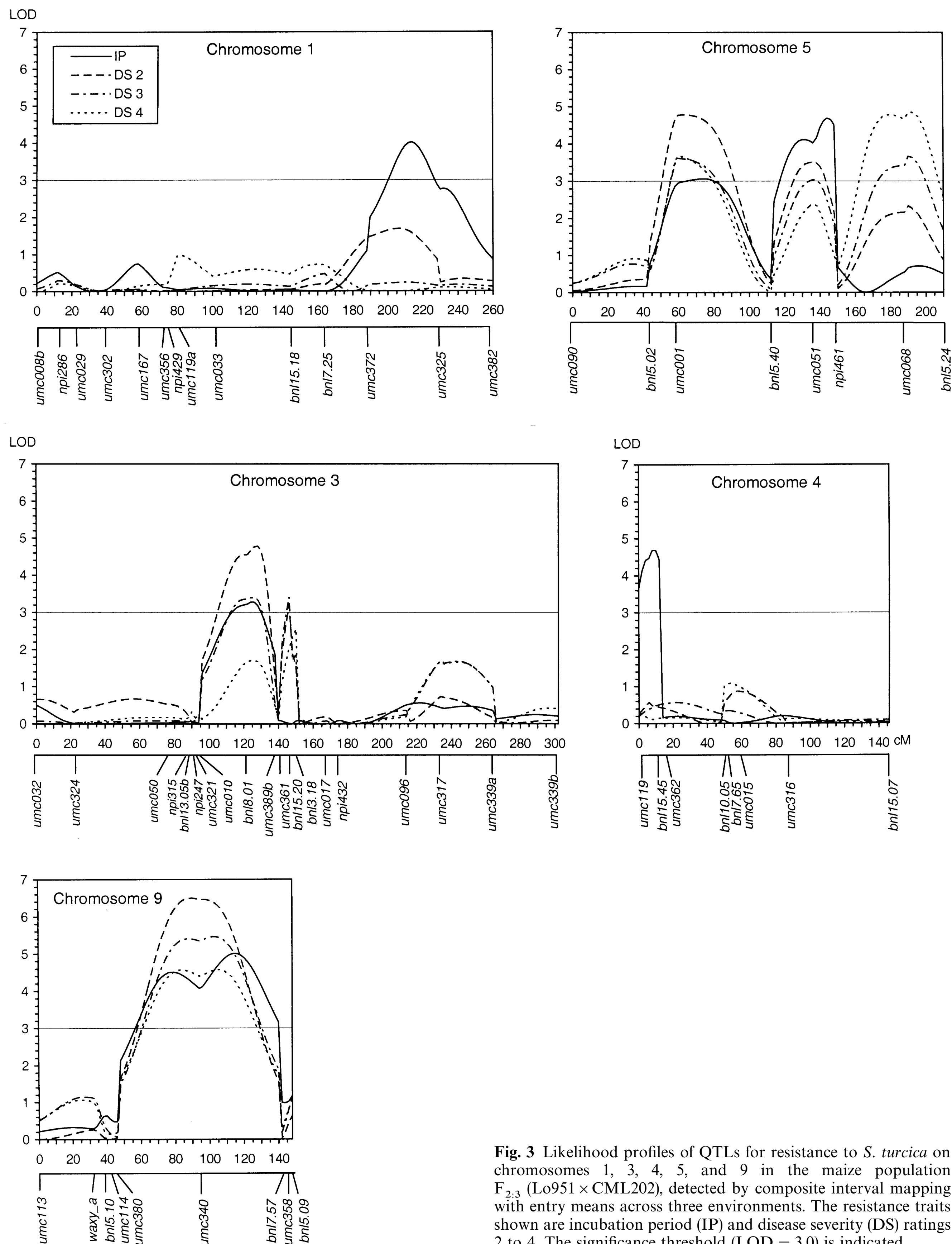

Fig. 3 Likelihood profiles of QTLs for resistance to *S. turcica* on chromosomes 1, 3, 4, 5, and 9 in the maize population $F_{2:3}$ (Lo951 × CML202), detected by composite interval mapping with entry means across three environments. The resistance traits shown are incubation period (IP) and disease severity (DS) ratings 2 to 4. The significance threshold (LOD = 3.0) is indicated

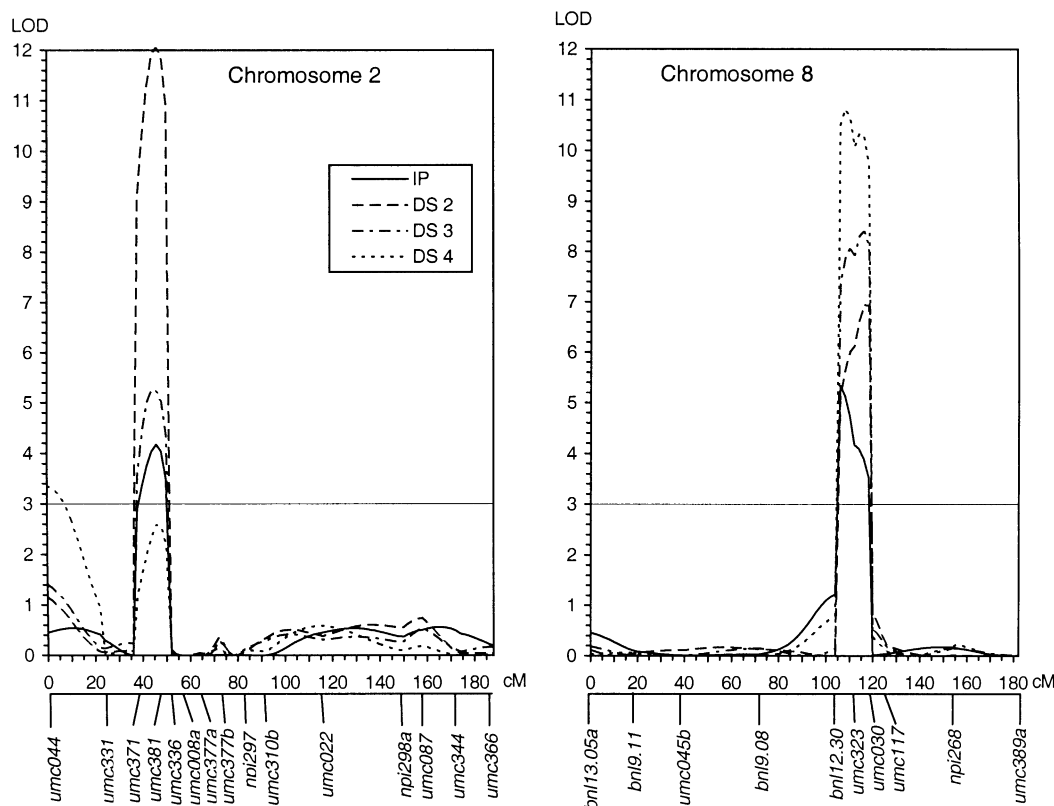

Fig. 4 Likelihood profiles of QTLs for resistance to *S. turcica* on chromosomes 2 and 8 in the maize population $F_{2:3}$ (Lo951 × CML202), detected by composite interval mapping with entry means across three environments. The resistance traits shown are incubation period (IP) and disease severity (DS) ratings 2 to 4. The significance threshold (LOD = 3.0) is indicated

similarly for the dynamics of the QTL on chromosome 9 (*umc380 – bnl7.57*). A condensed picture of this location-specific dynamics is given by the summed absolute values of QTL effects, sorted by traits and environments (Table 3, bottom). In Embu-L, the total effect of QTLs was maximal for DS 5, a disease severity reduction of 30% (Table 3), at an average NCLB severity of 33% (Fig. 1). At Kakamega the total QTL effect was greatest for DS 2 making up 27%, at an average disease severity of 49%.

Discussion

The interpretation of the observed QTL dynamics needs to consider the fact that the estimate of a resistance gene's effect depends on the overall disease intensity, just as the effect of a yield QTL would depend on the average yield level. This pattern is exemplified by the Embu-L trial in which the average QTL effect steadily increased from 1.39 at DS 1 to 3.75 at DS 5. We did not standardize gene effects by relating their magnitude to the average disease severity because that would have obscured the different precision of trials: Embu-S had relatively low repeatabilities and yielded few QTLs because drought stress had caused heterogeneous crop and disease development (Schechert 1997). The later scores at Kakamega were less informative than the earlier scores, probably due to a scaling effect, as the frequency distribution of the population became increasingly skewed at means above 80% disease severity. Nevertheless, the results clearly demonstrated that QTLs for resistance to *S. turcica* may be either effective early or late, or throughout plant development. QTLs for juvenile-plant resistance were prevalent in this population. This may hold true for maize in general, considering the tight correlation between IP and AUDPC, or between IP and post-flowering disease scores, observed in tropical and temperate germplasm (Smith and Kinsey 1993; Carson 1995; Schechert et al. 1997, 1999; Welz et al. 1998). In contrast, barley infected by *Puccinia hordei* carried mostly QTLs for adult-plant resistance, expressing resistance in the flag leaf but not at the seedling stage (Qi et al. 1998).

QTL × E interaction affected more than half of all QTLs. Where statistical evidence was obtained for the interaction, the underlying cause was mostly a lack of QTL effectiveness at Embu-S compared to Embu-L and Kakamega (Table 3). When a QTL was

consistently significant in only a single environment, e.g. the QTL on chromosome 3 flanked by *umc096* and *umc339b*, the *F* test could not be applied; yet this sort of pattern is indicative of environmental specificity, too. Furthermore, environment-specific QTLs were not less effective than non-specific ones. For example, the three QTLs found at Kakamega, but not Embu-L or Embu-S, (on chromosomes 2: *umc044 – umc331*, 3: *umc096 – umc339b*, and 10: *npi264 – umc130*) were all highly effective and associated with large AUDPC reductions (Table 3). Experiments with a broad range of populations and environments are needed to better understand the importance of QTL × E effects. Greenhouse experiments using different pathotypes might be useful to partition the underlying cause into its abiotic and pathogenic components. Indeed, the two Kenyan locations Embu and Kakamega are climatically somewhat different, but Carson and Dyke (1994) showed under controlled conditions that the ranking in IP of quantitatively resistant maize inbred lines was largely unaffected by varying temperature and light conditions. It may be more relevant for the observed QTL × E interaction effects that Embu and Kakamega harbour genetically distinct *S. turcica* populations. Their DNA marker-allele frequencies were different (Borchardt et al. 1998a, b), and at Embu virulence to the major resistance gene *Htn1* was common (97%) while it was nearly absent (1%) at Kakamega (Welz 1998).

The finding of different developmental patterns of resistance gene expression and the necessity to concentrate on few QTLs in a marker-assisted breeding program emphasize the question which of the detected QTLs are agronomically most important. Theoretically, genes expressed in juvenile maize should be more useful than genes expressed only in adult maize because northern leaf blight reduces grain yield most when it occurs before flowering (Ullstrup and Miles 1957). Furthermore, useful QTLs should be effective in all target environments of a maize hybrid. According to these demands, the six resistance alleles from CML202 on chromosomes 2 (*umc371 – umc381*), 4 (*umc119 – bnl10.05*), 5 (*bnl5.40 – npi461; bnl5.40 – npi461*), 8 (*umc323 – umc030*), and 9 (*umc380 – bnl7.57*) may be of great practical interest. All were effective at Kakamega and Embu, and all significantly prolonged the incubation period, which means they were expressed at an early stage.

The QTL mapping approach has proven to be useful for identifying genomic regions controlling quantitative disease resistance in maize and other crops. The challenge remains to apply this genetic information in marker-assisted breeding programs.

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