Theor Appl Genet (2006) 113:321–330
DOI 10.1007/s00122-006-0298-y

ORIGINAL PAPER

A region of maize chromosome 2 affects response to downy mildew pathogens

Ahmed Sabry · Dan Jeffers · S. K. Vasal · Richard Frederiksen · Clint Magill

Received: 24 May 2005 / Accepted: 19 April 2006 / Published online: 20 May 2006

Abstract Quantitative trait loci (QTLs) for downy mildew resistance in maize were identified based on co-segregation with linked restriction fragment length polymorphisms or simple sequence repeats in 220 F2 progeny from a cross between susceptible and resistant parents. Disease response was assessed on F3 families in nurseries in Egypt, Thailand, and South Texas and after inoculation in a controlled greenhouse test. Heritability of the disease reaction was high (around 93% in Thailand). One hundred and thirty polymorphic markers were assigned to the ten chromosomes of maize with LOD scores exceeding 4.9 and covering about 1,265 cM with an average interval length between markers of 9.5 cM. About 90% of the genome is located within 10 cM of the nearest marker. Three putative QTLs were detected in association with resistance to downy mildew in different environments using composite interval mapping. Despite environmental and symptom differences, one locus on chromosome 2 had a major effect and explained up to 70% of the phenotypic variation in Thailand where disease pressure was the highest. The other two QTLs on chromosome 3 and chromosome 9 had minor effects; each explained no more than 4% of the phenotypic variation. The three QTLs appeared to have additive effects on resistance, identifying one major gene and two minor genes that contribute to downy mildew resistance.

Communicated by D. A. Hoisington

A. Sabry · R. Frederiksen · C. Magill (✉)
Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843, USA
E-mail: c-magill@tamu.edu

D. Jeffers · S. K. Vasal
International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, Mexico, DF 06600, Mexico

Introduction

Sorghum downy mildew (SDM) of maize is caused by the oomycete *Peronosclerospora sorghi* (Weston and Uppal) C.G. Shaw, an obligate pathogen that cannot be cultured in the laboratory. Other downy mildews in maize result from infection with closely related oomycetes, including *Peronosclerospora philippinensis*, *Peronosclerospora maydis*, *Peronosclerospora heteropogoni*, and *Peronosclerospora zeae*. SDM of maize is a disease of great destructive potential since systemically infected plants seldom produce an ear. The disease occurs in maize and sorghum in warm, humid areas of the world. In Egypt, sorghum species are the primary hosts of *P. sorghi* where large quantities of oospores are produced. SDM became a global disease of maize during the years of rapid expansion of the use of sorghum for grain and forage. Frederiksen and Renfro (1977), Williams (1984), and more recently, Craig and Odvody (1992) reviewed the work on conventional disease management. Overall, these management practices have been successful in controlling the downy mildews in most of the maize growing regions of the world. There are exceptions, however, including Egypt where SDM of maize remains a serious problem (Nazim et al. 1995) as a consequence of cultural practices that often leave plots of maize surrounded by sorghum. Further, a recent report of metalaxyl resistant *P. sorghi*

(Isakeit et al. 2003) suggests alternative control methods may become even more important. The other maize downy mildew pathogens cause even more severe disease symptoms, but generally are restricted in distribution to areas of southeast Asia (Frederiksen and Renfro 1977).

Genetic resistance to SDM in maize has been reported. A relatively simple mode of inheritance has been reported from a cross involving two Egyptian inbreds. Three quantitative trait loci (QTLs) that contribute resistance to *P. sorghi* were detected in a population of recombinant inbred lines derived from a cross between resistant (G62) and susceptible (G58) inbred lines (Agrama et al. 1999). Two of the loci map close together on chromosome 1 while the third one was on chromosome 9. Several potential sources of genetic resistance in maize to SDM and other downy mildews have been identified in advanced inbred lines from CIMMYT germplasm. Recently, six QTLs on five chromosomes (1, 2, 6, 7, and 10) were identified in a RIL population from the cross of Ki3 (resistant) by CML139 (susceptible) (George et al. 2003) based on tests in India, Indonesia, Thailand, and the Philippines. Nair et al. (2005) identified SDM resistance loci on maize chromosomes 2, 3, and 6 in the Indian maize line NAI116 and verified that the locus on chromosome 6 also contributed resistance to diverse downy mildews.

One of the major difficulties in working with downy mildew resistance in maize has been the difficulty in accurately scoring the disease reaction. Plant maturity and the amount of pathogen inoculum are among the factors influencing the disease expression. As a consequence, multiple evaluations made in different locations are required for accurate assessment of genetic contributions to resistance. Identification of simple and accurately scored molecular markers for genes that contribute to downy mildew resistance of maize could greatly benefit future efforts to prevent diseases losses, especially if there are differences in the pathogen populations or environment by genotype interactions in different locations. The objective of this study was to identify chromosomal regions that contribute to the downy mildew resistance seen in a previously identified maize line.

Materials and methods

Mapping population

Two inbred maize lines were selected for this study based on response to downy mildew in screening trials in the Philippines. The trials included 30 lines originating from Thailand, Indonesia, Mexico, and the Philippines. P345C4S2B46-2-2-1-2-B-B-B (yellow), which was consistently resistant, was selected as the resistant parent (RP) and SC-TEP5-19-1-3-1-4-1-1 (white) served as the susceptible parent (SP). An F1 from the cross SP × RP was self-pollinated and 220 F2 individuals were selected at random for analysis. Tissue samples were collected from individual F2 plants before they were self-pollinated to produce F3 families that were evaluated for SDM responses to determine the phenotypic character for each family.

Sample collection and preparation for DNA isolation

Young leaves without necrotic areas or lesions were collected from the parents and F2 individuals. Midribs were removed and 10–15 cm leaf sections were collected in fiberglass screen mesh bags. Samples were frozen in liquid nitrogen and lyophilized 72 h. A mechanical mill (Tecator Cyclotec Sample Mill, Model 1093, Herndon, VA, USA) was used for grinding dried leaf samples. The fine powder obtained from each sample was stored in air-tight vials and kept at –20°C until DNA extraction.

DNA protocols

DNA extraction, quantification, digestion, and Southern hybridization using digoxigenin-dUTP labeled probes followed established protocols (Hoisington et al. 1994). For restriction fragment length polymorphism (RFLP) detection, 90 μg of DNA from each of the 220 samples was aliquoted into two 1.5-ml labeled tubes to be digested separately with restriction endonucleases *Eco*RI and *Hin*dIII. Fragments of *Hin*dIII-digested λ DNA Gibco BRL (now part of Invitrogen, Carlsbad, CA) labeled with digoxigenin-dUTP (Boehringer Mannheim, Indianapolis, IN) were prepared for use as size standard. Following electrophoresis in 0.7% agarose (Seakem LE) gels using 1 × TAE buffer, DNA was blotted to non-charged MSI Magnagraph Nylon membranes 0.45 μm pore size (Gibco BRL) and fixed using a ''Uv Crosslinler'' (Stratagene, Torrey Pines, CA) set to deliver 120,000 $\mu J/cm^2$.

RFLP markers

A set of 160 cloned RFLP probes from the Brookhaven National Laboratory (bnl) and the University of Missouri at Columbia (umc) were used to detect polymorphism between parental lines. The F2

individuals were screened for polymorphism using 60 polymorphic RFLP probes.

SSR protocols

A set of 496 simple sequence repeat (SSR) primers covering various ''bin'' locations in the maize genome was obtained from Research Genetics (now part of Invitrogen). Additional primer pairs expected to fill mapping gaps were identified from the maize genome database http://www.maizegdb.org/ssr.php and were custom made in the Advanced DNA Technology Laboratory in the Biology Department, Texas A&M University. All the PCR reagents except primers and deoxynucleotide triphosphates (dNTPs) were purchased from Promega (Madison, WI). Mastermixes were prepared so that each 15 µl PCR reaction contained final concentrations as follow: 1 × reaction buffer, 2.5 mM $MgCl_2$, 200 µM each dNTPs, and 0.3 µM each primer. Each reaction also contained 40 ng of template DNA, 10% glycerol, and 1 U of *Taq* DNA polymerase and was overlaid with 15 µl of mineral oil. Amplification was performed in a 96-well GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA). The temperatures and times used for PCR were as follows: in the first cycle, the mixtures were denatured at 94°C for 3 min, followed by 2 min at 56°C for primer annealing, and 2 min at 72°C for primer extension. The conditions for the following 29 cycles were the same as the first, except the time for DNA denaturation was reduced to 1 min. The final extension time for PCR amplification was 5 min at 72°C. Three microliters of 5 × SGB were added to each amplified sample.

Resolution of SSR amplified products

A 4% large super fine resolution agarose (Amresco) gel, 20 cm × 28 cm, was used for resolution of the SSR amplified products. Ethidium bromide was added to the melted agarose to a final concentration of 2 µg/ml before pouring into gel trays. Three gels were used to contain the 220 F2 individuals in addition to the parents and the molecular weight marker ϕX174/*HAE*III.

Marker analysis

The program ''Map Manager QTX'' by Manly et al. (2001) was used to establish linked marker groups and to create a genetic map. Distances between markers are presented in centiMorgans derived using the Kosambi function (Kosambi 1944) with linkage criterion $P < 0.00001$.

Phenotyping of the mapping population in field trials

Seeds collected from each of the F3 families as well as the two parental lines were grown in four field trials to evaluate the responses to downy mildew. Evaluation was conducted at two sites in Thailand (Suwan Farm, Kasetsart University), one in Gemmeiza, Egypt, and in Corpus Christi, Texas. The field experimental design in the Thailand and Egypt trials was randomized block design, with single row plots in three replications. Each row was sown with about 20 seeds at a within-row spacing of 0.25 m in 6 m long rows spaced 0.75 m apart. Each replicate was partitioned to 15 blocks and each block contained 15 rows. Because of the limitation of seed quantity, the Corpus Christi location was planted in one replication. Disease was established by planting a highly susceptible, locally adapted sorghum variety to serve as a spreader in every third row throughout the field at least 3 weeks prior to the expected date of planting of the tested materials. In Corpus Christi, 20–30 seeds from each F3 family were planted between rows of systemically infected sorghum. All materials were subjected to natural infection via spores dispersed from the spreader rows. The percentage of infected plants was scored 3 weeks after planting. In Corpus Christi, the percentage of plants showing local lesions or symptoms of systemic infection (Craig 2000) was scored for analysis separately or in combination. At both Corpus Christi, Texas and Gemmeiza, Egypt, *P. sorghi* Pathotype 1 is predominant while in Thailand, *P. sorghi* Thai strain, which was suggested by Yao (1991) to be renamed *P. zeae*, is predominant.

Greenhouse trials

In addition to the field evaluation, F3 families were tested for susceptibility in the greenhouse of the Plant Pathology Department, Texas A&M University at College Station. The greenhouse trial was conducted in three replications. Each replication consisted of nine trays. Using two 1/4″ Jiffy Strips, each tray contained 25 F3 families in addition to three control pots of sweet corn ''Golden Bantam.'' Each pot was sown with five seeds on an average. Pathotype 3 of *P. sorghi* was used to evaluate the F3 families in the greenhouse. Test trays were inoculated at 6 days after emergence with a conidial suspension adjusted to approximately 1×10^5/ml H_2O and applied at a rate equivalent to approximately 1 ml per plant using an atomizer. Conidial suspension was prepared daily as described by Cardwell et al. (1994). The percentage of infected plants in each F3 family was

scored 7 and 14 days after inoculation. The first reading was based on local infection score, while the second reading was mainly systemic symptoms.

Statistical analysis

The phenotypic data from both the field and greenhouse trials were recorded as the percentage of plants showing SDM infection. The percentages covered a wide range of values between 0 and 100. The binomial distribution was normalized to make the means and variances independent and normally distributed using the arcsine transformation (Little and Hills 1978). For field and greenhouse data, analyses of variance were conducted on transformed phenotypic data for individual environments using PROC GLM, SAS Institutes. Analysis of variance was also conducted on transformed values of the sweet corn controls across all trays used in the greenhouse evaluations to test for significant difference between trays and to detect if there is any bias resulting from daily preparation of the inoculum.

Bartlett's test was used to test for homogeneity between environments before combining the data (Gomez and Gomez 1984). Components of variance for the F3 families in all locations and across the field locations were computed considering various effects (locations, replicates, and F3 families) as random in the statistical model. Transformed entry means were used to compute the combined analyses of variance and covariance across environments as described by Bohn et al. (1996). Estimates of variance components σ^2 (error variance), σ^2_{ge} [genotype-by-environment (G × E) interaction variance], and σ^2_g (genotypic variance) of F3 families were calculated as described by Searle (1971). Heritability (h^2) on a F3 family transformed mean basis was estimated as described by Hallauer and Miranda (1981), where r = number of replications and e = number of environments.

$$h^2 = \frac{\sigma^2 g}{\frac{\sigma^2}{re} + \frac{\sigma^2 ge}{e} + \sigma^2 g}$$

QTL analysis

The analysis of QTLs was performed on the means of F3 family replicates for the arcsine-transformed data within each trial as well as across trials. Means were subtracted from 100 to get means of resistance percentage for each F3 family. The program "Map Manager QTXb17" (Manly et al. 2001) was used to detect significant association between segregating molecular markers and SDM resistance as a quantitative trait using a threshold of $P < 0.00001$. Duplicated and very close markers were eliminated before mapping of the QTLs to avoid regression failure.

The "marker regression option" was used to identify a list of all loci associated with SDM as a quantitative trait, which also provided the likelihood ratio statistic (LRS) and P value for the association in addition to a confidence interval and separate regression coefficients for additive and dominance effects. LRS, described by Haley and Knott (1992), is the measure of the significance of a possible QTL. The LRS can be interpreted as a χ^2 statistic or as a LOD score, but the LOD differs from conventional base-10 LOD scores by a factor of 4.6. The LRS needed for significance is about 20 for an F2 cross as mentioned by Lander and Kruglyak (1995).

The interval mapping procedure was used to fit a regression equation for the effect of a hypothetical QTL at the position of each marker locus and at regular intervals between the marker loci. This procedure is based on the work of Haley and Knott (1992), Martinez and Curnow (1992), and Zeng (1993, 1994). This procedure also provided the resulting regression coefficient(s) and a LRS that measures the significance of the coefficient(s). Because the population is an intercross population, a "free" model was used by QTX to fit coefficients for both additive and dominance effects.

The "permutation test" described by Churchill and Doerge (1994), which estimates an empirical genome-wide probability for observing a given LRS score by chance, was used to establish the significance of the LRSs generated by the interval mapping procedure.

Composite interval mapping (CIM) as described by Bohn et al. (1996) was also used for mapping of QTLs and estimation of their effects. QTLs other than the one being mapped can be called "background" loci. These background QTLs have two effects. Those which are not linked to the QTL being mapped behave like additional environmental effects and reduce the significance of any association. Those which are linked to the QTL being mapped bias the estimated location of that QTL. QTX provided the option to include or exclude other markers in the regression to reduce the effects of background QTLs.

Results

Identification of DNA-based polymorphism in the parental lines

Initial screening to identify polymorphisms in the F2 population used 160 clones previously located

throughout the maize RFLP maps. Of these, 60 revealed useful polymorphisms. Subsequently, a total of 496 SSR primer pairs were used to screen the parent lines for polymorphism. Gaps in the map were filled by testing SSR primer pairs selected from the same or adjacent bins. Eventually, based on map location and clarity of the polymorphic bands, 99 of 150 polymorphic SSRs were selected for use with the entire F2 population. The combination of 130 informative RFLP and SSR markers, all of which fit 1:2:1 Mendelian segregation ratios, were used for linkage analysis.

Linkage mapping

Unlinked and dominant markers were not useful for linkage analysis, leaving 130 markers assigned to ten linkage groups using Map Manager QTX with linkage criterion $P < 0.00001$. All markers were linked to the map with LOD scores exceeding 4.9. The map covered about 1,265 cM with markers distributed over all chromosomal regions with an average interval length of 9.5 cM. Table 1 shows the number of markers used per chromosome and indicates the gaps. About 90% of the genome was within 10 cM of the nearest marker. The map was largely in agreement with the most recently published RFLP and SSR maps and database established for temperate maize by ''Maize GDB'' (http://www.maizegdb.org/maizedb.php).

Table 1 Numbers of informative markers for each maize chromosome and potential gaps in coverage

Chromosome	No. of markers	Largest internal gap in cM	Bins[a] at ends with no markers
1	19	35.3	1.00 and 1.12
2	18	29.5	2.00
3	11	23.7	3.00
4	11	28.7	4.00–4.04[b]
5	17	19.0	5.00 and 5.09
6	14	19.8	None
7	13	37.0	None
8	12	38.9	8.00
9	10	34.9	9.00 and 9.08
10	05	25	10.00–10.02

[a]The maize genome has been divided into 100 evenly spaced bins of approximately 17 cM each (Davis et al. 1999); multiple simple sequence repeats and restriction fragment length polymorphism (RFLP) markers have been located in each bin

[b]A secondary pair of *Eco*RI RFLP bands detected using probe *umc67* mapped 28.7 cM distal to markers in bin 4.05. The probe also detected a polymorphism in the center of chromosome 1 as expected from prior maps. A BLAST search using the sequence of *umc67* against all available maize sequences revealed two highly homologous sequences ($1 \times e^{-63}$) that have not been mapped

Disease assessment and heritability estimates

The percentage of diseased F3 plants for each of the 220 F2 families was determined in each test location. The frequency of infected plants in F3 progeny rows generally ranged from 0 to at or near 100%, although at site 2 in Thailand, the maximum was 37%. (All data are available in Sabry 2003.) Due to the binomial nature of the classification scheme (infected versus non-infected), all the data (local and systemic) were subjected to arcsin transformation (Steel and Torrie 1960; Gomez and Gomez 1984). Since no difference was detected among blocks within fields or between flats in greenhouse trials, this factor was eliminated from further statistical analysis. Similarly, no differences due to daily inoculation were detected between plants in greenhouse trays. Bartlett's test indicated that the only homogenous combination was site 1 in Thailand and Egypt; therefore, combined analysis was employed only for those two locations.

Table 2 shows heritability (h^2) percentages calculated for the individual and combined analysis along with variance components. The heritability value was as high as 93.3% in site 1 in Thailand, while it was 48.0% in site 2. Combined analyses (site 1 in Thailand and Egypt) of variance and covariance across environments indicated that heritability was 58.88%. Genotypic components of variance (σ_g^2) were highly significant for infection in all environments and across environments. This is indicative of the presence of relatively high genetic variance for resistance to SDM in the population. However, a partitioned genotype-by-environment component reduced the h^2 value.

QTL mapping

Marker regression analysis revealed one chromosomal region strongly associated with SDM resistance at the threshold value of $P < 0.00001$ (Fig. 1a). The same region was identified in all the trials and the combined data. More than one marker in this specific region (bin 9 of chromosome 2) significantly associated with resistance. The LRS for locus *bnlg1893* varied from 262.1 using combined data to 89.2 for systemic infection in Corpus Christi. In site 2 in Thailand, the adjacent locus *umc36*, which is 3.1 cM downstream of *bnlg1893*, had the highest LRS value of 47.4 (Table 3). The total trait variance explained by the QTL at locus *bnlg1893* ranged from 70% in site 1 in Thailand to 34% in Corpus Christi systemic infection (Table 3). In Thailand site 2, locus *umc36* explained 22% of the phenotypic variance. Confidence interval values for locus *bnlg1893* were 3 and 4 for combined data and site

Table 2 Parental line means, grand means, genetic variance (σ^2_g), genotype × environment (σ^2_{ge}), environment variance (σ^2), and heritability (h^2) of sorghum downy mildew infection (arcsine-transformed values) for all tested environments and with combined data for Thai-1 and Egypt

Environment	SP mean	RP mean	$F_{2:3}$ grand mean	Variance components			Heritability
				σ^{2a}_g	σ^2_{ge}	σ^2	% h^2
Thai, site 1	89.34	6.62	36.87	578.11		125.04	93.28
Thai, site 2	26.62	0.70	7.72	23.66		76.8	48.03
Egypt	–[b]	9.46	14.53	175.47		148.16	78.04
Greenhouse local infection	76.11	1.28	26.37	357.17		348.18	75.47
Greenhouse systemic infection	53.11	1.28	23.21	247.48		434.43	63.09
Combined	–[b]	8.04	25.74	267.12	327.60	136.53	58.88

SP Susceptible parent, *RP* resistant parent

[a]All are highly significant

[b]Missing value

1 in Thailand, respectively, and 7 at Corpus Christi for the systemic infection data. For Thailand site 2, the confidence interval value was 13. Table 4 presents a summary of the results from the permutation test along with the significance level of LRSs generated by interval mapping. The values show the maximum LRS that might be expected to occur simply due to chance once across the entire genome in 63% (suggestive), 5% (significant), and 0.1% (highly significant) of trials based on multiple random reshuffling of resistance values among the progeny.

In an attempt to detect other less prominent QTLs, CIM was applied with removal of the effects of the highly significant QTL on chromosome 2. This analysis revealed two additional QTLs. A QTL associated with locus *phi073* (chromosome 3 bin 5) was identified in the Corpus Christi test (Fig. 1b). This QTL explained 4% of the phenotypic variance where susceptible plants were identified by the appearance of local lesions (LRS = 19.1 where 15.7 is significant) and in total infection rates (LRS = 22.1, the exact cutoff for highly significant). Suggestion of a QTL in the same region was seen in greenhouse data with the adjacent locus, *bnlg1035*. However, the LRS value was just below the level of significance (15.4 where 16.8 is significant for local lesions). CIM analyses for site 1 Thailand, Corpus Christi total infection, and combined data detected a second QTL that was associated with locus *umc105* at chromosome 9 bin 2 (Fig. 1c) that explained 3% of the phenotypic variance (LRS significant values were 17.2, 16.1, and 19.7, respectively). This second QTL was also seen in Corpus Christi local infection and greenhouse local infection data, with LRS values of 11.4 and 16.0 respectively, are just below the 5% level of significance.

Discussion

Despite the use of chemical controls and past breeding efforts, downy mildew diseases can still lead to severe losses in maize production. In part, this reflects variability of the pathogen. Different *Peronosclerospora* species predominate in various regions of the globe

Table 3 Marker regression analysis for 220 F3 families using 130 markers

Data set	Chr	Bin	Locus	LRS	%	*P*	CI	Add
Thailand, site 1	2	9	*bnlg1893*	258.9	70	0.00000	4	27.94
Thailand, site 2	2	10	*umc36*	47.4	22	0.00000	13	4.26
Egypt	2	9	*bnlg1893*	143.1	48	0.00000	5	13.55
Corpus Christi local infection	2	9	*bnlg1893*	163.8	53	0.00000	5	25.41
Corpus Christi systemic infection	2	9	*bnlg1893*	89.2	34	0.00000	7	7.75
Corpus Christi total infection	2	9	*bnlg1893*	215.6	63	0.00000	4	31.06
Greenhouse local infection	2	9	*bnlg1893*	122.6	43	0.00000	6	19.38
Greenhouse systemic infection	2	9	*bnlg1893*	133.9	46	0.00000	5	18.57
Combined (Thai 1 and Egypt)	2	9	*bnlg1893*	262.1	70	0.00000	3	20.75

Data for each location include chromosome (Chr), bin number (Bin), locus name, likelihood ratio statistic (LRS), total trait variance explained by QTL at this locus (%), the probability of an association this strong happening by chance (P), an estimate of the size of a 95% confidence interval for a QTL of this strength (CI), and the additive regression coefficient for association (Add)

Table 4 Significance levels of likelihood ratio statistic (LRS) that resulted from the use of the permutation test based on 130 markers and 220 segregating progeny

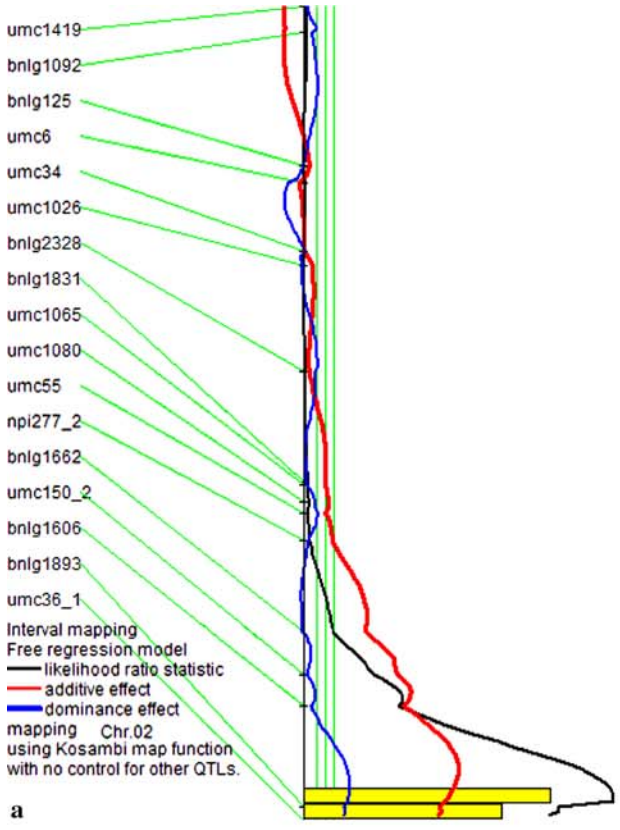
Data set	Suggestive LRS	Significant LRS	Highly significant LRS
Thailand, site 1	9.3	16.1	21.1
Thailand, site 2	9.5	16.2	21.5
Egypt	9.5	16.6	22.9
Corpus Christi local infection	9.4	15.7	22.1
Corpus Christi systemic infection	9.5	15.8	20.8
Corpus Christi systemic and/or local infection	9.4	15.9	22.3
Greenhouse local infection	9.7	16.8	22.3
Greenhouse systemic infection	9.5	16.5	24.3
Combined	9.4	16.3	25.1

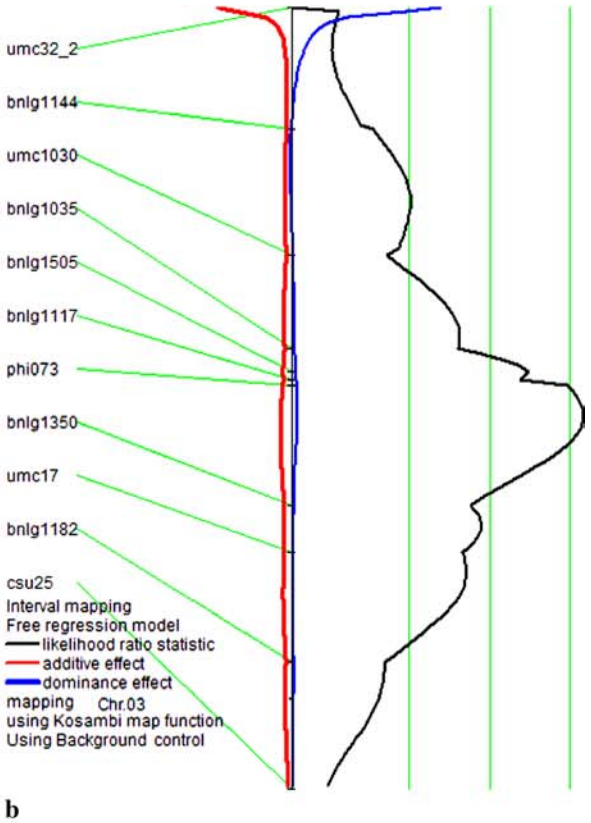
and different pathogenic races are also known to occur within a species. In this study, a CIMMYT line identified as resistant to downy mildew in the Philippines (where disease pressure is extremely high) was crossed with a susceptible line and the progeny were screened in subsequent generations for response to downy mildew in Thailand, Egypt, and the USA (Texas). The primary goal was to identify loci, if any, that might contribute toward resistance to *P. sorghi*, which has become a serious problem in Egypt. In Egypt, pathotype 1 is prevalent although traces of pathotype 2 are also found (Sabry 2003). Pathotype 1 of *P. sorghi* is also predominant in Corpus Christi, Texas, USA and response to pathotype 3 was assessed in greenhouse trials at TAMU, College Station, TX, USA. Although not confirmed experimentally, it is very likely that *P. zeae* was predominant in Thailand and *P. philippinensis* in the Philippines where initial screening was conducted. Because the nature of resistance in the RP was unknown and was to be evaluated against potentially different pathogen populations, a quantitative approach was used to identify potential contributing alleles in each environment. The relatively high values for heritability at each test location, even in the absence of high disease pressure, indicated one or more genes should be identified.

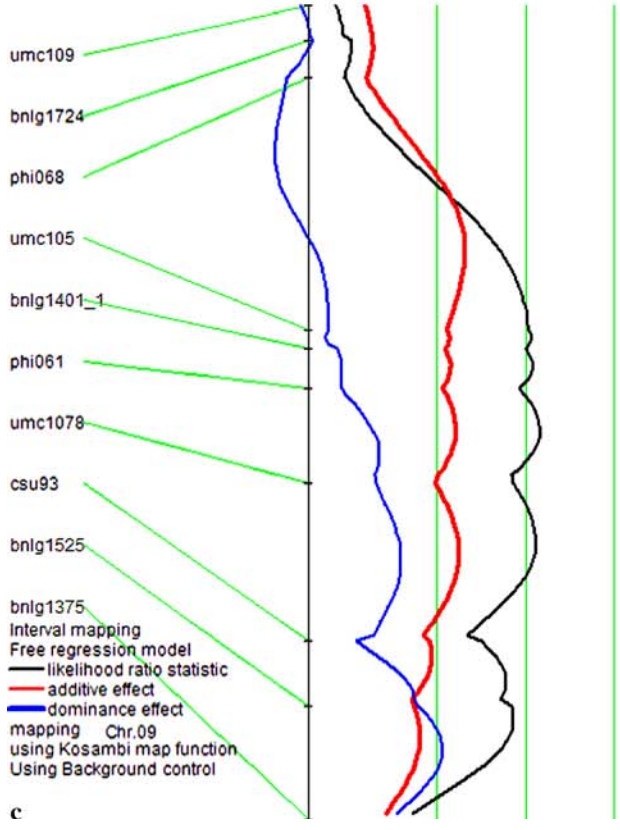
Selection of potential RFLP probes and SSR primers based on previous maps resulted in 130 useful markers for map coverage, of which only five were duplicates or very closely linked and so of limited value for QTL mapping. No internal gaps were present on any chromosome where a QTL could be more than 20 map units from a marker. For most chromosomes, no more than 1 ''bin'' at each end was unmarked. Chromosome 4 was an exception. No SSR primer pairs from bins 00 to 04 of chromosome 4 that were tested showed polymorphisms between the parents. With an average size of 17 cM per bin and relatively even spacing (Davis et al. 1999), 68 units at the end would be unmarked. Fortunately, a second pair of bands detected by RFLP probe *umc67* was located to this region. Overall, it is thus likely that a QTL almost anywhere in the genome that made a significant contribution to resistance would be detected by the mapping methods used.

Differences in heritability values measured in the different locations can be attributed to differences in disease pressure. Misclassification of susceptible plants that escaped infection would lower H^2 while increasing the difficulty of detecting linked markers. Thus the fact that the same region of chromosome 2 was associated with downy mildew resistance in all test environments suggests a major gene or resistance gene complex may occupy this region. Circumstantial evidence suggests this region from the RP contributes resistance to two species of downy mildew that affect maize, in addition to *P. sorghi*, for which resistance against two races was verified. It is interesting to note that George et al. (2003) detected a QTL in the same region that contributed resistance to both *P. heteropogoni* and *P. philippinensis* in a recombinant inbred population derived from Ki3 (resistant) by CML139 (susceptible). However, in their cross, no QTL in this region was detected for *P. sorghi*, *P. maydis*, or *P. zeae* resistance whereas a region of chromosome 6 conferred resistance to five different downy mildews. In the present study, no QTL was detected near chromosome 6 marker *nc013*, one of those associated with resistance in the Ki3 × CML139 cross, or with the markers in adjacent bins. These results suggest that molecular marker-assisted selection could be used to develop a line with enhanced or stable resistance by combining the two resistance QTLs.

The use of CIM revealed two additional QTLs with less effect once the effects of the major QTL on chromosome 2 were masked, but only in specific environments. These QTLs, one located on chromosome 3 (bin 3.05) and the other on chromosome 9 (bin 9.02), had minor effects and were not constant in all environments. The QTL on chromosome 3 was seen in


umc1419
bnlg1092
bnlg125
umc6
umc34
umc1026
bnlg2328
bnlg1831
umc1065
umc1080
umc55
npi277_2
bnlg1662
umc150_2
bnlg1606
bnlg1893
umc36_1
Interval mapping
Free regression model
likelihood ratio statistic
additive effect
dominance effect
mapping Chr.02
using Kosambi map function
with no control for other QTLs.
a


umc32_2
bnlg1144
umc1030
bnlg1035
bnlg1505
bnlg1117
phi073
bnlg1350
umc17
bnlg1182
csu25
Interval mapping
Free regression model
likelihood ratio statistic
additive effect
dominance effect
mapping Chr.03
using Kosambi map function
Using Background control
b


umc109
bnlg1724
phi068
umc105
bnlg1401_1
phi061
umc1078
csu93
bnlg1525
bnlg1375
Interval mapping
Free regression model
likelihood ratio statistic
additive effect
dominance effect
mapping Chr.09
using Kosambi map function
Using Background control
c

◀

Fig. 1 Printouts of the Mapmaker QTX program for chromosomes, 2 (**a**), 3 (**b**), and 9 (**c**) showing the significant quantitative trait locis (QTLs) that contributed to downy mildew resistance. *Vertical lines* reading outward mark suggestive, significant, and highly significant likelihood ratio statistic (LRS) values for each chromosome. *Histograms* in (**a**) show confidence interval for location of the QTL(s). Additive effects, dominance effects, and the calculated LRS are color coded as indicated

trials conducted in Corpus Christi, Texas, and in the greenhouse, and so could reflect a difference in the pathogen populations. The fact that Nair et al. (2005) also found a QTL for downy mildew resistance in the same region strongly suggests the observation is not an artifact. The position of the QTL on chromosome 9 is similar to that for a SDM resistance QTL reported by Agrama et al. (1999) based on a mapping population derived using germplasm from Egypt, but no evidence for contributions from chromosome 1 matching those they reported were detected here.

Studies on the inheritance of Philippine downy mildew resistance made before molecular mapping indicated that only a few genes controlled the reaction and resistance was partially dominant (Gomes et al. 1963). The results of Handoo et al. (1970), Borges (1987), and Singburaudom and Renfro (1982) all indicated that both additive and non-additive gene actions were important in the control of resistance. The current studies fit well with those observations, but also reveal that multiple sources of resistance can be identified and presumably combined to enhance stable resistance.

References

Agrama HA, Moussa ME, Naser ME, Tarek MA, Ibrahim AH (1999) Mapping of QTL for downy mildew resistance in maize. Theor Appl Genet 99:519–523

Bohn M, Khairallah MM, Jiang C, Gonzalez-De-Leon D, Hoisington DA, Utz HF, Deutsch JA, Jewell DC, Mihm JA, Melchinger AE (1996) QTL mapping in tropical maize: I. Genomic regions affecting leaf feeding resistance to sugarcane borer, other traits. Crop Sci 36:1352–1361

Borges OL (1987) Diallel analysis of maize resistance to sorghum downy mildew. Crop Sci 27:178–180

Cardwell KF, Bock C, Akinnioye OF, Onukwa D, Adenle V, Adetoro AO (1994) Improving screening methods for resistance to downy mildew of maize in Nigeria. Plant Health Manage Res Monogr 22:22–25

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Craig J (2000) Sorghum downy mildew. In: Frederiksen RA, Odvody GN (eds) Compendium of sorghum diseases, 2nd edn. APS, St Paul, pp 25–27

Craig J, Odvody GN (1992) Current status of sorghum downy mildew control. In: de Milliano WAJ, Frederiksen RA, Bengston GD (eds) Sorghum and millets diseases: a second world review. ICRISAT, Patancheru, Andhra Pradesh, pp 213–217

Davis GL, McMullen MD, Baysdorfer C, Musket T, Grant D, Staebell M, Xua G, Polacco M, Koster L, Melia-Hancock S, Houchins K, Chao S, Coe EH Jr (1999) A maize map standard with sequenced core markers, grass genome reference points and 932 expressed sequence tagged sites (ESTs) in a 1736-locus map. Genetics 152:1137–1172

Frederiksen RA, Renfro BL (1977) Global status of maize downy mildew. Annu Rev Phytopathol 15:249–275

George ML, Prasanna BM, Rathore RS, Setty TA, Kasim F, Azrai M, Vasal S, Balla O, Hautea D, Canama A, Regalado E, Vargas M, Khairallah M, Jeffers D, Hoisington D (2003) Identification of QTLs conferring resistance to downy mildews of maize in Asia. Theor Appl Genet 107:544–551

Gomes AA, Aquilizan FA, Payson RM, Galub AG (1963) Preliminary studies on the inheritance of corn to downy mildew disease. Philipp Agric 47:113–117

Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research. Wiley, New York

Haley CS, Knott SA (1992) A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69:315–324

Hallauer AR, Miranda JB (1981) Quantitative genetics in maize breeding. Iowa State University Press, Ames

Handoo MI, Renfro BL, Payak MM (1970) On the inheritance of resistance to *Sclerophthora rayssiae* var. *zeae* in maize. Indian Phytopathol 23:231–249

Hoisington D, Khairallah M, Gonzalez-de-Leon D (1994) Laboratory protocols: CIMMYT applied molecular genetics laboratory. CIMMYT, Mexico

Isakeit T, Odvody G, Jahn R, Decanini L (2003) *Peronosclerospora sorghi* resistant to metalaxyl treatment of sorghum seed in Texas. Phytopathology 93:S39

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander E, Kruglyak L (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 11:241–247

Little TM, Hills FJ (1978) Agricultural experimentation design and analysis. Wiley, New York

Manly KF, Cudmore JRH, Meer JM (2001) Map Manager QTX, cross-platform software for genetic mapping. Mamm Genome 12:930–932

Martinez O, Curnow RN (1992) Estimating the locations and the sizes of the effects of quantitative trait loci using flanking markers. Theor Appl Genet 85:480–488

Nair SK, Prasanna BM, Garg A, Rathore RS, Setty TAS, Sing NN (2005) Identification and validation of QTLs conferring resistance to sorghum downy mildew (*Peronosclerospora sorghi*) and Rajasthan downy mildew (*P. heteropogoni*) in maize. Theor Appl Genet 110:1384–1392

Nazim M, Khalifa EZ, El-Mersawy EM, Sadoma MT (1995) Downy mildew on maize in Egypt. Egypt J Phytopathol 23:53–67

Sabry AM-B (2003) QT mapping of resistance to sorghum downy mildew in maize. Plant pathology and microbiology. A&M University, College Station, 118 pp

Searle SR (1971) Linear models. Wiley, New York

Singburaudom N, Renfro BL (1982) Heritability of resistance in maize to sorghum downy mildew (*Peronosclerospora sorghi* (Weston and Uppal) C.G. Shaw). Crop Prot 1:323–332

Steel RGD, Torrie JH (1960) Principles and procedures of statistics. McGraw-Hill, New York

Williams RJ (1984) Downy mildews of tropical cereals. Adv Plant Pathol 2:1–103

Yao C (1991) Classification and detection of *Peronosclerospora* species on the basis of DNA southern hybridization and the polymerase chain reaction. Plant pathology and microbiology. Dissertation, Texas A&M University, 94 pp

Zeng ZB (1993) Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. Proc Natl Acad Sci USA 90:10972–10976

Zeng ZB (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468