Theor Appl Genet (2003) 107:544–551
DOI 10.1007/s00122-003-1280-6

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Identification of QTLs conferring resistance to downy mildews of maize in Asia

Received: 22 July 2002 / Accepted: 13 January 2003 / Published online: 21 May 2003

Abstract Downy mildew is one of the most destructive diseases of maize in subtropical and tropical regions in Asia. As a prerequisite for improving downy mildew resistance in maize, we analyzed quantitative trait loci (QTLs) involved in resistance to the important downy mildew pathogens – *Peronosclerospora sorghi* (sorghum downy mildew) and *P. heteropogoni* (Rajasthan downy mildew) in India, *P. maydis* (Java downy mildew) in Indonesia, *P. zeae* in Thailand and *P. philippinensis* in the Philippines – using a recombinant inbred line population derived from a cross between Ki3 (downy mildew resistant) and CML139 (susceptible). Resistance was evaluated as percentage disease incidence in replicated field trials at five downy mildew 'hotspots' in the four countries. Heritability estimates of individual environments ranged from 0.58 to 0.75 with an across environment heritability of 0.50. Composite interval mapping was applied for QTL detection using a previously constructed restriction fragment length polymorphism linkage map. The investigation resulted in the identification of six genomic regions on chromosomes 1, 2, 6, 7 and 10 involved in the resistance to the downy mildews under study, explaining, in total, 26–57% of the phenotypic variance for disease response. Most QTL alleles conferring resistance to the downy mildews were from Ki3. All QTLs showed significant QTL × environment interactions, suggesting that the expression of the QTL may be environment-dependent. A strong QTL on chromosome 6 was stable across environments, significantly affecting disease resistance at the five locations in four Asian countries. Simple-sequence repeat markers tightly linked to this QTL were identified for potential use in marker-assisted selection.

Keywords Downy mildews · Maize · Quantitative trait loci · Disease resistance · Molecular markers

Communicated by D.J. Mackill

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Introduction

Downy mildews, caused by several species in the genera *Peronosclerospora* and *Sclerophthora*, represent a destructive systemic disease of major economic importance to maize in Asia and in other maize growing areas. The major downy mildews that infect maize in Asia include the sorghum downy mildew [*Peronosclerospora sorghi* (Weston & Uppal)], Philippine downy mildew [*P. philippinensis* (Weston) Shaw], Java downy mildew [*P. maydis* (Raciborski)], sugarcane downy mildew [*P. sacchari* (Miyabe) Shirai and Hara] and brown stripe downy mildew [*Sclerophthora rayssiae* var. *zeae* Payak

and Renfro]. While *P. sorghi* causes downy mildew in both sorghum and maize, the maize strain of *P. sorghi* in Thailand rarely infects sorghum. On the basis of isozyme analysis, epidemiological studies and DNA banding patterns, this strain was reclassified as *P. zeae* (Yao 1991; Yao et al. 1992). In Rajasthan, India, the *P. sorghi* which forms oospores in the wild grass *Heteropogon contortus* (speargrass) was renamed *P. heteropogoni* (Siradhana et al. 1980) and the disease, caused when maize is infected by the conidial stage of the fungus, is referred to as Rajasthan downy mildew (White 1999).

Worldwide, the percentage of area with reported economic losses to downy mildew is 30%, both in tropical lowland maize and in subtropical, mid-altitude, transition zone and highland maize (Jeffers et al. 2000). Heavy losses (as high as 100%) have been recorded in maize from one or the other of the downy mildew pathogens in the Philippines, Taiwan, Indonesia, Thailand, India, Japan, Australia, Venezuela, North America, Europe, West Africa and other parts of the world (Bonde 1982; Rifin 1983; Rathore and Siradhana 1987). In the Asian region, where yield losses of 50% or more are common, downy mildew is considered as the top priority biotic constraint limiting maize productivity (Pingali 2001). Despite the introduction of downy mildew-resistant cultivars and the use of metalaxyl fungicide, severe incidence of the disease still occurs in localized areas (Dalmacio 2000). Seed treatment with fungicide by commercial companies makes the seeds expensive and generally beyond the financial reach of resource-poor farmers. These cost concerns, and the emerging problem of buildup of chemical resistance in the pathogen (Raymundo 2000), point to the use of resistant varieties as a more cost-effective and environmentally safe alternative for controlling the downy mildews.

Studies on the genetics of downy mildew resistance suggest it has a complex and polygenic nature and that additive effects predominantly contribute to the resistance (Borges 1987; De Leon et al. 1993; Kaneko and Aday 1980; Singburaudom and Renfro 1982). The mapping of quantitative trait loci (QTLs) make feasible the detection, localization and characterization of genetic factors contributing to the variation of polygenically inherited traits (reviewed by Young 1996). In maize, QTLs that contribute towards variation in disease resistance against sorghum downy mildew (Agrama et al. 1999), gray leaf spot (Bubeck et al. 1993; Saghai-Maroof et al. 1996), northern corn leaf blight (Dingerdissen et al. 1996; Freymark et al. 1993) and stalk and ear rot (Pe et al. 1993; Perez-Brito et al. 2001) have been identified.

As a first step towards the development of durably resistant, high-yielding maize varieties, a region-wide activity was undertaken by the Asian Maize Biotechnology Network (http://www.cimmyt.org/ambionet) to locate and characterize the QTLs involved in resistance to downy mildew disease and identify associated markers for further use in marker-assisted selection. The objective of study reported here was to map the genomic regions involved in resistance to downy mildews in different locations in Asia.

Materials and methods

Mapping population

A mapping population consisting of recombinant inbred lines (RILs) from the cross Ki3 × CML139 (Groh et al. 1998) was used in this study. The downy mildew resistant parent, Ki3, is a tropical yellow flint line with late maturity. This inbred line was derived after four cycles of self-progeny recurrent selection from Suwan1, a popular cultivar developed in Thailand for resistance to sorghum downy mildew (Aekatasanawan et al. 2000; Henderson 1984). The susceptible parent, CML139, is a subtropical yellow-red semi-flint line with intermediate maturity, which was developed for tropical corn borer resistance from Caribbean germplasm (Khairallah et al. 1998). Groh et al. (1998) used the $F_{7:8}$ RILs from this cross to map QTLs for resistance to southwestern corn borer (SWCB) and sugarcane borer (SCB). Using a population of 135 RILs, Groh et al. (1998) constructed a molecular map consisting of 143 restriction fragment length polymorphism (RFLP) markers, with a total length of 2,117 cM and an average spacing of 14.4 cM. The present study evaluated the same set of 135 RIL families for downy mildew reaction in the field. The parental lines and RILs were kindly provided by D. Bergvinson of the CIMMYT Maize Program.

Field trials

Field trials in this study were conducted in five tropical environments in four countries: at Mandya in southern India (12°N; 76°E; 695 m.a.s.l. elevation; 705 mm/year average rainfall) against *P. sorghi* (sorghum downy mildew); at Udaipur in western India (23°46′N; 73°09′E; 577 m.a.s.l. elevation; 633.3 mm/year average rainfall) against *P. heteropogoni* (Rajasthan downy mildew); at Maros in Indonesia (5°S, 119°38′E; 5 m.a.s.l. elevation; 2,400 mm/year average rainfall) against *P. maydis* (Java downy mildew); at Farm Suwan in Thailand (14.5°N, 101°E, 360 m.a.s.l. elevation; 1,200 mm/year average rainfall) against *P. zeae*; at Los Baños in the Philippines (14°08′N; 121°15′E; 62 m.a.s.l. elevation; 194 mm/year average rainfall) against *P. philippinensis*. The field experiments were carried out during August–September 1999 at Mandya, India; September–November 1999 in Thailand; August–September 2000 at Rajasthan, India; May–June 2000 in Indonesia; January–March 2002 in the Philippines.

Experimental design

The 135 RILs, along with parental lines Ki3 and CML139, were evaluated for their responses to respective downy mildew disease strains in field experiments using a 14 × 14 alpha lattice design with two replications per genotype. At Mandya and Udaipur in India, test entries were planted in 4-m-long rows having 15–20 plants per row. The experimental rows were 2.5 m long in Indonesia and in Thailand, containing 10 and 22 plants, respectively. In the Philippines, the rows were 5 m long containing 20 plants. The space between rows was 0.75 m except in Indonesia where it was 0.60 m.

Inoculation method and disease assessment

Field tests were conducted using the 'spreader row technique' (Mandya, India; Indonesia; Thailand), artificial infection using the 'whorl inoculation technique' (Udaipur, India) or modified spreader row with artificial infection (Philippines) to provide sufficient and uniform disease pressure. The susceptible genotypes (open-pollinated varieties, OPV) CM500 in Mandya, Antasena in Indonesia,

Tuxpeño in Thailand and Supersweet in the Philippines were used in the spreader rows. The local downy mildew pathogens, maintained in the maize OPVs in disease nurseries (except in case of the Philippines where the pathogen was maintained in sugarcane plants), served as sources of inoculum for the spreader rows.

At Mandya, India, seeds of CM500 were infected before planting using the 'sandwich method'. For this, the seeds were spread between layers of downy mildew-infected maize leaves with visible conidial growth and incubated for 2–3 days to facilitate infection of the fungus in the germinating seedlings. The infected seedlings were planted as spreader rows on all sides of the experimental block 30 days prior to the planting of test entries. One bed of spreader row was planted for every two beds of test entries. As a susceptible check, uninfected CM500 seeds were planted after every tenth row of test materials. Severe infection (98–100% downy mildew incidence) in the check rows across the experimental block indicated good pathogen pressure.

The spreader plants in Indonesia, consisting of two rows with 50 × 25-cm spacing, were planted 3 weeks earlier surrounding the test materials. After emergence of the spreader plants, downy mildew-infected maize plants in plastic pots were transferred into the inner rows at locations about 10 m apart. The OPV Antasena was also planted after every ten entries. In Thailand, one row of the susceptible genotype was planted for every ten rows of test materials and inoculated 1 week after emergence. The test materials were planted 2 weeks after inoculation of the spreader rows. At the planting date, the downy mildew infection in the spreader row plants was 30–40% (Indonesia) and 60–70% (Thailand); at later stages, the spreader rows showed higher infection (85–100% in Indonesia and 90–100% in Thailand). For the modified spreader row in the Philippines, two rows of the susceptible genotype were planted after every 20 rows of test materials and inoculated at the same time as the test materials five days after emergence. After 16 days, the downy mildew infection in the spreader rows which served as internal checks was 20–30%, reaching 100% at later stages.

At Udaipur, India and the Philippines, artificial infection was carried out by putting up to 1 ml of a conidial suspension in the whorl of each seedling of the test entries 5–7 days after germination. The suspension was prepared by collecting conidia from the downy mildew-infected plants (susceptible maize cvs. Kiran and Surya infected by *P. heteropogoni* conidia from *Heteropogon contortus* in Udaipur, India and cv. Supersweet infected by *P. philippinesis* from sugarcane in the Philippines) and suspending these in water to a concentration of approximately 40,000–50,000 (India) or 100,000 conidia/ml (Philippines). In India, the inoculation procedure was repeated for 3 consecutive days to ensure no escape of plants from artificial infection.

Disease reaction was assessed at 12 and 21 days after emergence (Indonesia; Thailand) or 21 and 35 days after emergence (Mandya, India; Philippines) or after inoculation of test entries (Udaipur, India) by scoring for systemic infection in the individual plants. Percentage disease incidence was determined by the ratio of the number of plants with infection to the total number of plants multiplied by 100. Inoculated plants that did not show systemic symptoms of downy mildew (emergence of characteristic chlorotic leaves) 1 month after artificial infection were considered to be resistant.

Data analysis

Phenotypic data

Least square means for the data from each location were calculated, and standard analysis of variance was performed using the SAS program (SAS Institute, Raleigh, N.C.) to determine variation in the phenotypic data among locations. The components of variance were estimated using a complete random effects model. Broad-sense heritability (H), defined as the ratio of genotypic to phenotypic variance, was estimated according to Liu (1998). Heritability was calculated using the mixed models procedure (PROC MIXED) of the SAS program. Heritability of downy mildew resistance under each environment as well as heritability of disease resistance across the various environments were calculated.

QTL mapping

QTL analysis was carried out on the set of 135 RILs for which both genotypic data (provided by S. Groh; Groh et al. 1998) and phenotypic data were available. The genotypic data consisted of 143 RFLP marker loci, and the phenotypic data comprised downy mildew disease incidence data from individual environments as well as pooled data across environments. The method of composite interval mapping (CIM) (Zeng 1994) was used to perform joint analysis of data across environments to map QTLs and estimate their genetic effects. A series of four models was used in the analysis. Model 1, which is simple interval mapping (Lander and Botstein 1989 or Model III of Zeng 1994), was used for the selection of cofactors; Model 2, which is CIM with unlinked markers as cofactors (Model II of Zeng 1994), was used to maximize QTL detection; Models 3 and 4 (Model I of Zeng 1994) with selected markers as cofactors, were used to confirm the QTLs detected in Model 2. Model 3 used two markers flanking the interval with a minimum map distance of 30 cM (window size = 30 cM), while Model 4 was with a minimum map distance of 20 cM (window size = 20 cM).

The threshold used for QTL detection was set at a likelihood ratio (LR) value that was greater than the threshold value for LOD ($\log_{10}$ of the likelihood odds ratio) value of 3.0 in a single environment (11.51) and/or joint analysis over all environments (19.66) or at a slightly lower threshold of a LOD value of 2.5 in a single environment (9.21) and/or joint analysis over all environments (17.83). A QTL was considered as present when the LR exceeded the threshold in Model 2 and a peak was also detected in Models 3 and 4. A QTL was also considered as present even if a peak was not detected in Model 2 (possibly due to a linked QTL), when the LR exceeded the threshold in Model 3. When separated by at least two markers and a minimum distance of 20 cM, two peaks on one chromosome were considered as two different QTLs. Otherwise, the higher peak was chosen to represent the QTL.

The critical values for QTL × environment (E) interactions for five environments were 9.49 and 13.28, at the 0.05 and 0.01 significance levels, respectively. The additive effect of QTLs from each environment and across environments was obtained under the assumptions of Model 3. An overall additive effect was estimated using mean values across environments. Multiple regression analysis was performed to estimate the total proportion of phenotypic variation due to the additive effects (R^2).

Genotypic data

To identify simple sequence repeat (SSR) markers tightly linked to a major QTL detected on chromosome 6 in the present study, 65 SSR markers located on chromosome 6 were selected (MaizeDB, http://www.agron.missouri.edu) for a survey of polymorphism between Ki3 and CML139. DNA used in the SSR analysis was extracted from leaves bulked from five to eight 1-week-old RIL plants according to the procedure of Saghai-Maroof et al. (1984) and as modified by Hoisington et al. (1994). Approximately 30 ng of DNA was used as template for the polymerase chain reaction (PCR) in a 15-μl reaction volume following the procedure of Hoisington et al. (1994). DNA was amplified using the following thermal cycle: an initial denaturation at 94 °C for 2 min; 30 cycles of a 1-min denaturation at 94 °C, a 2-min annealing at 56 °C, a 2-min extension at 72 °C; a final extension at 72 °C for 4 min (MJ Research DNA Engine Tetrad System Thermocyclers, Waltham, Mass.). Amplification products were separated on 3% agarose gel consisting of 2:1 Metaphor (BioWhitaker Molecular Applications, Rockland, Me.): SeaKem LE (Karlan Research Products, Santa Rosa, Calif.) agarose. Electrophoresis was done at 100 V for 2–3 h.

Table 1 Means of resistant (Ki3) and susceptible (CML139) parents and recombinant inbred line (RIL) population, range of the RIL population and estimates of variance components and heritabilities for percentage disease incidence for downy mildew in 135 $F_{7:8}$ RIL population Ki3 × CML139 in five locations (*SD* standard deviation)

Location	Ki3 Mean (%) ± SD	CML139 Mean (%) ± SD	RILs					Heritability
			Mean (%) ± SD	Range	σ^2_g	σ^2_{ge}	σ^2_e	
Mandya, India	32 ± 14	100 ± 0	93 ± 14	25–100	249.9**	–	82.7**	0.75
Udaipur, India	0 ± 0	83 ± 20	38 ± 30	0–100	595.8**	–	353.0**	0.63
Indonesia	24 ± 13	94 ± 12	90 ± 18	0–100	332.2**	–	152.4**	0.69
Thailand	1 ± 2	50 ± 29	20 ± 20	0–100	254.6**	–	185.8**	0.59
Philippines	0 ± 0	90 ± 12	60 ± 27	0–100	597.6**	–	232.9**	0.72
Across	11 ± 16	84 ± 24	60 ± 36	0–100	308.0**	121.5**	185.1**	0.50

** Variance component significant at the 0.01 level

Molecular mapping

Seven SSR markers located within the QTL region at bin 6.05 near the RFLP marker *bnl5.47* were used to genotype the 135 RILs. Segregation at each marker locus was tested by the X^2 goodness-of-fit test for the expected Mendelian segregation ratio 1:1 with a significance level of 0.01. Genotype data from five SSR marker loci that fit the segregation ratio were appended to the previous map file created for the Ki3 × CML139 RIL population (Groh et al. 1998). Linkage analysis was done using the MAPMAKER software (Lander et al. 1987). A LOD value of 3.0 and a maximum recombination frequency of 0.40 were used to declare linkage between two markers. Genetic map distances between the markers were estimated using the recombination frequencies and transformed into centiMorgan using Haldane's mapping function. Data from three evenly spaced SSR markers were added to the previous RFLP map, and phenotypic data of the 135 RILs were analyzed with CIM to confirm the location and tight linkage of the SSR markers to the QTL on chromosome 6.

Results

Phenotypic data

The parental lines exhibited contrasting phenotypes for field disease assessment, differing significantly in their reaction to downy mildew in each location and across locations (Table 1). The means for percentage disease incidence of the RILs differed among locations. At Mandya (India) and Indonesia, where the disease pressure was high, the distribution of the RILs was skewed towards the susceptible parent (RIL mean of 92.9% and 90.5% disease incidence, respectively), with the resistant parent Ki3 showing only moderate resistance. In contrast, the disease pressure at Udaipur (India) and Thailand was lower, and the distribution of disease scores was generally skewed towards the resistant parent (RIL mean of 38.2% and 20.5% disease incidence, respectively). Transgressive segregation, with a few RILs exceeding parental resistance in Indonesia, and a few exceeding parental susceptibility in Thailand, was observed (Table 1). Estimates for σ^2_g and σ^2_e for the RILs in each environment and for σ^2_g, σ^2_{ge}, and σ^2_e across environments were highly significant ($P < 0.01$). Heritability estimates of individual environments ranged from 0.59 in Thailand to 0.75 in Mandya (India), with an across environment heritability of 0.50 (Table 1), showing a reasonably high amount of genetic control of resistance. This and the highly significant differences between the RIL families indicate that resistance was segregating in the population and that much of the variation in the phenotype was attributable to genetic variation.

QTL analyses

QTL locations

Six QTLs for downy mildew resistance were detected in the Ki3 × CML139 RIL population in the joint analysis across five environments (Table 2, Fig. 1). Analysis of the data from India (Mandya and Udaipur), Indonesia, Thailand and Philippines detected two significant peaks on chromosome 2 (bins 2.06 and 2.09) and one each on chromosome 1 (bin 1.08), chromosome 6 (bin 6.05), chromosome 7 (bin 7.02) and chromosome 10 (bin 10.03) (Table 2). The three putative QTLs of smaller effects located on chromosome 1, 2 (bin 2.06) and 10 were detected in the joint analysis with an LR value slightly lower than the threshold value for LOD 3.0 (19.66) but higher than the threshold value for a LOD of 2.5 (17.83).

Two QTLs seemed to be pathogen-specific, significantly influencing resistance only to particular pathogen populations in India. The QTL on chromosome 1 was specific to *P. heteropogoni* in Udaipur and, more strongly, one QTL on chromosome 2 (bin 2.06) was specific to *P. sorghi* in Mandya. The second QTL on chromosome 2 (bin 2.09) and that on chromosome 7 were less specific, influencing resistance to both the Rajasthan and Philippine downy mildews and additionally for the QTL on chromosome 7, to Java downy mildew. Two QTLs contributed broad-based resistance to the different downy mildews in the five locations, albeit in contrasting ways. While the QTL on chromosome 10 only had small effects, that on chromosome 6 had a large effect, strongly influencing resistance to all the different downy mildews in the five locations. This QTL, having the highest LR values in the analysis of individual locations as well as in the joint analysis, was consistently detected across environments.

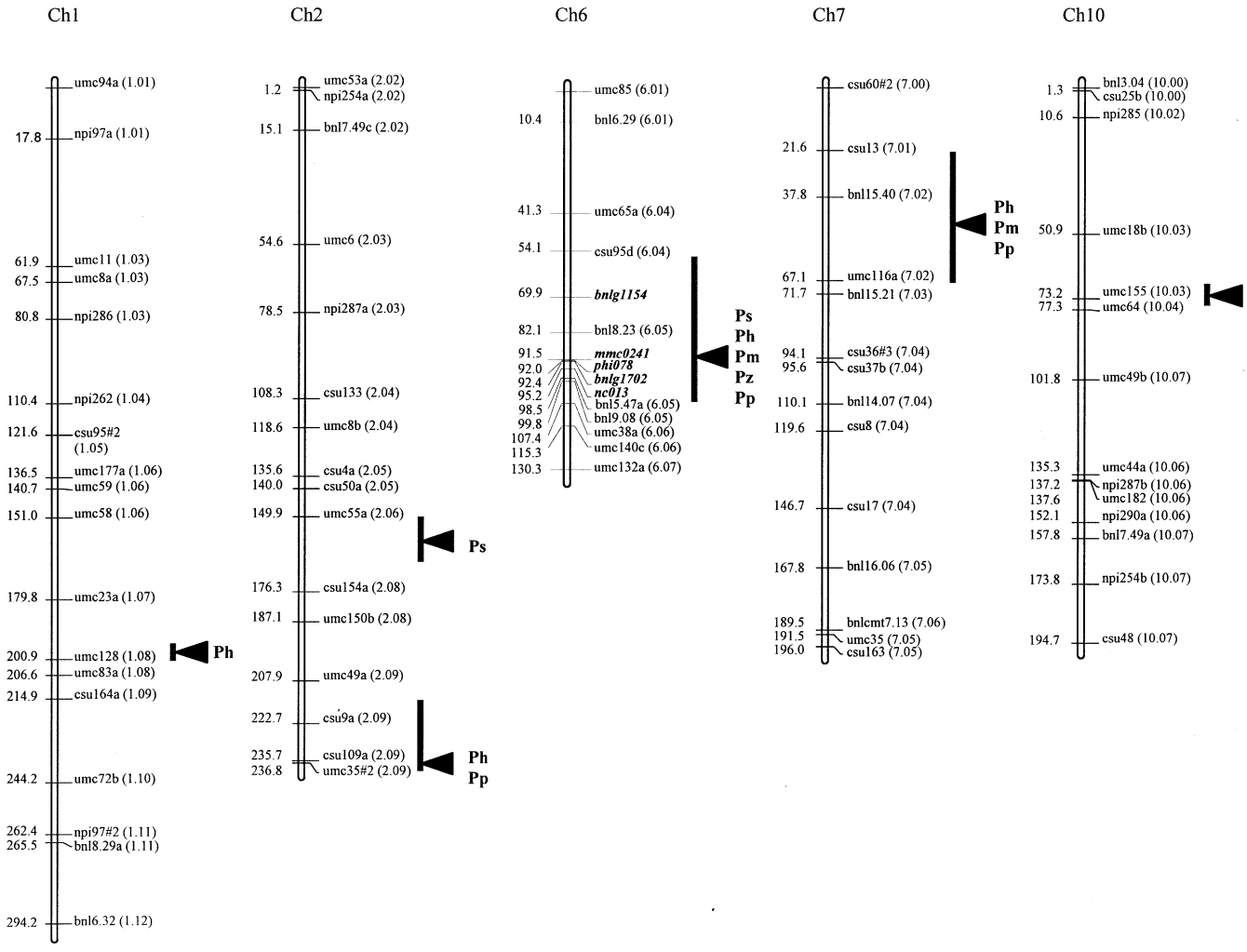

Fig. 1 Linkage map based on a $F_{7:8}$ RIL population from the Ki3 × CML139 cross showing SSR markers (*italics*), QTL positions (*length of line* based on significant LR values) and LR peaks (*triangles*). *Letters adjacent* to the *triangles* (Ps *Peronosclerospora sorghi*, Ph *P. heteropogoni*, Pm *P. maydis*, Pz *P. zeae*, Pp *P. philippinensis*) indicate the pathogen population in the individual environments for which the QTL was significant

All QTL × E interactions were significant (Table 2). This, and the large estimate of σ^2_{ge} observed across the locations, indicates that the expression of the QTLs was environment-dependent. The environmental effects, partly due to the characteristic pathogen populations in each location as well as other factors such as the inoculation methodology used and soil and climatic conditions, could have played an important role in the expression of downy mildew resistance.

QTL effects

Additive mean effects due to the alleles of the resistant parent (Ki3) in the second QTL on chromosome 2 (bin 2.09) and in the QTLs on chromosomes 1, 6, 7 and 10 are were responsible for the reduction in disease incidence (Table 3). The QTL for downy mildew resistance detected on chromosome 6 had the largest mean effect, with Ki3 alleles in this locus decreasing percentage disease incidence by an average of 5.1%. In each of the five locations, Ki3 alleles in this QTL reduced susceptibility, ranging from 5.0% in Indonesia to 13.6% in Udaipur. Alleles from the susceptible parent CML139 on the first QTL on chromosome 2 (bin 2.06) contributed to the resistance to *P. sorghi* in Mandya and, to a lesser degree, against *P. maydis, P. philippinensis* and *P. heteropogoni*, while alleles from the resistant parent Ki3 contributed to the resistance against *P. zeae* in Thailand (Table 3).

The proportion of the phenotypic variance explained by the QTLs varied across environments (Table 3). The amount of variation in disease susceptibility explained by the six QTLs in each location ranged from 25% (Thailand) to 57% (Udaipur, India). The variation explained by each major QTL (LOD 3.0) ranged from 3.4 (chromosome 2 at 158 cM in Mandya, India) to 31.2% (chromosome 6 QTL in Udaipur, India). Among the QTLs, the one on chromosome 6 had the largest contribution,

Table 2 Significant and putative QTL for percentage downy mildew incidence in Mandya and Udaipur, India, Indonesia, Thailand and Philippines by composite interval mapping (CIM, Model 3, swindow = 30) based on the RFLP linkage map of Groh et al. (1998)

Chromosome	QTL position (cM)	Marker interval[a]	Likelihood ratio (LR)[b]						
			Mandya *P. sorghi*	Udaipur *P. heteropogoni*	Indonesia *P. maydis*	Thailand *P. zeae*	Philippines *P. philippinensis*	Combined	QTL × E
1	197	*umc23a*–umc128	5.8	10.1*	2.8	2.7	0.0	18.2*	11.9*
2	158	umc55a–*csu154a*	14.1**	0.2	1.3	1.0	0.1	19.2*	12.4*
	234	*csu9c*–csu109a	2.6	32.5**	5.8	3.9	11.5*	35.0**	30.0**
6	89	bnl8.23–*bnl5.47a*	26.2**	47.5**	14.7**	17.8**	28.1**	56.7**	29.7**
7	48	bnl15.40–*umc116*	8.6	24.6**	10.4*	6.7	21.4**	31.7**	18.5**
10	73	umc155–*umc64a*	2.8	4.5	2.5	0.6	5.8	18.4*	18.3**

*, ** Significance levels of 0.05 and 0.01, respectively

[a] Markers selected as cofactors, by chromosome, are: 2 (*csu109a*), 6 (*bnl5.47a*), 7 (*bnl15.40*) and 10 (*umc155*). Nearest marker is underlined

[b] Significant if: LR is greater than the critical value of 11.51 (**, LOD 3.0) or 9.21 (*, LOD 2.5) for single environments; LR is greater than the critical value of 19.66 (**, LOD 3.0) or 17.83 (*, LOD 2.5) for combined environments; LR is greater than the critical value of 9.4877 or 13.28 for QTL × E interactions

Table 3 Summary of QTL effects for resistance to downy mildew estimated from phenotypic data of 135 RILs from a cross between Ki3 × CML139

Chromosome	QTL Position (cM)	QTL additive effects (% disease incidence)[a]						R^2 (%)[b]				
		Mandya	Udaipur	Indonesia	Thailand	Philippines	Mean	Mandya	Udaipur	Indonesia	Thailand	Philippines
1	197	–2.5	–5.8	–2.2	–2.7	0.5	–2.2	2.8	2.0	1.2	1.0	0.0
2	158	4.0	1.0	1.6	–2.6	1.0	2.7	3.4	0.1	0.0	1.7	0.1
	234	–1.8	–11.1	–3.2	–3.2	–6.1	–2.1	2.0	15.0	4.8	3.5	6.3
6	89	–5.4	–13.6	–5.0	–6.5	–9.8	–5.1	20.0	31.2	13.4	15.1	22.2
7	48	–3.0	–9.8	–4.3	–4.1	–8.6	–3.5	4.5	7.4	6.0	4.7	11.6
10	73	–1.7	3.8	2.0	1.1	4.2	–0.2	0.4	5.1	2.1	1.6	5.3
Total								35.2	57.2	26.5	25.3	42.6

[a] Estimation of the additive QTL effects with CIM (model 3); negative values indicate that the effect of the Ki3 (resistant) parent allele is to increase resistance

[b] Percentage of phenotypic variation (R^2) explained by markers linked to QTL

accounting for more than half of the phenotypic variance explained by all the QTLs detected in each of the five environments. The QTLs with lower significance (LOD 2.5) explained only a small proportion (2–6.3%) of the variation in the trait in each environment.

Identification of SSR markers linked to the QTL for downy mildew resistance on chromosome 6

Twenty-nine SSR markers were found to be polymorphic between Ki3 and CML139 out of the 65 markers located on chromosome 6 that were screened. Seven markers on the QTL location in bin 6.05 were used to genotype the RIL population. Four of the five markers which fit the 1:1 segregation ratio (*mmc0241, phi078, bnlg1702* and *nc013*) mapped between the RFLP markers *bnl5.47* and *bnl8.23* at bin 6.05, while one marker (*bnlg1154*) mapped between *bnl8.23* and *csu95d* (bin 6.04) (Fig. 1). The remapped chromosome 6 was in agreement with the previously published RFLP map by Groh et al. (1998). The position of the major QTL on chromosome 6 was confirmed by CIM with additional genotype data derived from three SSR markers (*bnlg1154, mmc0241* and *nc013*). The LR peak on chromosome 6 was located nearest the SSR marker *mmc0241* (Fig. 1).

Discussion

Using a set of 135 RILs derived from a cross between Ki3 (resistant) and CML139 (susceptible), we identified six QTLs for resistance to five important downy mildew diseases affecting maize production in the Asian region. The QTLs that were detected highlighted the differences among the pathogen populations in the five locations. The QTLs were associated with varying degrees of pathogen specificity. That pathogen specificity is a major mechanism of downy mildew resistance was demonstrated in pearl millet (Jones et al. 1995). In their study, different QTLs for resistance to each of four populations of *Sclerospora graminicola* were detected, indicating a gene-for-gene host pathogen system. However, Jones et al. (1995) did not find QTLs that were effective against all the pathogen populations. In a study by Agrama et al. (1999), three significant QTLs involved in mediating resistance to *Peronosclerospora sorghi* in maize in Egypt, including two QTLs located on chromosome 1 and a third on chromosome 9, were detected. Interestingly, although their study involved a different mapping population and, possibly, a different pathogen population as well, their study detected a QTL on chromosome 1 located near the RFLP marker *umc23a*, as in this study. Our findings indicate that both specific as well as nonspecific reactions may be involved in resistance to the downy mildews. Thus, while some loci may play a role in the specific recognition of the pathogen, other loci may have a more broad-spectrum function affecting fungal growth and symptom expression.

The phenotypic variation explained by the individual QTLs in each environment was generally lower than the R^2 values reported for controlling resistance to downy mildew in the maize (Agrama et al. 1999) and pearl millet (Jones et al. 1995) studies where higher heritabilities were also observed. As in the two studies, there were also putative QTLs with lower LOD scores that showed only small effects in the individual environments. Jones et al. (1995), assuming that there is a major gene mechanism involved in downy mildew resistance, hypothesized that a QTL effect may appear small if the pathogen population is made up of diverse strains because of the presence of several avirulence genes in the pathogen population. Thus, the magnitude of the effect of the individual QTL may depend on the make-up of the pathogen population against which the RILs have been screened, and these QTL that are of minor importance in these environments could have large effects in others. In this study, although the pathogen may not be as different as it would be with pathogens from different locations, there is some heterogeneity in the pathogen population based on the fact that the OPV plants, which served as sources of inoculum, are not genetically uniform.

The major QTL on chromosome 6 (bin 6.05) is located in a region containing other QTLs for disease and pest resistance. It is interesting to observe that chromosome 6 has clusters of genes influencing resistance to various biotic stresses; for instance, two QTL for SWCB leaf feeding damage, one on bin 6.04 (Khairallah et al. 1998) and another on bin 6.06 (Groh et al. 1998); two QTLs for resistance to *Fusarium moniliforme* on bins 6.04 and 6.06 (Perez-Brito et al. 2001); an array of resistance genes on bin 6.01, including the gene *mdm1* which confers resistance to the maize dwarf mosaic virus (MDMV) (Simcox et al. 1995), *wsm1* which confers resistance to a potyvirus, wheat streak mosaic virus (WSMV) (McMullen and Louie 1991); *rhm1*, which confers resistance to the fungal pathogen *Cochlilobus heterostrophus* (Zaitlin et al. 1993); a QTL for resistance to sugarcane mosaic virus (SCMV) in China (S. Zhang and X. Li, personal communication). The clustering of genes and QTLs for resistance against diseases and pests appears to be a widespread phenomenon in maize, being reported in all ten chromosomes (McMullen and Simcox 1995).

Our work identified SSR markers *mmc0241, phi078, bnlg1702* and *nc013* that are tightly linked to the major QTL on chromosome 6, indicating their possible use for marker-assisted selection. Beyond the possible use in marker-assisted selection another potential application of these results would be the identification and analysis of candidate genes to deduce information about the nature and function of the detected loci in determining resistance to downy mildews in Asia.

Acknowledgements This study was a collaborative effort of the Asian Maize Biotechnology Network (AMBIONET) with support from the Asian Development Bank. We thank Govindraju and R. Kumar for help in the field trials in India; S. Zhang and X. Li of the

AMBIONET-China Team for permission to cite unpublished data; S. Groh for the RFLP genotypic data, D. Bergvinson for providing seeds of the RILs, and G. Yongming (IRRI) for assistance in the statistical analysis.

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