View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by ICRISAT Open Access Repository

Mapping Quantitative Trait Loci for Resistance to Downy Mildew in Pearl Millet: Field and Glasshouse Screens Detect the Same QTL

E. S. Jones, W. A. Breese, C. J. Liu, S. D. Singh, D. S. Shaw, and J. R. Witcombe*

ABSTRACT

Downy mildew, caused by the pathogen *Sclerospora graminicola* (Sacc.) J. Schröt, can cause devastating yield losses in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Breeding for resistance to downy mildew is facilitated by an artificial glasshouse screening method that can be used worldwide. Quantitative trait loci (QTLs) mapping was used to determine whether resistance QTLs identified under field conditions in India were also detected in glasshouse screens carried out in India and the UK. Quantitative trait loci were mapped using 114 individual pearl millet progeny derived from a resistant × susceptible cross: molecular marker mapping was carried out in an F_2 population with restriction fragment length polymorphisms (RFLPs), and disease incidence was assessed on F_4 families. Composite interval mapping (CIM) was used to detect associations between F_4 family means and marker genotypes. Despite key environmental and methodological differences between the disease screens, the same two QTLs were detected in each screening environment. One QTL had a major effect and explained up to 60% of the phenotypic variation, while the other had a minor effect and explained up to 16% of the phenotypic variation. Two additional QTLs were also consistently detected across screens by examining pair-wise marker interactions. Multiple-trait interval mapping detected all of the QTLs that had been detected in individual screens, including the QTLs that had only been detected by examining pair-wise marker interactions, demonstrating its increased power over single trait mapping. Quantitative trait locus × environment interactions were significant at each QTL due to differences in the magnitude, rather than direction, of QTL effects. The differences in magnitude appeared to be a consequence of the degree of normality of the disease distribution, rather than any differences between screening methods.

Pearl millet is a staple food crop of the semiarid tropics. It is particularly important in regions with 200 to 800 mm of annual rainfall, in which no other cereal will yield grain (de Wet, 1987). Downy mildew, caused by the obligate biotrophic pathogen *S. graminicola*, can cause devastating yield losses in pearl millet and is a major constraint to productivity (Jeger et al., 1998; Singh et al., 1993).

Breeding for resistance is the only practical and economically feasible way to control downy mildew in pearl millet (Singh et al., 1997). To screen for resistance, a reliable downy mildew field screen has been developed that imitates the natural field situation (Williams et al., 1981). Inoculum is provided by oospores ploughed into the soil and airborne sporangia from preplanted rows of infected pearl millet genotypes. Glasshouse screens have also been developed, in which pot-grown seedlings at the coleoptile stage are spray-inoculated with a suspension of sporangia (Singh and Gopinath, 1985). Compared with field screens, a larger quantity of material may be screened in a smaller space and across a shorter period of time (Singh et al., 1997). However, despite key differences in the screening methodology, there have been no published data to indicate that disease scores in the glasshouse screen reflect those that would be obtained in the field. One major methodological difference is that the soilborne oospores used as inoculum in the field are absent from glasshouse screens. Oospores can play a significant role in infection, and genes controlling resistance to sporangial inoculum can not be assumed to be the same as those that confer resistance to oosporic inoculum (Hash et al., 1997).

The inheritance of resistance to downy mildew is a quantitative character, which has hindered the study of resistance as well as effective breeding. The advent of molecular markers has enabled QTLs for resistance to be mapped and has shown that the quantitative nature of resistance is due to the segregation of QTLs for pathotype-specific resistance of major effect, along with QTLs of minor effect (Jones et al., 1995). Breeders can now use marker-assisted selection to incorporate specific resistance QTL into parental lines of existing high yielding lines (Jones et al., 1995). To increase the durability of resistance, several gene pyramiding and deployment strategies have been proposed (Hash et al., 1997; Witcombe and Hash, 2000), all of which require the availability of a pool of QTLs with different pathogen specificities. These QTLs may be mapped using multilocational field evaluations against a range of *S. graminicola* pathogen populations, but heritabilities from such field evaluations are often low (Hash et al., 1997). Alternatively, evaluation against a multinational range of pathogen populations can take place under glasshouse conditions in a region where *S. graminicola* does not pose any threat to crop production (Hash et al., 1997). To provide the latter resource, a glasshouse screening center has been established in the UK. To determine the effectiveness of this center, it is important to test whether these glasshouse results are informative relative to the field environment.

Quantitative trait locus analysis has been used to study the stability of fungal-resistance QTL across environments (e.g., Saghai Maroof et al., 1996). In a similar

E.S. Jones, Plant Biotechnology Centre, Agriculture Victoria, La Trobe Univ., Bundoora VIC 3083, Australia; W.A. Breese and J.R. Witcombe, Centre for Arid Zone Studies, and D.S. Shaw, School of Biological Sciences, Univ. of Wales, Bangor, Gwynedd LL57 2UW, UK; C.J. Liu, CSIRO Plant Industry, Long Pocket Lab., 120 Meiers Road, Indooroopilly QLD 4068, Australia; and S.D. Singh, Int. Crops Research Inst. for the Semi-Arid Tropics, Patancheru AP 502 324, India. Received 13 July 2001. *Corresponding author (j.r.witcombe@bangor.ac.uk).

Published in Crop Sci. 42:1316–1323 (2002).

Abbreviations: BLUP, best-linear-unbiased-prediction; CIM, composite interval mapping; cM, centiMorgan; India/Ghs, glasshouse screen in India; LG, linkage group; QTL, quantitative trait locus; REML, restricted maximum likelihood; RFLP, restriction fragment length polymorphism; UK/Ghs, glasshouse screen in the UK.

way, QTL analysis can be used to determine the validity of a glasshouse screen in relation to a field screen. In this case, the principal question is not whether QTLs are stable across environments, but are the QTLs detected in the field also detected under glasshouse conditions, and, are the direction and magnitude of QTL effects consistent? Here we test, by the use of QTL mapping, whether the glasshouse screens established in the UK and India are effective indicators of resistance in the field.

MATERIALS AND METHODS

Plant Material

The parents of the mapping population were 7042(S)-1 (Hash and Witcombe, 1994), a downy mildew susceptible inbred line selected at ICRISAT-Patancheru, India, from germplasm accession IP 2696 that originated from an oasis in Chad; and P 7-3 (Singh et al., 1990), a downy mildew resistant inbred line selected at ICRISAT-Patancheru from germplasm accession IP 6118 that originated from Mali. 7042(S)-1 (paternal parent) was crossed to P 7-3 (maternal parent) to produce an F_1 population. Several F_1 plants were selfed to give an F_2 population of 175 individuals, from which only 125 individuals were sampled for DNA extraction and RFLP analysis. Each F_2 was selfed across two generations to derive 175 F_4 families on which downy mildew resistance was assessed.

Insufficient seed of the P 7-3 resistant parent meant that for the disease screen in the UK, the sister line, P 7-4, was used in its place. In test screens, P 7-3 and P 7-4 were not significantly different in their resistance response to a number of pathogen populations (data not shown), suggesting that they are genetically similar in terms of their resistance. The susceptible 7042(S)-1 parent was used in all screens. The hybrid HB 3 (Dave, 1987), which was known to have a high degree of susceptibility to the ICRISAT-Patancheru pathogen population, was used as an additional susceptible check.

For the screens at ICRISAT-Patancheru, the pathogen was maintained in the field on the highly susceptible host-genotypes 7042(S) (an inbred line from which 7042(S)-1 was derived by selfing) and HB 3. In the UK, pathogen material was maintained on 7042(S).

Disease Screens

Field Screen

The field screen was carried out in the downy mildew field nursery (Singh et al., 1997; Williams et al., 1981) at ICRISAT-Patancheru, India. Approximately 20 seeds of each entry were sown in alfisol soil in 2-m-long rows, 0.75 m apart. Inoculum was provided by soilborne oospores, the levels of which had been built up over the years by ploughing infected plants into the soil following each screen, and airborne sporangia from rows of infected 7042(S) and HB 3 genotypes planted every 9th row. Entries were randomized within each of six replicates. Parents and checks were represented twice within each replicate. Plants were drip-irrigated daily. Disease incidence (percentage of plants showing disease symptoms) was assessed 21 (India/Field-1) and 40 (India/Field-2) d after sowing. Symptoms were seen as distinct chlorosis of infected leaves and, at 40 d after sowing, green ears, where panicle seed was replaced by leaf-like structures (phylloidy).

Glasshouse Screen in India

The glasshouse screen in India (India/Ghs) was carried out at ICRISAT-Patancheru, India. Between 40 and 60 seeds of each genotype were sown into a dry compost consisting of equal proportions of alfisol, farmyard manure, and fine sand. Each pot represented a replicate of a pearl millet genotype, and there were three pot replicates per genotype. Pots were placed in a completely randomized design in a glasshouse, where cool air blowers were initiated when the temperature exceeded 25°C. Irrigation was performed daily with an overhead hose. When the seedlings were at the coleoptile to one-leaf stage the inoculum was prepared. Infected leaves from mature 7042(S) and HB 3 plants growing in the downy mildew field nursery at ICRISAT-Patancheru were detached, wiped clean of existing sporangiophores using moist cotton wool and incubated in darkness in plastic boxes for 8 h at 20°C and 100% relative humidity. The resulting sporangia were harvested into tap water at room temperature and the concentration was assessed and adjusted to $\approx 1.5 \times 10^5$ sporangia mL^{-1}. Each pot of seedlings was sprayed with ≈5 mL of inoculum using a hand-pumped sprayer. Disease incidence (percentage of plants showing chlorotic leaf symptoms) was assessed 14 d later, when the plants had four to five leaves.

Glasshouse Screen in the United Kingdom

The glasshouse screen in the UK (UK/Ghs) was carried out at Pen-y-Fridd Experimental Station, Bangor, UK, according to the protocols described by Jones et al. (1995). The UK screen differed from the Indian glasshouse screen as follows: between 30 and 40 seeds of each pearl millet genotype were sown into a low-nutrient peat and sharp sand compost (Chempak Seed Base, Chempak Products, Hoddesdon Herts., UK; NPK 25-39-30 mg L^{-1}). Four pot-replicates of each entry were flood-irrigated daily in a glasshouse providing a 16-h daylength (0600–2200 h) with supplementary lighting (to give a light intensity of between 500 and 1200 $\mu mol\ m^{-2}\ s^{-1}$) and a temperature of 25 to 30°C from 0600 to 1800 h and 20°C from 1800 to 0600h. For the inoculum, pearl millet leaves containing abundant oospores were collected from diseased 7042(S) and HB 3 plants growing in the downy mildew field nursery at ICRISAT-Patancheru 2 yr prior to the screen. This leaf material was dried, ground, and stored at room temperature. In the UK, seed of 7042(S) was sown into compost mixed with the ground leaf material. Sporangia produced on infected plants were used to infect further 7042(S) plants to supply sufficient inoculum for the screens (Jones et al., 1995). The sporangia produced following incubation of infected leaves were harvested into chilled (<2°C) distilled water and the concentration was adjusted to 9×10^4 sporangia mL^{-1}. Each pot of seedlings was sprayed with ≈4 mL of inoculum using a compressed-air cylinder-fed sprayer. The inoculum was maintained on ice to delay zoospore release until after inoculation and to ensure a uniform sporangial concentration with time (Jones et al., 2001).

Restriction Fragment Length Polymorphism Analysis and Map Construction

Restriction fragment length polymorphisms were detected with low copy probes derived from a *Pst*I-restricted genomic library of the pearl millet genotype 7042(S)-11, sister line to 7042(S)-1 (Liu et al., 1994). Southern hybridization analysis was performed as described by Liu et al. (1994). Probes detecting polymorphism were hybridized to the restricted DNA of 125 F_2 progeny. Some RFLPs contained a low to moderate frequency of nonparental alleles. Such RFLPs caused prob-

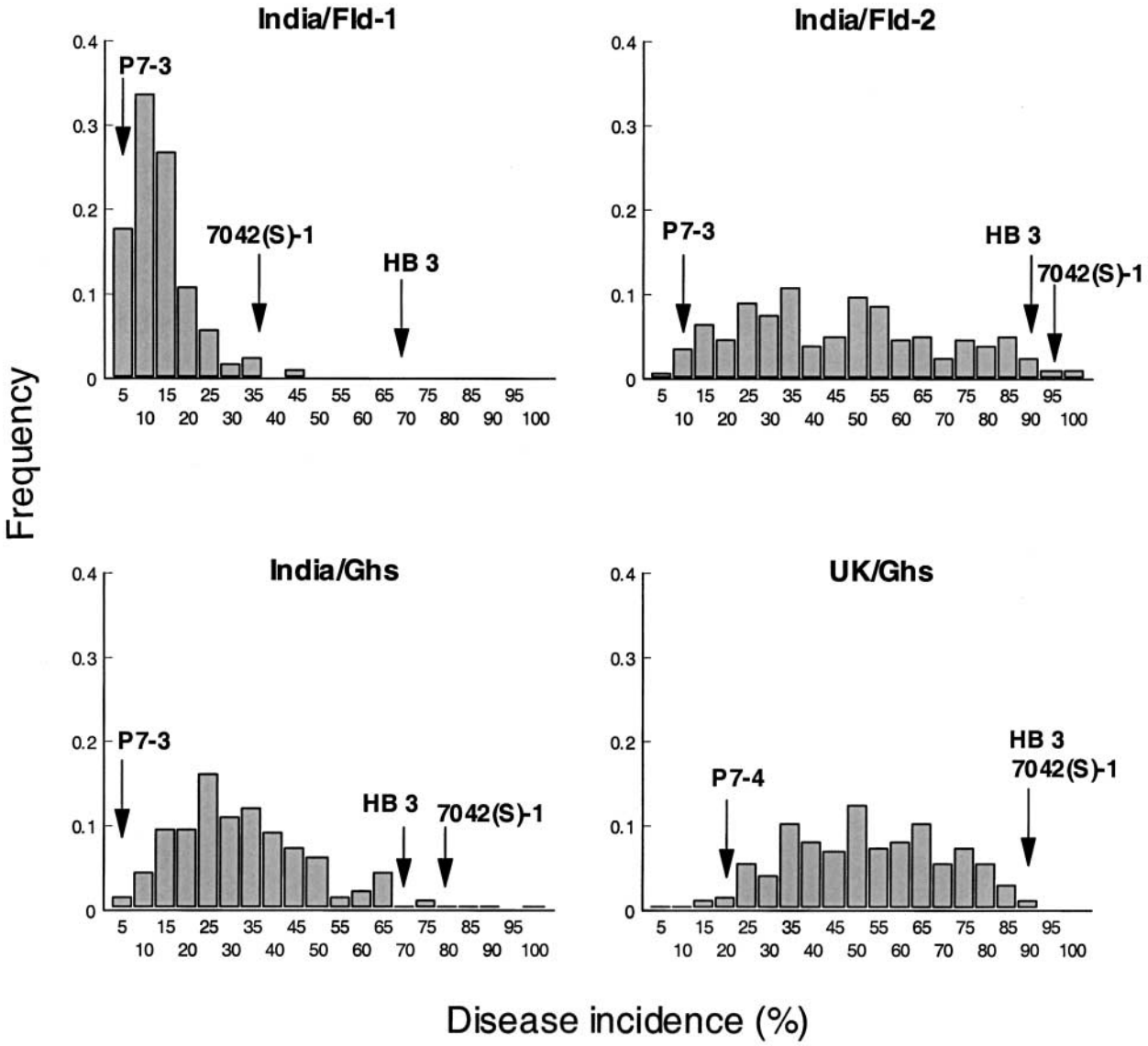

Fig. 1. Frequency distribution of disease incidence in F_4 families.

lems when ordering markers during map construction and were therefore removed from the mapping data set.

Linkage analysis was carried out using MAPMAKER 3.0 (Lander et al., 1987). Markers were grouped at LOD 4.0 and marker orders determined at LOD > 2.0. Orders were tested using the ripple command. Map distances were calculated using the Haldane mapping function (Haldane, 1919). Eighteen markers common to the pearl millet consensus map (Devos et al., 1995; Liu et al., 1994) were used to designate and orientate linkage groups (LGs).

Statistical Analysis of Disease Data

Disease scores made on genotypes with less than eight plants per pot-replicate were removed to avoid inaccurate assessment of percentage disease incidence. Data analyses were carried out in SAS (SAS Institute, 1985). Data were transformed (square root) to improve the distribution of residual-fitted data. Each screen was analyzed separately using general linear modeling (Proc GLM). Broad sense heritabilities were calculated for F_4 family means according to Wricke and Weber (1986). All screens were analyzed together using restricted maximum likelihood (REML; Proc MIXED). F_4 families and screens (i.e., environments) were treated as random effects.

The Shapiro-Wilk statistic (*W*-test) was used to assess the normality of the disease distributions for F_4 family means (normal option in Proc UNIVARIATE). Spearman's rank-order correlation coefficients were determined for pair-wise comparisons of screens (spearman option in Proc CORR).

Quantitative Trait Locus Analysis

QTL analysis was carried out using CIM (Jansen, 1993; Zeng, 1993) in QTL/Cartographer 1.14 (Basten et al., 1994, 2000). One hundred and fourteen lines had both genotypic and phenotypic information, and 38 mapped markers were used. Marker cofactors were selected by forward–backward regression with a critical *P*-value of 0.10. A window size of 10 centiMorgans (cM) and a maximum of 10 marker cofactors per model were used. Threshold levels were estimated using $P < 0.05$ for the comparison of 1000 data permutations without re-selection of background markers (Churchill and Doerge, 1994). For each screen, QTL analysis was carried out using

Table 1. General descriptions and statistics for screens.

Screen	Reps	Inoculum concentration	W‡	Skew§	Mean ± SE				GLM†				
									F_4 family		Replicate		
					P 7-3	7042(S)-1	HB 3	F_4 family	df	MS	df	MS	H^2#
		spores mL^{-1}											
India/Fld-1	6	Natural¶	0.89***	1.59	2.3 ± 4.5	35.1 ± 20.5	65.6 ± 11.7	12.0 ± 12.3	172	6.2***	5	25.9***	0.75
India/Fld-2	6	Natural¶	0.96**	0.34	8.1 ± 7.6	95.1 ± 6.1	89.9 ± 19.5	44.7 ± 27.9	172	19.7***	5	11.0***	0.90
India/Ghs	3	1.5×10^5	0.92***	1.20	1.8 ± 2.2	78.2 ± 17.1	70.0 ± 26.4	31.8 ± 16.9	184	7.8***	2	3.0*	0.79
UK/Ghs	4	9×10^4	0.97ns††	−0.07	12.2 ± 5.6	81.4 ± 3.5	84.6 ± 4.2	50.1 ± 18.2	189	9.8***	3	2.7ns	0.86

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level. ns, not significant.
† Results from general linear modelling (GLM).
‡ Deviation from normality of the disease incidence distribution for untransformed F_4 family means tested using the Shapiro-Wilk statistic (W).
§ Skewness of the disease incidence distribution for untransformed F_4 family means. Positive deviations from zero indicate the distribution is skewed towards resistance.
# Broad sense heritabilities for F_4 family means.
¶ Plants infected by air-borne sporangia and soil-borne oospores in the field.
†† ns = $P > 0.05$.

nontransformed F_4 family means, square-root-transformed means, and predicted means [derived using best-linear-unbiased-predictions (BLUP) of means adjusted for replicate using REML]. To determine average effects across all screens, across-screen predicted means were derived using BLUP within REML. Multiple interval mapping (Jiang and Zeng, 1995) in QTL/Cartographer 1.14 (Basten et al., 1994, 2000) was used to carry out joint analysis of all screens and to test for QTL × screen (environment) interactions.

For each screen, the presence of digenic interactions were investigated using two-way analyses of variance (proc GLM) using all possible pair-wise marker combinations. Significant ($P < 0.01$) interactions were placed in a regression model with markers having a significant main effect to determine whether the model fit was improved.

RESULTS

General Statistics

For each screen, the disease incidence among the F_4 families was continuously distributed (Fig. 1). There were no progeny with disease values significantly larger than the susceptible parent or smaller than the resistant parent demonstrating an absence of transgressive segregation. Deviation from normality for F_4 family disease distributions was greatest for India/Field-1 and India/Ghs, where a low level of disease resulted in the disease distributions being skewed towards the resistant parent (Table 1, Fig. 1).

For each screen, variation between F_4 family means was highly significant (Table 1). Heritabilities were high and ranged from 0.75 to 0.9 (Table 1). The lowest heritability was for India/Field-1 and was probably a consequence of poor separation of individuals due to the low disease incidence and a skewed disease distribution (Table 1).

Phenotypic correlations between screens ranged between 0.51 and 0.75, and were all highly significant (Table 2). The highest correlation was for resistance assessed in India/Field-2 and UK/Ghs. When all screens were analyzed together using REML, the F_4 family and F_4 family × screen interaction variance components were highly significant (values were substantially larger than their standard errors; Table 3).

Table 2. Spearman rank correlation coefficients for pair-wise comparisons of screens.

	India/Fld-2	India/Ghs	UK/Ghs
India/Fld-1	0.74***	0.60***	0.58***
India/Fld-2		0.51***	0.75***
India/Ghs			0.48***

*** Significant at the 0.001 probability level.

Map Construction

The mapped markers covered a total map distance of 280 cM. This is comparable to the value of 303 cM derived for the reference map for pearl millet (Liu et al., 1994), although this study also estimated total genome coverage to be 400 cM. Some map regions may therefore not be covered here. The maximum distance that occurred between markers was 30 cM and the average distance was 5 cM. Ripple analysis within MAPMAKER determined that there were only two regions that could not be ordered at LOD > 2.0, and these both occurred between pairs of markers separated by <2 cM. Nine out of 38 (24%) of the mapped markers showed significant deviation from the expected 1:2:1 segregation ratio at $P < 0.05$. Six of these were concentrated on LGs 3 and 6, and all showed distortion against alleles of the female resistant parent (P 7-3).

Quantitative Trait Locus Analysis

Comparison of Data Transformations

Square-root transformations of F_4 family means within each screen resulted in an increase in the normality of the distributions, but did not affect the LOD profiles (data not shown). Predicted (BLUP) means were more normally distributed (only India/Field-1 still

Table 3. Variance component analysis across screens using restricted maximum likelihood.

	Variance component ± SE
F_4 family	143.6 ± 24.5
Screen	277.1 ± 228.1
F_4 family × screen	110.5 ± 12.0
Rep (screen)	4.5 ± 2.3
Error	199.4 ± 6.9

Table 4. Quantitative trait loci (QTLs) detected using composite interval mapping.

Trait	LG†	+cM‡	Marker interval	LOD	R^2	W§	D#	4D¶
India/Fld-1	1	2	M413–M93	10.7	0.31	−6.4	−0.7	−2.8
	2	14	M543–M380	6.2	0.16	−4.8	0.6	2.4
India/Fld-2	1	4	M413–M93	22.7	0.60	−24.8	−6.0	24.0
	2	15	M543–M380	5.5	0.10	−10.1	−0.2	−0.8
India/Ghs	1	0	M413–M93	21.2	0.51	−19.6	−2.6	−10.4
	2	18	M543–M380	3.7	0.10	−5.5	7.6	30.4
UK/Ghs	1	0	M413–M93	18.4	0.55	−17.3	−2.3	−9.2
	2	15	M543–M380	3.1	0.10	−4.7	5.6	22.4
Predicted means	1	2	M413–M93	17.9	0.50	−13.1	−0.64	−2.56
	2	10	M322–M543	6.3	0.10	−6.4	1.8	7.2
Multiple interval mapping	1	0	M413–M93	27.4				
	2	18	M543–M380	7.2				
	3	8	M37–M248	5.0				
	5	0	M390–M318	5.4				

† LG = linkage group.
‡ Distance in centiMorgans (cM) from the end of the linkage group to the maximum likelihood position of the QTL.
§ W = weight at the maximum likelihood position for the QTL. This is (aa − bb)/2, where aa and bb are the phenotypic means of F_2 progeny individuals homozygous for the resistant parent alleles and susceptible parent alleles, respectively. A negative value indicates that resistance is inherited from the resistant parent.
# D = dominance value at the maximum likelihood position for the QTL.
¶ Estimated true value for dominance to compensate for heterozygotes in F_2 individuals segregating over two generations before phenotyping in F_4 families.

significantly deviated from normality) and resulted in quite different LOD profiles for the more skewed traits, India/Field-1 and India/Ghs. QTLs were detected in the same locations, but the variances explained by each QTL were considerably altered, with values becoming more similar to those of the less skewed traits, India/Field-2 and UK/Ghs. The analysis presented here is based on predicted means.

Comparison of Quantitative Trait Loci Detected in Each Screen

Two QTLs were detected in the same locations on LGs 1 and 2 in both assessments in the field (Table 4; Fig. 2A). The same two QTLs were also detected in the glasshouse screens carried out in India and in the UK (Table 4, Fig. 2B). The QTL on LG1 explained the highest proportion of the phenotypic variation in each of the screens (Table 4). For India/Field-2, India/Ghs, and UK/Ghs this was between 50 and 60% of the total phenotypic variation, whereas for India/Fld-1 the variation explained was about half of this at 31%. The QTL on LG2 explained between 10 and 16% of the total phenotypic variation. At each of the two QTLs detected in each of the screens, resistance was always inherited from the resistant parent (Table 4). The estimated inheritance of resistance varied between being partially dominant to completely dominant for the QTL on LG1, and between being additive and recessive for the QTL on LG2 (Table 4).

Quantitative Trait Locus Mapping with Across-screen Predicted Means and Multiple Interval Mapping

Quantitative Trait Locus analysis using across-screen predicted means resulted in the same two QTLs being detected on LGs 1 and 2 (Table 4, Fig. 2C). LOD scores, inheritance of resistance, and the phenotypic variation explained by these QTLs reflected the approximate average effects of the QTLs detected in individual screens (Table 4). The estimated inheritance of resistance for the QTL on LG1 was partially dominant and on LG2 was recessive. No additional QTLs were detected.

Analysis with multiple interval mapping detected the same two QTLs plus an additional two QTLs on LGs 3 and 5 (Fig. 2C). The locus on LG3 had been apparent just below the threshold level for QTL detection for the screen India/Field-2 (Fig. 2A). All QTLs had a significant QTL × environment component (data not shown).

Digenic Interactions

The number of pair-wise marker interactions that were significant at $P < 0.01$ was three times greater than that expected by chance. In each screen, and for the across-screen predicted means, the most significant of these occurred between M248 on LG3 and M390 on LG5. The same dominant × additive and dominant × dominant interactions were consistently observed (data not shown). These markers were in the same locations as the additional QTL detected with multiple interval mapping. An additional interaction of smaller effect was significant for India/Field-1, between the same marker, M248 on LG3, and M515 on LG1. Here, interactions were dominant × additive and additive × additive.

The interaction terms improved the significance of the overall model for each screen and increased the total phenotypic variation explained by up to 10% (Table 5).

DISCUSSION

Similar QTLs were detected in each of the screens, despite fundamental differences in screening methodologies. One QTL of major effect on LG1 (R^2 of 0.31–0.60) and one of minor effect on LG2 (R^2 of 0.10–0.16) were detected in the same locations. Resistance at each QTL was consistently inherited from the resistant parent, and the modes of inheritance were similar. Two additional genomic regions on LGs 3 and 5 were only detected following analysis of pair-wise marker interactions, yet were also consistently detected in each screen and increased the total phenotypic variation explained by up

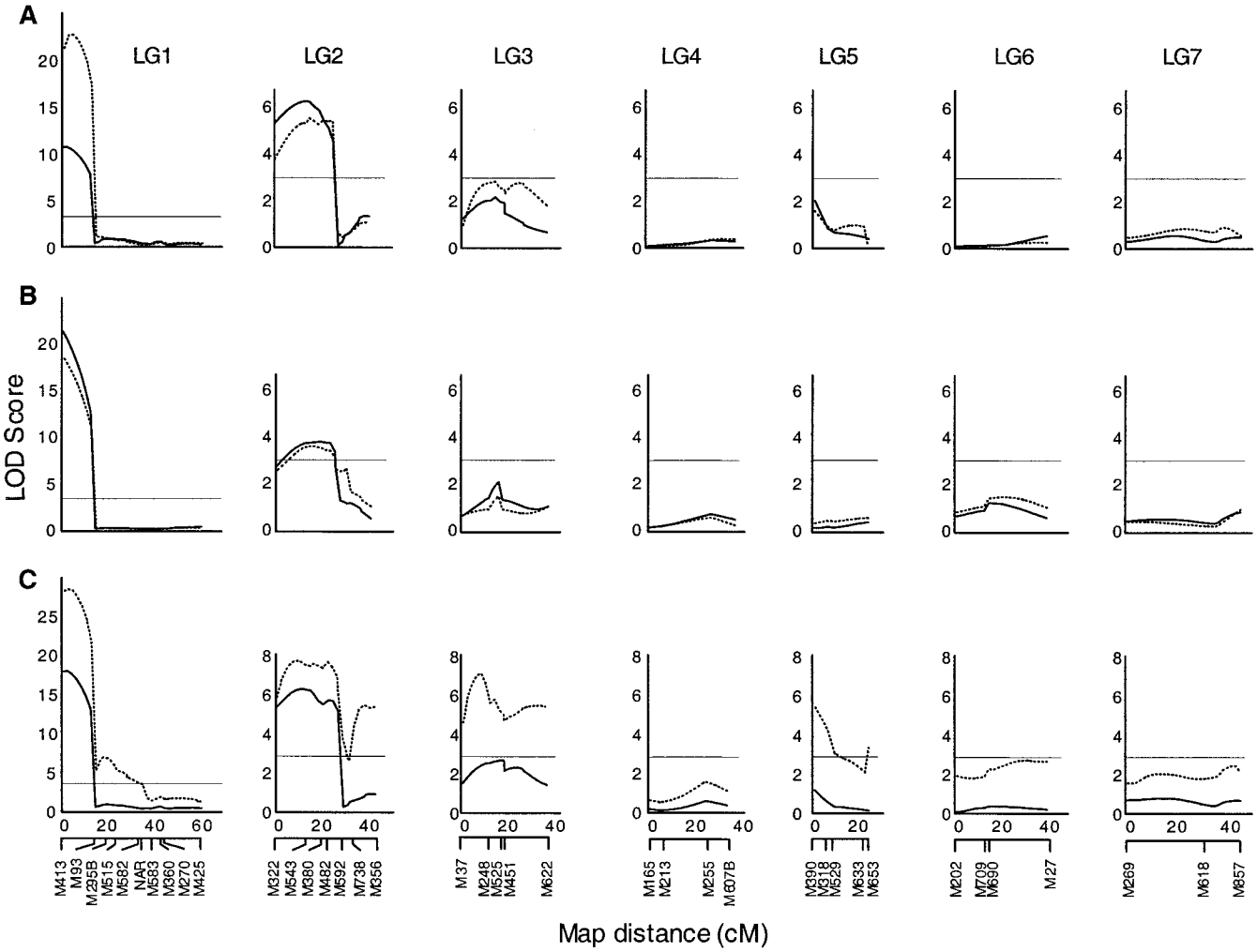

Fig. 2. Log of the odds (LOD) profiles for associations between molecular markers and disease incidence analyzed using composite interval mapping for (A) field screens (solid line = India/Field-1; dashed line = India/Field-2), (B) glasshouse screens (solid line = India/Ghs, dashed line = UK/Ghs), and (C) average effects across all screens (solid line = predicted means, dashed line = multiple interval mapping). Each graph represents a linkage group. The horizontal line across each graph indicates the level of significance for the presence of a quantitative trait locus estimated from permutation analyses. Note that the *y*-axis for LG1 is on a different scale to accommodate the high LOD.

to 10%. A further interaction of smaller effect was found in only one screen (India/Field-1).

The consistency of the resistance phenotype was further demonstrated by highly significant correlations between data collected in different screens. The highest correlation coefficient was found for comparisons between India/Field-2 and UK/Ghs, despite these screens being the least similar in terms of screening methodology.

Minor differences between the screens that could have affected the expression of QTLs included soil type, day and night temperatures, and irrigation regimes. Major differences were the type and method of inoculation, plant age at assessment, and the virulence composition of the inoculum. Several studies have shown that different inoculation techniques can result in different QTLs being detected (Albar et al., 1998; Lübberstedt et al., 1999; Setiawan et al., 2000; Zhu et al., 1999). For example, in sugar beet, out of eight QTLs detected in field and leaf-disc screens for resistance to *Cercospora beticola*, only one was common to both screens (Setiawan et al., 2000). Many studies have also found that plant age at inoculation and disease assessment result in different QTLs being detected (Clements et al., 2000; Qi et al., 1999; Shäfer-Pregl et al., 1999; Steffenson et al., 1996; Welz et al., 1999). Here, in the field, inoculum was in the form of soilborne oospores and airborne sporangia, and plants were exposed to inoculum from seedling emergence to grain filling. In the glasshouse, inoculum was solely in the form of a spray-inoculated suspension

Table 5. Proportion of the total phenotypic variance (R^2) explained by quantitative trait loci, excluding and including interaction terms.

Independent variables in model	India/Fld-1	India/Fld-2	India/Ghs	UK/Ghs	Predicted means
M413, M380	0.39	0.56	0.56	0.55	0.53
M413, M390, M248 × M390	0.49	0.62	0.63	0.61	0.61
M413, M390, M248 × M390, M248 × M515	0.54	0.64	0.63	0.61	0.63

of sporangia at the coleoptile-1 leaf stage and disease assessment was carried out at the 4-5 leaf stage, long before flowering was initiated. Sporangia and oospores have different modes of infection, with sporangia producing zoospores that penetrate leaf epidermal cells and stomata (Subramanya et al., 1983; Mauch-Mani et al., 1989) and oospores infecting the meristematic region of the root (Safeeulla, 1976). The relative roles of oospores and sporangia of *S. graminicola* on disease progression where both are present is not clear, but probably depends on environmental conditions (Jeger et al., 1998). In this study, if oospores are assumed to be infecting plants in the field, the detection of the same QTLs in field and glasshouse screens suggests that oospores incur a similar response to infection as the zoospores that are released from sporangia. Similarly, these results suggest that inoculation at the coleoptile-1 leaf stage elicits a similar disease response as inoculation via continual exposure as the plant matures.

A major factor that could have affected the disease response was the virulence composition of the inoculum used within the different screens. For the screen in the UK, oospores were collected from the field 2 yr before inoculum was collected for the screens carried out in India. The virulence structure of pathogen populations of *S. graminicola* can rapidly shift at a single location (Singh and Singh, 1987; Thakur and Rao, 1996), but the pathogen components affecting resistance in this cross appear to have remained stable across these 2 yr. Changes in host-pathogen interactions due to shifts in inoculum pathogenicity is an additional factor that has to be considered in repeated disease screens, and may explain inconsistencies in QTL detection that have occurred in other resistance QTL studies.

One explanation for the consistent detection of QTLs across different environments in this study is that resistance to downy mildew in pearl millet has an unusually high heritability for a quantitative disease trait. This, in turn, is probably due to resistance being controlled by a small number of QTLs which are likely to be race-specific (Jones et al., 1995). Inconsistencies have often been found in other host-pathogen systems where many QTLs of small and medium effect were segregating, so that screen-specific QTLs were more likely to occur. Here, the consistent detection of a digenic interaction across screens was more surprising, as the effect of interacting QTLs may be expected to be more environmentally variable. Consistent detection of interactions across screens has rarely been reported, but was found by Saghai Maroof et al. (1996) for gray leaf spot in maize, where consistency was attributed to adequate disease pressures across screens.

Two methods for analyzing data across all screens were tested. Analysis of QTLs using across-screen predicted means resulted in the same two QTLs of main effect being detected that had been found in individual screens. Multiple trait analysis using CIM resulted in an additional two QTLs being detected, which were in the same location as the two regions that were found to be involved in digenic interactions in individual screens. As has been predicted (Jiang and Zeng, 1995; Korol et al., 1995), multiple trait analysis using correlated trait complexes appears to increase the power of QTL detection compared with single trait analysis.

Quantitative trait locus × environment (QTL × E) interactions were highly significant at each of the QTLs detected. However, the sign of the additive effect did not change between screens demonstrating that the QTL × E interactions observed were due to changes in magnitude, rather than direction, of QTL effects. The change in magnitude of the phenotypic variance explained by QTL between screens appeared to be associated with the normality of the disease distribution, rather than any differences in screening methodology: Improving the normality of the distribution for the more skewed data greatly affected the variance explained by detected QTL.

One aim of this study was to detect new resistance QTL for marker-assisted selection. However, the QTL with the largest effect on LG1 mapped to the same location as a QTL of major effect detected against this same pathogen population in a different pearl millet cross (Jones et al., 1995), suggesting that the QTLs are due to effects at the same resistance gene. Although the resistance sources for each cross were derived from different African germplasm collections, both had origins in the highly genetically diverse Souna landrace (Singh et al., 1990; Talukdar et al., 1998). Both host genotypes were selected at ICRISAT-Patancheru during the late 1970s and early 1980s, so that the same QTL could have been selected. The QTL on LG2 has not previously been mapped, but the apparent recessive inheritance of resistance at this QTL would render its use impractical in hybrid breeding. Equally, the two QTL that were only detected following analysis of pair-wise interactions are unlikely to be used. The possible incomplete genome coverage provided by RFLP in this study may mean that additional QTLs remain undetected, although the relatively high total phenotypic variation already explained (up to 64%) would suggest that any additional QTL do not have a major effect.

Further QTLs need to be detected for resistance gene-deployment or pyramiding strategies to be effective against downy mildew in pearl millet. This will require methodical screening of multiple mapping populations against a range of pathogen populations of *S. graminicola* under controlled conditions. We have shown that such screens carried out in the glasshouse in the UK and India are likely to result in the detection of QTLs that control resistance under field conditions.

ACKNOWLEDGMENTS

The authors would like to thank Kevin Smith and Gavin Kearney for statistical advice, and Tom Hash, John Forster, and two anonymous reviewers for helpful comments on the manuscript. This work was funded by the Department for International Development (DFID) Plant Sciences Research Programme for the benefit of developing countries. The views expressed are not necessarily those of DFID.

REFERENCES

Albar, L., M. Lorieux, N. Ahmadi, I. Rimbault, A. Pinel, A.A. Sy, D. Fragette, and A. Ghesquière. 1998. Genetic basis and mapping of the resistance to rice yellow mottle virus. I. QTLs identification

and relationship between resistance and plant morphology. Theor. Appl. Genet. 97:1145–1154.

Basten, C.J., B.S. Weir, and Z.-B. Zeng. 1994. Zmap—A QTL cartographer. p. 65–66. *In* C. Smith et al. (ed.) Proc. World Congr. on Genetics Applied to Livestock Production: Computing Strategies and Software, 5th, Guelph, ON, Canada. 7–12 Aug. 1994. Dep. of Animal and Poultry Sci., Univ. of Guelph, ON, Canada.

Basten, C.J., B.S. Weir, and Z.-B. Zeng. 2000. QTL Cartographer. Version 1.14. Dep. of Statistics, North Carolina State Univ., Raleigh, NC.

Churchill, G.A., and R.W. Doerge. 1994. Empirical threshold values for quantitative trait mapping. Genetics 121:185–199.

Clements, M.J., J.W. Dudley, and D.G. White. 2000. Quantitative trait loci associated with resistance to gray leaf spot of corn. Phytopathology 90:1018–1025.

Dave, H.R. 1987. Pearl millet hybrids. p. 121–126. *In* J.R. Witcombe and S.R. Beckerman (ed.) Proc. Int. Pearl Millet Workshop, Patancheru, India. 7–11 Apr. 1986. ICRISAT Asia Centre, Patancheru, India.

Devos, K.M., T.S. Pittaway, C.S. Busso, M.D. Gale, J.R. Witcombe, and C.T. Hash. 1995. Molecular tools for the pearl millet nuclear genome. Int. Sorghum Millets Newsl. 36:64–66.

Haldane, J.B.S. 1919. The combination of linkage values and the calculation of distances between loci of linked factors. J. Genet. 8:299–309.

Hash, C.T., and J.R. Witcombe. 1994. Pearl millet mapping populations at ICRISAT. p. 69–75. *In* J.R. Witcombe and R.R. Duncan (ed.) Use of molecular markers in sorghum and pearl millet breeding for developing countries—Proc. ODA Plant Sci. Res. Programme Conf., Norwich, UK. 29 Mar.–1 Apr. 1993. Overseas Development Administration, London.

Hash, C.T., J.R. Witcombe, R.P. Thakur, S.K. Bhatnagar, S.D. Singh, and J.P. Wilson. 1997. Breeding for pearl millet disease resistance. p. 337–372. *In* Proc. Int. Conf. on Genetic Improvement of Sorghum and Pearl Millet, Lubbock, TX. 22–27 Sept. 1996. INTSORMIL, University of Nebraska, Lincoln, NE.

Jansen, R.C. 1993. Interval mapping of multiple quantitative trait loci. Genetics 135:205–211.

Jeger, M.J., E. Gilijamse, C.H. Bock, and H.D. Frinkling. 1998. The epidemiology, variability and control of the downy mildews of pearl millet and sorghum, with particular reference to Africa. Plant Pathol. 47:544–569.

Jiang, C., and Z. Zeng. 1995. Multiple trait analysis of genetic mapping for quantitative trait loci. Genetics 140:1111–1127.

Jones E.S., W.A. Breese, and D.S. Shaw. 2001. Infection of pearl millet by the downy mildew fungus *Sclerospora graminicola*: chilling inoculum to prevent zoospore release and subsequent spray damage to zoospores. Plant Pathol. 50:1–8.

Jones, E.S., C.J. Liu, M.D. Gale, C.T. Hash, and J.R. Witcombe. 1995. Mapping quantitative trait loci for downy mildew resistance in pearl millet. Theor. Appl. Genet. 91:448–456.

Korol, A.B., Y.I. Ronin, and V.M. Kirzhner. 1995. Interval mapping of quantitative trait loci employing correlated trait complexes. Genetics 140:1137–1147.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln, and L. Newburg. 1987. MAPMAKER: an interactive computer package for constructing primary linkage maps of experimental and natural populations. Genomics 1:174–181.

Liu, C.J., J.R. Witcombe, T.S. Pittaway, M. Nash, C.T. Hash, C.S. Busso, and M.D. Gale. 1994. An RFLP-based genetic map of pearl millet (*Pennisetum glaucum*). Theor. Appl. Genet. 89:481–487.

Lübberstedt, T., X.C. Xia, G. Tan, X. Liu, and A.E. Melchinger. 1999. QTL mapping of resistance to *Sporisorium reilana* in maize. Theor. Appl. Genet. 99:593–598.

Mauch-Mani, B., F.J. Schwinn, and R. Guggenheim. 1989. Early infection stages of the downy mildew fungi *Sclerospora graminicola* and *Peronosclerospora sorghi* in plants and cell structures. Mycol. Res. 92:445–452.

Qi, X., G. Jiang, W. Chen, R.E. Niks, P. Stam, and P. Lindhout. 1999. Isolate-specific QTLs for partial resistance to *Puccinia hordei* in barley. Theor. Appl. Genet. 99:877–884.

Safeeulla, K.M. 1976. Biology and control of the downy mildews of pearl millet, sorghum and finger millet. Wesley Press, Mysore, India.

Saghai Maroof, M.A., Y.G. Yue, Z.X. Xiang, E.L. Stromberg, and G.K. Rufener. 1996. Identification of quantitative trait loci controlling resistance to gray leaf spot disease in maize. Theor. Appl. Genet. 93:539–546.

SAS Institute. 1985. SAS users guide: basics. 1985 ed. SAS Inst., Cary, NC.

Schäfer-Pregl, R., D.C. Borchardt, E. Barzen, C. Glass, W. Mechelke, J.F. Seitzer, and F. Salamini. 1999. Localisation of QTLs for tolerance to *Cecospora beticola* on sugar beet linkage groups. Theor. Appl. Genet. 99:829–836.

Setiawan, A., G. Koch, S.R. Barnes, and C. Jung. 2000. Mapping quantitative trait loci (QTLs) for resistance to *Cercospora* leaf spot disease (*Cercospora beticola* Sacc.) in sugar beet (*Beta vulgaris* L.). Theor. Appl. Genet. 100:1176–1182.

Singh, S.D., and R. Gopinath. 1985. A seedling inoculation technique for detecting downy mildew resistance in pearl millet. Plant Dis. 69:582–584.

Singh, S.D., S.B. King, and P.M. Reddy. 1990. Registration of five pearl millet germplasm sources with stable resistance to downy mildew. Crop Sci. 30:1164.

Singh, S.D., S.B. King, and J. Werder. 1993. Downy mildew disease of pearl millet. Inf. Bull. no. 37. Int. Crops Res. Inst. for the Semi-Arid Tropics, Patancheru, India.

Singh, S.D., and G. Singh. 1987. Resistance to downy mildew in pearl millet hybrid NHB 3. Indian Phytopathol. 40:178–180.

Singh, S.D., J.P. Wilson, S.S. Navi, B.S. Talukdar, D.E. Hess, and K.N. Reddy. 1997. Screening techniques and sources of resistance to downy mildew and rust in pearl millet. Inf. Bull. no. 48. ICRISAT, Patancheru, India.

Steffenson, B.J., P.M. Hayes, and A. Kleinhofs. 1996. Genetics of adult plant resistance to net blotch (*Pyrenophora teres* f. *teres*) and spot blotch (*Cochliobolus sativus*) in barley. Theor. Appl. Genet. 92:552–558.

Subramanya, S., H.S. Shetty, and K.M. Safeeulla. 1983. Pearl millet downy mildew: biology of systemic infection by zoospores. Proc. Indian Nat. Sci. Acad. B49:385–394.

Talukdar, B.S., P.P. Prakash Babu, A.M. Rao, C. Ramakrishna, J.R. Witcombe, S.B. King, and C.T. Hash. 1998. Registration of ICMP 85410 dwarf, downy mildew resistant, restorer germplasm line of pearl millet. Crop Sci. 38:904–905.

Thakur, R.P., and V.P. Rao. 1996. Evidence for a new virulent pathotype in *Sclerospora graminicola* on pearl millet. Indian J. Mycol. Plant Pathol. 26:133.

Welz, H.G., A.W. Schechert, and H.H. Geiger. 1999. Dynamic gene action at QTLs for resistance to *Setosphaeria turcica* in maize. Theor. Appl. Genet. 98:1036–1045.

de Wet, J.M.J. 1987. Moderator's overview: Pearl millet (*Pennisetum glaucum*) in Africa and India. p. 3–4. *In* J.R. Witcombe and S.R. Beckerman (ed.) Proc. Int. Pearl Millet Workshop, Patancheru, India. 7–11 Apr. 1986. ICRISAT Center, Patancheru, India.

Williams, R.J., S.D. Singh, and M.N. Pawar. 1981. An improved field screening technique for downy mildew resistance in pearl millet. Plant Dis. 3:239–241.

Witcombe, J.R., and C.T. Hash. 2000. Resistance gene deployment strategies in cereal hybrids using marker-assisted selection: Gene pyramiding, three-way hybrids, and synthetic parent populations. Euphytica 112:175–186.

Wricke, G., and W.E. Weber. 1986. Quantitative genetics and selection in plant breeding. Walter de Gruyter & Co., Berlin.

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

Zhu, H., L. Gilchrist, P. Hayes, A. Kleinhofs, D. Kudrna, Z. Liu, L. Prom, B. Steffenson, T. Toojinda, and H. Vivar. 1999. Does function follow form? Principal QTLs for *Fusarium* head blight (FHB) resistance are coincident with QTLs for inflorescence traits and plant height in a double-haploid population of barley. Theor. Appl. Genet. 99:1221–1232.