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 F₂ population with restriction fragment length polymorphisms (RFLPs), and disease incidence was assessed on F₂ families. Composite interval mapping (CIM) was used to detect associations between F₂ 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 economical 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 multilocalional 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

*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 F1 population. Several F1 plants were selfed to give an F2 population of 175 individuals, from which only 125 individuals were sampled for DNA extraction and RFLP analysis. Each F2 was selfed across two generations to derive 175 F3 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 days 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 1.5 × 10⁵ 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 days 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 μmol 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 × 10⁵ 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 through 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 PsI-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 F2 progeny. Some RFLPs contained a low to moderate frequency of nonparental alleles. Such RFLPs caused prob-
Fig. 1. Frequency distribution of disease incidence in F₄ families.

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 F₄ family means according to Wricke and Weber (1986). All screens were analyzed together using restricted maximum likelihood (REML; Proc MIXED). F₄ 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 F₄ 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...
nontransformed \( F_4 \) family means, square-root-transformed means, and predicted means [derived using best-linear-unbi-
ased-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, 
was used to carry out joint analysis of all screens and to test 
for QTL \( \times \) 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 
makers 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 segre-
gation. 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 herita-
bility was for India/Field-1 and was probably a conse-
quence of poor separation of individuals due to the 
low disease incidence and a skewed disease distribution 
(Table 1).

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

**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 there-
fore 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 normal-
ity 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

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### Table 1. General descriptions and statistics for screens.

<table>
<thead>
<tr>
<th>Screen</th>
<th>Reps</th>
<th>Inoculum concentration</th>
<th>W‡</th>
<th>Skew§</th>
<th>Mean ± SE</th>
<th>F(_4) family</th>
<th>Replicate</th>
</tr>
</thead>
<tbody>
<tr>
<td>India/Fld-1</td>
<td>6</td>
<td>Natural§§</td>
<td>0.89***</td>
<td>1.59</td>
<td>2.3 ± 4.5</td>
<td>35.1 ± 20.5</td>
<td>65.6 ± 11.7</td>
</tr>
<tr>
<td>India/Fld-2</td>
<td>6</td>
<td>Natural§§</td>
<td>0.96**</td>
<td>0.34</td>
<td>8.1 ± 7.6</td>
<td>95.1 ± 6.1</td>
<td>89.9 ± 19.5</td>
</tr>
<tr>
<td>India/Ghs</td>
<td>3</td>
<td>1.5 ( \times 10^5 )</td>
<td>0.92***</td>
<td>1.20</td>
<td>1.8 ± 2.2</td>
<td>78.2 ± 17.1</td>
<td>70.0 ± 26.4</td>
</tr>
<tr>
<td>UK/Ghs</td>
<td>4</td>
<td>9 ( \times 10^4 )</td>
<td>0.97ns††</td>
<td>-0.07</td>
<td>12.2 ± 5.6</td>
<td>81.4 ± 3.5</td>
<td>84.6 ± 4.2</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Screen</th>
<th>India/Fld-2</th>
<th>India/Ghs</th>
<th>UK/Ghs</th>
</tr>
</thead>
<tbody>
<tr>
<td>India/Fld-1</td>
<td>0.74***</td>
<td>0.60***</td>
<td>0.58***</td>
</tr>
<tr>
<td>India/Fld-2</td>
<td>0.74***</td>
<td>0.51***</td>
<td>0.75***</td>
</tr>
<tr>
<td>India/Ghs</td>
<td>0.48***</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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### Table 3. Variance component analysis across screens using re-
stricted maximum likelihood.

<table>
<thead>
<tr>
<th>Variance component</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>( F_4 ) family</td>
<td>143.6 ± 24.5</td>
</tr>
<tr>
<td>Screen</td>
<td>277.1 ± 228.1</td>
</tr>
<tr>
<td>( F_4 ) family ( \times ) screen</td>
<td>110.5 ± 12.0</td>
</tr>
<tr>
<td>Rep (screen)</td>
<td>-4.5 ± 2.3</td>
</tr>
<tr>
<td>Error</td>
<td>199.4 ± 6.9</td>
</tr>
</tbody>
</table>
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/Field-2 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 significantly 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.

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 $\times$ additive and dominant $\times$ 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 $\times$ additive and additive $\times$ 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; Lübbertstedt 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; Schäfer-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.

<table>
<thead>
<tr>
<th>Independent variables in model</th>
<th>India/Fld-1</th>
<th>India/Fld-2</th>
<th>India/Ghs</th>
<th>UK/Ghs</th>
<th>Predicted means</th>
</tr>
</thead>
<tbody>
<tr>
<td>M413, M380</td>
<td>0.39</td>
<td>0.56</td>
<td>0.56</td>
<td>0.55</td>
<td>0.53</td>
</tr>
<tr>
<td>M413, M390, M248 × M390</td>
<td>0.49</td>
<td>0.62</td>
<td>0.63</td>
<td>0.61</td>
<td>0.61</td>
</tr>
<tr>
<td>M413, M390, M248 × M590, M248 × M515</td>
<td>0.54</td>
<td>0.64</td>
<td>0.63</td>
<td>0.61</td>
<td>0.63</td>
</tr>
</tbody>
</table>
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.

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