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ORIGINAL PAPER

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Identification and validation of QTLs conferring resistance to sorghum downy mildew (*Peronosclerospora sorghi*) and Rajasthan downy mildew (*P. heteropogoni*) in maize

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Abstract We have mapped the quantitative trait loci (QTLs) conferring resistance to sorghum downy mildew (*Peronosclerospora sorghi*; SDM) and Rajasthan downy mildew (*P. heteropogoni*; RDM), two species of DM prevalent throughout India. QTL mapping was carried out on a backcross population of 151 individuals derived from a cross between CM139 (susceptible parent) and NAI116 (highly resistant to both SDM and RDM). Heritability estimates were 0.74 for SDM and 0.67 for RDM. Composite interval mapping combined with a linkage map constructed with 80 simple sequence repeat (SSR) markers resulted in the identification of three QTLs (one each on chromosomes 2, 3 and 6) for SDM resistance and two QTLs (one each on chromosomes 3 and 6) for RDM resistance, all of which were contributed by NAI116. The significance of the major QTL on chromosome 6 (bin 6.05) that confers resistance to diverse DMs in tropical Asia, including SDM and RDM in India, was also verified. The results confirmed that some common QTLs contribute to both SDM and RDM resistance, while additional loci might specifically govern resistance to SDM. The QTL information generated in this study provide information that will aid in undertaking an integrated breeding strategy for the transfer of resistance to SDM and RDM in maize lines using marker-assisted selection.

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Introduction

Among the various pathogens affecting maize (*Zea mays* L.) production and productivity worldwide, the downy mildews (DMs) are considered to be highly important in terms of their geographical distribution and potential ability to cause significant yield reduction. Heavy losses in maize have been recorded from infestation of DM pathogens systemic to a region, such as in the Philippines, Taiwan, Indonesia, Thailand, India, West Africa, Venezuela, Japan, Australia, Europe, North America and other parts of the world (Bonde 1982; Rifin 1983). Worldwide, 30% of the area planted in tropical lowland, subtropical, mid-altitude transition zone and highland maize has suffered economic losses due to DM attack (Jeffers et al. 2000). In the Asian region, DM diseases are considered to be important biotic stresses limiting maize productivity (Pingali and Pandey 2001).

The important species causing DM of maize in the Asian region are *Peronosclerospora sorghi* (Weston & Uppal) Shaw (sorghum DM), *P. philippinensis* (Philippine DM), *P. maydis* (Java DM), *P. sacchari* (sugarcane DM) and *Sclerophthora rayssiae zeae* (brown stripe DM) (Sharma et al. 1993), of which the sorghum DM (SDM) is the most prevalent of the DMs observed in India. Spencer and Dick (2002), in a detailed analysis of various aspects of graminicolous DMs, indicated that there are two major centers of diversity for *Peronosclerospora*, one in the Indian sub-continent (*P. dichanthiicola*, *P. heteropogoni* and *P. westonii*) and another in eastern Melanesia and Australasia (*P. globosa*, *P. maydis*, *P. miscanthii*, *P. noblei*, *P. sachari* and *P. spontanea*). *P. philippinensis* and *P. sorghi* are widespread in distribution, and their origins are

conjectural. Considering the diversity of the DM pathogens and their potential to lead to systemic infection, there is need to develop host resistance to control them.

The pathogen *P. sorghi* was first reported in India by Butler in 1907 (Frederiksen et al. 1973). Since the 1960s, damage to maize and sorghum directly attributable to *P. sorghi* infestation has been observed to have a cosmopolitan distribution. Three strains have been identified in *P. sorghi*: the sorghum strain, infecting only sorghum, the maize strain, attacking only maize, and a strain infecting both the crops. These three strains are widely distributed throughout Asia, and all have been reported in India. In the Rajasthan state of India, the disease cycle of the prevalent DM, which at the time was considered to be a variant of SDM, was seen to pass through a collateral host, *Heteropogon contortus*. This pathogen was found to produce oospores on maize, a phenomenon absent in SDM (Siradhana et al. 1980). On the basis of significant differences in the morphological characteristics, disease cycle, host range and other traits, this DM was re-designated as 'Rajasthan downy mildew' (RDM; Schmidt and Freytag 1999; Spencer and Dick 2002).

With the increasing appreciation of the need for cost-effective and environmentally sound means for disease management and with the availability of powerful tools and techniques to genetically analyze disease resistance, there is now a renewed emphasis on host-controlled resistance for economically important diseases in crop plants, including maize. The cost concerns and the emerging problem of chemical resistance build-up in the DM pathogens have been earlier highlighted (Raymundo 2000). Genetic analysis of host resistance to SDM and RDM in India has proven that resistance to these diseases is under polygenic control; additive effects are the predominant contributors to resistance, though in the case of RDM, dominance effects also play an important role (Nair et al. 2004a). In addition, the recent observations that genotypes resistant to SDM are invariably resistant to RDM, while the RDM-resistant genotypes might show differential responses to SDM (Nair et al. 2004b; Yen et al. 2004) suggest that some common genetic factors might be controlling resistance to both SDM and RDM.

The QTL mapping analysis for SDM resistance in the Egyptian maize germplasm (Agrama et al. 1999) using single-factor analysis revealed three quantitative trait loci (QTLs) on two chromosomes (1L and 9), cumulatively explaining 53.6% of the phenotypic variance. All three QTLs were contributed by the resistant parent G62 and have an additive gene action. Under the Asian Maize Biotechnology Network (AMBIONET), facilitated by CIMMYT (International Maize and Wheat Improvement Center), Mexico, the Indian team in collaboration with partners in Indonesia, Philippines and Thailand, has recently carried out a study on QTL mapping of resistance against diverse DMs in the Asian region, including SDM and RDM (George et al. 2003). The study, utilizing recombinant inbred lines (RILs) derived from Ki3 (resistant) and CML139 (susceptible), led to the identification of five QTLs with significant effects on resistance to the five important DM diseases in Asia. Most significantly, a QTL on chromosome 6 at bin 6.05 was found to confer resistance to all five DM pathogens studied.

The major aim of the study reported here was to validate the QTL information generated through the AMBIONET on SDM and RDM resistance using a mapping population that can be effectively utilized in the Indian maize-breeding program. Also, by using a mapping population derived from the Indian germplasm, this study sought to identify other major QTLs, if any, conferring resistance to these diseases. We also wished to explore the parallelism in the genetic basis of resistance to SDM and RDM.

Materials and methods

Mapping population

We carried out a resistance screening trial for both SDM and RDM using 47 Indian maize inbred lines under artificial inoculation conditions for two seasons (1999 and 2000) to select the resistant and susceptible parents for developing the mapping population (Nair et al. 2004b). NAI116 is an Indian maize line that is highly resistant to both SDM and RDM. CM139, an elite parental line (female parent of a popular single-cross hybrid, Parkash) is highly susceptible to SDM at Mandya and moderately resistant to RDM at Udaipur (Nair et al. 2004b). Analysis of simple sequence repeat (SSR) polymorphisms in a set of resistant and susceptible lines revealed high genetic divergence between CM139 and NAI116. The mapping population developed was a backcross population using CM139 as the recurrent parent [(CM139×(CM139×NAI116)]. The BC_1F_1 progeny from a single ear were planted during the winter of 2000–2001 at the Maize Winter Nursery, Hyderabad. DNA samples from 177 individual BC_1F_1 plants were collected, and BC_1F_2 families were obtained by selfing each individual plant from which a DNA sample was collected. DNA samples from these 177 plants were used for genotyping the mapping population, and the BC_1F_2 families were used for phenotyping.

Field trials

Field trials were conducted from August to September 2001 in the 'hot spots' for SDM and RDM in India, Mandya in southern India (12°N; 76°E; 695 m a.s.l.; 705 mm/year average rainfall) and Udaipur in western India (23°46′N; 73°09′E; 577 m a.s.l.; 633.3 mm/year average rainfall), respectively.

Experimental design

The 177 BC_1F_2 families, along with the parental lines, were evaluated using a randomized block design with

two replications, with two rows per replication, in the disease screening nurseries at Mandya (against SDM) and Udaipur (against RDM). The test entries were planted in 4-m-long rows, with 15–20 plants per row and 0.75 m between rows.

Inoculation methods and disease assessment

Field tests were conducted using the 'sandwich method and spreader row technique' for SDM (Craig et al. 1977), and artificial infection was carried out using the 'whorl inoculation technique' (Rathore and Siradhana 1987) for RDM.

In the sandwich method, seeds of maize line CM500 were inoculated with SDM before planting: the seeds were first spread out between layers of DM-infected maize leaves with visible conidial growth and then incubated for 2–3 days at room temperature (100% relative humidity under light) to facilitate entry and establishment of the fungus into the germinating seeds. The infected seedlings were planted as spreader rows on all sides of the experimental block 30 days prior to the planting of the 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% DM incidence) in the check rows across the experimental block indicated uniform and strong pathogen pressure, leaving no possibility for 'disease escapes'.

For the whorl inoculation technique, the whorl of each seedlings was artificially infected with RDM: a 1-ml conidial suspension of RDM was placed in the whorl of each seedling of the test entries (5–7 days after germination) between 4:00 a.m. and 6:00 a.m. The suspension was prepared by collecting conidia from DM-infected plants (susceptible maize cultivars Kiran and Surya infected by *P. heteropogoni* conidia from *Heteropogon contortus* in Udaipur) at 2:00–3:00 a.m. and suspending them in water to a concentration of approximately 40,000–50,000 conidia/ml. The inoculation procedure was repeated for three consecutive days to ensure that no plant escaped artificial infection.

The disease reaction was assessed at 35 days after plant emergence (SDM)/inoculation (RDM) by scoring for systemic DM infection in the individual plants. Percentage disease incidence in each test entry was determined. Inoculated plants that did not show systemic symptoms of DM (emergence of characteristic chlorotic leaves) one month after artificial infection were considered to be resistant.

Molecular marker assays and linkage mapping

Molecular marker assay

DNA was extracted from 177 BC_1F_1 individuals using the CTAB procedure developed by Saghai-Maroof et al. (1984) with suitable modifications by Hoisington et al. (1994). Approximately 30 ng of DNA was used as the template for PCR in a 15-μl reaction volume. PCR was carried out in a MJ Thermal Cycler, USA (Model PTC-100; MJ Research, Waltham, Mass.) with the following cycling profile: an initial denaturation at 94°C for 2 min, followed by 30–35 cycles of amplification at 94°C for 1 min, 55–65°C (based on the annealing temperatures standardized for different SSR primers) for 2 min and 72°C for 2 min, with a final extension step at 72°C for 7 min followed by termination of the cycle at 4°C. The amplification products were separated on 3.5% Super Fine Resolution (SFR) agarose gel. Electrophoresis was at 100 V for 2–3 h. A total of 470 SSR loci uniformly spaced in the genome (MaizeDB; http://www.agron.missouri.edu) were assessed for SSR polymorphism in the parental lines of the mapping populations. Of the 470 loci, 89 were found to be polymorphic based on high-resolution agarose gel electrophoresis. These polymorphic SSR markers were used for genotyping the 177 BC_1F_1 individuals.

Linkage mapping

The standard χ^2-test was used to test the segregation pattern at each marker locus for the deviations from expected Mendelian segregation ratio of 1:1 (for a backcross mapping population). The overall mean frequencies for the alleles from the recurrent (CM139) and non-recurrent parent (NAI116) were calculated, and the deviations, if any, from the expected allele frequencies (0.75:0.25) were tested. Linkage analysis was carried out using MAPMAKER ver. 2.0 (Lander et al. 1987). A LOD score ($\log_{10}$ of the likelihood odds ratio) of 3.0 and a maximum recombination frequency of 0.40 were used to declare linkage between two markers. Only in those marker intervals spanning more than 40 cM was an LOD of 2.0 employed. After we had determined linkage groups and the correct linear arrangement of marker loci along the chromosomes, we estimated the recombination frequencies between marker loci by multi-point analysis.

Data analysis

Phenotypic data

Of 177 BC_1F_2 families, phenotypic data could be recorded for 172 families for SDM and 152 families for RDM. For each family, data were recorded on at least 15–20 plants per replication, and any entry with data from fewer than ten plants was not used for subsequent analysis. Least square means for the dataset from each location (Mandya for SDM and Udaipur for RDM) were calculated, and standard analysis of variance (ANOVA) was performed.

Analyses of phenotypic data were conducted on arc-sin-transformed phenotypic data for SDM and RDM, separately, considering all effects in the statistical model as random. Orthogonal contrasts among the means of

the BC_1F_2 families versus those of the original parental lines (CM139 and NAI116) as well as among the means of the BC_1F_2 families versus those of the parents of the mapping population [CM139 and (CM139×NAI116)] were computed. Estimates of the variance components for σ^2_e (error variance), σ^2_g (genotypic variance) and H^2 (heritability) of the respective trait (SDM resistance or RDM resistance) in the BC_1F_2 families were calculated, following the procedure described by Searle (1971), using SAS ver. 8.0 (SAS Institute, Cary, N.C.). Heritability values and confidence limits were calculated on a progeny mean basis as a function of the F ratio, as per the procedure suggested by Knapp et al. (1985).

QTL mapping

The QTL analysis was carried out on the set of 151 backcross individuals with phenotypic data for both SDM and RDM. The genotypic data consisted of 80 SSR marker loci, and the phenotypic data comprised SDM and RDM percentage incidence data from Mandya and Udaipur, respectively. The method of composite interval mapping (CIM; Zeng 1994) as implemented in QTL CARTOGRAPHER ver. 2.0 (Wang et al. 2004) was used to map QTLs and estimate their genetic effects. For CIM, we applied Model 6 of the ZMAPQTL procedure stipulating a BC population for which the genome was scanned every 2 cM. Five markers that explained most of the variation for the traits were specified as cofactors following single marker analysis. Empirical threshold levels for declaring the significance of a QTL at a Type-I error rate of 0.05 were obtained by performing 1,000 permutations of the data according to the method suggested by Churchill and Doerge (1994). The best estimate of QTL location was assumed to correspond to the position having the peak significance level. Additive effects of the detected QTLs were also estimated by the ZMAPQTL procedure. The R^2 values obtained through this analysis indicate the percentage phenotypic variance explained by each QTL.

Following the procedure suggested by Melchinger et al. (1998) and Groh et al. (1998), QTL positions identified in the present study were compared with those for SDM resistance (Agrama et al. 1999; George et al. 2003) and for RDM resistance (George et al. 2003). QTLs within a marker interval size of less than 20 cM (falling in the same bin) are considered to be common across the experiments.

Results

Phenotypic data analysis

The parental lines exhibited contrasting phenotypes for disease incidence and differed significantly in their reaction to both SDM and RDM. ANOVA revealed significant differences among the BC_1F_2 families in the SDM and RDM datasets, respectively (Table 1). The mean percentage SDM/RDM incidence and the ranges observed in the parental lines and various generations under study are given in Table 1. CM139 showed 100% SDM disease incidence and moderate resistance to RDM. NAI116, in contrast, was found to be highly resistant to both SDM and RDM (disease incidence of 3.3% and 0%, respectively). The F_1 progeny recorded 71.4% SDM incidence and 4.7% RDM incidence, while the BC_1F_1 showed 100% SDM incidence (highly susceptible) and 2.8% RDM incidence (resistant). The disease incidence in the BC_1F_2 families ranged from 42.9% to 100% (ranging from moderate to severe susceptibility) for SDM, and from 0% to 52.4% (ranging from resistance to susceptibility) for RDM. Broad-sense heritability (H^2) values for SDM and RDM incidence were estimated to be 74% (lower 95% confidence limit: 65%; upper 95% confidence limit: 80%) and 67% (lower 95% confidence limit: 55%; upper 95% confidence limit: 76%), respectively (Table 2). Despite the differences in the relative complexity of inheritance, both SDM and RDM were found to have comparable genotypic variances and heritability.

While the responses of the parents were consistent and as expected (based on previous evaluations in 1999 and 2000), the BC_1F_2 families were not normally distributed with respect to their responses to either SDM or RDM. The distribution for SDM was skewed towards susceptibility, and for RDM, towards resistance. This pattern was consistent with our expectations based on the genetic analyses of resistance to SDM and RDM carried out by Nair et al. (2004a). This absence of a normal distribution in the phenotypic values of the mapping populations is not uncommon, particularly for diseases such as DMs. Agrama et al. (1999) and George et al. (2003) also reported the absence of a normal distribution in phenotypic data on RILs, with the distribution also skewed towards the susceptible parent. This may have been particularly accentuated in the present study since the mapping population was a backcross with the susceptible recurrent parent. Although most quantitative genetic analyses assume that the trait of interest is normally distributed, it is important to note that some important traits (for example, disease scores or counts of flowers) do not generally show normal distributions, and this is particularly true for backcross progenies. However, QTL analysis does allow non-normal phenotypic data to be utilized (for example, Lubberstedt et al. 1998a, b).

SSR data analysis and linkage mapping

For linkage mapping, we used a genotypic dataset based on our analysis of 177 BC_1F_1 individuals using 89 polymorphic SSR markers. Two lines in the mapping population were found to have non-parental alleles for the majority of the SSR loci analyzed and hence were discarded from further analysis. Missing data in the

Table 1 SDM and RDM incidence in the parental lines and in the F_1 and backcross population under artificial inoculation conditions at Mandya and Udaipur (2001), respectively

Generation	Number of replications	SDM		RDM	
		Number of individuals	Susceptible (%)	Number of individuals	Susceptible (%)
P_1 (CM139)	6	89	100.0	154	23.4
P_2 (NAI116)	6	117	3.3	160	0.0
F_1 (CM139×NAI116)	2	24	71.4	28	4.7
BC_1F_1 [CM139×(CM139×NAI116)]	2	39	100.0	30	2.8

Table 2 Estimates of the variance components (σ^2_g, σ^2_e) and heritabilities (H^2) for percentage SDM and RDM disease incidence

Variance components/heritability	SDM	RDM
σ^2_g	107.85 ± 8.67*	84.12 ± 8.98*
σ^2_e	75.03	80.57*
H^2	0.74	0.67

*Variance components are significant at $P = 0.05$

genotyping of the mapping population across the 89 SSR loci were negligible (4.08%). For each of the loci analyzed, χ^2-tests were conducted to analyze the goodness-of-fit of segregation for the observed homozygous and heterozygous genotypes with the expected segregation ratio of 1:1 for a backcross population. Ten markers showed a deviation from this 1:1 ratio; three of these, *dupssr34*, *umc1573* and *umc1560*, showed such a large segregation distortion that they were not used for linkage mapping and the subsequent QTL analysis. Of the ten loci showing segregation distortion, seven displayed a lower than expected frequency of homozygotes, while three showed a lower than expected frequency of heterozygotes. Overall, the contribution of SSR alleles from the non-recurrent parent (NAI116) in the genotypes of individuals of the mapping population was 25.2%, with a range from 20.8% to 31.7%, while the contribution of SSR alleles from the recurrent parent (CM139) was 74.8%, ranging from 68.2% to 79.1%.

Of the original 89 polymorphic SSR markers, three markers were discarded due to a large segregation distortion (mentioned above) and an additional six were dropped during mapping due to high 'segment break distances' coupled with high map distances (exceeding

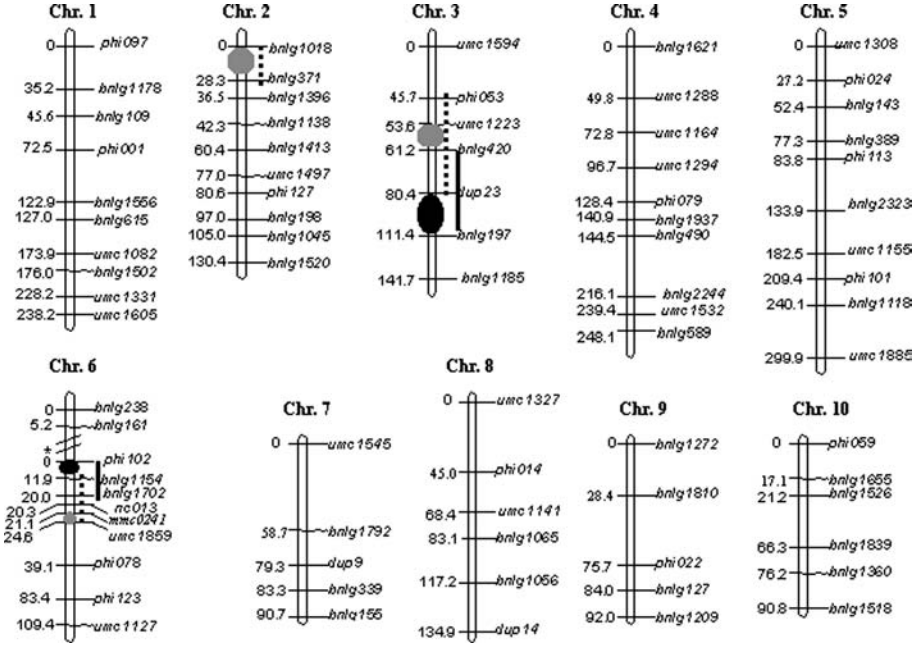

Fig. 1 Linkage map based on genotyping of the maize BC_1F_1 mapping population [(CM139×(CM139×NAI116)] using 80 polymorphic SSR markers. The *numbers* to the *left* of the chromosomes indicate the distance in centiMorgans relative to the first marker. The SSR marker names are given to the *right* of each chromosome. *Indicates the break in chromosome 6 due to a lack of polymorphic loci in the intervening region. The *solid bars* to the *right* of the chromosomes 3 and 6 indicate the marker intervals with LOD values equal to or more than the threshold values for RDM (2.63), the *dashed bars* to the *right* of chromosomes 2, 3 and 6 indicate marker intervals with LOD values more than the threshold value for SDM (2.51). The LR peaks are indicated as the *gray circles* for SDM and the *dark circles* for RDM

Table 3 Parameters associated with QTLs significantly affecting the percentage of SDM and RDM incidence in the BC_1F_1:F_2 plants analyzed using CIM

Chromosome bin location	Position	SSR marker interval	LOD at QTL position	Additive effect	R^{2a}
Sorghum downy mildew (*Peronosclerospora sorghi*)					
2.04–2.05	0.01	bnlg1018[b]-*bnlg371*	2.37	−4.98*	5.4
3.04–3.05	55.6	umc1223-*bnlg420*	4.22	−7.13*	14.9
6.05	21.1	mmc0241-*umc1859*	3.82	−6.41*	12.8
Rajasthan downy mildew (*P. heteropogoni*)					
3.06–3.07	80.4	dupssr23-*bnlg197*	2.6	−4.81*	7.2
6.05	0.01	phi102-*bnlg1154*	2.7	−4.38*	6.1

*Significant at $P = 0.01$
[a] Percentage phenotypic variance explained by the QTL
[b] Underlined markers indicate those nearest to the detected QTL

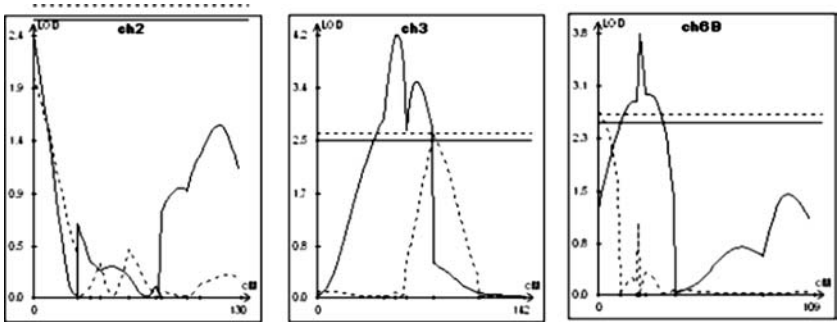

Fig. 2 The QTL likelihood maps indicating LOD scores for SDM and RDM incidence on chromosomes 2, 3, and 6. *Horizontal solid line* Threshold LOD score for SDM, *horizontal dashed line* threshold LOD score for RDM. *Solid line* LOD plots for SDM, *dashed line* LOD plots for RDM

60 cM) with adjacent markers in a linkage group, which significantly expanded the genetic map distances. Due to the non-availability of polymorphic SSR loci in bin locations 6.01–6.04, chromosome 6 was split into two sub-groups, 6A and 6B. The final map generated using 80 SSR markers had 11 linkage groups, spanning a length of 1,681.5 cM at an average marker interval of 24.4 cM (Fig. 1).

All ten maize chromosomes are represented in this linkage map. Although we used only 80 SSR markers for mapping, careful selection of the polymorphic markers resulted in a coverage of 68 bin locations among the approximately 100 bin locations spanning all ten chromosomes. Some of the bin locations in maize, particularly at the terminal regions of the chromosomes, have very few SSR markers, and we were unable to detect polymorphism at such bin locations in the present study, particularly on chromosomes 2 and 3. Eleven marker intervals, each larger than 40 cM, were identified in some linkage groups. However, the mapping data supported utilization of these intervals, as was verified by MAPMAKER.

The linkage map obtained in the present study was compared with (1) the widely referred to, intermated B73×Mo17 (IBM) molecular marker linkage map developed at the University of Missouri, Columbia, Mo., USA (MaizeDB; http://www.agron.missouri.edu/SSR/images.html) and (2) a SSR linkage map published by Wang et al. (2001) based on 83 markers. The SSR map generated in this study is largely congruent with both the maps, particularly in terms of relative order of the loci.

Interestingly, the seven SSR loci at bin 6.05 that we included for genotyping, based on the report of the presence of a major QTL controlling DM resistance in maize (George et al. 2003), were found to be polymorphic between the susceptible parental line CM139 and the resistant line NAI116. It is important to note that these seven SSR loci at bin 6.05 (*phi102*, *bnlg1154*, *bnlg1702*, *nc013*, *umc1859*, *mmc0241* and *phi078*) were also polymorphic between the resistant parent Ki3 and the susceptible parent CML139, which were the parental lines of the RILs used for the QTL mapping of resistance to diverse DMs in tropical Asia (George et al. 2003).

QTL analyses

Three QTLs for SDM resistance and two QTLs for RDM resistance were detected in the backcross mapping population [CM139×(CM139×NAI116)] by CIM using QTL CARTOGRAPHER ver. 2.0 (Table 3). The three QTLs for SDM resistance were located on chromosomes 2 (bin 2.04/05), 3 (bin 3.04/05) and 6 (bin 6.05), while the two QTLs for RDM were located on chromosomes 3 (bin 3.06/07) and 6 (6.05) (Fig. 2). The QTL on chromosome 2 appeared to confer resistance specific to *P. sorghi*. All of the resistance alleles were contributed by the resistant parent, NAI116 (Table 3). Additive effects were significant for all of the QTL detected. The QTL for SDM resistance detected on chromosome 3 had the largest effect, with the NAI116 allele at this locus decreasing the percentage of disease incidence by 14.9%, whereas the QTL on chromosome 3 that con-

tributed to RDM resistance reduced the percentage of disease incidence by only7.2% (Table 3). The QTL on chromosome 6 contributed 12.8% to phenotypic variance in SDM, while the one on chromosome 2 contributed 5.4%.

Discussion

Parallelism in the QTLs conferring resistance to SDM and RDM

The present study has been successful in identifying four QTLs (Table 2), all contributed by NAI116, of which three (one each on chromosomes 2, 3, and 6) were significant with respect to SDM resistance, while two QTLs (one each on chromosomes 3 and 6) were significant for RDM resistance. Noteworthy is that the QTLs detected on chromosome 6 (bin 6.05) were consistently significant for both SDM and RDM resistance. The analysis also clearly revealed that the number of QTLs contributing exclusively for resistance to RDM may be negligible relative to the number exclusively influencing SDM resistance. The QTLs contributing to SDM and RDM resistance detected on chromosome 3 were in adjacent bins. In earlier experiments, we were able to clearly demonstrate that the genotypes resistant to SDM were invariably resistant to RDM but that the reverse was not true (Nair et al. 2004b; Yen et al. 2004). While Nair et al. (2004b) studied a large set of purely Indian maize inbred lines against both SDM and RDM, Yen et al. (2004) evaluated a set of genotypes from different Asian countries (including Thailand, Philippines and India) in addition to those developed by the CIMMYT-Asian Regional Maize Program, Thailand and CIMMYT, Mexico. Both studies confirmed the parallelism in the responses of the genotypes to SDM and RDM. The possible reasons for SDM-resistant genotypes also being RDM-resistant, but not vice versa, could be due to a combination of three factors: (1) involvement of some major loci controlling resistance to both SDM and RDM; (2) the number of genetic factors controlling resistance to RDM being relatively fewer than those controlling resistance to SDM; (3) *P. heteropogoni* having a lower level of virulence than *P. sorghi*. The first two possibilities have been verified by the results presented here: QTL mapping showed that a number of common QTLs could confer resistance to both SDM and RDM, whereas resistance to SDM would possibly require additional QTLs.

Comparison of QTL data from different mapping populations/environments

Apart from identifying major QTLs contributed by the resistant source (NAI116), the results we obtained in present study support the significance of the QTLs controlling resistance to diverse DMs in tropical Asia, including SDM and RDM, that were identified in an earlier analysis of RILs (George et al. 2003) and the QTL for SDM resistance identified by Agrama et al. (1999).

George et al. (2003) evaluated RILs against diverse DMs in tropical Asia (*P. sorghi* and *P. heteropogoni* in India; *P. zeae* in Thailand; *P. philippinensis* in Philippines; *P. maydis* in Indonesia) and identified six genomic regions on chromosomes 1, 2, 6, 7, and 10 involved in resistance to the different DMs studied. Most significantly, these investigators identified a major resistance QTL on chromosome 6 (at bin 6.05) derived from the resistant parent (Ki3) that contributed significantly to all the DMs, including SDM and RDM. This QTL alone accounted about 20% and 30% of the phenotypic variation for SDM and RDM incidence, respectively.

We also detected a significant QTL on chromosome 6 at bin 6.05 that contributed to both SDM and RDM resistance. The six SSR markers at bin 6.05 (*phi102*, *mmc0241*, *bnlg1154*, *bnlg1702*, *nc013* and *phi078*) analyzed in this study were polymorphic between the resistant line NAI116 and the susceptible line CM139. Polymorphism for the same SSR markers was also observed between Ki3 (resistant) and CML139 (susceptible), the parental lines of the RILs analyzed in an earlier QTL mapping study (George et al. 2003). Significantly, the LR peak on chromosome 6 was located nearest to SSR marker *mmc0241* in both the present study and in that of George et al. (2003), thereby verifying the significance of the major QTL on chromosome 6 (bin 6.05) in conferring resistance to both SDM and RDM in maize. The percentage phenotypic variance explained by this QTL was lower in the present study than in that of George et al. (2003) in which RILs with a different resistant parent were investigated, possibly because RILs account for additive variance more efficiently than backcross families (Luo and Kearsey 1991). Validation of this major QTL on chromosome 6 (bin 6.05) has considerable significance in the implementation of marker-assisted selection (MAS) for the transfer of resistance to both SDM and RDM in elite, but DM-susceptible, maize germplasm in tropical Asian countries such as India. In this context, the deliberate choice of a backcross mapping population, despite some constraints, was appropriate for this study, since the QTL information can be immediately applied for improving specific genotype(s) important to the National Agricultural Research System (NARS) through marker-assisted backcrossing for line conversion.

A second, significant QTL on chromosome 2 (between bins 2.04 and 2.05) for SDM resistance detected in this study also appears to be same as that reported by George et al. (2003). Significantly, in both these studies, the QTL was found to contribute only to SDM resistance but not to RDM resistance. Using the resistant source, NAI116, we also identified QTLs on chromosome 3 that conferred resistance to both SDM and RDM; these QTLs were not detected in the earlier study employing the resistant parent, Ki3.

Agrama et al. (1999) mapped three QTLs, two on chromosome 1 and one on chromosome 9, conferring resistance to SDM in Egypt using a mapping population derived from 94 RILs (generated from a cross of the SDM-resistant line, G62, and the susceptible line, G58). QTL mapping was undertaken using single marker analysis. Two of the three QTLs revealed high QTL × environment interaction. The investigators concluded that SDM resistance is controlled by one major QTL and two minor genes. The results from both the present study and that of George et al. (2003) are not in agreement with those of Agrama et al. (1999). This is not surprising given that the parental lines involved, the type and size of mapping populations and the methodology employed for QTL detection were different, in addition to the possible differences in SDM pathotypes in India and Egypt.

Despite some encouraging results with respect to the consistency of QTLs influencing specific traits over different genetic backgrounds and locations/environments, considerable variation with respect to QTL locations and effects are often reported. The lack of a common element between the QTLs identified in different populations could be due to a combination of various factors, including: (1) the type and size of the mapping population used; (2) segregation of different sets of QTLs in different crosses; (3) detection of a QTL in a segregating population only if both parental lines contributed different alleles of the QTL; (4) epistatic interaction between QTLs in each of the mapping populations (Beavis and Keim 1996; Kearsey and Pooni 1996; Bohn et al. 1997). Beavis et al. (1991) recorded that a comparison of data for QTL localization in different segregating population for characters such as disease resistance reveals only a few QTLs that are common across populations. This is particularly relevant because of the fact that different climatic and growing conditions at individual environments might affect the expression of QTL involved in developmental, morphological and chemical characters affecting resistance against specific pathogens. Despite these possibilities, the detection of major QTLs for resistance against specific pathogen(s) contributed by diverse resistant lines could provide an opportunity for pyramiding multiple genes that might control different components of resistance.

An integrated breeding approach for SDM and RDM

For ecological and economical reasons, it is essential to emphasize the improvement of host-controlled resistance rather than to depend heavily or solely on chemical control. The identification of highly consistent and stable sources of resistance to DMs in Asia offers an important opportunity to launch a focused program on improving DM resistance in the elite maize germplasm and to develop resistant cultivars. In this context, the observation that inbred lines resistant to SDM are invariably resistant to RDM (Nair et al. 2004b; Yen et al. 2004) offers an opportunity for undertaking an integrated breeding strategy for improving resistance to both SDM and RDM in the elite maize germplasm. Since phenotypic selection for SDM and RDM resistance is cumbersome and could be hampered by the occurrence of 'disease escapes' (due to non-conducive conditions or low/non-uniform disease pressure) and because there is only a limited capacity for field experiments under artificial infection, integration of MAS at specific stages in breeding for DM resistance could be highly effective. A combination of phenotypic selection and MAS at appropriate stages would not only be cost-effective and time-effective but could also aid in recovery of a large proportion of the recurrent parent genome along with favorable alleles from the donor parent with significantly reduced linkage drag (Ribaut and Betran 1999).

The prospects for MAS in breeding programs depend heavily on the number of QTLs detected, the magnitude of the QTL effects and the possibility of utilizing the marker-QTL associations in different genetic backgrounds. The cumulative knowledge derived from the present study and from the recent QTL mapping experiments undertaken through the AMBIONET (George et al. 2003) would facilitate setting up a MAS program that may lead to an improvement of SDM as well as RDM resistance in the Indian maize germplasm. The major QTL detected on chromosome 6 (bin 6.05) is an excellent candidate for incorporating resistance to both SDM and RDM in maize using MAS.

QTL mapping also offers an opportunity for marker-assisted 'gene stacking'. In this case, MAS would be significantly superior to conventional phenotypic selection alone. With rapid advances in molecular marker mapping as well as increasing emphasis on marker validation, the coming few years might unveil many such useful markers for tagging and pyramiding traits of interest in maize. The integration of QTL mapping information into applied plant breeding programs will become more of a reality when QTL information is generated using elite germplasm relevant to the NARS.

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