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Mapping of QTL for downy mildew resistance in maize

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Abstract Quantitative trait loci (QTLs) of maize involved in mediating resistance to *Peronosclerospora sorghi*, the causative agent of sorghum downy mildew (SDM), were detected in a population of recombinant inbred lines (RILs) derived from the *Zea mays* L. cross between resistant (G62) and susceptible (G58) inbred lines. Field tests of 94 RILs were conducted over two growing seasons using artificial inoculation. Heritability of the disease reaction was high (around 70%). The mapping population of the RILs was also scored for restriction fragment length polymorphic (RFLP) markers. One hundred and six polymorphic RFLP markers were assigned to ten chromosomes covering 1648 cM. Three QTLs were detected that significantly affected resistance to SDM combined across seasons. Two of these mapped quite close together on chromosome 1, while the third one was on chromosome 9. The percentage of phenotypic variance explained by each QTL ranged from 12.4% to 23.8%. Collectively, the three QTLs identified in this study explained 53.6% of the phenotypic variation in susceptibility to the infection. The three resistant QTLs appeared to have additive effects. Increased susceptibility was contributed by the alleles of the susceptible parent. The detection of more than one QTL supports the hypothesis that several qualitative and quantitative genes control resistance to *P. sorghi*.

Key words Downy mildew · Marker-assisted selection · Quantitative trait loci · *Zea mays*

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Introduction

Maize (*Zea mays* L.) breeding programs generally focus on yield improvement. However, several diseases are responsible for major economic losses. Sorghum downy mildew [*Peronosclerospora sorghi* (Weston and Uppal) C.G. Shaw] is one of the most serious diseases in maize-producing areas throughout the world. *P. sorghi* (SDM) is a factor that limits maize production in several countries of Asia (Rifin 1983). Frederiksen and Renfro (1977) compiled a list of several Latin American countries where the disease has been reported. The high frequency of susceptibility among the inbred lines that are widely used as parents for hybrid seed production indicated the possibility of a serious vulnerability to SDM in midwestern corn hybrids in USA, which are derived from these parental lines (Craig et al. 1977). The disease has very probably also reached other areas of the continent (Borges 1987). In Egypt, *P. sorghi* has, in the last few years, been considered a major disease of maize in areas where sorghum is grown as a forage crop (Nazim et al. 1995).

The most efficient, effective, environmentally safe and economical means to control SDM of corn is the use of resistant varieties. The mode of inheritance of resistance to SDM in maize depends on the specific interaction between the host and the pathogen species involved and on conditions prevailing during evaluation of the disease reaction (Borges 1987). Studies on the host-pathogen interaction of maize and *P. sorghi* have shown that resistance is polygenically controlled. Additive gene effects control resistance only with a suggested polygenic system for resistance (Jinahyon 1973) or by many genes that have both additive and nonadditive effects

(Singburaudom and Renfro 1982). However, Sifuents and Frederiksen 1988, revealed that one or two dominant genes controlled resistance in sorghum segregates. Other sources of genetic resistance are known but have not been defined (Gowda et al. 1995).

Reports on linkage between quantitative trait effects and marker genotypes have been available in the literature for quite a long time (Sax 1923; Thoday 1961). The application of this strategy was in general not possible however, until the later development of isoenzyme, restriction fragment length polymorphism (RFLP), and the random applified polymorphic DNA (RAPD) markers that could be used to develop saturated genetic maps for many organisms. DNA-based molecular markers have been made for disease resistance genes in miaze using RFLP analysis (Bentolila et al. 1991; Zaitlin et al. 1993; Dingerdissen et al. 1996; Saghai-Maroof et al. 1996; Ming et al. 1997) and RAPD assay (Bubeck et al. 1993; Agrama and Moussa 1996a). Gowda et al. (1995) have applied RFLP and RAPD marker techniques to identify genes for resistance to downy mildew in sorghum. The use of quantitative trait loci (QTLs) mapping to identify quantiative disease resistance genes in plants is discussed in detail by Young (1996).

In QTL studies the use of recombinant inbred lines (RILs) has many advantages over F_2 or backcross populations (Burr and Burr 1991; Knapp and Bridges 1990). In maize, RILs have been used to identify QTLs for thermotolerance (Frova and Gorla 1993), pollen competitive ability (Ottaviano et al. 1991) and grain yield and its components (Austin and Lee 1996).

Progress has been made in mapping agriculturally important genes with molecular makers, which forms the foundation for marker-aided selection (MAS). The use of MAS can expedite such difficult screening procedures such as the testing for disease or insect resistance. However, when several resistance genes are initially present in a donor parent, some of them may be lost during th breeding programs. The chance of losing resistance genes can be reduced if they are detected early. This is particularly useful when the breeding process is time-consuming, e.g. when exotic germplasm is used as the resistant parent. Our approach to the location of QTLs for resistance to *P. sorghi* in maize is based on RFLP mapping a population derived from 94 RILs.

Materials and methods

The genetic association of polymorphic DNA fragments with sorghum downy mildew (SDM) caused by *Peronosclerospora sorghi* was assessed using RILs. A survey of downy mildew disease in the maize (*Zea mays* L.) inbreds and in hybrids from Maize Research Program of the Agricultural Research Center of Egypt was carried out in 1990. Two inbred maize lines were selected for this initial study; an inbred parent, G62 (P_1), which had been identified as being highly resistant, and an inbred, G58 (P_2), that was highly susceptible to the *P. sorghi* pathogen (Nazim et al. 1995). The F_2 population (G62×G58) consisted of 112 individuals and segregated for SDM genes. The reaction of each F_2 plant to SDM was tested in the greenhouse by spraying each plant at the seedling stage with a conidial suspension containing 4×104 conidia/ml (Craig 1987). Susceptible plants were rescued by spraying downy mildew-infected plants with 2 μg a.i. (active ingredient) metalaxyl/ml (Gowda et al. 1995).

Seeds from self-pollinated plants in the F_2 population were inbred for six generations by ear-to row propagation. To minimize selection, we generally chose the first ear in a row for propagation by selfing it for the next generation (Burr and Burr 1991). By 1995, 94 inbreds had reached the sixth generation (F_6) of self-pollination.

The 94 RI lines, together with the two parental lines and four lines related to the two parents, were evaluated. The experimental design was a 10×10 lattice of single-row plots in two replications. The lines were evaluated in the field in both the 1996 and 1997 seasons at the Alexandria University Research Station near Alexandria, Egypt. The planting density in each experiment was 56.000 plants per hectare. Maize was sown at a within-row spacing of 0.25 m in 6-m-long rows spaced 0.75 m apart and thinned to one plant per hill. The seedlings of each RIL were inoculated with conidia of *P. sorghi* (Craig 1987). Resistance was assessed 28 days after inoculation by scoring for systemic SDM on inoculated lines as shown by Craig et al. (1977). The data were recorded as the percentage of plants showing SDM infection. The percentages covered a wide range and were transformed using arcsine transformation as suggested for binomial data (Steel and Torrie 1960). This transformation was intended to make the means and variances independent and normally distributed. The heritability (h^2) based on the entry mean, as proposed by Fehr (1993), can be written as follow:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2/rt + \sigma_{ge}^2/t + \sigma_g^2}$$

Here, σ^2_g denotes the genetic variance; σ^2_{ge} is genotype×environment interaction; σ^2_e is experimental error; r and t are the number of replications and number of test environments, respectively.

From each of the 94 F_6 lines, 20–25 seeds were sown in the greenhouse and the subsequent seedlings bulked (Xiao et al. 1996) for DNA extraction. Genomic DNA of each line was digested with the restriction endonculeases *Eco*RI, *Hind*III, and *Bam*HI. Digested DNA was fractionated on 0.7% agarose gels. After electrophoresis, the gels were denatured, neutralized and Southern-blotted onto uncharged nylon membranes (MSI Magnagraph). DNA probes were labeled by polymerase chain reaction (PCR) amplification with 2.5% or 5.0% digoxigenin-dUTP (Boehringer-Mannheim). The probes were detected using the antidigoxigenin-alkaline phosphatase-AMPPD chemiluminescent protocol previously described in detail by Agrama and Moussa (1996b). Around 185 RFLP probes from Brookhaven National Laboratory (bnl) and the University of Missouri at Columbia (umc) were used to screen the two parental lines and an F_6 progeny sample consisting of six individuals. Probes that detected polymorphism between the parents with at least one enzyme were used to contruct the linkage map of the population of RILs. Segregation ratios at each marker locus were tested by the chi-square goodness-of-fit test for the expected Mendelian segregation ratio 1:1 (Austin and Lee 1996).

The program MAPMAKER/EXP 3.0 (Lander et al. 1987; Lander and Botstein 1989) was used to establish the RFLP map using the "ri self" setting. Distances between markers are presented in centiMorgans (cM) derived using the Kosambi function units (Kosambi 1944). Mapping of QTLs was performed using the single-factor analysis as described by Tuinstra et al. (1997). The analysis of QTLs was performed on the adjusted F_6 line means for the arcsine-transformed data within each trial as well as across trial. The PROC GLM procedure in SAS (SAS Institute, Raleigh, N.C.) was used to detect significant associations between segregating markers and the resistance as a quantitative trait. Associations between markers and the 2-year mean were declared significant, on per marker basis, at a significance threshold of $P<0.01$. An estimate of percentage of phenotypic variation explained by a marker associated with resistance was determined by regression analysis using multiple regression (PROC GLM and PROC REG, SAS Institutes,

Table 1 Means, ranges, genetic variance (σ^2_g), genotype×environment (σ^2_{ge}), environmental variance (σ^2_e), and heritabilities of systemic infection by *Peronosclerospora sorghi* (arcsine-transformed values) for 94 recombinant inbreds (F_6) of G62×G58

Experiment	G62 (P_1) Mean±SE	G58 (P_2) Mean±SE	RI lines						
			Mean±SE	Range	Variance components			Heritability	
					σ^2_g	σ^2_{ge}	σ^2_e	h^2	C.I.[a]
1996	5.47±1.14	62.12±3.67	47.01±6.31	4.09–77.48	42.5±6.2**		31.7	72.9	68.3–85.5
1997	6.02±1.03	58.24±4.08	49.43±7.48	5.20–74.11	46.3±7.0**		36.2	71.8	66.8–75.4
Combined	5.75±0.92	60.07±3.47	44.89±6.06	4.38–76.59	49.7±5.1**	26.4±2.3*	29.8	70.6	67.7–76.6

* Significant at the 0.05 level, ** significant at the 0.01 level
[a] Lower and upper boundary of 95% confidence interval of heritability estimate

Raleigh, N.C.). The coefficient of determination (R^2) from the multiple regression estimates the total proportion of phenotypic variation due to the additive effects. Herein, heritability (h^2) represents an estimate of the proportion of phenotyipic variation attributable to genetic sources. The R^2 value was divided by the h^2 to calculate the proportion of genetic variation due to additive effects of loci linked to all QTL for the SDM resistance (Austin and Lee 1996).

Results

Downy mildew (SDM) infection means of the inbred parents (G62 and G58) and their recombinant inbred progeny are presented in Table 1. Standard errors of the means were generally small. The F_6 families were not normally distributed for susceptibility, and the distribution was skewed towards the susceptible parent (G58). An arcsine transformation normalized the data. Parental line means, expressed in transformed values, ranged from 5.47 for 0.91% infection (G62), to 62.12 for 78.3% infection (G58). The mean of the inbred parent G62 (resistant) was significantly smaller than that of G58 (susceptible) in each individual season and also across seasons. The means of the RILs cover the range defined by the means of the two parents, with a combined mean of 44.89 for 49.7% infection. Individual RILs varied widely, with some showing only high susceptibility to SDM. Data collected during the two seasons were pooled to obtain a single value for each inbred, and these values were used for combined QTL analysis. Genotypic components of variance (σ^2_g) were significant for infection in both seasons and across seasons in the population (Table 1). This is indicative of the presence of genotypic variability in the population. Heritability (h^2) estimates based on entry means (Allard 1960; Fehr 1993) were generally high, indicating the presence of relatively high genetic variance for resistance to SDM (Table 1). However, a partitioned genotype-by-environment component reduced the h^2 value (70.6%) for the combined data.

A total of 114 RFLP markers were used to construct a genetic map of this population. The map covered about 1648 cM with 106 markers distributed over all chromosomal regions and classified into ten linkage groups. The average distance between adjacent marker loci was about

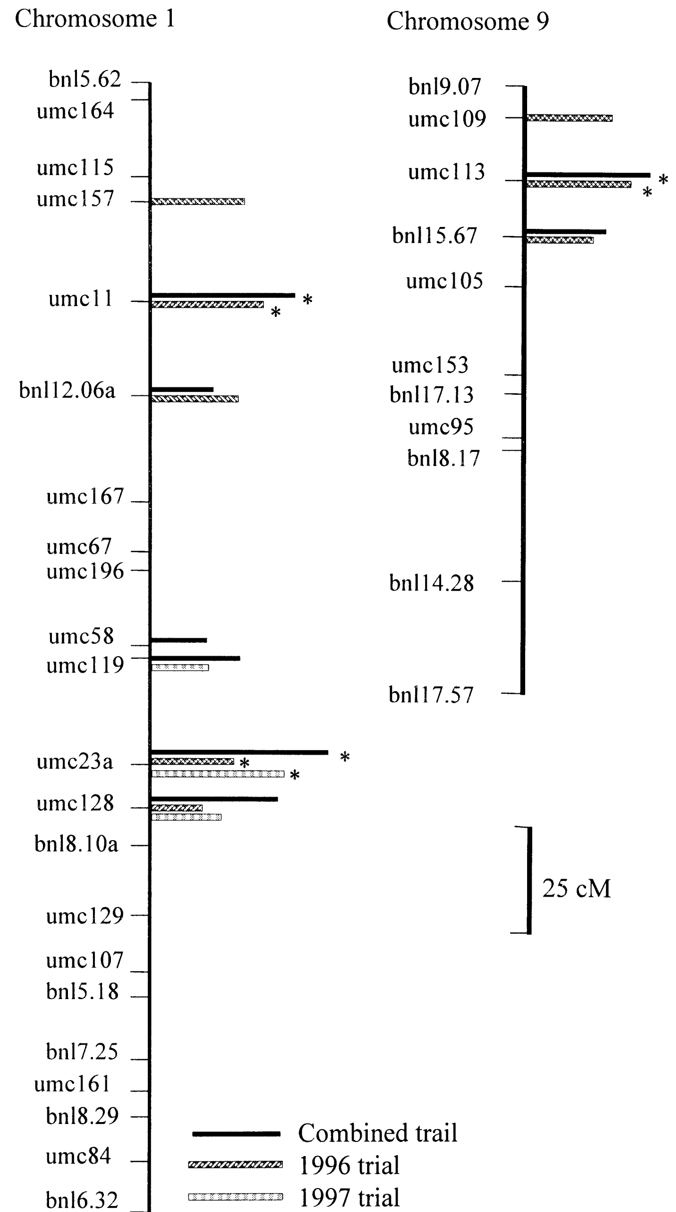

Fig. 1 RFLP analysis for sorghum downy mildew (*P. sorghi*) resistance in 94 recombinant inbreds from G62 (resistant)×G58 (susceptible). *Horizontal bars* indicate significant degree (R^2) of correlation between RFLP loci and resistance. * Significant values (P<0.05) for single QTLs

Table 2 Chromosomal locations and mode of gene action of QTL for resistance of maize to *P. sorghi*, based on data from 94 RI of the cross G62×G58 in two seasons. The resistant parent, G62, contributed all QTLs

Trail	Chromosome	QTL position (cM)	Nearest RFLP locus	Additive effect[a]	Phenotypic variance, R^2 [b]
1996	1	48.1	*umc11*	–11.2	18.0
	1	155.7	*umc23a*	– 6.4	13.2
	9	21.4	*umc113*	– 7.5	16.9
					35.3 (48.4%)
1997	1	151.8	*umc23a*	– 8.9	21.1 (27.9%)
Combined	1	48.6	*umc11*	–14.1	23.4
	1	155.7	*umc23a*	–19.8	28.5
	9	21.4	*umc113*	– 8.6	20.7
					53.6 (75.9%)

[a] A negative sign indicates that the G62 alleles decrease the value forsusceptibility
[b] Percentage of phenotypic variation (R^2) is explained by markers linked to QTL. Genetic variation estimates are shown in parentheses. Estimates were obtained by regression analysis using markers significantly associated with resistance

16 cM. Eight of the markers remained genetically unlinked. All markers were located to the linkage groups using the MAPMAKER "Group" command. The map is largely in agreement with previously published RFLP maps established for temperate maize (e.g., Maize DB, Polacco 1996; Agrama and Moussa 1996b; Agrama et al. 1997).

Only two chromosomes were associated with variation in SDM. The map for QTLs effects on SDM resistance in the RILs both during the two seasons and combined across seasons, computed using the transformed disease data, is shown in Fig. 1. More than 1 marker in a specific region was significantly associated with a phenotypic measurement (indicated in horizontal bars). Only the marker most strongly associated with each QTL was used (indicated with *). The incidence of infection was significantly affected by loci associated with two genomic regions on chromosome 1 and with one region on chromosome 9. The R^2 of the QTLs linked to *umc23a* on chromosome 1L was significant during both seasons, indicating that detection of the genetic factor contributing to SDM resistance is repeatable. There was also evidence of a genotype×environment interaction, as the chromosomes 1S and 9S have QTL effects that were significant in the 1996 season but not significant in the 1997 eason (Fig. 1). The results suggest that the expression of these two QTLs may be environment dependent. A significant R^2 was found in all three regions for data combined across the two seasons. Thus, three QTLs control the quantitative resistance to SDM in this mapping population.

Table 2 presents the genetic map locations and mode of action of SDM resistance with variation associated with each QTL. The highest SDM phenotypic variation in the combined disease data was observed for the QTL on chromosome 1L. This R^2 value for combined data was 28.5% for the QTL linked with *umc23a*. Other significant phenotypic variations for combined data were 20.4% at *umc113* on chromosome 9 and 23.4% at *umc11* on chromosome 1 (Fig. 1 and Table 2). Cumulatively, the three QTLs explained 53.6% of the phenotypic variance and 75.9% of the genotypic variation in the combined data analysis (Table 2). At the three QTLs, the resistance genes were contributed by the more resistant parent G62, and they acted in an additive fashion.

Discussion

The development of a reliable method for evaluating maize plants for the expression of SDM was crucial to the success of this study. Consistent heavy disease pressure is required to assess accurately the potential of plant genotypes to resist the onset and progress of SDM and to determine the magnitude of the effect of genetic factors that contribute to resistance. The two parents (G62 and G58) used to develop the RILs exhibited the most extreme phenotypes to SDM reaction. The inbred parent G62 had been identified earlier as being high resistant and the inbred G58 was highly susceptible to *P sorghi* pathogen (Nazim et al. 1995). RILs have several intrinsic advantages for use in mapping QTLs, that have been described by several authors (Burr and Burr 1991; Knapp and Bridges 1990; Austin and Lee 1996). Also, the heavy disease pressure maintained in field plots, combined with replicated disease evaluation in two growing seasons, mean that our assay was sufficiently sensitive to detect all QTLs effects on SDM resistance in the RI population.

Multiple regression analysis indicated that small number of QTLs controlled resistance to SDM disease. QTLs showing large and consistent effects on *P. sorghi* resistance in combined analysis were detected on chromosomes 1 (two QTLs) and 9. The finding that SDM resistance is determined by a relatively small number of QTLs agrees with the study of Gowda et al. (1995), who reported that the inheritance to SDM resistance in sorghum is governed by two Mendelian factors.

The additivity effect of these QTLs is consistent with results from previous field studies based on classical quantitative genetic analysis (Rifin 1983). However, other studies have demonstrated that such an assumption might not be valid in all cases. The results of Borges

(1987) and Singburaudom and Renfro (1982) showed that both additive and nonadditive gene effects are significant in determining resistance to SDM in maize, although additive effects are more important.

The strategy followed here allowed us to identify tight linkage of the RFLP markers *umc11, umc23a,* and *umc113* to genes conferring resistance to *P. sorghi* in maize. Two of the three QTLs were not always constant across seasons. However, only one QTL was stable in both seasons. These results suggest that one major gene and two minor genes control SDM resistance. These markers should be very useful in breeding programs in facilitating the introgression of the resistance genes into commercial varieties. DNA markers in genomic regions of interest enable breeders to select on the basis of genotype rather than phenotype, which can be especially helpful if a target trait is time-consuming to score. Marker-based breeding will revolutionize the process of cultivar development (Young 1996). Another interesting application of these results would be the use of these linked markers as a starting point for molecular approaches, such as chromosome walking, to clone the resistance genes (Bentolila et al. 1991). Marker-assisted selection for these loci should be productive for enhancing the expression of SDM resistance genes in maize across environments.

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