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ORIGINAL PAPER

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Identification of quantitative trait loci controlling resistance to maize chlorotic dwarf virus

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Abstract Ineffective screening methods and low levels of disease resistance have hampered genetic analysis of maize (*Zea mays* L.) resistance to disease caused by maize chlorotic dwarf virus (MCDV). Progeny from a cross between the highly resistant maize inbred line Oh1VI and the susceptible inbred line Va35 were evaluated for MCDV symptoms after multiple virus inoculations, using the viral vector *Graminella nigrifrons*. Symptom severity scores from three rating dates were used to calculate area under the disease progress curve (AUDPC) scores for vein banding, leaf twist and tear, and whorl chlorosis. AUDPC scores for the F_2 population indicated that MCDV resistance was quantitatively inherited. Genotypic and phenotypic analyses of 314 F_2 individuals were compared using composite interval mapping (CIM) and analysis of variance. CIM identified two major quantitative trait loci (QTL) on chromosomes 3 and 10 and two minor QTL on chromosomes 4 and 6. Resistance was additive, with alleles from Oh1VI at the loci on chromosomes 3 and 10 contributing equally to resistance.

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Introduction

Maize chlorotic dwarf virus (MCDV) incites a disease infecting maize (*Zea mays* L.) in the southeastern and south central United States (Knoke and Louie 1981). The range of MCDV is determined by the ranges of its overwintering host, johnsongrass [*Sorghum halepense* (L.) Pers.], and its principal insect vector, the blackfaced leafhopper [*Graminella nigrifrons* (Forbes)]. The virus is transmitted semipersistently by the vector and can be transmitted mechanically by vascular puncture inoculation (Louie 1995), but cannot be transmitted by leaf-rub inoculation or through seed.

Previous studies of MCDV resistance, using natural transmission under field conditions, gave conflicting results, suggesting that dominant (Dollinger et al. 1970), additive (Rosenkranz and Scott 1986, 1987), or additive and dominant (Naidu and Josephson 1976) gene action was important for controlling resistance. The variability in the results of these studies can be attributed to a number of factors including disease escape, fluctuation in disease incidence, environment by genotype interactions, and the synergistic effects of coinfection with other viral diseases, which occur in field studies using natural infection. Evaluations of MCDV resistance have also been conducted by placing potted seedlings in screen cages containing viruliferous leafhoppers or by mixing viruliferous leafhoppers with corn grits and placing them in the whorls of field grown plants. These methods may eliminate mixed infections, but disease escape is not prevented (Louie et al. 1990).

Tertiary vein banding, leaf twist and tear, and whorl chlorosis are symptoms of MCDV infection (Gordon and Nault 1977). Louie et al. (1974) identified vein banding as the diagnostic symptom of MCDV infection, which facilitated identification of resistant germplasm. In addition, screening methods were improved by using pure virus isolates, improved design of screen cages, and growing plants in controlled environments. A highly effective screening procedure was developed that uses

multiple viruliferous leafhopper infestations to inoculate young seedlings under controlled environmental conditions (Louie and Anderson 1993). This robust multiple-inoculation method for MCDV transmission, coupled with use of a virus isolate that produced severe symptoms on susceptible maize (Hunt et al. 1988) and evaluation of disease severity rather than incidence, has allowed for identification of new sources of MCDV resistance and reduced the variation in genetic studies (Pratt et al. 1994; Louie et al. 2002).

Inbred lines were evaluated using the multiple inoculation protocol to identify lines with high levels of resistance for use as potential parents for a mapping population (R.J. Anderson and R. Louie, unpublished results). Results of this preliminary study indicated that the inbred line Oh1VI (Louie et al. 2002) was highly resistant to MCDV. This line was crossed to the susceptible inbred line Va35, and a mapping population of F_2 progeny was developed. Based on the responses of F_1 and F_2 plants to MCDV inoculation, quantitative trait loci (QTL) mapping analysis was used to identify regions of the maize genome that control resistance to MCDV in Oh1VI.

Materials and methods

Plant and virus material

The maize (*Z. mays* L.) inbred line Oh1VI was developed from a Virgin Island population (PI 504148, Louie et al. 2002). The MCDV-resistant Oh1VI and the MCDV-susceptible inbred line Va35 were maintained at the Ohio Agriculture Research and Development Center. Va35 was crossed with Oh1VI, and F_1 plants were self-pollinated to create F_2 progeny. Three hundred sixteen F_2 plants were evaluated for MCDV resistance and genotyped as outlined below.

An MCDV isolate that produces severe symptoms on susceptible maize, MCDV-severe, was originally isolated from infected corn in southern Ohio. This isolate was previously referred to as MCDV-white stripe or MCDV-WS (Hunt et al. 1988). The isolate was maintained on the susceptible maize inbred line Oh28 by serial transmission from characteristically symptomatic plants using *G. nigrifrons*.

Disease evaluation

MCDV transmission was carried out using the multiple-inoculation protocol described by Louie and Anderson (1993). Seeds were germinated on moist filter paper for 30 h at 30°C and planted individually in 16.4×2.5 cm Cone-tainers (Stuewe and Sons, Corvallis, Ore., USA) containing sterilized greenhouse soil. The seedlings were randomized in racks (30.5×30.5 cm) and placed in Dacron organdy-covered cages (38×38×38 cm) inside a growth chamber with a 14/10-h light/dark cycle at a light intensity of 250 μmol m^{-2} s^{-1} and a 24/18°C temperature cycle. Viruliferous leafhoppers were obtained by exposing young adult *G. nigrifrons* to 1 to 3-week-old MCDV-infected maize Oh28 plants for a 48-h acquisition access period. Beginning 3 days after planting, the test plants were exposed to 3 inoculation access periods (IAP) of 48 h each, with 1,000 viruliferous leafhoppers. After the third IAP, the seedlings were fumigated and moved to a greenhouse for symptom development. Disease severity for individual plants was scored on a scale of 1 to 5 (1 = no symptoms, 5 = severe symptoms) for vein banding (chlorosis of the small leaf veins), twisting and tearing at the leaf margin, and leaf whorl chlorosis symptoms 6, 12, and 19 days after the first exposure to inoculative leafhoppers as described by Pratt et al. (1994). After the last rating, the plants were transplanted into 10-cm pots and placed in a greenhouse to allow growth of sufficient tissue for DNA extraction.

Experimental design

The disease screening of 316 F_2 plants was conducted in six cages, divided between two planting dates. A randomized block design integrating four or five plants each of the susceptible inbred line Oh28, the resistant parent (Oh1VI), the susceptible parent (Va35), and the F_1 cross with the F_2 plants in each cage was used. Planting date was used as a block effect and the cages were used as replications.

Genotypic analysis

Approximately 24 days after transplanting, seedling leaf tissue was frozen in liquid nitrogen, lyophilized, then ground in a Wiley mill. DNA was extracted using the CTAB procedure (Saghai-Maroof et al. 1984), then digested with the restriction enzymes *Eco*R1, *Hin*dIII, *Bam*H1, *Eco*RV, and *Dra*1, according to the supplier's recommendations (New England Biolabs, Beverly, Mass., USA). Digested DNA was separated by electrophoresis on 0.8% agarose gels in Tris-acetate-EDTA buffer (Sambrook et al. 1989) and transferred to Genescreen Plus membranes (Dupont NEN, Boston, Mass., USA), using a modified "dry blot" procedure (Kempter et al. 1991). RFLP probes from the UMC core set (Davis et al. 1999) were obtained from the University of Missouri–Columbia. Hybridization and autoradiography were carried out as described by McMullen and Louie (1989). Simple sequence repeat (SSR) markers were used to provide additional genotypic information in regions of interest or low marker density. Primer sequences were obtained from the maize genetics and genomics database (http://www.maizegdb.org/). PCR methods were as described by Davis et al. (1999), and products were resolved on super-fine resolution agarose gels (Ameresco, Solon, Ohio, USA) at concentrations from 4% to 5.5%. F_2 plants (314 individuals), the

parental inbred lines, and F_1 plants of Va35 × Oh1VI were genotyped with 108 RFLP markers and 46 SSR markers.

Data analysis

Area under the disease progress curve (AUDPC) scores, which combine disease severity with the timing of disease development, were calculated for each symptom on individual plants and used in all genetic analyses. Variation between cages and planting dates was evaluated by analysis of variance (ANOVA), using SAS PROC GLM (SAS Institute, Cary, N.C., USA).

A linkage map was constructed from the RFLP and SSR genotypes using MAPMAKER/EXP, version 3.0 (Lander et al. 1987). Marker order was determined by sequential use of the *group*, *try*, and *compare* commands of MAPMAKER. For the linkage map, $\log_{10}$ of the likelihood ratio (LOD) scores over 3.0 were considered significant, and the maximum recombination distance allowed was 50 cM. Recombination frequencies were converted to map distances (centiMorgan) with the Kosambi map function of JoinMap, version 3.0 (van Ooijen and Voorrips 2001).

Composite interval mapping (CIM), using Windows QTL Cartographer, version 2.0 (Wang et al. 2003), was used to further analyze the association between markers and traits. Model 6 was used to scan the genome at 2-cM intervals, using a window size of 10 cM. Five markers were selected as cofactors, using the forward–backward regression method of stepwise regression. One thousand permutations (Doerge and Rebai 1996) were used to determine LOD significance levels ($P=0.01$). Single factor ANOVA was used to confirm associations between RFLP markers and traits, using PROC GLM. A significant *F*-test ($P<0.001$) indicated cosegregation of the marker locus genotypic class, with the AUDPC score of the phenotype.

Regression (R^2) values taken at the peak LOD score of a QTL were used to indicate the percentage of the phenotypic variation explained by the QTL. The type of gene action (additive/dominant) was estimated using QTL Cartographer. The effect of an allele substitution was calculated using the formula $\alpha = a + d(q-p)$ as described by Falconer (1981). Broad-sense heritability estimates were made using the formula $h^2 = (\{\sigma^2F_2 - [(\sigma^2P_1 + \sigma^2P_2 + \sigma^2F_1)/3]\}/\sigma^2F_2)$ (Mahmud and Kramer 1951). PROC GLM was used to conduct two-way ANOVA to evaluate interactions between the QTL.

Results

MCDV resistance evaluation

Subsequent to multiple leafhopper inoculation with MCDV, using the protocol developed by Louie and Anderson (1993), susceptible plants showed the three symptom types characteristic of MCDV infection: vein banding comprising chlorotic streaks along small veins, twisting and tearing along the leaf margin, and general chlorosis of the leaf whorl (Pratt et al. 1994). Within 12 days postinoculation (dpi) 100% of the virus-susceptible Oh28 and Va35 seedlings showed symptoms (data not shown). In contrast, only 15% of the Oh1VI seedlings showed any symptoms of virus infection, and these appeared late (19 dpi) in the evaluation period. In the susceptible lines, vein banding was apparent and became severe sooner than twist and tear or chlorosis, and there was less variability among individuals of a given genotype for the vein-banding symptom ratings (Fig. 1). The F_1 plants developed symptoms more slowly than did the susceptible inbred lines, but showed severe symptoms by 19 dpi. These results were consistent with a previous study showing that symptom severity was an important component of the MCDV resistance response (Pratt et al. 1994). AUDPC scores were used to evaluate MCDV infection of the F_2 population, because they would reflect both reduced disease severity and delayed symptom development.

Because disease screening was done in six different cages with two different planting dates, mean cage AUDPC scores were compared to ensure that environmental differences did not influence disease ratings (Table 1). Mean disease scores differed significantly between cages for specific entries and disease symptoms (e.g., vein banding in Va35 and chlorosis in Oh28). However, there was no consistent skewing of scores between the cages or planting dates. In particular, there was no statistically significant difference among the six cages in the mean AUDPC scores for vein-banding symptoms in the F_1 and F_2 progeny of the Va35 × Oh1VI cross. Scores of the F_1 generation are important, as the generation is uniform in genotype and intermediate in disease resistance. Vein banding is the primary diagnostic trait for MCDV infection in maize, and leaf twist and tear and whorl chlorosis symptoms may be inconsistent or delayed (Pratt et al. 1994). The relative consistency of vein-banding symptoms among plants indicated that data for individual plants in different cages could be pooled for QTL analysis.

In the rating system used, a symptomless plant would have an AUDPC score of 13, and a plant showing very severe symptoms (a rating of 5) on the earliest rating date would have an AUDPC score of 65. Both susceptible inbred lines showed severe vein banding, leaf twist and tear, and leaf whorl chlorosis symptoms, with similar and relatively high mean AUDPC scores of more than 57 for vein banding, 35 for leaf twist and tear, and 43 for whorl chlorosis (Table 1). In contrast, symptoms in the resistant Oh1VI were limited to very mild, barely visible vein banding and chlorosis, with mean AUDPC scores near 13.

The mean AUDPC scores of the F_1 progeny were close to the calculated midparent values for leaf twist and tear (24.5) and whorl chlorosis (29.2) symptoms, but the mean score for vein banding (44.3) was higher than

the calculated midparent value (35.2). AUDPC scores of the F_2 plants for vein banding, leaf twist and tear, and whorl chlorosis showed a continuous distribution (Fig. 2). For each symptom type, scores for F_2 individuals were between those of the parents, suggesting that there was no transgressive segregation. Broad-sense heritabilities, calculated from symptom variance for parents and progeny, were 0.87, 0.35, and 0.60 for vein banding, leaf twist and tear and whorl chlorosis, respectively.

Linkage map

DNA was obtained from 314 of the 316 Va35 × Oh1VI F_2 progeny for which phenotypic data were taken. Initially, 201 RFLP probes were hybridized to blots of the parental inbred lines and F_1 plant DNA digested with five restriction endonucleases (data not shown). The 154 probes that showed clear polymorphisms between parental alleles were used to determine the genotypes of the F_2 individuals. For the remaining 47 probes, either insufficient data were obtained or segregation deviated significantly from the predicted 1:2:1 as determined by χ^2 analysis, and these markers were not used for constructing the linkage map. However, data for four linked markers (*umc167*, *umc133*, *umc67*, and *bnl5.59*) on chromosome 1 were retained, even though segregation ratios were skewed. Skewed segregation of markers in this region of chromosome 1 has been noted previously (Lu et al. 2002). SSR markers were used to increase genotypic information in regions of interest or low coverage. Ultimately, 108 RFLP and 46 SSR markers were used to construct a genetic linkage map with MAPMAKER. A 1,602-cM map with ten linkage groups and an average interval of 10.4 cM between markers was constructed (Fig. 3). Although map distances varied, no significant differences in marker order were noted between this map and the maize IBM2 neighbors map (http://www.maizegdb.org).

QTL mapping

CIM, using QTL Cartographer (Wang et al. 2003), indicated that LOD scores of 1.42, 1.44, and 1.47 were significant for resistance to MCDV-induced vein banding, leaf twist and tear, and whorl chlorosis, respectively. Two major and two minor QTL for MCDV resistance were identified in Oh1VI (Fig. 3; Table 2). The presence and location of QTL were nearly coincident for resistance to expression of all three symptoms. The major QTL were located on chromosomes 3 (*umc102*) and 10 (*umc44*), and we will designate these loci *mcd1* and *mcd2*, respectively. Minor QTL were located on chromosomes 4 (*umc52*) and 6 (*umc85*). For *mcd1*, resistance to vein banding and leaf twist and tear was localized to a 32.4-cM interval (*umc92* to *bnl8.01*), and resistance to whorl chlorosis was significant in a 35.5-cM interval between *umc1223* and *bnl15.2*. For *mcd2*, resistance to all three symptoms was found in the 31-cM interval between *npi232* and *npi290*. ANOVA showed significant association between marker genotypes and AUDPC scores in the chromosomal regions containing the major QTL identified by interval mapping, although the regions identified by ANOVA were slightly larger than those identified by CIM (Fig. 3). The two minor QTL identi-

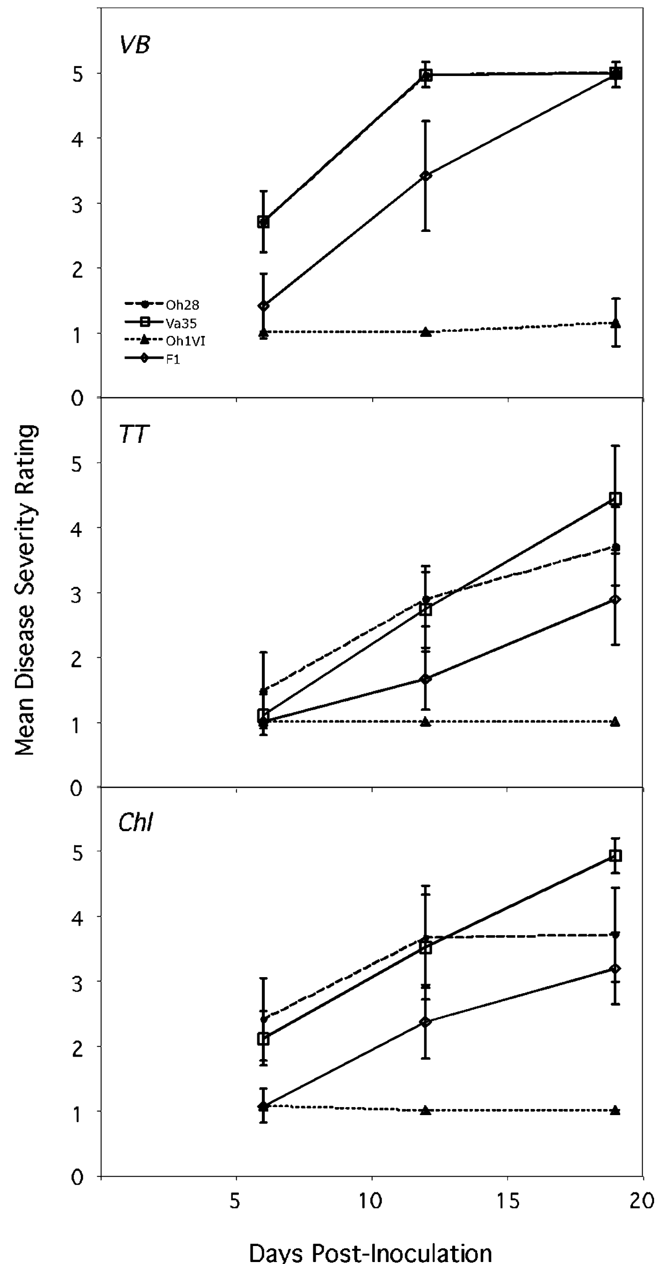

Fig. 1 Symptom development in maize after inoculation with maize chlorotic dwarf virus (MCDV). Seedlings were inoculated with MCDV, and disease severity for vein banding (*VB*), twist and tear (*TT*), and chlorosis (*Chl*) was rated 6, 12, and 19 days postinoculation as outlined in Materials and methods. The mean symptom severity rating (±SD, $n=27$) for Oh28 (*circles*), Va35 (*squares*), Oh1VI (*triangles*), and F_1 (*diamonds*) seedlings are shown. Note that the curves for Oh28 and Va35 overlap completely in the VB panel

Table 1 Maize chlorotic dwarf virus (MCDV) symptoms in resistant (Oh1VI) and susceptible (Va35 and Oh28) inbreds and their progeny

Symptom	Cage 1[a]		Cage 2		Cage 3		Cage 4		Cage 5		Cage 6		Block 1[b]		Block 2[c]		All data[d]	
/Entry	AUDPC	n[e]	AUDPC	n	AUDPC	n	AUDPC	n	AUDPC	n	AUDPC	n	AUDPC	n	AUDPC	n	Mean	SD
Vein banding																		
Oh28	57.5a	4	58.2a	4	58.2a	4	57.8a	5	58.4a	5	54.7b	5	58.0a	12	56.9a	15	57.4	2.2
Va35	59.0a	4	57.5b	4	59.0a	4	59.0a	5	55.6c	5	54.5d	5	58.5a	12	56.3b	15	57.3	2.0
Oh1VI	13.0b	4	13.0b	4	13.0b	4	13.9a	5	13.3ab	5	13.0b	5	13.0b	12	13.4a	15	13.2	0.5
Va35 × Oh1VI F_1	41.5	4	40.6	4	48.2	4	48.2	5	48.1	5	44.3	5	42.0	12	46.1	15	44.3	6.2
Va35 × Oh1VI F_2	36.1	26	34.8	25	35.9	25	34.7	80	34.3	80	32.1	80	35.6	75	33.7	240	34.2	10.7
Twist and tear																		
Oh28	31.5c	4	41.8a	4	33.0bc	4	34.2bc	5	36.3b	5	34.9bc	5	35.4a	12	35.1a	15	35.3	4.4
Va35	36.6abc	4	30.8c	4	33.2bc	4	35.7abc	5	41.2a	5	37.6ab	5	33.5a	12	38.1a	15	36.1	5.2
Oh1VI	13.0	4	13.0	4	13.0	4	13.0	5	13.0	5	13.0	5	13.0a	12	13.0a	15	13.0	0.0
Va35 × Oh1VI F_1	22.2ab	4	19.8b	4	25.0ab	4	23.1ab	5	26.4a	5	23.7ab	5	22.3ab	12	24.4ab	15	23.5	4.1
Va35 × Oh1VI F_2	19.3ab	26	19.2ab	25	18.9b	25	21.1a	80	21.1a	80	18.6b	80	19.1ab	75	20.2ab	240	20.0	4.7
Chlorosis																		
Oh28	36.7cd	4	51.5a	4	46.6ab	4	36.4d	5	45.4ab	5	43.1bc	5	44.9bc	12	41.6bc	15	43.1	6.8
Va35	46.1	4	42.2	4	45.3	4	43.4	5	46.5	5	47.2	5	44.5	12	45.7	15	45.2	4.3
Oh1VI	14.5a	4	13.0b	4	13.0b	4	13.0b	5	13.0b	5	13.0b	5	13.5b	12	13.0b	15	13.2	0.8
Va35 × Oh1VI F_1	34.6a	4	27.3bc	4	25.6c	4	30.9bc	5	31.1ab	5	27.4bc	5	29.2b	12	29.8b	15	29.5	4.5
Va35 × Oh1VI F_2	23.8	26	24.5	25	24.9	25	23.9	80	24.4	80	23.1	80	24.5a	75	23.8a	240	24.0	5.7

[a] Mean AUDPC (Area under the disease progress curve) scores were calculated for each entry in the six cages used for disease ratings. Plants in cages 1–3 were inoculated on the same dates, and those in cages 4–6 were inoculated on a second set of dates. Means within a row followed by the same letter or no letter are not significantly different ($P = 0.05$)

[b] Block 1 consisted of cages 1–3

[c] Block 2 consisted of cages 4–6

[d] The mean and standard deviation (SD) for all plants[e] The number of plants in the cage

fied by CIM were not significant in ANOVA. These were on chromosome 4 between *umc127* and *umc52*, and on chromosome 6 between *jc1270* and *umc85* (Fig. 3; Table 2).

Gene effects

The QTL on chromosome 3 explained 23–25% of the variation in symptom expression, while the locus on chromosome 10 explained 13–20% of the variation as shown by the R^2 values (Table 2). Each of the minor QTL on chromosomes 4 and 6 explained 3–5% of the resistance. The gene action was primarily additive for the two major loci, and all of the alleles conferring resistance came from Oh1VI (Table 2). Addition of alleles from Oh1VI at *umc102* or *umc44* produced a linear decrease in the mean AUDPC score for all three symptom types (data not shown). These data suggest that the contributions from the major loci are similar, and that there is no interaction between alleles from the two loci. The lack of significant interaction between the major loci was confirmed in a two-way ANOVA. A significant interaction for resistance to vein banding ($P < 0.01$) and twist and tear ($P < 0.06$) between the major QTL on chromosome 10 and the minor QTL on chromosome 6 was identified by two-way ANOVA (data not shown).

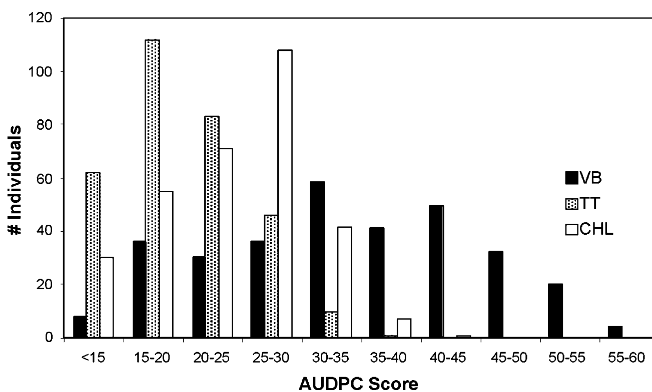

Fig. 2 Symptoms of MCDV infection in F_2 individuals derived from Va35 × Oh1VI. The number of F_2 individuals within the specified range of area under the disease progress curve scores (*AUDPC scores*) is shown for the VB (*dark bars*), TT (*gray bars*), and CHL (*white bars*) symptoms

Discussion

Previous attempts to identify and genetically characterize MCDV resistance in maize were hampered by several factors: variable rates of virus transmission under field and greenhouse conditions, the presence of symptomatic plants infected with other viruses in field experiments, and the presence of plants co-infected with MDMV and MCDV in field experiments. (Scott and Rosenkranz 1981; Guthrie et al. 1982; Rosenkranz and Scott 1986, 1987; Louie et al. 1990). A preliminary study using the multiple-inoculation protocol to transmit an MCDV isolate that produced severe symptoms on susceptible maize identified Oh1VI as resistant and Va35 as susceptible inbred lines (Pratt et al. 1994; R. Louie and R.J. Anderson, unpublished). A further refinement over

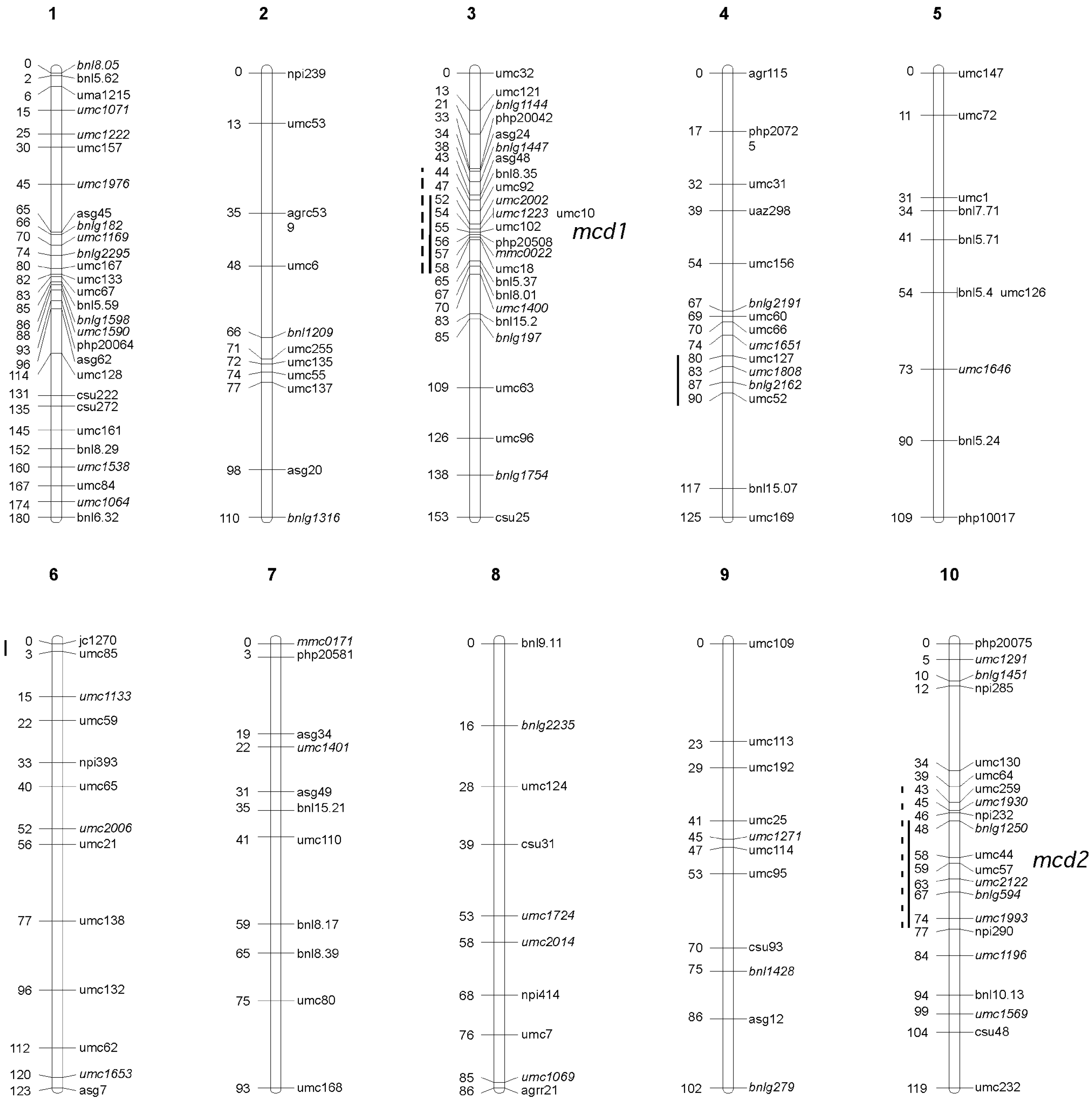

Fig. 3 Linkage map for Va35 × Oh1VI F_2 progeny. The genotypes of 314 F_2 individuals were determined using 108 RFLP (*plain text*) and 46 SSR (*italics*) markers, and a linkage map was made using MAPMAKER/EXP, version 3.0 (Lander et al. 1987). The positions of the markers are at the *right* of the ten linkage groups, and distances between markers are indicated in centiMorgans to the *left* of the linkage groups. Markers with significant $\log_{10}$ of the likelihood ratio (>1.42) scores for resistance to vein banding determined by composite interval mapping are indicated with *solid lines* to the *left* of the chromosome, and those significantly ($P<0.001$) associated with resistance to vein banding by ANOVA are indicated with *dotted lines*. The positions for the two major QTL associated with MCDV resistance, *mcd1* and *mcd2*, are noted to the *right* of chromosomes 3 and 10, respectively

previous studies was to use AUDPC scores for resistance evaluation. Severity of disease symptoms and timing of symptom appearance are evaluated simultaneously, allowing plants with intermediate resistance responses to be identified.

In the current study, the high levels of MCDV infection that occurred at early screening dates in the susceptible control (Oh28) and the susceptible parent Va35 resulted in mean AUDPC scores that were nearly 90% of the theoretical maximum for the vein-banding symptom. In contrast, mean AUDPC scores for all symptoms were very close to the theoretical minimum in the resistant parent Oh1VI. Thus, the multiple-inoculation screening protocol was highly effective, and parents

Table 2 Location of quantitative trait loci for resistance to MCDV in maize

Chromosome	Trait[a]	Marker[b]	cM[c]	LOD[d]	ANOVA[e]		R^{2f}	Gene effects[g]	
					F	*P*		Additive	Dominant
3	VB	*umc102*	47–64	23.74	24.61	< 0.001	0.248	−7.74	0.02
	TT	*umc102*	47–64	14.73	19.19	< 0.001	0.226	−2.81	0.80
	CHL	*umc102*	55–82	20.49	20.82	< 0.001	0.253	−3.63	0.18
10	VB	*umc44*	46–77	21.41	19.94	< 0.001	0.236	−6.37	1.52
	TT	*umc44*	46–77	11.72	10.49	< 0.001	0.131	−2.10	0.08
	CHL	*umc44*	46–77	12.53	11.16	< 0.001	0.176	−2.67	0.18
4	VB	*umc52*	81–117	3.49	2.05	0.106	0.031	−2.06	2.69
	TT	*umc127*	81–117	3.47	3.13	0.025	0.030	−1.10	−0.19
	CHL	*umc52*	81–117	3.05	2.19	0.089	0.048	−1.27	0.79
6	VB	*umc85*	0–4	3.60	1.80	0.148	0.039	−2.28	1.76
	TT	*umc85*	0–4	4.27	3.52	0.015	0.053	−1.18	0.47
	CHL	*umc85*	0–4	2.87	2.18	0.090	0.044	−1.23	1.14

[a]Traits associated with susceptibility to MCDV: *VB* Vein banding, *TT* leaf twist and tear, *CHL* whorl chlorosis
[b]*LOD* Log_{10} of the likelihood ratio. The marker associated with the peak of the LOD curve (not shown)
[c]The interval associated with significant LOD scores 1.42, 1.44, and 1.47 for VB, TT and CHL, respectively, calculated using the *Zmapqtl* function in QTL Cartographer (1,000 permutations)
[d]The LOD score at the peak marker
[e]*ANOVA* Analysis of variance. Association of the peak marker with the resistant phenotype determined by ANOVA
[f]Portion of the genetic variance for resistance associated with marker calculated using QTL Cartographer
[g]Additive and dominance effects were calculated as described in Materials and methods. A negative sign (−) indicates the contribution from Oh1VI

with divergent responses to MCDV infection were identified. AUDPC scores were lower for leaf twist and tear and whorl chlorosis in both susceptible inbred lines (about 55% of the maximum). These results are consistent with previous studies that showed that vein banding is the most reliable and rapidly appearing symptom of MCDV infection, while the appearance and severity of leaf twist and tear and whorl chlorosis are dependent on plant age, growing conditions, and host genotype (Louie et al. 1974; Gordon and Nault 1977; Pratt et al. 1994). As a result of the reliable screening protocol and identification of highly resistant and susceptible inbred lines, we were able to accurately detect and score disease symptoms in F_2 individuals to identify the loci controlling the resistance response.

Symptom development in the Va35 × Oh1VI F_1 was similar to or slightly greater than the calculated midparent AUDPC values, indicating that the traits were not controlled by dominant genes. In addition, the distribution of AUDPC scores in F_2 progeny was approximately normal, and no transgressive segregation was seen. Further, the continuous distribution of phenotypes indicated a quantitatively inherited trait. CIM (Wang et al. 2003) was used to identify two major QTL for MCDV resistance in Oh1VI: *mcd1* near *umc102* on chromosome 3 and *mcd2* near *umc44* on chromosome 10. In addition, minor QTL for resistance were identified near *umc52* on chromosome 4 and near *umc85* on chromosome 6. The presence and location of the major, but not the minor, QTL were confirmed by ANOVA. A linear additive response of MCDV resistance to the number of Oh1VI alleles at the *mcd1* and *mcd2* loci was seen with no interaction between the loci. These data are consistent with previous results that indicated the presence of multiple loci for MCDV resistance and quantitative inheritance (Scott and Rosenkranz 1981; Rosenkranz and Scott 1986, 1987; Louie et al. 1990).

The loci identified in this study account for 55.4, 44, and 52.1% of the genetic variability for vein banding, leaf twist and tear and whorl chlorosis, respectively. QTL identified for resistance to maize streak virus and maize mosaic virus were responsible for similar portions of the genetic variance (Ming et al. 1997; Pernet et al. 1999). It is possible that the relatively low portion of resistance explained by *mcd1* and *mcd2* is due to factors in the susceptible line that prevent expression of resistance. This is corroborated by the appearance of symptoms in Va35 × Oh1VI BC_4F_1 plants with Oh1VI alleles at *mcd1* (*umc102*) or *mcd2* (*umc44*) (M.W. Jones et al., unpublished results). Homozygous near-isogenic lines carrying *mcd1* and *mcd2* alone and in combination are being developed to test these QTL for their effectiveness in controlling resistance. Alternatively, minor QTL not detected in this study could explain a portion of the resistance not accounted for by *mcd1* and *mcd2*. Another possibility is that heterotic effects, or complex interactions between genotype and environment that are common in experiments involving virus diseases and insect vectors, account for the observed results (Louie et al. 1990).

Clustering of disease resistance genes in maize and other plants is notable (McMullen and Simcox 1995; Collins et al. 1998; Redinbaugh et al. 2004). *Mcd1* and *mcd2* both map to chromosomal regions reported to contain genes or QTL for other phylogenetically dissimilar viral and fungal pathogens (Collins et al. 1999; Redinbaugh et al. 2004). Similarl to *mcd1*, QTL or genes conferring resistance to maize mosaic virus (*Mv1s*, Ming et al. 1997), sugarcane mosaic virus (*Scm2*, Melchinger et al. 1998), and wheat streak mosaic virus (*Wsm2*, McMullen et al. 1994) are all tightly linked to *umc102*.

In addition to virus resistance, this region carries genes encoding proteins with the nucleotide-binding site–leucine-rich repeat (NBS–LRR) motifs characteristic of resistance genes and is associated with the complex *Rp3* locus for rust resistance (Collins et al. 1999; Webb et al. 2002). The region of chromosome 10 near *umc44* that carries *mcd2* is also associated with a dominant gene for resistance to wheat streak mosaic virus (*Wsm3*, McMullen et al. 1994), a QTL for maize streak virus resistance (Pernet et al. 1999) and a QTL for sugarcane mosaic virus resistance (Xia et al. 1999). Interestingly, the minor QTL for MCDV resistance on chromosome 6 is associated with a major locus for resistance to members of the *Potyviridae* (McMullen and Louie 1989; Melchinger et al. 1998; McMullen et al. 1994). The interaction seen between *mcd2* and the minor QTL on chromosome 6 is interesting as resistance genes to several other unrelated viruses are linked to the same RFLP markers. Interactions between the loci on chromosomes 6 and 10 have been reported for resistance to sugarcane mosaic virus (Xia et al. 1999).

Although a current model for clustering of NBS–LRR type resistance genes in maize would suggest that resistance to multiple viruses at a specific locus is the result of the clustering of independent resistance genes (Hulbert et al. 2001), resistance to the different maize viruses has not been separated genetically. One reason for this is that loci associated with virus resistance are located in chromosomal regions with suppressed recombination, particularly those on chromosomes 3 and 6 (McMullen and Simcox 1995). *umc102* on chromosome 3 is located near the centromere, where suppression of recombination reduces map distances between loci, and *umc85* is located on the short arm of chromosome 6, adjacent to the highly heterochromatic nucleolus organizer region. For example, no separation of the tightly linked loci *Mdm1* and *Wsm1* at *umc85* was found in 82 F_3 families derived from a cross of a near-isogenic line carrying the loci and the susceptible line Oh28 (M.W. Jones, unpublished results). Another factor is the lack of inbred lines in which multiple virus resistance has been identified. Interestingly, Oh1VI is resistant to a number of other maize-infecting viruses, including three members of the Potyviridae, a rhabodivirus (maize fine streak virus), a tombusvirus (maize necrotic streak virus), and High Plains virus (M.G. Redinbaugh et al., unpublished results).

Currently, little is known about maize resistance to MCDV at a biochemical or molecular level, and no genes encoding virus resistance have been isolated from maize. However, virus resistance genes have been identified in other systems. One type of virus resistance includes dominant genes that trigger the hypersensitive response (HR) to virus infection. These genes include the *N* gene of tobacco, the *Rx1* and *Rx2* genes of potato, the *Sw-5* gene from tomato, and the *HRT* gene of *Arabidopsis*, which encode NBS–LRR proteins similar to resistance genes characterized for bacterial and fungal pathogens (Hulbert et al. 2001). Other virus resistance genes are not associated with the HR. For example, two genes (*RTM1* and *RTM2*) from *Arabidopsis* that confer dominant non-HR resistance to the potyvirus turnip crinkle virus. These genes encode phloem localized proteins that lack the NBS–LRR motif (Chisholm et al. 2000, 2001; Whitham et al. 2000). The resistance mechanism associated with these genes remains to be elucidated. A significant portion of non-HR virus resistance genes is genetically recessive. In lettuce and *Arabidopsis*, recessive resistance to potyvirus infection was conferred by genes encoding the cap-binding translation initiation factor, eIF-4E (Ruffel et al. 2002; Nicaise et al. 2003). Because the eIF-4E protein interacts with the potyvirus genomic protein (VPg) during infection (Leonard et al. 2000), resistance is likely to result from an incompatible reaction between eIF-4E and the VPg. Waikaviruses use a genome strategy similar to potyviruses and are thought (but not shown) to have a VPg. It is intriguing to hypothesize that a similar disruption of virus and maize protein interactions might be associated with the QTL for MCDV resistance.

Rice tungro spherical virus (RTSV), a waikavirus related to MCDV, causes significant disease problems in rice. RTSV resistance segregated with a 3:1 ratio in rice ARC11554 × TN1 F_2 plants, indicating a single dominant gene is responsible for resistance (Sebastian et al. 1996). RTSV resistance mapped to the end of rice chromosome 4 and was linked to leafhopper resistance. Notably, regions of rice chromosome 4 are syntenous with maize chromosome 10 (Ahn and Tanksley 1993). However, the *umc44* marker associated with *mcd2* maps to a different location on rice chromosome 4 than the RTSV resistance locus (near RZ262) in a comparison of the maize IBM neighbors 2003 and rice Cornell 2001 RFLP maps. Nonetheless, it would be interesting to determine whether markers associated with RTSV resistance in rice are associated with MCDV resistance in maize.

Transmission of viruses under controlled conditions in the laboratory offers the advantage of consistently achieving high and uniform rates of pathogen transmission that simplifies phenotypic analysis of populations segregating for resistance. For other maize viruses, including MDMV and WSMV, laboratory-based methods successfully identified resistance that is fully expressed under field conditions (M.W. Jones et al., unpublished results). Because the two major QTL for MCDV resistance were identified under controlled conditions where disease escapes were minimized, it is likely that these QTL will be effective in the field. Thus, the markers associated with *mcd1* and *mcd2* may be useful for breeders incorporating MCDV resistance into maize.

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