The *Mdm1* Locus and Maize Resistance to *Maize dwarf mosaic virus*

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ABSTRACT

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Previously, *Mdm1*, a gene controlling resistance to *Maize dwarf mosaic virus* (MDMV), was identified in the inbred line Pa405. The gene was tightly linked to the restriction fragment length polymorphism marker umc85 on the short arm of chromosome 6. This chromosomal region is also the location of resistance genes to two other viruses in the family Potyviridae, *Sugarcane mosaic virus* (SCMV) and *Wheat streak mosaic virus* (WSMV). A diverse collection of 115 maize inbred lines was evaluated for resistance to MDMV and SCMV, and for MDMV resistance loci on chromosome 6S. Forty-six resistant inbred lines were crossed to three MDMV-susceptible inbred lines to develop F₂ populations. The F₂ populations were inoculated with MDMV and scored for infection and symptom type. Environmental factors influenced both the rate and type of symptom development. Bulk segregant analysis of each F₂ population indicated that, in 42 of 43 MDMV-resistant lines, chromosome 6S markers found in the resistant parent also were present in the bulked resistant but not the susceptible tissue. Markers previously associated with resistance to both SCMV and WSMV on chromosome 3 and to WSMV on chromosome 10 were associated with resistance in nine and seven of the F₂ populations, respectively. These data suggest that *Mdm1* or closely linked genes on chromosome 6S are associated with MDMV resistance in most germplasm, but that other loci also may affect resistance.

Additional keywords: potyvirus, restriction fragment length polymorphism (RFLP), simple sequence repeat (SSR)

Maize dwarf mosaic and sugarcane mosaic, the diseases incited by *Maize dwarf mosaic virus* (MDMV) and *Sugarcane mosaic virus* (SCMV), are among the most important maize viral diseases worldwide (28). The two viruses are related members of the genus *Potyvirus* that are nonpersistently transmitted by aphids (16) and can be mechanically transmitted experimentally. They are differentiated from one another by host range, geographic distribution, and genome sequence. Sources of resistance have been identified in tropical (1), European (13), and U.S. corn belt (8) germplasm.

MDMV resistance was linked with a locus on the short arm of chromosome 6 in studies using reciprocal translocations (4,7,24,25), morphological markers (22),

and restriction fragment length polymorphism (RFLP) analysis (11,12). The genetically dominant locus identified in maize inbred line Pa405 was named *Mdm1* (11). However, these early genetic analyses showed variation in both the numbers of genes involved in resistance to MDMV and their modes of action. This variation was attributed to the use of different resistance sources, disease escapes, mixtures of viruses and virus strains, different classification systems for resistant and susceptible plants, and the type of analysis used (6). In addition, McMullen and Louie (11) suggested that other genes modify the activity of *Mdm1*.

SCMV resistance was associated with a dominant gene, *Scm1*, on the short arm of chromosome 6 in maize inbred line D32 (3,13). In contrast to MDMV, a second dominant locus for SCMV resistance, *Scm2*, was found near the centromere of chromosome 3 (2,3). Although allelism tests indicated the presence of a common dominant gene for SCMV resistance in European inbreds and Pa405 (30), it is not known whether *Mdm1* and *Scm1* are tightly linked genes or the same gene.

The goal of this study was to determine the responses of a widely based collection of germplasm to inoculation with MDMV and SCMV, and to test the hypothesis that *Mdm1* is associated with resistance to MDMV in this diverse germplasm.

MATERIALS AND METHODS

MDMV and SCMV isolates. The MDMV and SCMV isolates used were originally collected in Ohio and have been maintained at Wooster, OH by serial passage on susceptible maize (*Zea mays* L.; 6). Virus identity was verified at each passage by bioassay on sorghum (*Sorghum bicolor* L. Monenchen) cv. Atlas and Sart, wheat (*Triticum aestivum* L.) cv. Monon, and the susceptible maize inbred line Oh28 (6). Atlas is resistant to MDMV and *Wheat streak mosaic virus* (WSMV), but susceptible to SCMV. Sart is susceptible to MDMV and SCMV, but resistant to WSMV. Wheat is susceptible only to WSMV, and Oh28 is susceptible to all three viruses. Enzyme-linked immunosorbent assay (9) was used to confirm virus identity prior to these experiments using antisera raised against MDMV strain A and SCMV (MDMV strain B) (10).

Plant growth, inoculation, and symptom evaluation. A collection of 115 maize inbred lines was screened for response to MDMV and SCMV inoculation in 1999 to 2000. The collection consisted of 83 open-pedigree inbred lines with tropical backgrounds (1) obtained from J. L. Brewbaker (Department of Tropical Plant and Soil Sciences, University of Hawaii at Manoa, Honolulu). In previous analyses, most of these lines were resistant or partially resistant to MDMV and/or SCMV (1). In addition, 32 temperate inbred lines previously reported to have resistance to MDMV or other viruses (8) maintained at the Ohio Agricultural Research Center, Wooster were used. Seed of all lines was increased in Wooster prior to these experiments.

Experiments to monitor germplasm response to virus inoculation were conducted under greenhouse conditions. Natural light was supplemented with 400-W high-pressure sodium lights (P. L. Light Systems, Beamsville, ON, Canada) between October and April to attain photoperiods of 12 h of light and 12 h of darkness. Temperature was maintained at 25 and 18°C (day and night). Twelve seed of each inbred line were planted into one of four rows in perforated plastic trays (30 by 46 by 7 cm) containing autoclaved greenhouse soil. At 14 days after planting, leaves were rub inoculated four times at 2-day intervals as previously described (6). Plants were scored for the presence of mosaic, chlorotic streak, and limited delayed chlorotic streak symptoms 11, 14, 19, 21, and 24 days post inoculation (dpi)

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*The e-Xtra logo stands for “electronic extra” and indicates the HTML abstract available on-line contains a table, detailing the responses of inbred lines, that is not included in the print edition.

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with MDMV and 11, 14, 17, 23, 27, and 30 dpi with SCMV. Results presented are from three independent experiments for each virus.

F₂ population development and phenotype evaluation. F₂ populations were developed from 46 inbred lines that either had less than 25% MDMV or SCMV infection at the last rating date in initial experiments, previously were reported as MDMV resistant, or previously were used as the resistant parent in genetic crosses. Each line was crossed to the MDMV-susceptible inbred lines Oh28, yM14, and K55. F₁ progenies were self pollinated to create F₂ populations.

Twenty-five seedlings from each F₂ population were tested for their response to MDMV inoculation as described above. Inbred lines Oh28, yM14, K55 (susceptible), and Pa405 (resistant) served as controls. Numbers of symptomatic plants were determined at 10, 15, 20, and 25 dpi. Plants with limited symptoms were scored as infected when detected. The data presented are for four independent experiments.

Genotypic analysis. Leaf tissue was collected from the F₂ populations from one replicate of the test for MDMV susceptibility for bulked segregant analysis (14). For each population, plants with clear mosaic symptoms at 15 dpi were used for the susceptible pool and plants that were asymptomatic at 25 dpi were used for the resistant pool. Tissue was frozen in liquid nitrogen and lyophilized, and DNA was extracted by the cetyltrimethylammonium bromide method (21). For each population, DNA from each of the three susceptible parents and the F₂ resistant and susceptible pools was analyzed. RFLP and simple sequence repeat (SSR) analyses were carried out as previously described (5), using markers described in the Maize Genetics and Genomics Database. Markers (and their bins) evaluated in this study include RFLP probes umc85 (6.01), jc1270 (6.0), umc102 (3.05), umc44 (10.06), and umc167 (1.05); and SSR probes bnlg1422

(6.01), umc1133 (6.01), bnlg1867 (6.01), bnlg1505 (3.05), mmc0132 (3.04), and bnlg1360 (10.07). Enzyme-probe combinations were chosen to ensure that polymorphisms were present among the RFLP or SSR alleles for the three susceptible parents. This facilitated identification of polymorphic alleles between the resistant parent (allele unknown) and at least one of the susceptible parents.

Data analysis. Variation among experiments in the percentage of infected plants was evaluated at each scoring date by analysis of variance (ANOVA). Mean infection levels were calculated for inbred lines using the number of infected plants at the last scoring date. Area under the disease progress curve (AUDPC) scores (27) were based on a simplified disease severity rating system (asymptomatic plant = 0, delayed or limited symptoms = 1, and systemic mosaic symptoms = 2). The mean disease severity score for each scoring date was used to calculate the AUDPC. The relative rankings for inbred lines and F₂ populations among experiments were compared using Spearman's rank correlation coefficients using the CORR function of SAS (ver. 9.1; SAS Institute, Cary, NC). For the F₂ populations, χ^2 tests were used to examine goodness of fit to a 3:1 (resistant:susceptible) ratio, indicating resistance controlled by a single dominant gene at each scoring date using SAS.

RESULTS

Response of maize germplasm to MDMV and SCMV. In experiments to characterize the responses of 115 maize inbred lines to inoculation with MDMV and SCMV, the susceptible inbred line Oh28 and resistant inbred line Pa405 were 100 and 0% infected, respectively, at all scoring dates for each of the three independent experiments with each virus. This indicates that the experimental conditions were suitable for identification of resistant and susceptible responses. Consistent with previous studies (1,6), mean infection levels for SCMV were higher than those

for MDMV at all scoring dates, with a mean incidence of $74 \pm 39\%$ (mean $\pm$ standard deviation.) and $53 \pm 43\%$ for SCMV and MDMV, respectively, at the last scoring date (*data not shown*). For both viruses, ANOVA indicated that the mean incidence varied significantly among experiments on some scoring dates, as did AUDPC scores (*data not shown*). However, there was a strong correlation in the relative responses of the inbred lines to MDMV and SCMV, as indicated by highly significant Spearman's rank correlation coefficients for both disease incidence and AUDPC score (Table 1). Therefore, the data were pooled for further analysis. Possible sources of variation among experiments were the effects of seasonal differences in greenhouse environmental conditions on plant growth, virus titer of the inoculum used, and difficulties detecting symptoms in genetically diverse lines under variable light conditions.

Of the 115 inbred lines tested, 15 and 10 had no symptoms at the last rating date with MDMV and SCMV, respectively, in any of the experiments. However, virus-like symptoms were observed on a few plants of lines HIX4231 and Pa405 at earlier rating dates, resulting in positive AUDPC scores. Incidences of infection between 0 and 10% were found for another 11 (MDMV) and 5 (SCMV) lines. In contrast, 29 and 34% of the lines inoculated with MDMV and SCMV, respectively, were completely susceptible. In most cases, lines infected with MDMV were infected at equal or higher incidence with SCMV. However, eight lines had substantially lower incidence (>1 standard deviation) of SCMV infection than MDMV infection and 38 lines had lower incidence of MDMV than SCMV infection.

To determine whether some lines that ultimately had a high incidence of infection expressed resistance in the form of delayed or reduced symptom development, a modified disease severity rating system was used to calculate AUDPC scores. T256 inoculated with SCMV had the highest AUDPC score (31.5 ± 7), and the susceptible Oh28 had AUCPC scores of 29.2 ± 1.1 and 30.5 ± 8.1 for inoculation with MDMV and SCMV, respectively. Several lines that had over 80% disease incidence had AUDPC scores less than 5 (e.g., Tx5855, FLA 2 BT 106, and Pa884 P with MDMV, and A632 with SCMV). These data suggest that, at least in these lines, resistance can be expressed as either limited symptoms or as a delay in symptom appearance. The relative AUDPC scores were similar for MDMV and SCMV inoculation as indicated by the Spearman's rank correlation coefficient ($\rho = 0.552$; $P < 0.0001$).

Association of MDMV resistance with loci on chromosomes 1, 3, 6, and 10. Forty-six of the inbred lines were selected for further analysis based on their resist-

Table 1. Spearman's rank correlation among experiments for *Maize dwarf mosaic virus* (MDMV) and *Sugarcane mosaic virus* (SCMV) incidence and area under the disease progress curve (AUDPC) scores

Virus ^w	Score ^x	SRCC ^y	P ^z
Inbred lines			
MDMV	Infection (%)	0.790–0.876	<0.0001
	AUDPC	0.890–0.923	<0.0001
SCMV	Infection (%)	0.775–0.813	<0.0001
	AUDPC	0.806–0.840	<0.0001
F ₂ populations			
MDMV	Infection (%)	0.166–0.702	<0.0513
	AUDPC	0.364–0.685	<0.0001

^w Maize inbred lines or F₂ populations were inoculated with MDMV or SCMV as outlined in the Materials and Methods.

^x Data presented are the correlations between experiments for the % infected plants at each rating date or area under the disease progress curve (AUDPC).

^y Range of Spearman's rank correlation coefficients (SRCC) for comparisons between three (inbred lines) or four (F₂ populations) independent experimental replications.

^z Highest probability that the ranks of inbred lines or populations between experimental replications were not correlated.

tance to MDMV infection in initial experiments or previous reports of MDMV resistance (4,8,15,22,23). Of these, the responses for three of the inbred lines (Mp448, DR, and Narrino 330) were not included because of limited seed availability and difficulties with growing the lines in the greenhouse. F₂ populations derived from crosses of the 46 lines with three different susceptible parents were tested for their response to MDMV inoculation. ANOVA indicated significant variation in mean MDMV incidence at all rating dates as well as in mean AUDPC scores among the experiments ($P < 0.0001$, *data not*

shown). However, the relative rankings scored by the different F₂ populations by AUDPC were quite similar among experiments, as indicated by Spearman's rank correlation coefficients (Table 1). Therefore, the data were pooled for further analysis. In all experiments, the susceptible inbred lines Oh28, K55, and yM14 were 100% infected by the last rating date and the resistant line Pa405 was asymptomatic at all rating dates, indicating that experimental conditions were suitable to demonstrate susceptible and resistance host responses (*data not shown*).

A range of responses to MDMV inoculation occurred among the F₂ populations (Table 2). Some populations (e.g., those derived from Oh1VI, ICA L219, and DR crosses) had a low incidence of MDMV infection at all rating dates. All populations showed some resistance to MDMV, with significantly lower AUDPC scores than the susceptible parent controls. Interestingly, there was significant variability among F₂ populations derived from the same resistant parent for half of the lines. On average, F₂ populations using Oh28 as the susceptible parent had significantly higher AUDPC scores than those derived from yM14 or K55.

Table 2. Maize dwarf mosaic virus (MDMV) disease severity and chromosomal association of resistance with markers on chromosomes 1, 3, 6, and 10 for 130 F₂ populations derived from 46 maize inbred lines^x

Inbred line	Basis ^z	MDMV response ^y		Inbred X yM14		Inbred X Oh28		Inbred X K55	
		AUDPC		AUDPC	Chr.	AUDPC	Chr.	AUDPC	Chr.
Oh1VI	O	274 ± 19		248	6	288	...	287	6
Narrino330	P	311 ± 108		448 b	3	183 a	...	301 ab	6
ICA L219	I	314 ± 61		228	6	356	6	357	6
DR	O	325 ± 7		331	6	316	6, 3	329	...
Mo18W	P	342 ± 167		120 a	...	521 b	6, 3	385 b	6, 3
Cuba	O	342 ± 87		255 a	6, 3	461 ab	6, 3	310 b	...
KU 1403	I	351 ± 28		379	...	361	6	313	6
HIX4231	I	355 ± 40		326	6	411	6	328	6
KP 291	I	373 ± 37		397	6	402	6	321	6
Oh1EP	I	394 ± 47		352	6	459	6	371	...
CI44	I	409 ± 42		353	1	455	6	419	10
Pa405	I	410 ± 81		437	6	300	6, 10	494	10
HIX4243	I	427 ± 45		447	6	469	...	364	10
Mp448	P	432 ± 79		330	...	523	6	443	6
Tzi3	P	440 ± 41		399	...	nd	...	481	6
CIMMYT-A21	I	442 ± 35		453	6	478	...	395	...
INV 534	I	450 ± 67		360	6	520	6	470	6
MIT-2-S6	I	453 ± 120		406 a	...	617 b	...	335 a	6
Hi27	I	457 ± 157		273 a	...	657 ab	3	440 ab	6
INV 36	P	466 ± 235		753 c	6	177 a	...	469 b	...
Fla2AT113	P	476 ± 32		507	6	nd	...	444	10, 1
Phil-DMR6	I	479 ± 149		335 a	6	417 a	6, 3	684 b	...
Tzi4	O	480 ± 147		308 a	6	666 b	6	467 ab	...
CG1	I	483 ± 57		442	6	564	6	443	...
KU 1414	I	491 ± 67		527	...	549	6	398	6
Fla2AT114	I	497 ± 7		490	6	nd	...	503	6
INV 302	I	503 ± 117		607 b	6	nd	10	340 a	6
IIIA	P	512 ± 88		545 ab	6	599 b	6	392 a	6
MIT-11-S3	P	527 ± 290		201 a	10	473 b	6	906 c	...
ICA Nic 108	I	537 ± 77		449	nd	636	nd	525	nd
Mp705	O	537 ± 163		513 a	6	747 b	...	351 a	...
ICA L210	I	540 ± 166		676 b	...	637 b	...	307 a	3
A239	P	543 ± 112		418 a	6	690 b	...	522 ab	6
Ga209	P	543 ± 46		nd	...	589	nd	497	nd
TL-2	P	552 ± 45		507	...	597	6	nd	...
Fla2AT98	I	567 ± 34		568	...	607	...	525	6
Oh7B	I	583 ± 128		468 a	3	762 ab	6	519 a	...
Pa32	I	583 ± 85		583 a	6	752 b	6	nd	...
Pa11	I	584 ± 470		446 b	nd	90 a	nd	1216 c	nd
Ky61:2335	P	602 ± 217		317 a	6	844 b	...	645 b	6
B68	I	609 ± 86		682	...	657	6, 3	489	3
CIMMYT-A6	P	628 ± 113		740 b	...	nd	...	515 a	6
Oh07	P	639 ± 133		550 a	...	827 b	6	541 a	...
KS 22	P	649 ± 282		367 b	1	nd	...	930 a	6, 10
Ph 102-28	P	685 ± 159		587 a	...	909 b	...	560 a	6
EMBRAPA 282	P	711 ± 328		1174 b	...	506 a	...	454 a	6

^x Disease severity (area under the disease progress curve [AUDPC]) for F₂ populations derived from the cross of the indicated inbred line crossed to each of three MDMV-susceptible parents. The data represent the means of four independent experiments. Values within a row followed by the same letter or no letter are not significantly different ($P < 0.05$). For each cross, markers from the resistant inbred on chromosome (Chr.) 6S, 3L, 10, or 1 were associated with the asymptomatic pool as indicated; nd = not determined.

^y Disease severity for F₂ plants inoculated with MDMV. The data presented are AUDPC scores averaged over the three F₂ populations for four independent experiments. Data are expressed as the mean ± standard deviation.

^z The basis for selecting inbred lines for F₂ population development was performance in the inbred screening experiments (I), known resistance to other maize viruses (O), or use as the resistant parent in previous genetic studies of virus resistance (P).

The χ^2 tests were used to determine the goodness of fit of MDMV incidence in the F₂ populations to a 3:1 (resistant:susceptible) ratio for a single dominant gene model at each scoring date (Table 3). More than 80% of the populations having yM14 or K55 as the susceptible parent fit the model at 15 dpi. For Oh28, 62% of the populations fit the 3:1 segregation model at 15 dpi. On day 10, all but one population that did not fit the model had an excess of asymptomatic plants, indicating that the disease had not developed fully in this period. By the last rating date (25 dpi), all

populations that did not fit the model had an excess of susceptible plants.

Virus resistance in the F₂ populations was associated with RFLP and SSR markers located on chromosomes 6S, 3, 10, or 1 (Table 2) that previously were shown to be tightly linked to virus resistance (18). For markers associated with resistance, alleles from both the resistant and susceptible parents were found in DNA from the pool of asymptomatic plants, and the allele from the susceptible parent alone was found in DNA from the symptomatic pool (Fig. 1). Markers located on chromosome

6S were associated with MDMV resistance in 42 of the 43 inbred lines tested (Table 2). Markers on chromosome 3 were associated with MDMV resistance in nine inbred lines and 12 F₂ populations. Markers on chromosomes 10 and 1 were associated with resistance in seven and four inbred lines, respectively. Resistance in ICA L210 was not associated with chromosome 6S; however, the locus from the resistant parent on chromosome 3 was present in this line. In all cases where the markers were polymorphic, DNA from the resistant pool had markers from both the resistant and

Table 3. Segregation of *Maize dwarf mosaic virus* (MDMV) resistance in maize F₂ populations^y

Lines	Days post inoculation											
	Inbred line X yM14				Inbred line X Oh28				Inbred line X K55			
	10	15	20	25	10	15	20	25	10	15	20	25
Oh1VI	89/9	82/16	80/18*	75/23*	70/9	64/15*	61/18*	54/25*	95/6	82/19*	78/23*	70/31*
Narrino 330	91/7	77/21*	59/39	40/58	95/3	88/10	86/12	71/27*	89/9	80/18*	74/24*	69/29*
ICA L219	93/8	86/15	83/18*	80/21*	93/8	79/22*	74/27*	57/44	91/6	74/23*	69/28*	59/38
DR	89/10	76/23*	75/24*	68/31*	87/11	80/18*	74/24*	65/33	89/6	73/22*	70/25*	65/30*
Mo18W	95/3	95/3	92/6	67/31*	83/16	75/24*	52/47	44/55	95/5	77/23*	69/31*	52/48
Cuba	98/2	83/17*	79/21	71/29*	80/18*	70/28*	65/33	52/46	87/9	76/20*	75/21*	64/32
KU 1403	92/5	76/21*	68/29*	47/50	85/13	75/23*	64/34	36/62	89/11	85/15	77/23*	59/41
HIX4231	89/7	77/19*	72/24*	60/36	91/9	76/24*	68/32*	46/54	93/6	80/19*	75/24*	53/46
KP 291	81/18*	73/26*	70/29*	63/36	84/15	79/20*	73/26*	41/58	88/10	79/19*	76/22*	58/40
Oh1EP	94/5	76/23*	73/26*	58/41	83/17*	76/24*	63/37	50/50	87/9	72/24*	69/27*	59/37
C144	94/6	82/18*	73/27*	50/50	86/14	79/21*	59/41	50/50	90/9	79/20*	69/30*	37/62
Pa405	52/14*	46/20*	45/21*	45/21*	81/8	70/19*	69/20*	65/24*	69/26*	65/30*	57/38	46/49
HIX4243	91/9	74/26*	64/36	46/54	93/6	79/20*	58/41	32/67	93/6	80/19*	71/8	48/51
Mp448	90/10	79/21*	77/23*	63/37	90/9	68/31*	57/42	36/63	85/11	79/17*	59/37	38/58
Tzi3	92/7	75/24*	67/32*	57/42	...	...	...	...	92/7	69/30*	62/37	45/54
CIMMYT-A21	88/12	78/22*	63/37	43/57	92/10	76/20*	59/28*	19/56	79/17*	65/31*	28/68	12/84
INV 534	81/17*	76/22*	73/25*	65/33	80/17*	61/36	59/38	51/46	84/14	68/30*	58/40	56/42
MIT-2-S6	81/18*	74/25*	70/29*	59/40	71/27*	61/37	49/49	43/55	83/12	76/19*	70/25	63/32*
Hi27	90/9	82/17*	78/21*	69/30*	80/15	60/35	45/50	20/75	82/15	71/26*	67/30*	48/49
INV 36	85/10	57/38	30/65	9/86	100/0	95/5	89/11	59/41	94/5	84/15	55/44	27/72
Fla2AT113	92/8	80/20*	49/51	31/69	...	...	...	...	89/10	80/19*	61/38	41/58
Phil-DMR-6	93/7	80/20*	74/26*	61/39	82/16	70/28*	69/29*	59/39	91/8	56/43	41/58	28/71
Tzi4	91/7	89/9	75/23*	45/53	63/20*	57/26*	35/48	28/55	88/13	81/20*	63/38	37/64
CG1	68/12	59/21*	54/26*	45/35	82/13	64/31*	51/44	35/60	98/6	78/22*	61/39	44/56
KU 1414	81/15	72/24*	54/42	33/63	94/5	74/25*	50/49	22/77	95/4	81/18*	65/34	44/55
Fla2AT114	90/8	68/30*	57/41	48/55	...	...	...	...	84/16	71/29*	61/39	42/58
INV 302	87/10	66/31*	50/47	20/77	87/9	64/32*	54/42	26/70	93/4	78/19*	72/25*	52/45
IIIa	86/12	71/27*	52/46	34/64	89/11	67/33*	48/52	36/64	90/6	81/15	65/31*	38/58
MIT-11-S3	93/5	85/13	84/14	76/22*	80/18*	68/30*	63/35	53/45	82/16	39/59	22/76	14/84
ICA Nic 108	91/8	79/20*	60/39	36/63	89/11	70/30*	44/56	16/84	92/8	69/31*	60/40	31/69
Mp705	83/7	69/21*	47/43	36/54	89/8	57/40	28/69	27/70	86/8	76/18*	72/22*	44/50
ICA L210	79/19*	64/31*	44/54	17/81	88/8	69/27	39/57	13/83	87/13	81/19*	78/22	69/31*
A239	87/10	79/18*	65/32*	40/57	83/17*	55/45	43/57	31/69	85/11	66/30*	58/38	34/62
TL-2	47/9	42/14*	36/20	33/23	87/8	69/26*	35/60	41/54	...	...	...	...
Ga209	...	...	...	...	90/7	68/29*	43/54	35/62	86/11	74/23*	58/39	32/65
Fla2AT98	91/9	72/28*	51/49	23/77	88/10	64/34	49/49	21/77	89/10	69/30*	55/44	37/62
Oh7B	83/13	71/25*	60/36	42/54	90/10	54/46	31/69	24/76	91/9	73/27*	57/43	34/66
Pa11	86/10	77/19*	61/35	37/59	99/0	98/1	92/7	79/20*	55/42	8/89	8/89	4/93
Ky61:2335	87/6	72/21*	69/24*	63/30*	81/18*	48/51	26/73	18/81	86/12	59/39	45/53	28/70
B68	86/10	64/32*	39/57	9/87	80/14	55/39	45/49	28/66	87/10	78/19*	55/42	31/66
CIMMYT-A6	79/17*	65/31*	28/68	12/84	...	...	...	...	88/10	74/24*	53/45	35/63
Oh07	62/8	48/22*	40/30	23/47	81/19*	55/45	27/73	13/87	86/14	70/30*	56/44	39/61
Ph102-28	90/5	64/31*	46/49	23/72	85/15	48/52	17/83	1/99	77/16*	74/19*	50/43	22/71
KS 22	85/11	79/17*	68/28*	55/41	...	...	...	...	82/17*	42/57	17/82	8/91
EMBRAPA282	52/47	21/78	6/93	3/96	79/13	67/25*	55/37	33/59	78/21*	75/24*	63/36	52/47
Pa32	71/12	54/29	39/44	20/63	63/13*	47/29	19/57	13/63	...	...	...	...
Percentages ^z												
All	13	82	44	20	24	63	20	10	2	82	36	11
Chr6	14	81	62	24	31	81	19	12	10	86	43	14
Single gene	12	81	58	23	29	82	18	12	12	88	42	15

^y The F₂ populations were derived from a cross of the inbred line noted at the left crossed to the indicated susceptible line. Numbers indicate the number of asymptomatic individuals/number of symptomatic individuals. Data are the sum of four independent replications; * indicates that the ratio of asymptomatic/symptomatic plants fits a χ^2 goodness of fit ($P < 0.05$) for a 3:1 segregation model for a single dominant gene.

^z The percentage of F₂ populations that fit the model at each rating date for all populations, those populations in which a gene on chromosome 6 (Chr. 6) was associated with resistance, and those populations for which a single gene on chromosome 1, 3, 6, or 10 was associated with resistance.

susceptible parents, and the susceptible pool had only the marker from the susceptible parent. These data are consistent with dominant gene action, although the presence of disease "escapes" in the resistant pool cannot be ruled out.

DISCUSSION

The inbred lines used in this study were selected from a diverse set of germplasm to find new alleles for MDMV and SCMV resistance, and to determine the role of *Mdm1* in the resistance response of the lines. Inbred lines of tropical origin that previously were screened for resistance to multiple diseases, including MDMV and SCMV (1), were included, as were U.S. lines used in earlier virus resistance studies (4,8,15,23). The experiments were designed to minimize the variability due to environment and inoculation method by screening seedlings after multiple MDMV or SCMV leaf-rub inoculations in the greenhouse. Although susceptible lines were uniformly 100% infected with MDMV and SCMV by 10 dpi, there was significant variation in disease incidence and AUDPC score among experiments. However, there was a relatively strong correlation in the ranks of the inbred lines between experiments, and this allowed us to pool the results for further analyses. These results indicate that there is an effect of environmental conditions on disease development in the greenhouse. Our results are consistent with previous studies indicating an environmental influence on the expression of MDMV resistance (17,23).

The responses of the inbred lines to inoculation with MDMV and SCMV were consistent with those from previous studies (1,6,8). More plants were infected with SCMV than with MDMV (74 versus 52%). In addition, more lines (54 versus 24) were 100% infected with SCMV compared with MDMV and fewer lines (10 versus 15) had no symptoms after SCMV inoculation. Some inbred lines were identified that were highly resistant to MDMV and fully susceptible to SCMV (e.g., Mp705 and ICA L210). However, no lines were found that were susceptible to MDMV but highly resistant to SCMV. These results suggest that either multiple loci (e.g., *Scm1* and *Scm2*) are required for SCMV resistance or that different factors are required. For lines such as Fla2BT 106 or Pa884 P inoculated with MDMV, a high percentage of plants were symptomatic, but symptoms were either limited or delayed in appearance, as indicated by a relatively low AUDPC score. This result suggests that quantitative as well as qualitative resistance to MDMV is present among the inbred lines tested, similar to that described for *Scm2* in the European line FAP1360A (2).

Molecular markers on the short arm of chromosome 6 were linked to the resistant

pool in 42 of the 43 inbred lines for which association could be determined (Table 2), confirming the hypothesis that the *Mdm1* gene or cluster of genes in this region is primarily responsible for MDMV resistance in these maize lines. However, in line ICA L210, resistance to MDMV was associated with chromosome 3 in the region where *Scm2* was identified (2,3,13). Markers from regions of chromosomes 3, 10, and 1 previously associated with potyvirus resistance (18) also were associated with MDMV resistance in a number of lines. The χ^2 analysis indicated that resistance fit a 3:1 segregation ratio at the 15 dpi rating date for most lines. Prior to this time, symptom development was not fully established, as reflected in the excess of asymptomatic plants. After 15 dpi, resistance apparently breaks down in some lines and populations (Table 3). Yuan et al. (32) suggested that host development regulates the expression of quantitative trait loci (QTL) for SCMV resistance in mapping populations derived from the European line FAP1360A. Similarly, it is possible that some alleles of *Mdm1* or other resistance genes on chromosome 6S may be developmentally regulated. Only the three F_2 populations derived from Oh1VI and DR maintained a 3:1 ratio from 15 dpi through the last scoring date. For Cuba, two of the three F_2 populations maintained a 3:1 ratio from 15 dpi through the last scoring date. It is interesting that segregation of resistance in DR and Cuba crosses fit a 3:1 ratio even though multiple loci were associated with resistance for these lines. However, if the genes on chromosomes 3 and 10 act as modifiers of *Mdm1* activity that delay infection or resistance breakdown, the observed patterns of marker and resistance segregation could occur. The high level of potyvirus resistance in Cuba, DR, and Oh1VI may make these tropical inbred lines useful for developing virus-resistant hybrids. Higher-than-expected levels of infection in maize inbred lines carrying resistance genes previously have been noted under greenhouse conditions (8,13). In fact, Scott and Louie (23) proposed that, because screening conditions allowed for selection of the most resistant genotypes, it was preferable to do first screenings under greenhouse conditions, prior to screening materials under field conditions.

The relative resistance of F_2 populations to MDMV generally was similar to that found for the inbred lines alone, although there were notable exceptions. For example, the B68 inbred line was completely resistant to MDMV and SCMV, but the F_2 population ranked 41 out of 46 populations tested for AUDPC score (Table 2). This line combined particularly poorly with yM14, with about 90% of the F_2 plants being infected at 25 dpi. Lines ICA L219, Mo18W, and ICA L210, for which MDMV resistance was associated with markers on

chromosome 3 near *Scm2*, were completely susceptible to SCMV. Further work to elucidate the nature of the MDMV resistance associated with chromosome 3 would be of interest.

The importance of the interaction between specific resistant and susceptible parents is seen in the wide variation in AUDPC scores of the F_2 populations having a common resistant parent (Table 2). F_2 populations derived from Pa11 had the lowest AUDPC scores for the cross to Oh28, and the highest of all values for the cross to K55. Although this is the most dramatic case, significant differences among F_2 population responses for different susceptible parents were found for 23 of the 42 inbred lines. For the three susceptible parents used in this study, mean AUDPC scores were significantly higher for crosses made to Oh28, suggesting that this line has the weakest genetic background for MDMV resistance. Differences in MDMV resistance among populations having the same resistant and different susceptible parents also were noted by Melchinger et al. (13) and McMullen and Louie (11).

Differences in the developmental expression, strength, and dominant character of *Mdm1* and other resistance alleles on chromosomes 6, 3, 1, and 10 were evident from both the χ^2 analysis and the variability of F_2 populations derived from resistant inbred lines (Tables 2 and 3). Most symptomatic plants had a systemic mosaic similar to that seen in the susceptible controls. However, symptom appearance was delayed in some plants relative to susceptible controls, and limited symptoms or limited symptoms that developed into full mosaics at later scoring dates were seen in other individuals. It is likely that plants with delayed or limited symptoms carry resis-

Fig. 1. Association of Maize dwarf mosaic virus (MDMV) resistance with loci on chromosomes 1, 3, 6, and 10. An example of a Southern blot used to show the association of loci on chromosome 6 with the pool of asymptomatic plants identified in MDMV-inoculated F_2 plants. The blot was constructed using *EcoRV*-digested DNA from plants derived from a cross of *Zea mays* Pa405 with the three susceptible lines yM14 (M), Oh28 (O), and K55 (K). DNA from each susceptible parent and the resistant (R) and susceptible (S) pools from each F_2 population was probed with the cDNA probe *umc85*.

tance alleles that are affected by environment, heterozygosity, or the presence or absence of minor resistance or effector genes. Xu et al. (31) showed that virus resistance from *Scm1* is four times stronger than that from *Scm2*, and that the dominance character of the genes depended on the environment. Unlinked minor genes could affect the durability of resistance or the rate and extent of symptom development (29). These results are consistent with the hypothesis that *Mdm1* is a dominant resistance gene, and that genes on chromosomes 3 and 10 may modify that resistance by delaying its breakdown, delaying or reducing infection, or limiting symptom expression. Others hypothesized that, in addition to *Mdm1*, modifying or minor genes are necessary to attain high levels of resistance to MDMV or SCMV, and the breakdown of resistance over time is a result of lack of these genes (7,15,19,20,25,26). However, these genes cannot be detected with the approach used here, and will require QTL analysis of F₂ plants.

The results presented here indicate that MDMV resistance is present in a wide variety of tropical and U.S. maize germplasm. This range of germplasm should facilitate development of maize hybrids that combine virus resistance with other desired traits. In addition, *Mdm1* or closely linked genes on chromosome 6S clearly are important for MDMV resistance in most of this germplasm. However, the complexity of the results suggests that other factors, including minor genes, dosage effects, and genotype–environment interactions, all may contribute to the expression of resistance. These results also indicate that multiple genomic regions can be important for MDMV resistance, and this may need to be taken into account when making selections based on phenotype alone.

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