Determination of the Number of Genes for Resistance to Maize Dwarf Mosaic Virus Strain A in Five Corn Inbred Lines

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ABSTRACT

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A recently devised method was used to determine the number of genes that condition resistance to maize dwarf mosaic virus strain A (MDMV-A) in corn inbreds Mp71:222, T232, GA203, AR254, and Pa405. The basic assumption of the method is that each allele for resistance delays symptom expression by some length of time. Only two classes of plants, diseased and symptomless, are counted in two or more segregating generations to determine the number of alleles for resistance that allow symptom expression at any given time after inoculation. In this work, the following three generations were used: (Resistant [R] × Susceptible [S]) F_2, (R × S) × S, and (R × S) × R. For each evaluation date, the observed ratios of MDM-diseased to total number of plants were compared to the expected ratios for the number of resistance alleles allowing symptom expression by calculating chi-square values for goodness-of-fit. The observed ratios in all generations fit simultaneously the expected ratios for the proposed gene hypothesis at least twice in the course of each experiment. Data obtained when zero and one resistance alleles and again when zero, one, and two resistance alleles allowed symptom expression indicated two and three genes for resistance to MDMV-A in Mp71:222 and T232, respectively. When zero, one, two, and three and again when zero, one, two, three, and four resistance alleles permitted symptoms to be expressed, disease incidence data suggested three genes for MDMV-A resistance in GA203. Data acquired both in 1981 and 1982 provided evidence for the existence of two genes for resistance to MDMV-A in AR254. Seven days after inoculation (when plants with zero, one, and two resistance alleles showed symptoms) and 14 days after inoculation (when plants with zero, one, two, and three resistance alleles showed symptoms), the numbers of diseased plants in all generations best fitted the five-gene hypothesis for MDMV-A resistance in Pa405 both in 1981 and 1982.

Additional key words: genetics of disease resistance, *Zea mays*.

Maize dwarf mosaic (MDM) is the most widely occurring viral disease of corn (*Zea mays* L.) in the United States, and it potentially could reduce grain yield by as much as 45% (12). Of the two major strains of the virus, strain A (MDMV-A) has a wider geographical occurrence and taxonomic distribution within the Gramineae than strain B (MDMV-B) (11). Since Johnson grass (*Sorghum halepense* (L.) Pers.) is the principal overwintering host of MDMV-A, but not of MDMV-B, the former strain tends to have a predominately southern distribution, whereas the latter strain, for unknown reasons, causes epiphytotics on corn mainly in northern latitudes of the USA.

Many studies have been conducted to elucidate the type of gene action that is operative in the inheritance of resistance to MDMV in corn, most of which involved evaluation of disease severity data from diallel crosses (4,7–9,18). The conclusions drawn from the results of these studies do not agree on the relative importance of additive gene action and dominance effects in the inheritance of the host response to MDMV. Some investigators (8,18) concluded that resistance to MDMV was attributable largely to additive gene action with nonadditive gene effects playing a minor role, whereas other investigators (4,7,9) deduced from their results that resistance to MDMV was largely or partially dominant.

A number of field tests, which relied on natural infection, were carried out to estimate the number of genes conditioning resistance to MDMV. Using means and variances of disease severity ratings of parental inbreds and their F_1, F_2, and backcross generations, Dollinger et al (1) estimated that there were two to three dominant genes responsible for resistance in inbred Oh07, whereas Josephson and co-workers (5,6) reported dominance for resistance that was controlled by one to two major genes and perhaps some minor genes in the resistant inbreds T115, T220, T222, and T224. Naidu and Josephson (9) calculated the number of effective factors, K, in a 10-inbred diallel cross, and concluded that there were as many as four different genes for resistance to MDMV present among the resistant parental lines T232, Tx601, Ky226, GA209, and Mo18W.

Some researchers used mechanical inoculation in studies that were intended to estimate the number of genes for resistance to MDMV. Findley et al (3) reported that the percentage of diseased plants in some of the generations tested fit the hypothesis for a single dominant gene for resistance to MDMV-A in inbred Pa405. In one instance, the data indicated two dominant genes for resistance to this virus in Pa405. Roane et al (10) based their conclusions that inbred Oh7B had a single dominant gene for resistance to MDMV-A on disease severity ratings in F_1, F_2, and F_3 generations. Scott and Rosenkranz (17) found that the number of genes for resistance to MDMV-A in the five resistant inbreds GA209, Mp339, Mp412, T240, and Va35 ranged between one and three, with none exhibiting dominance.

Reciprocal chromosomal translocations also have been used to determine the number of chromosomal arms carrying genes for resistance to MDMV (2,14,15). All studies were in agreement that each arm of chromosome 6 carries a gene for resistance to MDMV in a number of resistant inbreds.

In an attempt to simplify the procedure for generating genetic data and to improve the reproducibility of results as well as to standardize the interpretation of the data, we devised a new method for the determination of the number of genes for resistance to MDMV (17). The present study was intended to test this method on five corn inbred lines with varying degrees of resistance to MDMV-A while determining the number of genes that govern resistance to this virus in them. A preliminary report on part of this work has been made (13).

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MATERIALS AND METHODS

Corn inbreds Mp71:222 (78.9% diseased upon manual inoculation in the greenhouse), T232 (45.5%), GA203 (45.0%), AR254 (formerly known as Ark. 361) (75.0%), and Pa405 (0.0%), previously identified as resistant to MDMV-A, were chosen for this study. Inbred CI21 served as the common, highly susceptible parent (S). For each resistant inbred, seed of the three segregating progenies ([R × S] F_2, [R × S] × S, and [R × S] × R) was produced either at Mississippi State or in southern Florida during the winter. The experiments were conducted in a field on the Plant Science Farm of Mississippi State University in 1981 and 1982.

An experiment consisted of the three segregating progenies for each of two resistant inbreds plus a highly susceptible hybrid (Pioneer 3368A) as a check, so that there were seven entries per experiment. Each entry was planted at the rate of 35 seeds per row in three single-row plots in each replication, and three replications were grown as randomized complete blocks in each experiment. Rows were spaced 97 cm apart and a 51-cm alley was cut between plots within each row. The result was that there were usually 28–30 plants per plot available for classification. Experiments were planted 2 wk apart to facilitate classification of plants as often as possible.

At the three- to four-leaf stage, all plants were inoculated with MDMV-A using an artist's airbrush operated from a tractor-mounted air compressor at a constant pressure of 7.0 kg/cm^2 (100 psi). Two adjacent rows of plants were inoculated at a time by two persons sitting behind the tractor. To ensure that each plant received a dose of the virus, all plants were inoculated twice within a few hours, once from one direction and the second time from the opposite direction. The preparation of the inoculum was the same as previously described (12).

When the susceptible check plants first began to show symptoms (4–7 days after inoculation, depending on temperature) all plants were evaluated for the presence or absence of mosaic symptoms every day. The evaluation of plants continued until the daily increase in the number of diseased plants became insignificant. A final evaluation was made about 4 wk after inoculation. Uninoculated susceptible check plants provided information on the natural disease incidence in the experimental field.

The method used in this study to determine the number of genes for resistance to MDMV-A in a resistant inbred is based on a number of assumptions which are: genes for resistance to MDMV-A are inherited independently (no linkage), no epistasis is present (no gene affects the expression of another, nonallelic gene), and each allele for resistance has an equal effect (no dominance) and delays symptom expression by some length of time. This method uses only two classes of plants, diseased and symptomless, in segregating generations, and thus employs disease incidence rather than disease severity data.

To understand how Table 1 was developed, it is necessary to know how many combinations of different numbers of alleles for resistance are present among the plants of each segregating generation and at what frequency. For instance, assume that the resistant parent has two unlinked genes for resistance (*AABB*) and the susceptible parent has none (*aabb*), and that each resistant allele exerts an equal effect. By denoting the resistance alleles with capital letters and listing all possible genotypes, we find that the F_2 population contains the following genotypic ratios: 1/16 *AABB*, 2/16 *AABb*, 1/16 *AAbb*, 2/16 *AaBB*, 4/16 *AaBb*, 2/16 *Aabb*, 1/16 *aaBB*, 2/16 *aaBb*, and 1/16 *aabb*. Since each resistance allele has an equal effect, these F_2 ratios can be reduced to 1/16 (6%), 1/4 (25%), 3/8 (38%), 1/4 (25%), and 1/16 (6%) plants with 4, 3, 2, 1, and 0 alleles for resistance, respectively. Now we are ready to look at the percentages in the F_2 column in Table 1. With two genes for resistance present in the resistant parent, there are 6% plants with 0 resistance alleles, 31% (6 + 25) plants with 0 and 1 resistance alleles, 69% (6 + 25 + 38) plants with 0, 1, and 2 resistance alleles, 94% (6 + 25 + 38 + 25) plants with 0, 1, 2, and 3 resistance alleles, and 100% (6 + 25 + 38 + 25 + 6) plants with 0, 1, 2, 3, and 4 resistance alleles.

The same procedure can be applied to the two backcross generations to obtain the percentages of plants with all possible

TABLE 1. Expected percentages of plants in the F_2 and both backcross generations with symptoms for different number of resistance genes without dominance and different numbers of resistance alleles

Resistance genes in resistant parent (no.)	Resistance alleles allowing symptom expression (total no.)	Percent symptomatic plants in generation:[a]		
		(R × S) F_2	(R × S) × S	(R × S) × R
1	0	25	50	0
2	0	6	25	0
3	0	2	13	0
4	0	0	6	0
5	0	0	3	0
1	0 & 1	75	100	50
2	0 & 1	31	75	0
3	0 & 1	11	50	0
4	0 & 1	4	31	0
5	0 & 1	1	19	0
1	0, 1, & 2	100	100	100
2	0, 1, & 2	69	100	25
3	0, 1, & 2	34	88	0
4	0, 1, & 2	14	69	0
5	0, 1, & 2	5	50	0
1	0, 1, 2, & 3	100	100	100
2	0, 1, 2, & 3	94	100	75
3	0, 1, 2, & 3	66	100	13
4	0, 1, 2, & 3	36	94	0
5	0, 1, 2, & 3	17	81	0
1	0, 1, 2, 3, & 4	100	100	100
2	0, 1, 2, 3, & 4	100	100	100
3	0, 1, 2, 3, & 4	89	100	50
4	0, 1, 2, 3, & 4	64	100	6
5	0, 1, 2, 3, & 4	38	97	0

[a] R = resistant parent, and S = susceptible parent.

genotypes. Still using the example of two genes for resistance in the resistant parent, we find that in the backcross to the susceptible parent all plants have two or fewer alleles for resistance while in the backcross to the resistant parent all plants have two or more alleles for resistance. In a similar manner, we calculated the expected percentages of diseased plants in the F_2 and both backcross generations for one, three, four, and five resistance genes in the resistant parent and varying numbers of resistance alleles (Table 1).

The ratios of the number of diseased to total number of plants for each entry in an experiment were compiled daily, converted to percentages of diseased plants, and compared in Table 1 to the expected percentages of diseased plants for the different numbers of resistance alleles allowing symptom expression. When a similarity between the observed and the expected percentages of diseased plants was noticed in all three segregating generations, this not only indicated how many resistance alleles were permitting symptom expression, but also showed the number of genes for resistance present in the resistant parent. Chi-square (χ^2) values for the goodness-of-fit between the observed and expected numbers of diseased and healthy plants were calculated for each segregating generation of a resistant inbred. If the χ^2 test showed that differences between the observed and expected ratios of diseased to healthy plants were not significant at $P = 0.05$ ($\chi^2 < 3.841$, 1 df) for each segregating generation at least on two evaluation days, the hypothesis for the specific number of resistance genes in the resistant inbred was accepted.

Because the segregating generations of the studied inbreds were tested at different times during the growing season and hence at different temperatures, the various numbers of resistance alleles were allowing symptoms to be expressed on different evaluation dates for the individual inbreds, except those that were paired in the same experiment. This accounts for the difference in the number of days after inoculation when the observed numbers of diseased plants best fit the expected numbers of diseased plants in all segregating generations of the individual inbreds.

RESULTS

In 1981, we were able to fit our observed ratios of diseased to healthy plants to certain expected ratios, which enabled us to estimate the number of genes for resistance to MDMV-A in all five resistant inbreds. To corroborate our estimates, we tested inbreds AR254 and Pa405 again in 1982. Seed of all three segregating generations of Mp71:222, T232, and GA203 was not available for retesting. For convenience, we have included in the tables the number of diseased to the total number of plants as well as the percentages of diseased plants for each segregating generation.

For Mp71:222, the percentages of diseased plants 5 days (when plants with zero and one allele for resistance showed symptoms) and 9 days after inoculation (when plants with zero, one, and two alleles for resistance showed symptoms) supported a two-gene hypothesis of resistance, using the F_2 and backcross to the susceptible parent (BC_1) (Table 2). On the last evaluation date, 14 days after inoculation, the numbers of diseased plants were approaching those expected when zero, one, two, and three alleles for resistance would have allowed symptom expression. Had we made another evaluation a few days later, the data obtained then would most likely have fit again the data expected for a two-gene hypothesis. Unfortunately, we could not use the data from the backcross to the resistant parent because of questionable purity of the seed.

Data for T232 collected 4 days after inoculation showed that plants with zero and one allele for resistance were expressing symptoms, and the percentages of diseased plants in all three segregating generations corresponded closely to those expected when assuming three genes for resistance (Table 3). Four days later, the percentages of diseased plants indicated that plants with zero, one, and two alleles for resistance were showing symptoms, and the obtained ratios of diseased to total number of plants again were in accord with the three-gene hypothesis of resistance. A limitation of this test was the relatively small number of plants available for evaluation (due to paucity of seed) in the backcross to the susceptible parent.

In the early stages of disease development among plants of the three segregating generations involving inbred GA203, the numbers of diseased plants did not fit any genetic hypothesis. However, 13 days after inoculation, when plants with zero, one, two, and three resistance alleles exhibited symptoms, the observed percentages of diseased plants began to fit the percentages of diseased plants expected for a three-gene hypothesis of resistance (Table 4). Seven days later, when plants with zero, one, two, three, and four resistance alleles showed symptoms, the agreement

TABLE 2. The ratios of observed (O) and expected (E) numbers of maize dwarf mosaic-diseased to total numbers of corn plants inoculated in segregating generations involving resistant inbred Mp71:222 and susceptible inbred CI21 for a two-gene hypothesis of resistance

	Days after inoculation and number of resistance alleles allowing symptom expression					
	5 days (0 and 1 alleles)			9 days (0, 1, and 2 alleles)		
Generation	O	E	χ^{2a}	O	E	χ^{2a}
(Mp71:222 × CI21) F_2	90/272 (33.1)[b]	84/272 (31)[b]	0.555	190/272 (69.8)[b]	188/272 (69)[b]	0.093
(Mp71:222 × CI21) × CI21 (BC_1)	102/132 (77.3)	99/132 (75)	0.364	127/132 (96.2)	132/132 (100)	···
$F_2 + BC_1$	192/404 (47.5)	183/404 (45)	1.041	317/404 (78.5)	320/404 (79)	0.070

[a] Chi-square (χ^2) values were calculated by using the observed and expected numbers of diseased and healthy plants. $\chi^2 > 3.841$ indicates a significant difference between the observed and expected numbers of diseased plants at $P = 0.05$, 1 df.
[b] Numerals in parentheses are percentages of observed (with decimal) and expected (whole numbers) diseased plants.

TABLE 3. The ratios of observed (O) and expected (E) numbers of maize dwarf mosaic-diseased to total numbers of corn plants inoculated in segregating generations involving resistant inbred T232 and susceptible inbred CI21 for a three-gene hypothesis of resistance

	Days after inoculation and number of resistance alleles allowing symptom expression					
	4 days (0 and 1 alleles)			8 days (0, 1, and 2 alleles)		
Generation	O	E	χ^{2a}	O	E	χ^{2a}
(T232 × CI21) F_2	26/252 (10.3)[b]	28/252 (11)[b]	0.120	83/252 (32.9)[b]	86/252 (34)[b]	0.127
(T232 × CI21) × CI21 (BC_1)	20/43 (46.5)	22/43 (50)	0.209	34/43 (79.1)	38/43 (88)	3.247
(T232 × C121) × T232 (Bc_2)	0/208 (0.0)	0/208 (0)	···	10/208 (4.8)	0/208 (0)	···
$F_2 + BC_1$	46/295 (15.6)	50/295 (17)	0.414	117/295 (39.7)	124/294 (42)	0.663

[a] Chi-square (χ^2) values were calculated using the observed and expected numbers of diseased and healthy plants. $\chi^2 > 3.841$ indicates a significant difference between the observed and expected number of diseased plants at $P = 0.05$, 1 df.
[b] Numerals in parentheses are percentages of observed (with decimal) and expected (whole numbers) diseased plants.

between the observed and expected ratios of diseased to total number of plants was very close for three resistance genes in inbred GA203.

In 1981, AR254 gave a close fit between the observed and expected ratios in the F_2 generation for a two-gene hypothesis of resistance both 7 and 20 days after inoculation (Table 5). The agreement between observed and expected ratios for BC_1 was closer 20 days than 7 days after inoculation, although statistically there was no significant difference between observed and expected values on either evaluation day. The fit for the backcross to the resistant parent (BC_2) was good on both evaluation days. The data collected in 1982 confirmed the two-gene hypothesis of resistance for AR254 in all three segregating generations when evaluations were made 12 and 16 days after inoculation.

Inbred Pa405 proved to have the highest level of resistance to MDMV-A of any inbred studied by us so far. In 1981, the observed and expected ratios of diseased to symptomless plants agreed closely for the F_2 and reasonably well (difference still not statistically significant at $P=0.05$) for BC_1 both 7 and 14 days after inoculation when assuming five genes for resistance (Table 6).

TABLE 4. The ratios of observed (O) and expected (E) numbers of maize dwarf mosaic-diseased to total numbers of corn plants inoculated in segregating generations involving resistant inbred GA203 and susceptible inbred CI21 for a three-gene hypothesis of resistance

	Days after inoculation and number of resistance alleles allowing symptom expression					
	13 days (0, 1, 2, and 3 alleles)			20 days (0, 1, 2, 3, and 4 alleles)		
Generation	O	E	χ^{2a}	O	E	χ^{2a}
(GA203 × CI21) F_2	176/281 (62.6)[b]	185/281 (66)[b]	1.419	242/281 (86.1)[b]	250/281 (89)[b]	2.379
(GA203 × CI21) × CI21 (BC_1)	248/269 (92.2)	269/269 (100)	...	269/269 (100.0)	269/269 (100)	...
(GA203 × CI21) × GA203 (BC_2)	53/295 (17.9)	38/295 (13)	6.433*	152/295 (51.5)	148/295 (50)	0.275
F_2 + BC_2	229/576 (39.8)	223/576 (39)	0.139	394/576 (68.4)	398/576 (69)	0.096

[a] Chi-square (χ^2) values were calculated using the observed and expected numbers of diseased and healthy plants. $\chi^2 > 3.841$ indicates a significant difference between the observed and expected numbers of diseased plants at $P = 0.05$, 1 df.
[b] Numerals in parentheses are percentages of observed (with decimal) and expected (whole numbers) diseased plants.

TABLE 5. The ratios of observed (O) and expected (E) numbers of maize dwarf mosaic-diseased to total numbers of corn plants inoculated in segregating generations involving resistant inbred AR254 and susceptible inbred CI21 for a two-gene hypothesis of resistance in 1981 and 1982

	Days after inoculation and number of resistance alleles allowing symptom expression					
	7 days (0 alleles)			20 days (0, 1, 2, and 3 alleles)		
Generation	O	E	χ^{2a}	O	E	χ^{2a}
1981						
(AR254 × CI21) F_2	14/198 (7.1)[b]	12/198 (6)[b]	0.403	190/198 (95.9)[b]	186/198 (94)[b]	1.348
(AR254 × CI21) × CI21 (BC_1)	62/202 (30.7)	51/202 (25)	3.492	201/202 (99.5)	202/202 (100)	...
(AR254 × CI21) × AR254 (BC_2)	0/203 (0.0)	0/203 (0)	...	158/203 (77.8)	152/203 (75)	0.869
F_2 + BC_1	76/400 (19.0)	63/400 (16)	2.679	391/400 (97.7)	388/400 (97)	0.773
	12 days (0, 1, and 2 alleles)			16 days (0, 1, 2, and 3 alleles)		
1982						
(AR254 × CI21) F_2	140/216 (62.0)[b]	149/216 (69)[b]	1.769	203/216 (94.0)[b]	203/216 (94)[b]	0.000
(AR254 × CI21) × CI21 (BC_1)	266/299 (89.0)	299/299 (100)	...	291/299 (97.3)	299/299 (100)	...
(AR254 × CI21) × AR254 (BC_2)	30/114 (26.3)	29/114 (25)	0.105	81/114 (71.1)	86/114 (75)	0.947
F_2 + BC_2	170/330 (51.5)	178/330 (47)	2.701	284/330 (86.1)	289/330 (85)	0.290

[a] Chi-square (χ^2) values were calculated using the observed and expected numbers of diseased and healthy plants. $\chi^2 > 3.841$ indicates a significant difference between the observed and expected numbers of diseased plants at $P = 0.05$, 1 df.
[b] Numerals in parentheses are percentages of observed (with decimal) and expected (whole numbers) diseased plants.

TABLE 6. The ratios of observed (O) and expected (E) numbers of maize dwarf mosaic-diseased to total numbers of corn plants inoculated in segregating generations involving resistant inbred Pa405 and susceptible inbred C121 for a five-gene hypothesis of resistance in 1981 and 1982

	Days after inoculation and number of resistance alleles allowing symptom expression					
	7 days (0, 1, and 2 alleles)			14 days (0, 1, 2, and 3 alleles)		
Generation	O	E	χ^{2a}	O	E	χ^{2a}
1981						
(Pa405 × CI21) F_2	11/239 (4.6)[b]	12/239 (5)[b]	0.800	46/239 (19.2)[b]	41/239 (17)[b]	0.855
(Pa405 × CI21) × CI21 (BC_1)	106/242 (43.8)	121/242 (50)	3.719	188/242 (77.7)	196/242 (81)	1.727
(Pa405 × CI21) × Pa405 (BC_2)	7/319 (2.2)	0/319 (0)	...	10/319 (3.1)	0/319 (0)	...
F_2 + BC_1	117/481 (24.3)	133/481 (28)	3.224	234/481 (48.6)	237/481 (49)	0.024
1982						
(Pa405 × CI21) F_2	16/243 (6.6)[b]	12/243 (5)[b]	1.284	35/243 (14.4)[b]	41/243 (17)[b]	1.161
(Pa405 × CI21) × CI21 (BC_1)	136/274 (49.6)	137/274 (50)	0.015	223/274 (81.4)	222/274 (81)	0.027
(Pa405 × CI21) × Pa405 (BC_2)	0/264 (0.0)	0/264 (0)	...	0/264 (0.0)	0/264 (0)	...
F_2 + BC_1	152/517 (29.4)	149/517 (29)	0.040	258/517 (47.9)	263/517 (49)	0.169

[a] Chi-square (χ^2) values were calculated using the observed and expected numbers of diseased and healthy plants. $\chi^2 > 3.841$ indicates a significant difference between the observed and expected numbers of diseased plants at $P = 0.05$, 1 df.
[b] Numerals in parentheses are percentages of observed (with decimal) and expected (whole numbers) diseased plants.

However, there were 2–3% diseased plants in BC_2, where none were expected on both evaluation days. Data from 1982, also collected 7 and 14 days after inoculation, corroborated very well our assumption that Pa405 has five genes conditioning resistance to MDMV-A. The agreement between the observed and expected ratios was excellent in all three segregating generations.

In addition to the disease incidence data for the two evaluation days presented for each resistant inbred in its respective table, there were other data which, on certain evaluation days, supported the proposed hypotheses for the specific numbers of resistance genes. On other evaluation days, the obtained data fit neither the expected data for the proposed gene hypotheses nor the expected data for any other, alternative gene hypothesis. To illustrate the merit of our method by which the number of genes for resistance in a resistant inbred can be determined, disease incidence data for Pa405, collected in 1982, were used to obtain the best possible fit for the three-, four-, or five-gene hypothesis regardless of the date of plant evaluation and hence the number of alleles for resistance (Table 7). It is evident from the calculated chi-square values that the closest agreement between the observed and expected ratios of diseased to total number of plants in each segregating generation was obtained when one assumed that inbred Pa405 possesses five genes for resistance to MDMV-A.

DISCUSSION

Several problems beset earlier investigations on the nature of resistance to MDMV. Reliance on natural infection with MDMV caused susceptible plants that escaped infection to be classed as resistant. Some experiments were confounded by the presence of maize chlorotic dwarf virus so that disease ratings included the host reaction to both viruses. Another problem arose when investigators made only one evaluation of plants in the course of a genetic experiment in the field. The number of MDM-diseased plants increases while the number of plants with easily recognizable mosaic symptoms decreases with time. That mosaic symptoms become diffuse in many MDMV-infected plants with age may be due to a gradual increase in temperature as the season progresses. Therefore, it is advisable to evaluate each plant several times to obtain meaningful genetic ratios. Finally, almost all researchers used disease severity ratings (indices) rather than disease incidence to measure genetic variation among plants exposed to MDMV. This choice may represent another problem.

If one assumes that host response to MDMV-A can best be explained by additive gene effects, then our method for determining the number of genes for resistance is appropriate. The results we obtained so far support this assumption. Because data on percentages of diseased plants are as effective in identifying levels of resistance to MDMV-A in corn as data on disease severity ratings (16), it is advantageous to use the simpler and more reliable system in classifying MDMV-A-inoculated plants for their reaction to this virus. Using disease incidence means that only two classes of plants are involved: diseased and symptomless. With MDM, determination of whether an inoculated plant is diseased or symptomless is easy and certain when plants are inoculated in the three- to five-leaf stage and evaluated several times within 4–5 wk after inoculation. On the other hand, any method that uses a disease severity rating scale for MDM, no matter how elaborate, involves a judgment as to the severity of the mosaic symptoms in individual, diseased plants. The extent and intensity of the mosaic symptoms are the only criteria by which one can determine the severity of disease in immature corn plants, and these systemic symptoms do not lend themselves well to a qualitative classification. There is less room for error when only two classes of plants, diseased and symptomless, are involved. More importantly, for a disease severity rating scale to be validly used in the determination of the number of resistance genes to MDMV-A when assuming additive gene action, the scale must include as many disease severity classes as there are resistance alleles plus one class for plants that lack all resistance alleles. Thus, one would have to know in advance how many alleles for resistance were present in an inbred to devise a disease severity scale for rating plants in segregating generations of that inbred.

In an earlier study on the effectiveness of resistance to MDMV (16), we found in a diallel cross of MDMV-resistant (R) and MDMV-susceptible (S) inbreds that when the level of infection was low (susceptible checks 50–60% diseased), the R × S crosses indicated dominance for resistance. However, when the level of infection was high (susceptible checks 85–95% diseased), the R × S crosses pointed to additive gene action in the inheritance of resistance to MDMV-A. In another genetic study (17), we noticed that with some resistant inbreds, the observed ratios of diseased to symptomless plants in the segregating generations early in disease development indicated some dominance for resistance. These ratios suggesting dominance consistently disappeared on later evaluation dates, in favor of ratios indicating that each allele for resistance contributed an equal amount toward total resistance.

Means and variances of disease reaction in parental inbreds and their F_1, F_2, and backcross generations can be used to estimate the minimum number of genes for resistance to MDMV. Our method provides a means for determining the exact number of genes for resistance to MDMV more than once in the course of the same

TABLE 7. The ratios of observed (O) and expected (E) numbers of maize dwarf mosaic-diseased to total numbers of plants inoculated in segregating generations of corn inbred Pa405 and the corresponding chi-square (χ^2) values to indicate the closest goodness-of-fit for three-, four-, and five-gene hypotheses of resistance based on the 1982 data

	No. of alleles for resistance allowing symptom expression					
	0 and 1			0, 1, and 2		
Generation	O	E	χ^{2a}	O	E	χ^{2a}
Three-gene hypothesis						
(Pa405 × CI21) F_2	24/243 (9.9)[b]	27/243 (11)[b]	0.31	85/243 (35.0)[b]	83/243 (34)[b]	0.10
(Pa405 × CI21) × CI21	194/274 (70.8)	137/274 (50)	47.43**	271/274 (98.9)	241/274 (88)	30.86**
(Pa405 × CI21) × Pa405	0/264 (0.0)	0/264 (0)	···	2/264 (0.7)	0/264 (0)	···
Four-gene hypothesis						
(Pa405 × CI21) F_2	6/243 (2.5)	10/243 (4)	1.48	32/243 (13.2)	34/243 (14)	0.14
(Pa405 × CI21) × CI21	103/274 (37.6)	85/274 (31)	5.57*	207/274 (75.5)	189/274 (69)	5.49*
(Pa405 × CI21) × Pa405	0/264 (0.0)	0/264 (0)	···	0/264 (0.0)	0/264 (0)	···
	0, 1, and 2			0, 1, 2, and 3		
Five-gene hypothesis						
(Pa405 × CI21) F_2	16/243 (6.6)	12/243 (5)	1.28	35/243 (14.4)	41/243 (17)	1.16
(Pa405 × CI21) × CI21	136/274 (49.6)	137/274 (50)	0.01	223/274 (81.4)	222/274 (81)	0.03
(Pa405 × CI21) × Pa405	0/264 (0.0)	0/264 (0)	···	0/264 (0.0)	0/264 (0)	···

[a] Chi-square (χ^2) values >3.841 (*) indicate a significant difference ($P=0.05$, 1 df) and >6.635 (**), a highly significant difference ($P=0.01$, 1 df) between the observed and expected numbers of diseased plants.

[b] Numerals in parentheses are percentages of observed (with decimal) and expected (whole numbers) diseased plants.

experiment.

To ensure that the adopted hypothesis for the particular number of MDMV-A-resistant genes in an inbred was the most appropriate, we observed two self-imposed rules. First, the chosen hypothesis was based on the coincident ratios of diseased to total number of plants inoculated in all segregating generations tested on at least two evaluation days. Second, if segregating generations of two resistant inbreds were included in the same experiment, the observed ratios of diseased to total number of plants had to show that the same number of alleles for resistance allowed symptom expression in all segregating generations of both inbreds on the same recording day, regardless of the number of genes for resistance each inbred possessed.

There is a discrepancy in the number of genes for resistance to MDMV-A in inbred Pa405 reported by us earlier (13) and that reported in this paper. The reported three-gene hypothesis for resistance in Pa405 was based on 1981 data collected 9 days (which fit the expected data well) and 17 days after inoculation (which fit the expected data only marginally). In 1982, the observed ratios of diseased to symptomless plants fit exceedingly well the expected ratios for the five-gene hypothesis 7, 14, and 21 days after inoculation. When we reexamined the 1981 data, the agreement between the observed and expected ratios was also better for the five-gene hypothesis than for the three-gene hypothesis of resistance 7 and 14 days after inoculation. (Therefore, at the presentation of the paper (13), the five-gene hypothesis of resistance for Pa405 was reported, but the abstract regrettably still contained the erroneous three-gene hypothesis.)

In our experiments on the genetics of resistance to MDMV we use enough inoculum pressure so that all susceptible plants are infected and evaluate all inoculated plants frequently to record plants with incipient symptoms and those plants whose symptoms are transitory. If classification of inoculated plants is made only once, or even twice, the data on disease reaction may not only give incorrect genetic ratios, which, in turn, would result in the assumption of the wrong number of genes governing resistance, but may also lead to an erroneous conclusion regarding the type of gene action involved.

If the problems encountered in earlier studies on the genetics of resistance to MDMV can be overcome in future work by using thorough inoculation with a known isolate of the virus and frequent evaluation of inoculated plants, new data may confirm that each allele for resistance exerts an equal effect on the total resistance of an inbred. We found agreement in the number of genes conditioning resistance to MDMV-A in several corn inbreds when the determination was made with our new method and the reciprocal chromosomal translocation technique (15,17).

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