Type of Gene Action in the Resistance to Maize Chlorotic Dwarf Virus in Corn

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Journal Series Paper 6378 of the Mississippi Agricultural and Forestry Experiment Station.
Accepted for publication 26 January 1987.

ABSTRACT

Rosenkranz, E., and Scott, G. E. 1987. Type of gene action in the resistance to maize chlorotic dwarf virus in corn. Phytopathology 77:1293-1296.

A six-parent diallel cross, comprised of three maize chlorotic dwarf virus (MCDV)-resistant (Ky122, Mp444, and Tx29A) and three MCDV-susceptible (AR234, Ky21, and T131) corn inbred lines, was chosen to estimate the genetic variance of host response to MCDV, and thus gain information on the type of gene action involved in the resistance to MCDV in corn. The 15 possible crosses (without reciprocals) were grown during 2 yr in a screenhouse into which leafhoppers from viruliferous colonies of *Graminella nigrifrons* were released at the rate of six and 12 insects per plant in 1984 and 1985, respectively. Each year, about 125 plants of each cross were evaluated for the presence of tertiary vein clearing, the diagnostic symptom of maize chlorotic dwarf. The results were consistent under the two levels of inoculum pressure. In both years, the mean disease incidence of the nine resistant (R) × susceptible (S) crosses was equal or similar in magnitude to the mean disease incidence for the combined three R × R and the three S × S crosses. The diallel analysis of the disease incidence data showed a relatively large and highly significant mean square for general combining ability and a relatively small and statistically nonsignificant mean square for specific combining ability. Thus, our results indicate that the total genetic variance in host response to MCDV among the 15 crosses was contained in the general combining ability, suggesting additive gene action, and that nonadditive gene action (dominance variance) was absent because the variance for specific combining ability was insignificant.

Additional key words: disease resistance, vector inoculation, *Zea mays*.

The etiology of the "corn stunting disease" in the United States of the 1960s was complicated by the presence of at least three causal agents. In the lower Midwest, maize dwarf mosaic virus and maize chlorotic dwarf virus (MCDV), the latter not identified as a distinct pathogen until 1968 (7,8), were causing severe losses in corn (*Zea mays* L.). In the South, especially in Mississippi and Louisiana, corn stunt spiroplasma (thought to be a virus at the time) occurred in the same corn fields with the above two viruses. Before the etiology of the corn stunting disease complex was clarified, a few field studies, conducted under natural infection, were carried out in an attempt to elucidate the inheritance of resistance to what was believed to have been either corn stunt (3) or maize dwarf mosaic (2,4). In all these cases, however, there are now strong indications that these researchers were working primarily with maize chlorotic

dwarf (MCD), although maize dwarf mosaic was included in the studies conducted in Ohio (2) and Missouri (4).

Grogan and Rosenkranz (3) studied the effects of genes on host response by comparing the disease severity ratings of one susceptible and three resistant inbreds, their F_1, F_2, and both backcross populations obtained under heavy natural infection during three consecutive growing seasons. Their results indicated a strong additive influence, no dominance, and insignificant epistatic effects in the inheritance of host response to the prevalent corn stunting disease agent transmitted by the leafhopper, *Graminella nigrifrons* (Forbes), now believed to have been MCDV. However, also using disease severity ratings in a similar mating design with one resistant and two susceptible inbreds (but different statistical treatment of disease severity data), Dollinger et al (2) concluded that resistance was largely dominant. Although these researchers assumed that they were dealing with maize dwarf mosaic virus, the description of their disease severity rating scale indicates that they were working with natural infection by MCDV and maize dwarf mosaic virus.

Because no studies on the genetics of resistance to MCDV in corn have been conducted under controlled conditions of infection, the present study was designed to gain such information. The diallel cross was chosen in preference to other mating designs because it provides general information on the type of gene action involved in the resistance through calculation of the general combining ability and specific combining ability of the parental inbred lines. We decided on disease incidence over disease severity as a means of measuring variability in host response because disease severity of the diagnostic symptom of MCD, chlorotic striping in the tertiary veins of leaf laminae (5), used in this study did not show sufficient variation in crosses to devise a scale of discrete or definable classes. Furthermore, an earlier study showed that data on percentages of diseased plants were usually as effective in identifying levels of resistance to MCDV as were data on disease severity ratings (12).

MATERIALS AND METHODS

Three MCDV-resistant corn inbreds (Ky122, Mp444, and Tx29A) and three MCDV-susceptible inbreds (AR234, Ky21, and T131) were selected on the basis of previously secured susceptibility data to make the six-parent diallel cross (Table 1). The 15 possible crosses (reciprocal crosses excluded) were grown in each of 2 yr as a randomized complete block design with four replications. A replication consisted of a single row of each of the 15 crosses. Thirty-five seeds per row were planted every 5.5 cm directly into the soil of a screenhouse within rows that were 4.65 m long, spaced 61 cm apart. A perfect stand would have provided 140 plants per cross or 2,100 plants (35 plants × 60 rows) per experiment.

The isolate of MCDV originated from a severely diseased field corn plant collected at Mississippi State in 1972 and was maintained in plants of sweet corn cultivar Seneca Chief as well as in the leafhopper vector *G. nigrifrons*. The virus was transferred weekly to fresh sweet corn seedlings with viruliferous leafhoppers. *G. nigrifrons* were reared on plants of sweet corn cultivar Seneca Chief and rice (*Oryza sativa* L.), with both provided in the same cages. The rearing cages were constructed of wooden frames, 42 × 66 × 92 cm, covered with 55-mesh (22 strands per centimeter) nylon cloth and furnished with a solid wood base and a glass plate on top. These cages have two nylon cloth-covered doors opposite each other at the small sides and can hold six 15-cm clay pots inside a metal tray. Watering of plants is done with a hose through the nylon screen into the metal tray. Each cage can accommodate up to 2,000 leafhoppers on six medium-sized (80–90 cm) corn plants for 2 to 3 wk.

Leafhoppers were exposed to severely MCDV-infected corn plants for a minimum acquisition access period of 7 days before their release into the screenhouse. They were removed with an aspirator from viruliferous colonies and placed into the leaf whorl of each seedling, being in the three- to four-leaf stage, at the rate of three adult insects per seedling. After all seedlings had received the first three leafhoppers, the procedure was repeated. In the 1984 test, each seedling was exposed to a total of at least six leafhoppers and to twice that number in the 1985 test. Because only one in three adult *G. nigrifrons* is a transmitter of MCDV (1), each seedling received, on the average, two and four viruliferous leafhoppers in 1984 and 1985, respectively. During 1984, the test material was planted on 12 July, and the first inoculative leafhoppers were released into the screenhouse on 21 July. The next year, the same genotypes were planted on 1 July, and the first leafhoppers were released 9 days later.

The first evaluation of plants for disease reaction was made 40 and 30 days after planting in 1984 and 1985, respectively, which was followed by two additional ratings at intervals of 3 wk. On the last evaluation date, both disease incidence and disease severity of individual plants were recorded. The different number of days after planting when plants were evaluated in 1984 and 1985 reflects the faster rate of disease development in the second year. Susceptibility to MCDV was evaluated by the presence of chlorotic striping in the tertiary veins of leaf laminae, which is the diagnostic symptom of MCD (5). Each leaf on every plant was examined for this symptom. Data used for statistical analyses were percentages of diseased plants for each of the 15 possible crosses: three resistant (R) × R, nine R × susceptible (S), and three S × S hybrids. The analysis of variance of the combined data for the 2 yr was

TABLE 1. Reaction of parental corn inbreds in the diallel to vector-inoculation with maize chlorotic dwarf virus (MCDV) in a screenhouse[a]

			Unreplicated trial[b]			Replicated trial[c]		
Inbred	Endosperm color[d]	Response class[e]	Plants rated (no.)	Plants diseased[f] (%)	Disease severity index[g]	Plants rated (no.)	Plants diseased[f] (%)	Disease severity index[g]
Ky122	W	R	32	0.0	···	84	6.0	2.50
Mp444	Y	R	31	0.0	···	86	7.0	2.50
Tx29A	W	R	37	13.5	2.00	86	30.2	3.21
T131	W	S	32	100.0	3.50	92	66.3	2.61
Ky21	W	S	32	100.0	2.90	90	98.9	3.91
AR234	Y	S	33	100.0	4.52	93	100.0	3.69

[a] Six adults of *Graminella nigrifrons* from MCDV-inoculative colonies were placed into the leaf whorl of each seedling in the three- to four-leaf stage, and the seedlings grew directly in the soil in a screened enclosure.
[b] Plants of each inbred were grown in a single row.
[c] Plants were grown in a randomized complete block design with three replications.
[d] Abbreviations: W = white (colorless) endosperm; Y = yellow endosperm.
[e] Abbreviations: R = resistant inbred; S = susceptible inbred.
[f] Plants were evaluated (at least twice) only for the presence or absence of chlorotic striping in the tertiary veins, a diagnostic symptom of maize chlorotic dwarf in corn.
[g] Diseased plants were rated for disease severity on a scale of 2 (mild) to 5 (severe), and the disease severity index was calculated by adding the severity rating for each diseased plant and dividing the total score by the number of diseased plants.

performed according to a program that allows for missing entries (9) as one cross was missing in 1985.

RESULTS

In the 1984 test, 1,900 plants (90.5% stand) were evaluated for their response to vector inoculation with MCDV after the release of 11,450 leafhoppers (about six insects per plant). There were an average of 32, 31, and 34 plants per row for the R × R, R × S, and S × S crosses, respectively. The first diagnostic symptoms of MCD were noticed on two plants of the most susceptible genotype, AR234 × T131, 8 days after the first viruliferous leafhoppers were released. The created inoculum pressure, however, was only high enough to cause infection in a little more than one-half of the potentially susceptible plants. Even with this level of inoculum pressure, infection was reasonably uniform and proportional for the three types of crosses, i.e., R × R, R × S, and S × S. The diallel analysis of the disease incidence data showed a highly significant general combining ability and an insignificant specific combining ability among the single crosses (Table 2). Also, agreement between the mean disease incidence for the nine R × S crosses and that for the six combined R × R and S × S crosses was within one percentage point on the last evaluation date (Table 3). Both of these results indicated that a high level of additive variance was present among the crosses regarding their response to MCDV.

In the 1985 test, 1,680 plants (85.7% stand) of the same diallel cross were rated for their reaction to MCDV after the release of 19,950 leafhoppers from viruliferous colonies (about 12 insects per plant). The average number of plants per row was 28, 30, and 33 in R × R, R × S, and S × S crosses, respectively. The first symptoms were observed on a few plants of the most susceptible genotypes within 10 days from the start of vector release. The inoculum pressure in this test was sufficiently high to cause 95–100% infection of the most susceptible crosses. Seed of one of the R × S crosses, Mp444 × Ky21, was not available due to low inventory, so only eight of the nine possible R × S crosses were analyzed. Results from this test corroborated the findings obtained the previous year. The mean disease incidence for the R × S crosses equaled that for the combined R × R and S × S crosses on the last evaluation day (Table 3). The similarity between these two percentages of diseased plants improved with each successive evaluation.

When the mean for the percentages of diseased plants obtained in the 2 yr was calculated separately for each cross and the 14 means (data were missing for one R × S cross in 1 yr) statistically analyzed, the Student-Newman-Keuls multiple range test showed that the disease incidence for four of the R × S crosses fell within the range of the disease incidence of the R × R crosses, and the disease incidence for the other four R × S crosses fell within the range of that of the S × S crosses (Table 4). As a result, the mean percentage of diseased plants for the eight R × S crosses (55.8%) was essentially the same as that for the combined three R × R crosses and the three S × S crosses (56.3%). These results were based on an average of 250 plants evaluated for each of the 14 crosses during the 2 yr.

TABLE 2. Analysis of variance for incidence of maize chlorotic dwarf in a six-parent corn diallel cross after vector-inoculation in a screenhouse in 2 yr

Source of variation	df[a]	Incidence MS[b]	Incidence F[c]
Years (environment)	1	40,526.23	274.77**
Replications within years	6	344.97	2.34*
Entries (crosses)	14	1,893.69	12.84**
General combining ability (GCA)	5	4,854.66	32.92**
Specific combining ability	9	248.71	1.69
GCA × years	5	294.85	2.00
Error	89	147.49	
Total	115		

[a] df = Degrees of freedom.
[b] MS = Mean square.
[c] Asterisks: * significant F test, $P = 0.05$; and ** highly significant F test, $P = 0.01$.

DISCUSSION

Before an attempt is made to determine the number of resistance genes involved in the genetics of resistance to MCDV in various corn inbreds, it would be helpful to know the type of gene action that is operative in the response of corn genotypes to this virus. Such knowledge is also basic to the maximum efficiency of a breeding program that has as its goal the development of MCDV-resistant populations or hybrids. Previous investigations (2,3) on the inheritance of resistance to what now is believed to have been MCDV were carried out under the uncertain conditions of natural inoculation in the field. Ours is the first study to use vector inoculation with MCDV in a confined facility from which other interfering diseases and insects were excluded. Even with the differences in the inoculation conditions, corn genotypes, mating designs, types of disease data collected, and statistical treatment of data, our results corroborate the findings of Grogan and Rosenkranz (3), namely that additive variance is much more important than dominance variance in the inheritance of resistance to MCDV in corn. On the other hand, our results and conclusions differ from those of Dollinger et al (2) and Naidu and Josephson (6) who attributed the variation in host response to the combination of this virus and maize dwarf mosaic virus primarily to dominance variance.

Because of the nature of MCDV-transmission, which can be accomplished experimentally only with the leafhopper vector, this study was limited in scope. We used a diallel cross of three resistant and three susceptible parents with four replications in each of two experiments. A larger diallel with the same number of replications would not have fit into the existing screenhouse facility. For the same reason, we were unable to include reciprocal crosses in our diallel experiment. Another important consideration in research with MCDV is the availability of sufficient numbers of the leafhopper vector *G. nigrifrons*, which transmits the virus with an efficiency of only 34 to 35% (1). Thus, rearing of the vector becomes a major aspect in any research involving MCDV. In any genetic work on host plant resistance to a pathogen, it is essential to be able to infect all potentially susceptible plants. With MCDV and *G. nigrifrons*, three times as many leafhoppers are required to infect a given number of plants as with a virus-vector combination where each individual insect has the capacity to infect a host plant.

The results from this study provide two lines of evidence to support the supposition that the variability of host response to MCDV in corn is due largely to additive genetic variance and that nonadditive variance in the form of dominance is insignificant. First, the mean disease incidence of the R × S crosses equaled the mean disease incidence of the combined R × R and S × S crosses. On the last evaluation date in both years the difference between

TABLE 3. Summary of the mean response of a six-parent corn diallel cross to vector-inoculation with maize chlorotic dwarf virus (MCDV) in a screenhouse in 2 yr

Type of cross[c]	Crosses (no.)	Diseased plants on day after planting in each year (%)[a] 1984[b] 40	1984[b] 60	1984[b] 80	1985 30	1985 40	1985 60
R × R	3	10.8	21.3	22.3	30.1	35.9	56.3
R × S	9 (8)[d]	26.9	36.9	37.8	49.2	60.2	75.4
S × S	3	30.9	47.4	54.2	76.3	83.5	92.1
Midpoint between R × R and S × S		21.0	34.6	38.6	54.2	61.3	75.5

[a] During each year, plants were grown in a randomized complete block design with four replications, and 35 seeds were planted per replication (row).
[b] During 1984 six adults and during 1985 12 adults of *Graminella nigrifrons* from MCDV-inoculative colonies were placed into the leaf whorl of each seedling in the three- to four-leaf stage, and the seedlings grew directly in the soil in a screened enclosure.
[c] Abbreviations: R = resistant parent; S = susceptible parent.
[d] In the 1985 test, one R × S cross (Mp444 × Ky21) was missing due to lack of seed.

TABLE 4. Mean incidence of maize chlorotic dwarf among crosses from a six-parent corn diallel resulting from vector inoculation in a screenhouse in 2 yr[w]

Cross[x]	Plants rated (no.)	Symptomatic plants (%)
R × R		
Mp444 × Tx29A	217	49.9 c[z]
Mp444 × Ky122	257	35.9 c
Tx29A × Ky122	250	33.4 c
R × S[y]		
Mp444 × AR234	257	67.0 b
Mp444 × T131	264	68.7 ab
Tx29A × AR234	277	74.4 ab
Tx29A × T131	263	46.3 c
Tx29A × Ky21	236	44.3 c
Ky122 × AR234	249	61.8 b
Ky122 × T131	217	45.0 c
Ky122 × Ky21	217	39.0 c
S × S		
AR234 × T131	264	83.7 a
AR234 × Ky21	263	70.7 ab
T131 × Ky21	267	64.5 b

[w] During each year, plants were grown in a randomized complete block design with four replications in a screened enclosure; during 1984, six adults and during 1985, 12 adults of *Graminella nigrifrons* from maize chlorotic dwarf virus-inoculative colonies were placed into the leaf whorl of each seedling in the three- to four-leaf stage.

[x] Abbreviations: R = resistant parent; S = susceptible parent.

[y] During 1984, all nine R × S crosses were tested, but in the 1985 test one R × S cross (Mp444 × Ky21) was missing due to lack of seed.

[z] Percentages in the column followed by different letters are significantly different at the $P = 0.01$ level according to the Student-Newman-Keuls multiple range test.

these two means was a fraction of 1% (Table 3). Second, regarding the relative ability of the resistant parents to transmit MCDV resistance to their hybrids, the general combining ability was very large and highly significant, whereas the specific combining ability was very small and statistically nonsignificant. According to statistical genetic theory, this is interpreted to mean that additive variance (gene action) is much more important than dominance in the inheritance of resistance to the vein clearing component of the MCD syndrome. (Dominance is closely associated with large specific combining ability variance.)

Although the inoculum pressure of MCDV varied in the 2 yr, being twice as great in 1985 as in 1984, there was good agreement in results between the two tests. Under both levels of inoculum, the mean disease incidence for the R × S crosses was close to the midpoint between the mean disease incidence for the R × R and that for the S × S crosses. On the first evaluation date, the difference between the mean percentage of diseased plants for the R × S crosses and the midpoint was 4 to 5% points, became smaller on the next evaluation date, until on the third (last) reading it had narrowed to less than 1%. This trend was observed in both tests. The proportional degree of infection among the three groups of crosses under the two levels of inoculum indicated that there may be a direct relationship between virus dosage and subsequent disease incidence. Doubling the average number of viruliferous leafhoppers released per plant resulted in almost exactly twice the amount of disease. These results also suggest that in both tests the distribution of viruliferous leafhoppers was uniform and inoculation of the plants at random.

The selection of the parental lines for the diallel cross was based on the reaction of inbred lines to MCDV obtained in two previous tests conducted under similar experimental conditions as the diallel experiments. Although the resistant inbreds Ky122 and Mp444 were equally resistant in terms of both disease incidence and disease severity while inbred Tx29A was less so, in all possible crosses, Ky122 imparted the greatest amount of MCDV resistance and Mp444 the least. Among the susceptible parental lines, inbred AR234 contributed the greatest amount of susceptibility when crossed with either resistant or other susceptible lines, whereas inbred Ky21 contributed the least susceptibility. Thus, inbred lines with very similar levels of resistance to MCDV may contribute different amounts of resistance to their hybrids. This may be explained by assuming that the additive effect of a certain number of resistance genes is sufficient to render an inbred resistant (as in Mp444), and the presence of an additional gene for resistance in another inbred (as in Ky122) is not expressed phonotypically except in a cross with a susceptible inbred.

High levels of resistance to MCDV are probably conditioned by more than two or three genes, but the exact number is unknown (10). We know from a study with chromosomal translocations that corn inbred Mp412 has a gene for resistance to MCDV on the short arm of chromosome 1, the long arm of chromosome 3, and probably also on the short arm of chromosome 4 (11). The next step in the investigation of the genetics of resistance to MCDV should be an attempt to determine, under controlled conditions of inoculation, the number of genes for resistance present in several unrelated corn genotypes of varying levels of resistance.

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