Diallel Analysis for Reaction of Eight Corn Inbreds to Helminthosporium maydis Race T

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ABSTRACT

Eight inbred lines and 28 F_1 hybrids from a set of diallel crosses among the lines, each in both *cms*-T and normal (N) cytoplasms, were evaluated for reaction to *Helminthosporium maydis* race T. Plants were artificially inoculated in the field and disease ratings, lesion length, and lesion area measured.

All three disease reactions were highly intercorrelated. Disease reactions from *cms*-T plants were highly correlated with those from their N cytoplasm counterparts. Resistance in corn to race T was both cytoplasmic and nuclear-genic. All eight inbreds and F_1 hybrids with N cytoplasm exhibited a higher degree of resistance to race T than their *cms*-T cytoplasm counterparts. Mo17^T was the only inbred showing a relatively high level of resistance in *cms*-T cytoplasm. The most resistant crosses involved Mo17 as a parent.

Estimations of combining ability indicated that genetic variation for disease resistance in corn was associated with highly significant general combining ability (GCA) effects. The (Vr, Wr) graphic analysis indicated that nuclear gene resistance to race T had additive gene effects and was partially dominant. Estimations of degree of dominance showed the resistant inbred Mo17 in both types of cytoplasm had the most dominant genes.

The use of the restoration factor (*Rf*) is suggested to facilitate incorporation of high degrees of nuclear gene resistance into elite *cms*-T corn, allowing effective selfing, sib-crossing, or backcrossing. Recurrent selection in populations of *cms*-T cytoplasm having a degree of resistance is also suggested through the use of *Rf*.

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Additional key words: *cms*-T cytoplasm, N cytoplasm, combining ability.

The unexpected outbreak of southern leaf blight of corn (*Zea mays* L.) caused by *Helminthosporium maydis* (Nisikado & Miyake) race T in the United States in 1970 stimulated plant breeders and pathologists to seek sources of disease resistance to improve corn hybrids.

Races T and O of *H. maydis* can be identified by differential pathogenicity and by pathotoxin production (6,13). These races are morphologically similar; however, race T shows selective pathogenicity to different types of corn cytoplasm, while race O does not. Corn with normal (N) cytoplasm is resistant to race T, whereas corn with Texas male-sterile (*cms*-T or T) cytoplasm is susceptible. The genetic analysis of ascospore progeny obtained from the cross between race T and race O showed that both the selective pathogenicity and the production of the host-specific pathotoxin of race T are monogenic in inheritance (8). Resistance in corn to race O is due to nuclear genes and is expressed both qualitatively and quantitatively (5). Qualitative resistance to *H. maydis* is characterized by lesion types; resistant plants have small, circular chlorotic lesions (1,12). This chlorotic-lesion resistance is conditioned by the recessive gene *rhm* (12). Quantitative resistance is characterized by number and size of lesions, or by percentage of the entire plant or leaf area infected. Inheritance of quantitative resistance is partially dominant in single crosses among inbreds. Additive and/or complementary effects were also detected from backcross progenies involving resistant inbreds (11).

The precision of the estimate of genetic effects in

quantitatively inherited disease resistance depends on the method used to determine disease reactions. A study (9) on disease components of corn plants infected with race T showed that measurement of lesion sizes on infected leaf blades is the most useful measure of differences in disease reactions of *cms*-T corn plants to race T. The present study determined the combining ability and dominance relationships among eight corn inbreds, in *cms*-T and N cytoplasm, using different methods of evaluating disease reactions to race T.

MATERIALS AND METHODS.—Eight corn inbreds in both *cms*-T and N cytoplasms grown in paired rows were artificially inoculated with *H. maydis* race T in 1971 at the Agronomy South Farm, Urbana, Illinois. The inbreds with *cms*-T cytoplasm showed either resistant ($Mo17^T$), intermediate ($B37^T$ and $Oh43^T$), or susceptible ($W64A^T$, $Oh07^T$, $N28^T$, $B14A^T$ and $Hy2^T$) reactions to race T (Lim, *unpublished*). The inbreds with N cytoplasms exhibited a range of reaction to race T similar to that of their *cms*-T cytoplasm counterparts, but with a much lower amount of infection. Diallel sets of F_1 hybrids, each in *cms*-T and N cytoplasm, were made from the eight inbreds. Hybrids in *cms*-T cytoplasm were made by using *cms*-T versions of inbreds as female parents and N versions as male parents.

In 1972, the parents and one set of 28 F_1 hybrids, each in *cms*-T and N cytoplasm were grown in paired plots (rows) in a randomized complete block arrangement with two replications. A third replication contained only F_1 hybrids. Plots were spaced approximately 90 cm apart. Each plot contained 12 plants spaced about 30 cm apart. All plants were inoculated by the ground leaf method (4) at the 7- to 9-leaf stage and again 2 weeks later. Inoculum was prepared from infected leaf tissue collected in 1971 from an artificially inoculated field. Ten plants in each plot were rated for disease reaction approximately 2 weeks after mid-silk using a 0 to 100 scale based on infection of the entire plant. On this scale 0 designates no infection and 100 complete susceptibility. Clearly expressed lesions on the ear-leaf of 10 plants per plot were measured longitudinally in mm (lesion length) and lesion area (mm^2) was calculated from lesion length × width.

Analyses of variance were performed for disease rating, lesion length, and lesion area. The general combining ability (GCA) and the specific combining ability (SCA) effects of the parental genotypes were estimated in F_1 hybrids using Griffing's method 4, model I (fixed model–selected parent lines) (2). A computer program written by Littlewood et al. (10) was used to estimate combining abilities. Graphic analysis (3,7) was used to determine the genetic dominant effects and relations within the parental inbreds. The underlying assumptions were tested by performing a two-way analysis of variance on the (Wr-Vr) values calculated for each of the parents in the diallel table (3). Vr is the variance of all the offspring of each parent in each array (complete row or column), and Wr is the covariance of the parents with their offspring in each array of the diallel table. The slope of the regression line (Vr, Wr) was tested for deviation from unity by the "t" test (14).

RESULTS.— The artificial inoculation assured that all plants were infected. Disease ratings, lesion length, and lesion areas from *cms*-T plants were highly correlated with those from their N cytoplasm counterparts. The correlation coefficients (r) were 0.77, 0.77 and 0.73 on disease ratings, lesion length, and lesion areas, respectively. In all instances, disease on *cms*-T plants was much more severe than on their corresponding N cytoplasm counterparts. The r values among the three methods used to determine disease reaction were highly significant; 0.90 between disease ratings and lesion length, 0.87 between disease ratings and lesion areas, and 0.97 between lesion length and lesion area.

Highly significant differences among inbreds and F_1 hybrids in both types of cytoplasm were found for disease reactions (Table 1). Inbred $Mo17^T$ had significantly lower rating, shorter lesion length, and lesion area was less than other *cms*-T inbreds. This was reflected in crosses with $Mo17^T$. Inbreds $B37^T$ and $Oh43^T$ were less susceptible than $W64A^T$, $Oh07^T$, $N28^T$, and $B14A^T$. All crosses involving susceptible inbreds tended to be susceptible except crosses with the resistant inbred Mo17. The F_1 hybrid between the most susceptible inbreds $W64A^T$ and $N28^T$ was very susceptible. Differences in disease reaction among inbreds and F_1 hybrids having N cytoplasm were significant. Inbred Mo17 with N cytoplasm had the most resistant reaction of the inbreds. The inbreds W64A and N28 were less resistant than other inbreds and conferred less resistance in crosses. However, differences in disease reaction were much smaller than those obtained from their *cms*-T cytoplasm counterparts. The most susceptible N inbreds W64A and N28 were still more resistant to race T than the resistant *cms*-T inbred $Mo17^T$. Although differences were small, it was also noted that crosses between the most susceptible inbreds having N cytoplasm tended to be more resistant than crosses between resistant and intermediate inbreds having *cms*-T cytoplasm.

Combining ability.—Mean squares for both GCA and SCA effects for disease resistance were highly significant (Table 2). However, mean squares for GCA were much greater than those for SCA, indicating a preponderance of additive gene effects for disease resistance for these eight inbred genotypes in both types of cytoplasm. Estimates of GCA effects associated with each of eight inbreds in both *cms*-T and N cytoplasms for disease reaction as determined by three methods are given in Table 3. Resistant inbred Mo17 in both types of cytoplasm had the highest negative GCA effect of all inbreds for each method used to determine disease resistance. Inbred B37 in both types of cytoplasm showed negative GCA effects for all three traits similar to Mo17, but of much less magnitude. Inbred Oh43 in both types of cytoplasm exhibited a negative GCA effect as obtained by disease rating similar to that of B37. GCA effects obtained by lesion length and lesion areas of $Oh43^T$ were positive. All other inbreds in both *cms*-T and N cytoplasms generally showed positive GCA effects.

Gene action.—The degree of genetic dominance controlling disease reaction was estimated by using Vr, Wr, graphic analysis (3, 7). For each of the three methods used to determine disease reaction, the analysis of homogeneity of Wr-Vr over arrays was performed to test the validity of the assumptions (3). Two replications of parents and F_1 generations were used in this analysis, since there were only two replications for the parental lines. The genotype mean squares in the Wr-Vr analysis of variance were not significant and gave no indication of

TABLE 1. Means of disease rating, lesion length, and lesion area for eight inbreds, and for 28 hybrids involving these inbreds in *cms*-T and N cytoplasm infected with *Helminthosporium maydis* race T

Inbred and Hybrid	*cms*-T cytoplasm			N cytoplasm		
	Disease rating (0-100)	Lesion Length (mm)	Lesion area (mm^2)	Disease rating (0-100)	Lesion length (mm)	Lesion area (mm^2)
B37	36.0	9.4	33.6	3.1	1.2	1.2
W64A	69.9	14.8	45.7	16.9	5.9	8.8
Mo17	26.5	5.5	13.2	1.3	0.5	0.5
Oh07	65.7	14.5	54.2	8.9	4.2	6.1
N28	60.9	16.5	71.2	15.3	5.8	9.4
Oh43	33.8	11.5	41.6	2.9	2.7	2.8
B14A	57.7	15.2	71.6	17.5	3.5	3.5
Hy2	48.0	14.5	51.4	5.6	3.2	4.8
B37 × W64A	50.1	13.2	43.0	6.5	3.9	4.4
B37 × Mo17	15.8	4.9	12.2	1.1	1.1	1.1
B37 × Oh07	41.4	15.1	59.6	3.2	2.5	2.5
B37 × N28	40.8	16.0	79.7	5.5	4.3	4.6
B37 × Oh43	30.8	16.6	67.1	1.6	1.2	1.2
B37 × B14A	45.2	16.0	70.5	2.8	2.3	2.3
B37 × Hy2	35.7	14.5	54.9	3.1	2.4	2.9
W64A × Mo17	22.8	7.9	22.7	1.6	1.5	1.5
W64A × Oh07	47.7	16.8	69.5	6.5	4.5	8.8
W64A × N28	50.2	21.5	111.6	8.7	6.1	11.9
W64A × Oh43	45.2	14.8	65.5	3.5	3.8	4.4
W64A × B14A	47.5	16.7	57.0	7.2	4.4	6.3
W64A × Hy2	44.7	16.6	66.6	6.7	5.6	7.2
Mo17 × Oh07	24.0	8.2	23.4	1.5	1.5	1.5
Mo17 × N28	23.4	12.4	38.0	2.0	1.9	2.0
Mo17 × Oh43	16.8	6.0	14.8	0.9	1.5	1.5
Mo17 × B14A	19.2	6.7	19.1	1.3	1.5	1.5
Mo17 × Hy2	20.9	7.7	19.6	1.1	2.6	2.6
Oh07 × N28	56.4	22.0	112.1	9.4	6.9	12.5
Oh07 × Oh43	36.5	16.8	70.9	2.3	3.0	3.2
Oh07 × B14A	46.8	18.7	74.9	11.3	5.9	10.1
Oh07 × Hy2	46.4	17.2	78.6	6.8	5.1	7.4
N28 × Oh43	38.4	18.4	90.8	3.5	3.5	4.6
N28 × B14A	48.1	21.9	99.6	8.7	4.8	9.0
N28 × Hy2	39.4	20.0	94.9	8.7	7.1	14.6
Oh43 × B14A	46.1	17.0	76.3	1.9	1.8	2.0
Oh43 × Hy2	38.9	15.3	58.4	2.7	2.8	3.0
B14A × Hy2	43.3	20.3	96.6	7.6	6.0	9.4
SE[a]	1.29	0.20	1.40	0.49	9.14	0.28
SE[b]	1.82	0.28	1.97	0.69	0.21	0.39

[a]SE = standard error of any treatment mean.
[b]SE = standard error of the difference between two treatment mean.

failure of the assumptions.

Estimations of the degree of dominance and dominance relations of the parents, determined from F_1 data, are presented in Fig 1-3. The (Vr, Wr) graphs can be interpreted (7,15) as follows: (i) the parabola marks the limits within which the variance-covariance points (Vr, Wr) should lie; (ii) if the regression coefficient of the (Vr, Wr) graph is not significantly different from unity, the gene system can be deduced to be additive without the complication of gene interaction (epistasis); (iii) if dominance is partial, the regression line with b = 1 would pass through the Wr axis above the origin; (iv) the order of the array points on the line indicates the distribution of dominant and recessive genes among the common parents of the arrays. The common parent possessing the most dominant genes has the lowest (Wr, Vr) values, and this point will be closest to the origin. The (Vr, Wr) graphs in Fig. 1 for disease reaction determined by disease ratings showed the slope of regression lines to be not significantly different from 1, indicating no complications

TABLE 2. Mean squares from combining ability analysis of variance for disease reaction in the F_1 generation to *Helminthosporium maydis* race T

Source of variation	d.f.	Mean squares: Disease rating (1-100)	Lesion length (mm)	Lesion area (mm^2)
		cms-T Cytoplasm		
GCA[a]	7	488.07**[a]	88.51**	3039.01**
SCA[b]	20	20.77**	1.76*	92.67**
Residual	54	1.17	0.04	1.37
		N Cytoplasm		
GCA	7	30.55**	11.15**	47.47**
SCA	20	2.35**	0.71**	4.00**
Residual	54	0.09	0.02	0.04

[a]**indicated significant difference, $P < 0.01$.
* indicates significant difference, $P = 0.05$.
[b]GCA = General combining ability.
[c]SCA = Specific combining ability.

of gene interaction (epistasis). Disease resistance in eight parents, each containing *cms*-T and N cytoplasm, was predominantly additive with partial dominance. Inbred Mo17 seemed to have the most dominant genes in both types of cytoplasm, while in N cytoplasm B14A and in *cms*-T cytoplasm W64A had the most recessive genes. The order of the eight array points for N cytoplasm differed from that for *cms*-T cytoplasm except for inbreds Mo17 and Oh43, which were in the same order in both cytoplasms. The slopes of the regression lines for disease resistance determined by both lesion length and lesion area were significantly different from unit slope, suggesting that gene interactions of some arrays (epistasis) are present (Fig 2 and 3). However, these slopes were not significantly different from the slopes of disease rating graphs (for $P=0.05$) which agreed well with a slope of b = 1. The order of array points was quite different among three methods used to determine disease resistance, except for Mo17. Mo17 in both *cms*-T and N cytoplasm had the most dominant genes for both lesion

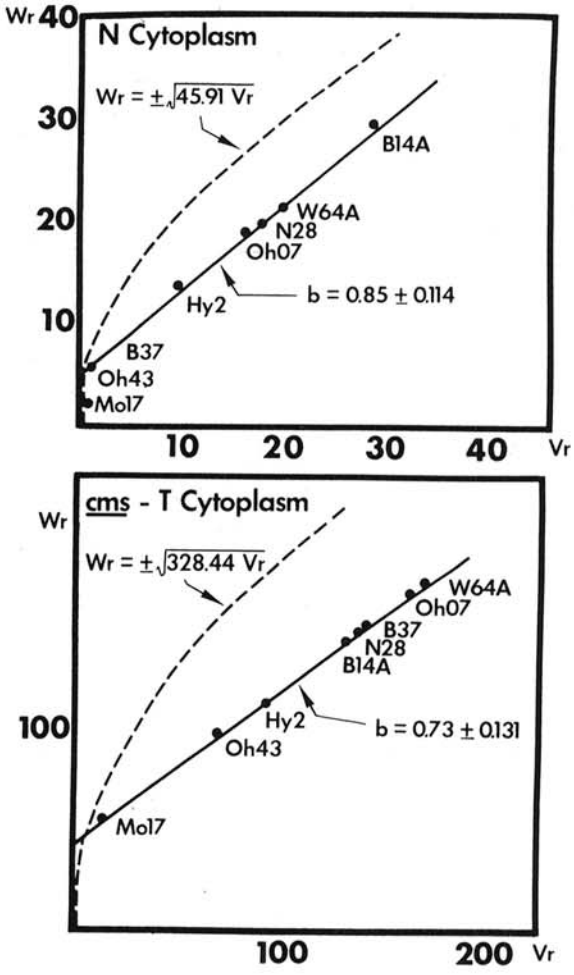

Fig. 1. Variance-covariance graphs for disease rating of eight F_1 parental arrays for corn relative to its resistance to *Helminthosporium maydis* race T. Vr is the variance of all the offspring of each parent in each array (complete row or column), Wr is the covariance of the parents with their offspring in each array of the diallel table.

TABLE 3. Estimates of general combining ability (GCA) effects of parental genotypes in the F_1 generation for disease reaction to *Helminthosporium maydis* race T

Inbred	GCA effects: N Cytoplasm Disease rating (0-100)	N Cytoplasm Lesion length (mm)	N Cytoplasm Lesion area (mm^2)	*cms*-T Cytoplasm Disease rating (0-100)	*cms*-T Cytoplasm Lesion length (mm)	*cms*-T Cytoplasm Lesion area (mm^2)
B37	−1.804	−1.417	−3.329	−1.354	−1.196	−2.833
W64A	7.929	0.450	0.179	1.462	0.875	1.417
Mo17	−19.621	−8.500	−47.862	−3.737	−2.212	−4.050
Oh07	6.429	1.667	8.671	1.512	0.754	1.667
N28	6.012	4.567	31.621	2.429	1.621	3.867
Oh43	−2.587	0.133	1.854	−2.587	−1.212	−2.683
B14A	5.529	1.967	8.787	1.479	0.304	0.767
Hy2	1.887	1.133	5.437	0.796	1.121	1.850
SE[a]	0.625	0.110	0.676	0.174	0.073	0.117

[a]SE = standard error of the difference between two effects.

length and lesion area as was also determined by disease ratings, whereas the parent possessing recessive genes differed, depending on the method used and the types of cytoplasm. The array points of B37 and Oh43 in N cytoplasm were in the same order on regression lines and showed a similar degree of dominance for all three methods, while in *cms*-T cytoplasm the positions of the points were changed.

DISCUSSION.—Results indicate that resistance in corn to race T in the inbreds and hybrids studied is mainly cytoplasmic and partially nuclear-genic. The *cms*-T cytoplasm-specific pathogenicity of race T is well known. All inbreds and F_1 hybrids having N cytoplasm exhibited a higher degree of resistance to race T than their counterparts in *cms*-T cytoplasm. Currently, breeding for additional resistance in N cytoplasm corn to race T may not have any practical value, because all current N cytoplasm hybrids seem to have adequate resistance to race T in the field.

Differences in disease reactions among parental inbreds and F_1 generations having *cms*-T cytoplasm were highly significant. Some crosses in *cms*-T cytoplasm between resistant inbreds showed a degree of resistance which was similar to disease reactions of crosses between the most susceptible inbreds having N cytoplasm. For instance, reactions of resistant Mo17^T × Oh43 or Mo17^T × B37 crosses were similar to those of Oh07 × N28 or W64A × N28 crosses. This indicates that *cms*-T hybrids can be produced that have a relatively high degree of resistance to race T. In this study, Mo17 was the only inbred having this level of resistance. A few other inbreds having *cms*-T cytoplasm are currently known to exhibit resistant reactions similar to the reaction of Mo17^T (Lim, *unpublished*).

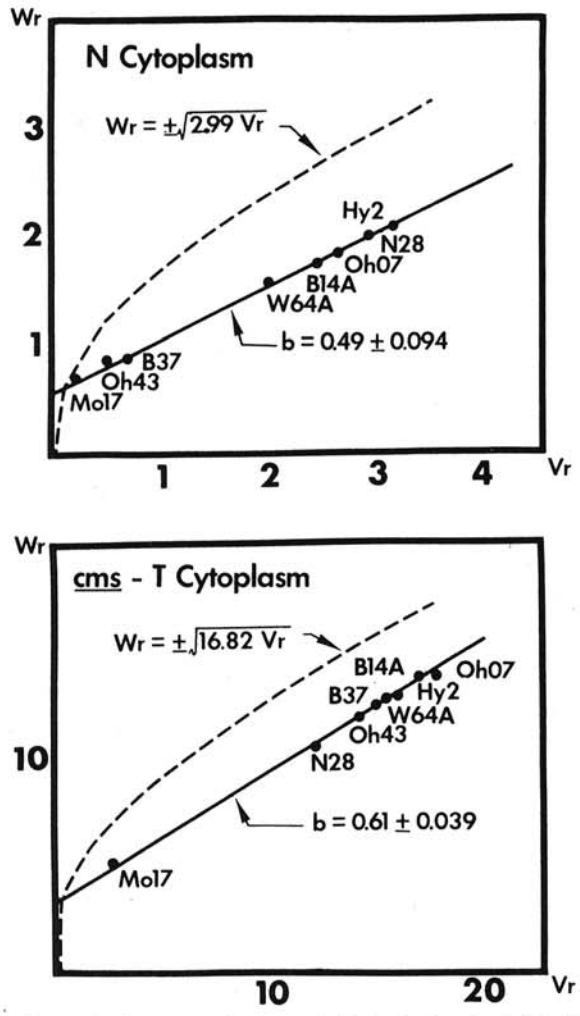

Fig. 2. Variance-covariance graphs for lesion length of eight F_1 parental arrays for corn relative to its resistance to *Helminthosporium maydis* race T. Vr is the variance of all the offspring of each parent in each array (complete row or column), Wr is the covariance of the parents with their offspring in each array of the diallel table.

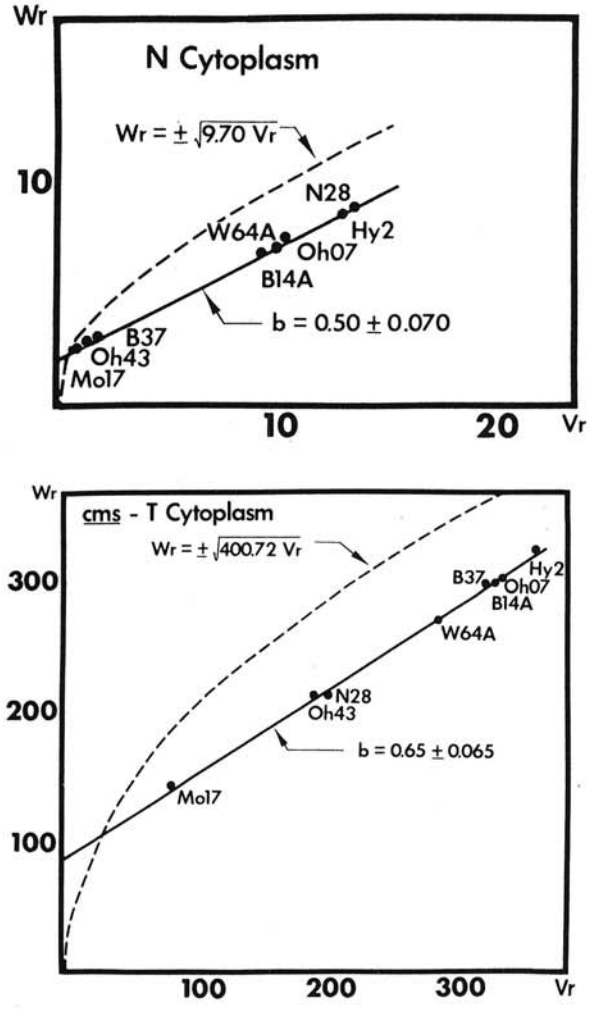

Fig. 3. Variance-covariance graphs for lesion areas of eight F_1 parental arrays for corn relative to its resistance to *Helminthosporium maydis* race T. Vr is the variance of all the offspring of each parent in each array (complete row or column), Wr is the covariance of the parents with their offspring in each array of the diallel table.

The three methods used to determine disease reactions generally showed similar results as is shown by the highly significant correlations. The disease rating based on the entire plant requires less time, is as reliable as measurements of lesion length or lesion area, and therefore, would be the most practical method for evaluating disease reaction.

The diallel analysis for combining ability indicated that genetic variation for disease resistance in corn plants having both *cms*-T and N cytoplasm was associated with highly significant GCA effects. If this observation holds true among elite lines, it should be possible to develop relatively resistant corn hybrids having *cms*-T cytoplasm from crosses involving the most resistant corn inbreds. SCA effects were also significant, but were much less so than GCA effects. Generally, SCA effects were found in crosses of susceptible lines. The (Vr, Wr) graphic analysis also indicated that nuclear gene resistance to race T has additive gene effects, and is partially dominant. The evidence of gene interactions in both graphs of lesion length and area could have resulted from specific interactions in crosses of susceptible genotypes as is in the case of SCA effects. The slopes of both graphs were not significantly different from the slopes of disease ratings for both types of cytoplasm. As the slopes of disease ratings agreed well with unity slope, evidence for suggesting gene interaction in the expression of resistance is very small. Estimations of the degree of dominance showed that resistant inbred Mo17 in both types of cytoplasm has the most dominant genes. Mo17 in *cms*-T cytoplasm was quite far removed from other genotypes along the regression line in the direction of greater dominance, suggesting that resistance was largely dominant and may be controlled by only a few genes. The order of array points for intermediately resistant inbreds Oh43 and B37 having N cytoplasm, was similar for all three methods used to determine disease reaction. However, with *cms*-T inbreds, the order of all array points except Mo17 changed, depending on the method of disease evaluation. These differences of order were probably due to variations among different methods, or possibly due to the amount of infection, lesion length, and lesion area each being determined by separate genetic systems in corn plants. In any event, it suggests that the finer detail of order of points along the regression line is not significant in breeding for resistance, particularly in the case of *cms*-T corn plants. The use of plants carrying the restoration factor (*Rf*) would greatly facilitate incorporations of a high degree of resistance into elite lines having *cms*-T cytoplasm by allowing for effective selfing, sib-crossing, or backcrossing. Recurrent selection could also be practiced in such populations having a degree of resistance.

It should be emphasized that results in this study only give information about those genes which control disease resistance and which are segregating in crosses of the eight parent lines used. Further exploration of parental combinations will be needed to obtain a high degree of resistance in corn plants having *cms*-T cytoplasm suitable for use in the Corn Belt.

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