Disease Response of Sweet Corn Hybrids Derived from Dent Corn Resistant to Maize Dwarf Mosaic Virus

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Contribution 1247, Department of Plant Pathology, authorized for publication 24 March 1981 as Journal Series Paper 6218 of The Pennsylvania Agricultural Experiment Station.

Accepted for publication 24 August 1981.

ABSTRACT

Anzola, D., Romaine, C. P., Gregory, L. V., and Ayers, J. E. 1982. Disease response of sweet corn hybrids derived from dent corn resistant to maize dwarf mosaic virus. Phytopathology 72:601-604.

Six sweet corn hybrids developed from a maize dwarf mosaic virus (MDMV)-resistant dent corn inbred were examined under greenhouse conditions for resistance to MDMV strains A and B. All hybrids responded to virus inoculation with a reduced rate of symptom development and some hybrids showed a lower disease incidence compared to plants with susceptible dent or sweet corn germ plasm. The altered disease response was most apparent in the sweet corn hybrid, Cr288 × Cr290. This hybrid developed symptoms in 14–28 days with a 50 to 100% disease incidence whereas susceptible hybrids invariably developed a 100% disease incidence within 7 days. In addition, at an early stage of infection, the level of virus was significantly lower in the six hybrids when compared to susceptible hybrids. The virus-host interaction characteristic of this sweet corn germ plasm is known to be correlated with the expression of disease resistance in dent corn. The results suggest that these hybrids might serve as a potential source of resistance to MDMV.

Maize dwarf mosaic virus (MDMV) in corn (*Zea mays* L.) is known to occur throughout the United States (4) and recent surveys indicate that it is steadily increasing in geographical distribution (3,11). For the most part, maize dwarf mosaic disease (MDM) is not considered a major problem in dent corn production due to the availability of resistant cultivars. The disease remains a more serious threat in sweet corn, however, because resistance has not been incorporated into acceptable hybrids.

Expression of disease resistance in dent corn has been shown to be correlated with specific features of the virus-host interaction. For example, resistance is associated with a delay in the appearance of symptoms, a lower disease incidence, and frequently, a reduced virus concentration in the plant (5–7). Kuhn and Smith (7) have formulated a disease index system to evaluate resistance in corn which considers both the rate of symptom development and the disease incidence. They observed that the index values obtained from greenhouse studies with over 500 dent corn lines were closely correlated with disease incidence and yield of diseased plants in the field.

In the present study, we have evaluated the resistance of sweet corn hybrids derived from crosses of sweet corn germ plasm with MDMV resistant dent corn germ plasm. These hybrids were thought to possess resistance since they developed little or no disease during natural spread of MDMV. An obvious shortcoming of a field evaluation which relies on natural infection is that the frequency of inoculation by the aphid vector represents an unknown variable. In an attempt to more precisely characterize and quantify the apparent resistance of these hybrids, we have mechanically inoculated plants in the greenhouse and examined those components of the virus-host interaction which are known to be correlated with disease resistance.

MATERIALS AND METHODS

Experimental sweet corn hybrids, Cr288 × Cr289, Cr288 × Cr290, Cr283 × Cr290, Cr284 × Cr289, Cr286 × Cr289, and Cr296 × Cr290, were obtained from the breeding program of Crookham Co., Caldwell, ID 83605. These hybrids were made by crossing inbred lines derived from crosses of the dent corn inbred line, B68, with the sweet corn hybrid, Silver Queen. The original cross of B68 × Silver Queen was made by V. E. Gracen, Department of Plant Breeding, Cornell University, Ithaca, NY 14853. These hybrids were evaluated for resistance relative to either the commercial sweet corn hybrid, Spring Gold (Harris Seed Co., Rochester, NY 14603) or the susceptible dent corn hybrid SX-60 (P-A-G, Aurora, IL 60507).

Propagation and maintenance of plants. Kernels were sown in 10-cm-diameter plastic pots containing equal volumes of steam-pasteurized soil, peat, and perlite. Plants were grown in a greenhouse at 21 ± 2 C supplemented with ~9 klux of fluorescent illumination for a 16-hr photoperiod.

Virus culture. Strains A and B of MDMV were provided by C. W. Boothroyd, Cornell University, Ithaca, NY 14853, and were propagated in *Sorghum bicolor* L. 'Sart' and 'Rio,' respectively.

Mechanical transmission. Inoculum for the transmission of MDMV was prepared by homogenizing symptomatic systemically infected leaves of cultivars Sart and Rio sorghum plants with a mortar and pestle in chilled 10 mM phosphate buffer, pH 7.2 (phosphate buffer) (1 ml/g tissue). Inoculum containing both virus strains was prepared by mixing equal volumes of infected leaf extracts from cultivars Sart and Rio. For inoculation, extracts were rubbed with a sterile cotton-tipped applicator stick onto the youngest leaf of plants in the two- to four-leaf stage. Leaves were dusted with 30-μm (500-mesh) Carborundum prior to inoculation and rinsed with water immediately following inoculation.

Determination of MDMV. An infectivity assay and enzyme-linked immunosorbent assay (ELISA) were used to determine the amount of virus in the corn plants at various time intervals after inoculation. Test samples for virus analysis by infectivity test consisted of a composite of 1-g tissue samples taken from the youngest leaf of each of three to five plants. Plants were assayed individually for virus by ELISA. A half-leaf sampling procedure was used for experiments in which the plants were to be analyzed by both methods.

Infectivity assay. Leaf tissue was homogenized with a mortar and pestle in phosphate buffer (1 ml/g tissue). The homogenate was filtered through two layers of cheesecloth and further diluted to 10^{-1}, 10^{-2}, and 10^{-3} with phosphate buffer. Three plants of SX-60 corn were mechanically inoculated with each dilution of the extract

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0031-949X/82/06060104/$03.00/0

and observed every other day for 20 days for symptom development. The amount of infectious virus in the plants is expressed as an "infectivity index," which is based on the number of SX-60 plants infected at each dilution, the dilution factor, and the incubation period for symptom expression. The index was calculated by multiplying the accumulative total of diseased plants at each dilution for the entire 20-day period by the negative log of the dilution factor and adding these products together for each dilution tested (9).

ELISA. Antisera for MDMV-A and MDMV-B were provided by D. T. Gordon, Ohio Agricultural Research and Development Center, Wooster, OH 46691. The procedure for the preparation of the γ-globulin and the protocol for the ELISA test were essentially as described by Clark and Adams (2) and Voller et al (14). ELISA test parameters consisted of a coating γ-globulin concentration of 1 μg/ml, a 10^{-3} dilution of the enzyme-conjugated γ-globulins, and an enzyme substrate reaction period ranging from 0.5 to 1.5 hr. One gram of leaf tissue was homogenized with a mortar and pestle in 10 ml of 0.02 M phosphate, 0.15 M NaCl, and 0.02% NaN_3, pH 7.4, containing 0.05% Tween-20 (Sigma Chemical Co., St. Louis, MO 63178) and 2% polyvinyl pyrrolidone (40,000 MW, Sigma). The virus-antibody complex was assessed by quantifying the yellow-colored reaction end-product by colorimetry at 405 nm ($A_{405\ nm}$). Statistical analysis was performed using Duncan's least significant difference test (DLSD).

RESULTS

Single-cross hybrid response to inoculation with MDMV-A and MDMV-B. The six sweet corn hybrids that were derived from a resistant dent corn inbred exhibited a delay in the appearance of symptoms and some hybrids showed a lower disease incidence when compared to susceptible SX-60 dent corn following inoculation with a mixture of MDMV strains A and B (Table 1). At 7 days postinoculation, all of the SX-60 plants had developed typical MDMV symptoms whereas the single-cross hybrids had disease incidence ranging from 0 to 75%. Symptoms failed to develop in some inoculated plants of hybrids Cr288 × Cr290, Cr288 × Cr289, and Cr283 × Cr290 when observed for up to 35 days after inoculation. The rate of symptom development and disease incidence was most dramatically reduced in hybrid Cr288 × Cr290. Throughout this study we observed that susceptible hybrids Spring Gold and SX-60 consistently developed a 100% disease incidence within 7 days, while symptoms in Cr288 × Cr290 appeared between 14 and 28 days with a 50 to 100% disease incidence.

TABLE 1. Disease incidence in sweet corn hybrids derived from maize dwarf mosaic virus (MDMV)-resistant dent corn germ plasm inoculated with a mixture of strains MDMV-A and MDMV-B[a]

Corn line	Disease incidence[b] at: Day 7	Day 21	Day 35
Cr288 × Cr289	1/12	4/12	9/12
Cr288 × Cr290	0/6	1/6	3/6
Cr283 × Cr290	1/6	4/6	5/6
Cr284 × Cr289	4/6	6/6	6/6
Cr286 × Cr289	9/12	12/12	12/12
Cr286 × Cr290	1/6	6/6	6/6
SX-60 dent corn	6/6	6/6	6/6

[a] Plants were mechanically inoculated with strains MDMV-A and MDMV-B. At 7, 21, and 35 days postinoculation, the number of the plants with disease symptoms was determined.
[b] Expressed as the number of plants with symptoms divided by the number of plants inoculated.

The rate of virus accumulation was significantly lower in some of the single-cross hybrids compared to susceptible SX-60 as judged by the reduced ability to detect MDMV-A and MDMV-B antigens by ELISA at an early stage in infection (Table 2). The maximal concentration of virus was detected at 7 days in SX-60 but was usually not reached until 35 days in the single-cross hybrids. Symptom development was delayed to the greatest extent in Cr288 × Cr290 and this was correlated with the most reduced rate of virus accumulation.

Cr288 × Cr290 response to inoculation with MDMV-A or MDMV-B. Further characterization of the virus-host interaction in sweet corn derived from dent corn was conducted with the hybrid Cr288 × Cr290. Since a mixed inoculum was used in the previous experiments, it was not possible to evaluate this hybrid for resistance to MDMV incited by strains A and B, separately. Plants of Cr288 × Cr290 and the commercial sweet corn hybrid, Spring Gold, were therefore inoculated with MDMV-A or MDMV-B. Spring Gold was used for comparison in these experiments because it was judged to yield well even though it readily develops disease symptoms when inoculated with MDMV (1). Symptoms developed more rapidly in this experiment than in the previous ones. The most probable explanation is this experiment was performed in late spring when environmental conditions were more favorable for symptom expression. The development of symptoms was significantly delayed in Cr288 × Cr290 relative to Spring Gold following inoculation with either virus strain (Table 3). Symptoms in Cr288 × Cr290 were first observed at 15 days after inoculation in 10% of the plants and in 60–80% at 30 days. In contrast, Spring Gold exhibited a disease incidence of 100% within 5 days of inoculation with either virus strain.

The rate of MDMV-A accumulation was also significantly reduced in Cr288 × Cr290 although the pattern of synthesis was similar to that in hybrid Spring Gold (Table 4). MDMV-A could not be detected in Cr288 × Cr290 at 7 days by infectivity assay and ELISA but had attained a near maximal level in Spring Gold at this time. The peak period for the accumulation of MDMV-A occurred in both hybrids at 14 days after inoculation which was followed by a

TABLE 2. Determination of the viral antigen concentration in sweet corn hybrids derived from maize dwarf mosaic virus (MDMV)-resistant dent corn germ plasm inoculated with a mixture of strains MDMV-A and MDMV-B[a]

Corn line	ELISA value (A_{405nm})[bc] at: Day 7 MDMV-A	Day 7 MDMV-B	Day 21 MDMV-A	Day 21 MDMV-B	Day 35 MDMV-A	Day 35 MDMV-B
Cr288 × Cr289	0.13	0.02	0.45	0.28	0.96	0.23
Cr288 × Cr290	0.13	0.00	0.09	0.16	0.42	0.35
Cr283 × Cr290	0.26	0.00	0.16	0.46	1.04	0.44
Cr284 × Cr289	1.03	0.93	0.41	0.57	1.16	0.59
Cr286 × Cr289	0.10	0.87	0.62	0.62	1.04	0.59
Cr286 × Cr290	0.55	0.22	0.52	0.40	1.17	0.55
SX-60 dent corn	1.18	0.96	0.56	0.43	0.82	0.48
DLSD[d]	0.14	0.10	0.13	0.13	0.26	0.12

[a] Plants were mechanically inoculated with strains MDMV-A and MDMV-B and the concentrations of MDMV-A and MDMV-B antigens were determined by ELISA at 7, 21, and 35 days postinoculation.
[b] Absorbance values are the means of six plants and two experiments.
[c] Absorbance values for uninoculated plants ranged from 0.01 to 0.27.
[d] Duncan's least significant difference, $P = 0.05$.

rapid decline in the amount of virus detectable thereafter. The level of infectious virus and viral antigen detected during this peak period was comparable but significantly higher in Spring Gold than in the hybrids. Similarly, a reduced rate of accumulation of MDMV-B was also detected in Cr288 × Cr290.

Disease symptoms in the single-cross hybrids. Discrete longitudinal chlorotic streaking symptoms developed in the leaves of the six single-cross hybrids following single infections with a mixture of MDMV-A and MDMV-B as well as in Cr288 × Cr290 following infection with either virus strain. The streaking symptoms remained confined to the areas of the leaves that were initially affected. The susceptible hybrids Spring Gold and SX-60, on the other hand, showed the typical diffuse yellow mosaic which became distributed uniformly throughout the leaf.

DISCUSSION

Resistance in corn to MDMV is expressed in several ways. Kuhn and Smith (7) observed resistance to be correlated with the length of time from inoculation to symptom development. They observed that a 6–10 day incubation period occurred in susceptible dent corn hybrids compared to 16–28 days in resistant hybrids. The same relationship between incubation period and resistance apparently exists in dent corn inbreds as well (10,12). Resistant corn lines are also more difficult to infect both mechanically (7) and through aphid feeding (8). Mechanical inoculation of resistant corn lines under greenhouse conditions usually results in a disease incidence of about 50%. This is generally regarded as an increase in the rate of aborted infections and not a variation in susceptibility within the population.

Dent corn varieties with resistance to MDMV are now used commercially, but relatively little progress has been made in developing resistant sweet corn. The sweet corn hybrids evaluated in this study were derived from a single cross with resistant dent corn and were thought to possess some resistance to the disease. We have determined that the virus-host relationship of these hybrids is similar to that observed with resistant dent corn lines. All of the single-cross hybrids exhibited a delay in the appearance of symptoms and some hybrids showed a lower disease incidence compared to susceptible corn. This response was most evident in Cr288 × Cr290. Using Kuhn and Smith's (7) disease index system for evaluating resistance, we have calculated disease index values (130–170) for this hybrid, which would classify it as resistant to either MDMV strain A or B in the field. Our empirical prediction of field resistance is corroborated by the low incidence of disease that has been observed in this hybrid during natural outbreaks of MDMV.

The dynamics of MDMV replication in the host has been investigated with respect to the expression of disease resistance in dent corn. Kuhn and Smith (7) reported a high correlation between resistance and the ability of the host to reduce the rate of virus accumulation, particularly in the early stages of infection. Tu and Ford (13) and Jones and Tolin (5) found comparable levels of virus in both susceptible and resistant dent corn lines. We observed a lower rate of virus synthesis in the single-cross hybrids, as judged by a reduced ability to detect virus shortly after infection, although the maximal level attained was often comparable to susceptible hybrids. It should be mentioned that in analyzing the hybrids for virus, inoculated plants were sampled without regard to symptom expression. Thus, it may be argued that the lower virus levels simply reflect the fact that not all of the sampled plants were infected. However, this does not appear to be a valid explanation since significantly reduced ELISA values were observed at 7 days after inoculation in hybrids that eventually developed a 75 to 100% disease incidence (Tables 1 and 2). Further, ELISA values at 7 days for individual inoculated plants of most hybrids were consistently lower than susceptible hybrids.

The precise host mechanism that acts to limit the rate of virus synthesis in the hybrids remains unclear. It has been previously suggested that the mechanism of resistance in dent corn involves an effect on virus evasiveness. Resistance in the dent corn hybrid Illinois A was related to a restricted systemic movement of virus out of the inoculated leaves (13). Jones and Tolin (5) elucidated a similar mechanism in the hybrid T8 × 07B but the effect was on the cell-to-cell movement of the virus. They observed that symptoms in T8 × 07B and several other resistant inbreds and hybrids appeared as a longitudinal chlorotic streaking in the leaves. Symptoms of this nature developed in the six experimental single-cross hybrids we have examined and may possibly implicate a similar mechanism of resistance.

In summary, we have characterized the virus-host relationship in sweet corn hybrids developed from resistant dent corn to often

TABLE 3. Disease incidence in the sweet corn hybrids Cr288 × Cr290 and Spring Gold inoculated with either maize dwarf mosaic virus (MDMV) strains MDMV-A or MDMV-B[a]

	Disease incidence[b] at:									
	Day 5		Day 15		Day 20		Day 25		Day 30	
Inoculum	Cr288 × Cr290	Spring Gold	Cr288 × Cr290	Spring Gold	Cr288 × Cr290	Spring Gold	Cr288 × Cr290	Spring Gold	Cr288 × Cr290	Spring Gold
MDMV-A	0/10	10/10	1/10	10/10	2/10	10/10	6/10	10/10	6/10	10/10
MDMV-B	0/10	10/10	1/10	10/10	3/10	10/10	8/10	10/10	8/10	10/10

[a] Plants of the hybrid derived from Cr288 × Cr290 and hybrid Spring Gold were mechanically-inoculated with either MDMV-A or MDMV-B. The number of the plants with disease symptoms was determined at 5, 15, 20, 25, and 30 days postinoculation.
[b] Expressed as the number of plants with symptoms divided by the number of plants inoculated.

TABLE 4. Determination of the virus antigen and concentration in the sweet corn hybrids Cr288 × Cr290 and Spring Gold inoculated with maize dwarf mosaic virus (MDMV) strains MDMV-A or MDMV-B[a]

	Time after inoculation							
	Day 7		Day 14		Day 21		Day 35	
Inoculum/Corn Line	Infectivity Index	ELISA[b]	Infectivity Index	ELISA	Infectivity Index	ELISA	Infectivity Index	ELISA
MDMV-A/Cr288 × Cr290	0	0.13	66	1.06	8	0.63	10	0.12
MDMV-A/Spring Gold	40	0.81	64	1.35	23	0.59	24	0.27
MDMV-B/Cr288 × Cr290	ND[c]	0.02	ND	0.19	ND	0.19	ND	0.22
MDMV-B/Spring Gold	ND	0.25	ND	0.40	ND	0.32	ND	0.34

[a] Plants of hybrid Cr288 × Cr290 and hybrid Spring Gold were mechanically inoculated with strains MDMV-A or MDMV-B. The concentration of MDMV-A was determined by infectivity assay and ELISA and that of MDMV-B by ELISA at 7, 14, 21, and 35 days postinoculation.
[b] Expressed as mean absorbance at 405 nm. Duncan's LSD = 0.13 at $P = 0.05$ for all values shown in the table. All values represent a mean of three replications. At each time interval, the mean absorbance values for uninoculated plants ranged from 0.02 to 0.09.
[c] Not determined.

involve a delay in the appearance of symptoms, a lower disease incidence, and a reduced rate of virus accumulation. Since these characteristics are known to be correlated with the expression of resistance in dent corn, it seems reasonable to conclude that these sweet corn hybrids also possess resistance to MDMV. Our findings corroborate the apparent resistance displayed by these hybrids during natural spread of MDMV in the field and thereby further establish the potential usefulness of this genetic material as a source of resistance.

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