Evaluation of Maize Germ Plasm for Resistance to Maize Rayado Fino Virus

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ABSTRACT

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Maize rayado fino virus (MRFV) causes severe yield losses in maize (*Zea mays*) in Latin America with reductions of 40–50% on indigenous genotypes and 100% in some new cultivars. A collection of 20 different maize genotypes were obtained from the Center for the Improvement of Maize and Wheat (CIMMYT, México), Estación Experimental Fabio Baudrit (EEFB-Alajuela), and the Consejo Nacional de la Producción (CNP-Costa Rica). Enzyme-linked immunosorbent assay (ELISA) and symptom severity were used to evaluate the responses of the maize genotypes to MRFV infection under greenhouse conditions. Two genotypes from the CIMMYT germ plasm collection (accession nos. 2980-93 and 3974) showed mild and delayed symptoms and low MRFV concentrations as determined by ELISA.

Maize rayado fino virus (MRFV) is the type member of the marafivirus group of plant viruses, which includes oat blue dwarf virus (OBDV) and Bermuda grass etched-line virus (BELV) (18,24). Virions are isometric particles ca. 30 nm in diameter, contain a single-stranded RNA of 2×10^6 mol. wt., and have two distinct capsid proteins of 2.2 and 2.8×10^4 kDa (9,11,13,23). MRFV is primarily transmitted by the leafhopper *Dalbulus maidis* (De Long & Wolcott) in a persistent manner and is propagative in its vector (12,14,29,30,32). Although *D. maidis* is the main vector, other leafhoppers, *D. elimatus* (Ball), *Baldulus tripsaci* (Kramer & Whitcomb), *Stirellus bicolor* (Van Duzee), and *Graminella nigrifrons* (Forbes), transmitted the virus under experimental conditions (30).

MRFV is the only maize-infecting virus existing solely in the center of origin of maize (11,14–17). MRFV most often occurs in plants co-infected with mollicutes (corn stunt spiroplasma and maize bushy

stunt phytoplasma [formerly mycoplasma-like organism]) that are transmitted by the same leafhopper vector (8,28). Although restricted to the Americas to date, MRFV is widespread and is becoming increasingly important in tropical areas (3,15,21). MRFV may cause 10–40% loss of the mature ear weight in local genotypes and nearly 100% mature ear weight loss in recently introduced cultivars (1,18,33). Lethal effects have been reported in Colombia and El Salvador (13). Susceptibility among maize cultivars to MRFV is variable, and symptoms consist of numerous short and long chlorotic stripes, wilting of young plants, general chlorosis, and stunting. Symptoms of MRFV are most noticeable at the base of leaves, with an increase in severity in the younger leaves (12,30).

There are no satisfactory control measures for MRFV, and no immune genotypes have been detected among representative races or germ plasm of maize (18,37). Locally adapted Central American landraces appear in general to be more tolerant to the virus and to show lower degrees of incidence within a population (17). Identifying new sources of resistance and finding suitable methods for characterizing MRFV resistance are very important for control of the disease.

Symptom severity is a common trait used to screen germ plasm for resistance to plant viruses. However, symptom severity is not always proportional to viral replication within the host and is influenced by environmental conditions (38,40). Enzyme-linked immunosorbent assay (ELISA) in conjunction with symptomatology has been used by others to evaluate wheat species for resistance to wheat streak mosaic virus (19,36). Here, we report the use of ELISA

and symptomatology to evaluate the response of 20 different genotypes of maize to MRFV infection.

MATERIALS AND METHODS

Host plants. Maize genotypes were obtained from the International Center for the Improvement of Maize and Wheat (CIMMYT-México). In addition, two hybrids were obtained from Estación Experimental Fabio Baudrit (EEFB-Alajuela, Universidad de Costa Rica) and three maize cultivars were obtained from the National Production Council (CNP, Consejo Nacional de la Producción, San José, Costa Rica) (Table 1). All seeds were assumed to be homogeneous. Genotype representatives of the different races of maize were selected according to the information provided by Bretting et al. (4).

Planting method and inoculation. The experimental design in the first evaluation for resistance was a randomized complete block with the 20 different genotypes and five replications grown under greenhouse conditions (20). Two seeds were sown per 6-in. pot and later plants were thinned to one per pot before virus inoculation. Ten individual plants were used for each replication: five plants were exposed to *D. maidis* (previously exposed to MRFV-infected plants; 10–12 leafhoppers were applied per plant, as determined in preliminary studies to be appropriate optimum leafhopper density) and kept at the CIBCM greenhouse, and five plants were exposed to nonviruliferous *D. maidis* and kept in similar conditions at the Facultad de Agronomía de Universidad de Costa Rica. The leafhoppers were placed in the whorl of 3- to 4-day-old individual plants and were kept caged on the plants for 3 days (transmission period, TP), after which the leafhoppers were removed and the plants fumigated with Tamaron. The plants were then kept under 12 h light with the temperature fluctuating between 20 and 30°C. Fluorescent lighting (10,800 lux) was used to supplement natural light. A total of 1,000 plants were analyzed in this first evaluation.

In the second evaluation, selected genotypes identified as the most “susceptible” and “resistant” in the first evaluation were further tested to examine the role of insect transmission in determining the resistance phenotype. Thirty susceptible plants (15 each of Guarare and HC-78) were exposed

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to 10–12 viruliferous insects per plant as in the first evaluation. After a TP of 3 days on susceptible genotypes, the insects were serially transferred at 3-day intervals to the resistant genotypes (2980-63 or 3974) for a second TP and then to the susceptible genotypes (Guarare or HC-78) for a third TP.

Inoculum preparation. An isolate of MRFV was obtained from the Guapiles location of Costa Rica (Guapiles-MRFV isolate) and maintained on a susceptible corn hybrid (HC-78), with frequent transfer. Nymphs of *D. maidis* from an infected colony were used to prepare the viruliferous leafhoppers following the procedure of Gámez (12).

Symptomatology assay. Symptom ratings were taken every 2 days and the fourth leaf was photographed 15 days after the TP. Preliminary studies showed that the response of the fourth leaf was representative of the whole plant to MRFV infection. Highly susceptible plants were monitored daily. Ratings of the fourth leaf were based on a five-point scale where 0 = absence of symptoms; 1 = presence of chlorotic dots encompassing 0–25% of the leaf area; 2 = presence of light chlorotic dots, covering between 25 and 50% of the leaf area; 3 = presence of continuous stripes along the axis of the leaves, covering between 50 and 75% of the leaf area; and 4 = presence of intense symptoms with necrosis of the leaf tissue, especially along margins and tip of the leaves, affecting 75–100% of the leaf area.

Tissue samples. Fifteen days after completion of the TP, samples from the proximal section of leaf 4 were removed with a scissor, finely cut, and stored in a small plastic bag at –20°C for use in ELISA. Because of practical limitations, samples were taken only from three randomly selected plants in three of the repetitions of inoculated plants within each accession in the first evaluation period, i.e., 180 plants from groups exposed to viruliferous and nonviruliferous insects were tested by ELISA. In the second evaluation, only plants that had symptoms in the first and third TP and no symptoms in the second TP were tested by ELISA.

Antiserum and immunoglobulin preparation. MRFV was purified and antiserum produced following previously published procedures (23,31). The immunoglobulin G (IgG) fraction was isolated by exchange column chromatography with protein A and quantified by the Lowry method (25). Alkaline phosphatase (type VII-S, Sigma Chemical Co., St. Louis, MO) was coupled to IgG with glutaraldehyde (2).

Double-antibody sandwich ELISA (DAS-ELISA). For DAS-ELISA, 500 mg of tissue were taken from the bag and homogenized in 2.5 ml of PBSAT, pH 7.2 (137 mM NaCl, 3 mM KCl, 10 mM Na₂HPO₄, 2 mM KH₂PO₄), containing 4% BSA and 0.05% Tween 20 (6) using a me-

chanical homogenizer. The ground samples were placed at –20°C for long-term storage. The samples were thawed and diluted 1:10, 1:100, and 1:1,000 in healthy plant sap for ELISA.

Plates were coated with a 1:500 dilution of MRFV-specific IgG (1 mg/ml) in carbonate buffer, pH 9.6, and the secondary antibody was alkaline phosphatase-conjugated IgG (1 mg/ml, Sigma) at a 1:400 dilution, both of which proved to be optimal in a previous evaluation. After incubation of the plates containing primary antibody for 2 days at 4°C, the wells were washed with 200 µl of PBSAT, and 200 µl of the diluted samples were added to the wells and the plates were incubated overnight at 4°C. After addition of the secondary antibody, plates were incubated for 2 h at 37°C. Finally, 200 µl of a 0.6 mg/ml solution of *p*-nitrophenyl phosphate (Sigma) were added to each well and color development was allowed to continue for 2 h at 37°C. ELISA Nunc (Inter Med., Denmark) microtiter plates were used and reactions were recorded at 405 nm in a MR700 Dynatech spectrophotometer (Dynatech, UK). Five-fold serial dilutions of purified MRFV capsid protein, diluted in healthy plant extracts, were used in all plates as a control. The readings were carried out at 1.5, 4, and 12 h after addition of the substrate; however, in most cases results were obtained in 1.5 h. In cases of doubt, the reading after 4 h was compared to the reading at 1.5 h.

A sample was judged as positive when the reading was greater than the average

absorbance value of a healthy plant extract reaction plus three standard deviations (31). The detection threshold was 1 ng of MRFV capsid protein in PBSAT. In comparison, extracts from healthy maize plants mixed with MRFV capsid protein diluted in PBSAT showed small differences in absorbance values at 405 nm; therefore, these values were used to establish a calibration curve that was linear between 1 and 40 ng of viral capsid protein (data not shown). Dilution of the plant samples was done to get a reading in the linear portion of the curve. The value obtained was then converted with the appropriate dilution factors to estimate the concentration of MRFV in samples; a formula derived from a correlation between the capsid protein concentration and absorbance at 405 nm was used.

Data analysis. Statistical analyses were performed at CIBCM, Costa Rica, and repeated at USDA/ARS, Beltsville, MD, using statistical packages Minitab (PC-Dos compatible), Geno.SAS (PC/SAS version 6.04), Statgraphic (PC-DOS compatible), and Statview (Macintosh). ANOVA, least statistical differences (LSD), Tukey, and linear regression analyses were performed to analyze the response of the different genotypes to MRFV infection (35).

RESULTS

Symptomatology distribution and capsid protein concentrations during the first evaluation. In the first evaluation, most genotypes developed chlorotic spots and streak symptoms within 2 to 3 days

Table 1. Ratings of different maize genotypes to maize rayado fino virus (MRFV) infection as determined by symptom severity and ELISA during the first evaluation

Genotype	Race (Source ^w)	Symptom Severity ^x	ELISA ^y
			(ng of CP-MRFV/mg tissue)
HC-78	cv. commer (CNP)	2.88 ± 1.05 a ^z	280.35 ± 200.85 abc ^z
5393	Chande 9 (CIMMYT)	2.64 ± 1.49 ab	118.27 ± 116.51 bc
Guarare	Hybrid (EEFB)	2.60 ± 1.12 ab	347.15 ± 299.56 ab
9823	Canill 9 (CIMMYT)	2.44 ± 1.04 bc	272.39 ± 170.17 abc
9063	Cannil (CIMMYT)	2.44 ± 1.38 bc	177.23 ± 179.84 abc
HC-43	cv. commer. (CNP)	2.28 ± 1.27 bcd	144.60 ± 126.93 bc
5431	Tuxpen (CIMMYT)	2.28 ± 1.27 bcd	325.86 ± 295.20 ab
3190	Costen 9 (CIMMYT)	2.28 ± 1.14 bcd	277.92 ± 193.04 abc
9887	Cubaam 9 (CIMMYT)	2.16 ± 1.25 cde	196.87 ± 193.92 abc
3195	Costen 9 (CIMMYT)	2.12 ± 1.01 cdef	244.78 ± 286.66 abc
2490	Tuson 9 (CIMMYT)	2.12 ± 1.05 cdef	166.81 ± 179.84 abc
9053	Chande 9 (CIMMYT)	2.08 ± 1.15 cdef	286.17 ± 261.33 abc
B-833	cv. commer. (CNP)	1.96 ± 1.24 def	152.84 ± 181.67 bc
3201	Puya 9 (CIMMYT)	1.88 ± 1.16 efg	253.98 ± 211.27 abc
2445	Ficuba 9 (CIMMYT)	1.88 ± 1.30 efg	280.96 ± 338.38 abc
2435	Crioll (CIMMYT)	1.84 ± 1.06 efg	432.45 ± 692.14 a
RPM-89	Hybrid (EEFB)	1.76 ± 1.16 fg	324.86 ± 272.51 ab
3175	Comun 9 (CIMMYT)	1.56 ± 1.04 g	104.93 ± 107.92 abc
3974	St. Croix (CIMMYT)	0.88 ± 1.09 h	144.60 ± 284.82 bc
2980-93	Hybrid (CIMMYT)	0.48 ± 0.65 i	17.30 ± 51.05 c
Healthy plant	cv. commer (CIBCM)	0.00 ± 0.00 j	0.02 ± 0.01 d

^w CNP = Consejo Nacional de la Producción; CIMMYT = Center for the Improvement of Maize and Wheat; EEFB = Estación Experimental Fabio Baudrit; CIBCM = Centro de Investigación en Biología Celular y Molecular.

^x Symptom ratings, 0 = no symptoms to 4 = severe symptoms. Average from five replications with five plants per replication.

^y Average from three replications with three plants per replication.

^z According to LSD's test at significance level of $\alpha = 0.05$.

after the completion of the TP. Symptom severity varied according to the genotype and ranged from moderate to severe (grades 1–4) (Table 1, Fig. 1). Symptoms were observed on the fourth leaf of individual plants, followed by leaves 5–8, except in some cases when they initially appeared on the third leaf. The symptoms were usually present over the full leaf but were more severe within the proximal region. This effect was more evident in younger leaves 7 and 8. Genotypes 3974 and 2980-93, unlike the others, developed symptoms 10–12 days after completion of the TP, and these symptoms were moderate

in severity and remained so up to 30 days after the TP when the experiment was terminated.

Symptom severity varied among genotypes, replications, and plants within the different genotypes. The ANOVA (factorial ANOVA) for this data showed significant differences in the responses of the different genotypes to MRFV infection. The least significant difference (LSD) analysis at a significance level of $\alpha = 0.05$ yielded various groups of genotypes when compared with the group of the healthy plants (Table 1).

Genotypes HC-78, 5393, and Guarare

showed the highest levels of disease incidence and severity and can be considered as highly susceptible to MRFV (Table 1). This can be clearly observed by analyzing the infected plant distribution because approximately 20–30% of the plants showed symptoms reaching grade 4 in the symptomatology scale (Fig. 1A). Nonetheless, approximately 10% of the plants from these genotypes were asymptomatic, and ELISA results indicate that these plants were not infected. In general, between 10 and 20% asymptomatic non-infected plants were observed in all the genotypes tested (Figs. 1A–D).

Fig. 1. Frequency distribution plot as percentages of infected plants according to symptom severity of the different maize genotypes. Symptom ratings correspond to response of the fourth leaf at 15 days after the transmission period, and are based on a five-point scale with 0 = absence of symptoms; 1 = presence of chlorotic dots encompassing 0–25 % of the leaf area; 2 = presence of light chlorotic dots, covering between 25 and 50% of the leaf area; 3 = presence of continuous stripes along the axis of the leaves, covering between 50 and 75% of the leaf area; and 4 = presence of intense symptoms with necrosis of leaf tissue, affecting 75–100% of the leaf area.

The genotypes presented in Figure 1B and C showed a higher percentage of infected plants with grades of infection 2 and 3 and can therefore be considered as moderately susceptible to MRFV infection. The response of genotypes HC-43 and B-833 to infection is rather interesting because they are commercial cultivars obtained from the National Production Council (CNP-Costa Rica). In HC-43, for example, 20 and 50% of the infected plants showed symptoms in grades 2 and 3, respectively. In a like manner, values of 30 and 40% of infected

plants with symptoms in grades 2 and 3, respectively, were found with genotype B-833.

The responses of genotypes RPM-89, 3175, 3974, and 2980-93 to MRFV infection are shown in Figure 1D. Approximately 50% of the plants in genotypes 3974 and 2980-93 showed no symptoms at all and only 10–20% of the plants showed symptoms in categories 2 and 3. These symptoms generally appeared 10–12 days after completion of the transmission period.

The response of different maize genotypes and individuals within each genotype to viral infection as determined by DAS-ELISA was variable. Genotype Guarare expressed the highest capsid protein level, which correlated with symptom severity (average 2.60 ± 1.12). Genotype 3974, on the other hand, scored a low rating in the symptomatology assay yet had capsid protein concentrations similar to those of genotypes determined to be moderately susceptible (Table 1). On average, however, genotypes Guarare and HC-78 had

Group of insect	"susceptible" Genotypes	Nº of plant	"resistant" Genotypes	Nº of plant	"susceptible" Genotypes	Nº of plant
X1	Guarare (S)	1001	2980-93 (S)	1061	HC-78(S)	1121
X2	Guarare (S)	1002	2980-93(NS)	1062	HC-78(S)	1122
X3	Guarare (S)	1003	3974 (S)	1063	HC-78(NS)	1123
X4	Guarare (NS)	1004	3974(S)†	1064	HC-78(S)	1124
X5	HC-78 (S)	1005*	2980-93 (ND)	1065*	Guarare (ND)	1125
X6	HC-78 (S)	1006	3974 (S)	1066	HC-78(S)	1126
X7	HC-78 (S)	1007	3974 (S)	1067	HC-78(S)	1127
X8	Guarare (S)	1008	3974†(ND)	1068	Guarare(S)	1128
X9	Guarare (NS)	1009	3974 (S)	1069	HC-78(S)	1129
X10	HC-78 (S)	1010	2980-93(NS)	1070	Guarare(S)	1130
X11	HC-78 (S)	1011	3974(NS)†	1071	Guarare(S)	1131
X12	HC-78 (S)	1012	2980-93(NS)†	1072	Guarare(S)	1132
X13	HC-78 (S)	1013	3974(NS)†	1073	HC-78(S)	1133
X14	HC-78 (NS)	1014	2980-93(NS)	1074	HC-78 (NS)	1134
X15	Guarare (S)	1015	3974(NS)	1075	Guarare(S)	1135
X16	HC-78 (S)	1016	3974(NS)	1076	HC-78 (NS)	1136
X17	Guarare (S)†	1017	2980-93 (S)	1077	Guarare (NS)	1137
X18	HC-78 (S)	1018	2980-93(NS)	1078	Guarare(S)	1138
X19	Guarare (S)	1019	3974 (ND)†	1079	HC-78 (NS)	1139
X20	Guarare (S)	1020	3974 (S)	1080	Guarare (NS)	1140
X21	Guarare (NS)	1021	2980-93(NS)	1081	Guarare(NS)	1141
X22	HC-78 (S)	1022	2980-93 (S)	1082	Guarare(NS)	1142
X23	HC-78 (S)	1023	3974(NS)	1083	Guarare (S)	1143
X24	Guarare (S)	1024	2980-93(NS)	1084	Guarare (S)	1144
X25	HC-78 (S)	1025	3974 (NS)	1085	HC-78 (S)	1145
X26	Guarare (S)	1026	2980-93 (NS)	1086	Guarare(NS)	1146
X27	Guarare (S)†	1027	2980-93 (S)	1087	HC-78 (NS)	1147
X28	Guarare (S)	1028	2980-93(S)	1088	HC-78 (S)	1148
X29	HC-78 (S)	1029	2980-93 (NS)	1089	Guarare (S)	1149
X30	HC-78 (S)	1030	3974 (NS)	1090	HC-78 (S)	1150

† = dead plant
* = dead insects

(S) = Symptomatic plant
(NS) = Non-symptomatic plant

ND = Not determined

Fig. 2. Schematic representation of the infection and symptom distribution in the "susceptible" and "resistant" genotypes to maize rayado fino virus infection in the second evaluation. TP = transmission period.

30- to 100-fold higher virus levels at low symptom ratings than did either 3974 or 2980-93 at the same symptom ratings. Virus concentration values were similar in plants expressing high symptom values, whether the plants were representatives of the susceptible (Guarare or HC-78) or resistant (2980-93 or 3974) genotypes.

Genotype 2980-93 had low capsid protein levels, being one order of magnitude lower than those found in genotypes 2435 and Guarare. There were no correlations between the capsid protein levels and symptomatology for the other genotypes. Some genotypes, such as RPM-89 and 2445, scored low values in the symptomatology rating yet accumulated high concentrations of the capsid protein.

The ANOVA for these data showed significant differences in the response of different maize genotypes to infection by MRFV. The LSD test at $\alpha = 0.05$ allowed the separation of the genotypes into several groups (Table 1). Linear regression analysis revealed low correlation between symptomatology and capsid protein concentration ($r = 0.18$) in the different genotypes tested.

Symptom distribution and capsid-protein concentrations in the second

phase. A second evaluation of selected genotypes was performed to discriminate host resistance from transmission frequency. In the second evaluation, the genotypes assayed were Guarare and HC-78 (considered susceptible) and 3974 and 2980-93 (considered resistant) according to the results of the first evaluation (Table 1). During the second evaluation, it was of particular interest to analyze plants inoculated with groups of insects determined to be transmitters in all three TPs.

In Figure 2 and Table 2, summaries of the interactions occurring during this second evaluation are presented. As shown in Figure 2, symptoms of MRFV infection were observed in 86.7 and 63.3% of the susceptible genotypes in the first and third TP, respectively, whereas only 36.7% of the plants of the resistant genotypes showed symptoms in TP2. Symptom production in TP3 where there was none in TP2 suggests that either the virus was not transmitted or symptoms were not visible, even though the vectors were clearly viruliferous. These data show that approximately 86% of the plants in genotypes 3974 and 2980-93 (2nd TP) were exposed to viruliferous insects, suggesting that

transmission efficiency was high. The unusual number of dead plants occurring in the second TP was due to fungal contamination in a new batch of soil used to grow these plants.

The genotypes Guarare and HC-78 displayed generally severe symptoms 2 to 3 days after completion of the transmission period. When plants of genotypes 3974 and 2980-93 showed symptoms, these appeared late (10–15 days after of the TP) and were mild (see example in Fig. 3). In the data presented in Table 2, only the plants corresponding to the groups of insects inducing symptomatic plants in the first and third TP but asymptomatic plants in the second TP were considered.

Genotype HC-78 showed the highest grade of symptoms with a mean of 3.75, which implies intense symptoms with approximately 75% of the leaf area affected. The genotype Guarare had a mean of 2.75, which represents about 50% of the leaf area with symptoms. The values for HC-78 and Guarare in this phase are higher than the 2.88 and 2.60, respectively, determined in the first phase (Table 1), which was expected since only symptomatic plants were rated, whereas values from the first evaluation included asymptomatic plants. The high standard deviation values observed confirm the great variability existing among plants in both genotypes.

The ANOVA analysis for these data showed the presence of significant differences between susceptible and resistant genotypes to MRFV infection. Tukey's multiple comparisons test at a significance level of $\alpha = 0.01$ allowed the separation of genotypes into two groups, with the resistant genotypes comparable to the group of healthy plants (Table 2).

Plants showing clear symptoms in the susceptible genotypes in TPs 1 and 3 and no symptoms in TP2 in the resistant plants were chosen for analysis of virus concentrations. Virus levels were determined in these experiments to evaluate the relationship of virus replication/accumulation to symptom production. The response to virus infection of the different maize genotypes in the second evaluation was as variable as it was in the first. The susceptible genotypes HC-78 and Guarare expressed very high levels of viral capsid protein (336.10 and 158.20 ng, respectively) (Table 2), and these values are in accordance with the symptomatology shown by those genotypes but different from 280.35 and 347.15 ng measured during the first phase (compare values in Tables 1 and 2).

On the other hand, the "resistant" genotypes 2980-93 and 3974 showed very low levels of virus capsid protein (14.1 and 0.39 ng, respectively). The concentration corresponding to genotype 2980-93 is very similar to that found in the first phase (17.3 ng), but for genotype 3974 it was significantly different from that found in the first phase (144.61 ng) (Table 2). A few plants

Table 2. Effect of maize rayado fino virus (MRFV) on "susceptible" and "resistant" genotypes of maize as determined by symptom severity and ELISA in the second evaluation

Genotypes ^x	Symptomatology ^y	DAS-ELISA (ng of CP-MRFV/mg tissue)
HC-78	3.75 ± 0.50 a ^z	336.10 ± 100.90 a
Guarare	2.75 ± 1.26 a	158.20 ± 100.40 a
2980-93	0.00 ± 0.00 b	14.10 ± 27.70 b
3974	0.00 ± 0.00 b	0.39 ± 0.52 b
Healthy plant	0.00 ± 0.00 b	0.04 ± 0.02 b

^x Average of four plants.

^y 0 = no symptoms to 4 = severe symptoms.

^z According to Tukey's test, different letters correspond to statistically different groups at significance level of $\alpha = 0.01$.

Fig. 3. Symptom response of genotypes Guarare and 2980-93 to maize rayado fino virus infection. The picture was taken of leaf 4 at 15 days post-inoculation. "Healthy" is represented by a genotype HC-78 plant that was not exposed to leafhoppers.

of genotype 3974 showed mild symptoms, but levels of MRFV-CP per milligram of fresh tissue were as high as the "susceptible" genotypes. The ANOVA for these data showed significant differences between susceptible and resistant genotypes to MRFV infection as determined by capsid protein concentration. Tukey's multiple comparisons test at a significance level of ($\alpha = 0.01$) allowed the separation of the genotypes into two groups, placing the resistant ones in the same category as healthy plants (Table 2). Linear regression analysis of the relationship of symptomatology ratings to capsid protein concentrations in the second phase revealed a significant correlation ($r = 0.8$).

DISCUSSION

We obtained up to 86% MRFV transmission by inoculation of maize plants with 10 to 12 viruliferous insects per plant, significantly higher than the 30% reported by Gámez (12) but similar to that reported by Toler et al. (37). The variability observed in symptom ratings among the different genotypes and plants within genotypes may be due to variability in leafhopper transmission or lack of homozygosity among the genotypes evaluated. In all of the genotypes we analyzed, the production of chlorotic symptoms correlated with MRFV capsid protein concentration, as was found by Stoddard et al. (36) in their evaluation of wheat germ plasm for resistance to wheat streak mosaic virus. In our study, a combination of ELISA and visual inspection of symptoms best determined resistant germ plasm.

In general, the reactions of the 20 maize genotypes to MRFV fell into five susceptibility groups based on symptomatology and three groups based on ELISA. Two genotypes, 3974 and 2980-93, exhibited statistically significant differences in the frequency of distribution of symptoms and concentrations of capsid protein when compared to the remaining 18 genotypes. The values of 0.88 and 0.48 on the symptomatology scale were not significantly different ($\alpha = 0.05$) from healthy plants. Therefore, we consider these genotypes to be "resistant" to infection as described by Cooper and Jones (7). It should be made clear that in the resistant genotypes there were some symptomatic plants (levels 1–3). The remaining genotypes are considered to be susceptible to virus infection, with the genotypes HC-78, 5393, and Guarare the most susceptible. HC-78 is a commercial cultivar of the CNP-Costa Rica; 5393 is a selection from the Chande 9 race obtained from CIMMYT, Mexico; and the genotype Guarare is a hybrid obtained from the EEFB, Alajuela, Costa Rica. Of particular interest was the response of genotype RPM-89, for which 50% of the plants showed symptoms in grade 2. This genotype is a hybrid about to be released commercially and was pro-

vided by I. C. Salas from the EEFB-UCR (Fig. 2D). There was no correlation between the race of maize from which the genotype was derived and susceptibility to MRFV.

For some virus strain-host species combinations, a relationship exists between the concentration of virus and the symptoms induced (38,40), however, in many instances, the degree of symptom severity is not always proportional to viral replication within the host and is influenced by the host genetic background and environmental conditions (27,34). Generally, streak and mosaic symptoms are characterized by areas of visible chlorotic symptoms interspersed with areas that appear normal. High concentrations of virus are usually found in the chlorotic areas, whereas the dark green areas are free or contain only small amounts of virus. Further studies will be necessary to determine the mechanism of resistance to MRFV in genotypes 3974 and 2980-93.

An historical examination of the relationship of maize to the corn stunt pathogens, which includes MRFV, may suggest the source of resistance in these genotypes. Nault et al. (30) examined the response of annual and perennial teosintes to MRFV. They found that all annual teosintes were highly susceptible, the perennial diploid teosinte *Zea diploperennis* was susceptible, but symptoms were mild or absent, and the perennial tetraploid teosinte *Zea perennis* was immune. Both *Z. perennis* and *Z. diploperennis* are natural hosts of *D. maidis*, but only *Z. diploperennis* will naturally hybridize with maize. In addition, *Z. diploperennis* is immune to maize bushy stunt phytoplasma and susceptible to corn stunt Spiroplasma (28,30). It is thought that these three pathogens may have played a role in the evolution of maize and its ancestors, including *Z. diploperennis* (28). A strong correlation was found between resistance to a virus disease of corn called "stunt" and teosinte introgression into 25 Mexican races of corn (5,39). Selection causes the population to become homozygous for teosinte introgression; therefore, MRFV may have been a selective force in the evolution of maize from its wild relatives (26,28).

Accession 3974 (P.I. 484036) is a selection of the Saint Croix 1 Caribbean Island collection and is a lowland tropical adaptation selected by L. Pollack, Iowa State University, Agronomy Department for use in increasing yield (*personal communication*). Accession 2980-93 (P.I. 488912) is a yellow-flint, mid-highland adaptation developed by S. Taba and members of the Ecuador maize program in 1982 and whose germ plasm includes parents from Guatemala, CIMMYT, Colombia, and Ecuador. These selections represent highly evolved corn relationships, and the nature and amount of teosinte introgression is unknown. Genetic crosses to closely related

susceptible genotypes and examination of virus resistance will need to be performed to identify the number of genes involved in the resistance phenotype (10,20,22).

In summary, we have developed the ability to index breeding lines and germ plasm collections for tolerance or resistance to MRFV. The future aims of this research are to use the resistant genotypes identified in this study in national programs to develop maize hybrids with increased resistance to MRFV and to study the nature of resistance to the virus in genotypes 3974 and 2980-93 under defined environmental conditions to reduce variability within a genotype.

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