Incidence and Geographic Distribution of Maize Rayado Fino Virus (MRFV) in Latin America

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ABSTRACT

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Samples of maize (*Zea mays* L.) exhibiting symptoms of the maize rayado fino virus (MRFV) were collected in eight Latin American countries during August to December of 1992, as part of a survey of virus distribution. Maize with MRFV symptoms were found in all countries and life zones sampled. Of the 103 collected plants tested for the presence of MRFV using hybridization with an MRFV cRNA probe, 75% were positive for the virus. Much variation in MRFV symptoms and symptom expression patterns was found.

ABSTRACTO

Muestras de *Zea mays* L. con síntomas del virus Maize Rayado Fino Virus (MRFV) se colectaron en ocho países de Latinoamerica durante 1992, para determinar la distribución y la abundancia de este virus. Plantas de maíz con síntomas del virus MRFV fueron encontradas en todos los países y las zonas de vida muestreada. De las 103 plantas evaluadas para detectar MRFV mediante el métado de hibridación con el marcador cARN. El 75% de las mismas resultaron infectadas con MRFV. La expresion de la sintomatología del MRFV presento grandes variaciones.

The leafhopper-borne maize rayado fino virus (MRFV) is the only known indigenous virus of maize in Meso America (9). MRFV is the best characterized member of the marafivirus group of plant viruses, which includes oat blue dwarf virus and Bermudagrass etched line virus (5). MRFV, which appears to be restricted to the Americas, is widespread and is becoming increasingly important in tropical areas (6,8). MRFV was first recognized in El Salvador and Costa Rica over 15 years ago and has since been detected in all Central American countries, including Guatemala, Honduras, Nicaragua, and Panama (3,4,8), in addition to Mexico (8,20), the southern U.S. (1), Colombia (17), Peru (8), Venezuela (15), Brazil (13), and Uruguay (8).

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MRFV virions are isometric, approximately 30 nm in diameter, and contain a single-stranded RNA genome with a molecular mass of 2.0 to 2.1 × 10^6 daltons (16). The virus is able to replicate in both maize and its insect vector (*Dalbulus maidis* Delong and Wolcott) (19). MRFV often occurs in field infections associated with mollicutes (corn stunt spiroplasma and maize bushy stunt phytoplasma) that are transmitted by the same vector (10). There is a close association among *D. maidis*, MRFV, and their maize host (11). Maize is considered the only natural host in areas where MRFV and its leafhopper vector are endemic (7). It has been proposed that MRFV, *D. maidis*, and *Zea mays* L. coevolved in a triad in which the parasitic members (virus and insect) display highly specialized interactions (9).

MRFV and *D. maidis* have successfully exploited the capacity of *Z. mays* to adapt to a broad range of environments. The virus and insect vector have been found in maize fields from sea level to altitudes of over 3,500 meters. They have been detected from the southern U.S. through Mexico and Central America to Northern Argentina and Uruguay in South America (7,8).

Only preliminary information is available about the existence of strains of MRFV. The Brazilian corn streak virus (13) and the maize rayado colombiano virus (17) are in the same virus group as MRFV (5,6,8).

This work describes the most comprehensive survey to date of the incidence and distribution of MRFV in maize fields of eight Latin American countries and uses molecular techniques. The distribution and different symptomatology patterns of MRFV in field infections are reported.

MATERIALS AND METHODS

Sample collection. Maize leaf samples were collected extensively in eight Latin American countries (Mexico, Guatemala, Honduras, Costa Rica, Colombia, Venezuela, Peru, and Bolivia) during August to December 1992 (Table 1). Various geographic zones were defined by physical distance and geographic barriers in order to group the collecting sites. Ecological zones

Table 1. Number of geographic zones and collecting sites in which symptoms of maize rayado fino virus (MRFV) infection were observed

	Geographic zones		Collecting sites	
	MRFV symptoms			
Country	Present	Absent	Present	Absent
Mexico	8	1	16	5
Honduras	2	0	4	2
Guatemala	3	1	5	5
Costa Rica	3	1	7	5
Colombia	4	0	6	0
Venezuela	1	1	3	2
Peru	5	1	21	3
Bolivia	2	1	6	4
Total number of sites	28	6	68	26
Percentage	82%	18%	72%	28%
Total	34 geographic zones		94 collecting sites	

were defined using Holdridge's parameters (12). A reference card was made describing characteristics of the environment, geography, and plant symptoms. A photograph was taken of each plant sampled and associated symptoms recorded.

Material was collected from the basal part of the leaves, which most frequently expressed symptoms. The leaves sampled were preserved by placing them in paper bags interspaced with paper sheets. Three to seven days after sampling, the leaves were air dried, packed in plastic bags and stored in a suitcase for a period of 2 to 3 months. The samples were lyophilized upon arrival at the laboratory in Costa Rica, followed by storage at –70°C.

Maize plants inoculated with a Guapiles (Costa Rica) isolate of MRFV and grown under controlled conditions at Centro de Investagación en Biología Celular y Molecular (CIBCM) were used as positive controls for the laboratory experiments. Uninoculated maize plants grown similarly were used as negative controls.

Nucleic acid extraction. Lyophilized corn leaves were ground to a powder with a mortar and pestle in liquid nitrogen; nucleic acids were extracted by a modification of the direct phenol method (2). One-half gram of the leaf powder was homogenized with 10 ml of 0.1 M Tris, pH 8.9 containing 0.1 M sodium diethyl-dithiocarbamic acid, 0.1 M sodium chloride, 0.01 M EDTA, 0.005 M dithiothreitol, and 1% sodium dodecyl sulfate (SDS), 10 ml of buffer-saturated phenol, pH 7.5 (Life Technologies, Gibco-BRL, Gaithersburg, MD), and an equal volume of chloroform. The mixture was shaken for 10 min and the aqueous and organic phases were separated by centrifugation for 10 min at 5,000 × *g*. Total nucleic acids were ethanol precipitated. Polysaccharides were removed by adding equal volumes of 2.5 M K_2HPO_4,

Table 2. Incidence of maize rayado fino virus (MRFV) infection in samples collected in eight Latin American countries in August to December, 1992

		Geographic location of collecting site[a]			MRFV-infected plants[b]	
Country	**Collecting site**	**Altitude**	**Latitude**	**Longitude**	**No.**	**Relative concentration**
Mexico	Francisco Villa, Veracruz	50	20°18′	97°18′	[1]	1(+++)
	Ojo de Agua, Veracruz	100	20°18′	97°18′	[1]	1(++)
	Cotaxtla, Veracruz	16	18°48′	96°21′	[1]	1(–)
	Ciudad Mendoza	1,400	18°45′	97°12′	[2]	1(+++)/1(++)
	Zumbilla, Puebla	2,100	18°39′	97°18′	[1]	1(+)
	Acatepec, Puebla	2,000	18°15′	97°33′	[1]	1(+++)
	Agua Dulce, Oaxaca	1,700	17°54′	97°48′	[1]	1(–)
	Chila, Puebla	1,700	17°57′	97°54′	[3]	1(+++)/2(+)
	Teloloapan, Guerrero	1,500	18°22′	99°54′	[1]	1(+)
	Coyuca, Guerrero	400	18°18′	100°48′	[1]	1(+)
Honduras	Sambrano, Francisco Morazan	1,550	14°17′	87°25′	[1]	1(+++)
	Ojo de Agua, El Paraiso	620	14°04′	86°53′	[3]	1(++)/1(+)/1(–)
	Chichicaste, El Paraiso	500	14°04′	86°23′	[1]	1(+)
	Santa Maria, El Paraiso	460	14°09′	86°17′	[1]	1(+)
Guatemala	La Máquina, Suchitepéquez	75	14°16′	91°35′	[3]	1(+)/2(–)
	Nueva Concepción, Escuintla	100	14°10′	91°10′	[2]	1(+)/1(–)
	Santa Lucía, Sacatepéquez	2,000	14°34′	91°40′	[1]	1(+)
	Mita, Jutiapa	800	14°21′	89°43′	[1]	1(+++)
Colombia	Uyamal, Antioquia	500	6°30′	75°48′	[2]	1(+)/1(–)
	Santa Fe, Antioquia	500	6°30′	75°48′	[2]	2(+)
	Ica-Bello, Medellín, Antiquia	1,440	6°19′	75°21′	[15]	3(+++)/4(++)/3(+)/5(–)
	Río Negro	2,120	6°10′	75°23′	[1]	1(+)
	ICA-Palmira, Valle del Cauco	1,000	3°32′	76°41′	[16]	1(+++)/3(++)/2(+)/10(–)
	ICA-Mosquera, Condinamarca	2,400	4°41	74°12′	[3]	2(+++)/1(–)
Venezuela	S. Juan de los Morros, Guarico	300	9°45′	67°21′	[2]	2(+)
	CENIAP-Maracay, Aragua	455	10°17′	67°36′	[4]	2(+++)/1(+)/1(–)
Peru	Chincha-INIAA, Ica	280	–13°30′	76°08′	[1]	1(+++)
	Guanami, Ica	600	–13°55′	75°38′	[1]	1(+)
	Nazca, Ica	250	–14°50′	74°57′	[1]	1(+)
	Jesús, Cajamarca	2,500	–7°12′	78°30′	[4]	2(+++)/1(+)/1(–)
	Namora, Cajamarca	2,850	–7°15′	78°20′	[2]	2(+++)
	Huayubamba, Cajamarca	2,000	–7°17′	78°17′	[2]	1(+++)/1(++)
	San Marcos, Cajamarca	2,000	–7°20′	78°15′	[2]	2(++)
	Urubamba, Cuzco	3,100	–13°23′	72°08′	[1]	1(++)
	Ollantaytambo, Cuzco	2,760	–13°12′	72°17′	[1]	1(++)
	Yucay, Cuzco	2,900	–13°20′	72°05′	[1]	1(–)
	Muyurina, Ayacucho	2,420	13°00′	74°10′	[1]	1(+++)
	Valle de Huatatas, Ayacucho	2,600	13°00′	74°10′	[1]	1(+++)
	Valle de Chaceo, Ayacucho	2,600	13°00′	74°10′	[1]	1(+++)
	Camoán, Ayacucho	2,720	13°00′	74°10′	[1]	1(+++)
Bolivia	Univ. Agraria, Cochabamba	2,560	–17°25′	66°05′	[1]	1(+++)
	Pairumani, Cochabamba	2,560	–17°25′	66°15′	[2]	1(+++)/1(++)
	Vallecito, UAA, Santa Cruz	2,560	–17°25′	66°15′	[1]	1(+)
	Torno, Santa Cruz	450	–18°02′	63°25′	[1]	1(+++)
Costa Rica	Invernadero, Guápiles	1,200	10°15′	83°50′	[1]	1(++)
	San Rafael de Alajuela	800	9°52′	84°15′	[3]	1(+++)/2(–)
	Fabio Baudrit, Alajuela	800	10°00′	84°17′	[1]	1(+++)
	Zarcero	1,500	10°12′	84°25′	[2]	1(++)/1(+)

[a] Geographic location of the collecting site is defined by latitude, longitude, and altitude (meters above sea level).

[b] [n] = number of plants sampled at a given site; n(+++), n(++), n(+), or n(–) designate the number of sampled plants with signal intensities exhibited in Figure 1 and corresponding to relative virus concentrations in the samples.

pH 8.0, and methoxyethanol to the water-suspended nucleic acids.

After the addition of 0.25 vol of 1% cetyltrimethylammonium bromide (CTAB) to the separated upper phase from the previous step, and 2 h at 4°C, nucleic acids were pelleted by centrifugation for 10 min at 5,000 × *g*. The pellet was washed three times with 70% ethanol/0.1 M sodium acetate pH 5.2 and was resuspended in water and ethanol precipitated. The final pellet was resuspended in 200 µl of sterile water.

Hybridization analysis. Hybridization of 103 samples was performed using an RNA probe produced by in vitro transcription of an 800-bp MRFV cDNA clone that encodes the capsid protein gene and 3′ untranslated region of the virus (18). The probe was labeled with ^{32}P-UTP (Amersham Corp., Arlington Heights, IL; specific activity = 800 Ci/mmol).

Nylon membranes (Nytran, Schleicher and Schuell, Keene, NH) equilibrated with 2× SSC (1× SSC = 3.0 M NaCl, 0.03 M sodium citrate, pH 7.0) and air dried, were spotted with approximately 6- to 7-µg samples of total nucleic acids. Nucleic acids were crosslinked to the membrane with a UV light Stratalinker (Stratagene Cloning Systems, La Jolla, CA).

Prehybridization was performed with a buffer consisting of 6× SSC, 4× Denhardt's (1× = 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin [BSA]), 0.1 % SDS, and 100 µg of denatured calf thymus DNA per ml at 65°C for 20 min.

For the hybridization, 1 µl (approx. 1,000,000 cpm) of the 4,65 RNA probe was added per ml of prehybridization mix, followed by incubation overnight at 65°C. The membranes were washed at room temperature with 2× SSC and 0.2% SDS for 30 min, followed by treatment with RNase A (final concentration of 1 µg/ml in 2× SSC) for 15 min at room temperature, followed by a final wash with 0.1% SSC and 0.1% SDS for 30 min at 50°C. Autoradiography was performed by exposing the filter to Kodak X-AR film.

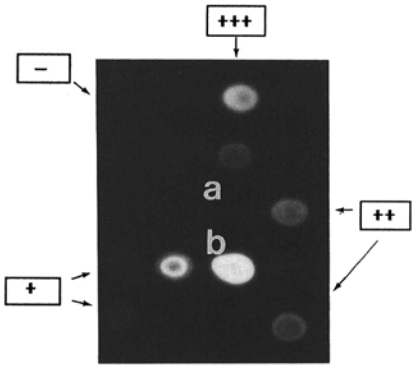

Fig. 1. Dot blot analysis of equivalent amounts of nucleic acids extracted from maize samples by means of the ^{32}-P-labeled 4,65 maize rayado fino virus (MRFV) cRNA probe. Signal intensity on the contact print of the autoradiogram is shown as follows: +++, when the hybridization signal was similar to a highly infected positive control; ++, when a relatively strong hybridization signal was observed; +, when a weak signal was observed; –, when the signal was similar to the negative control. a = negative control; b = MRFV positive control.

RESULTS

Geographical distribution. MRFV was detected in all eight countries where the sampling was done. Of the 34 geographic zones that were sampled, 28 (82%) had maize plants exhibiting symptoms of MRFV infection and 6 (18%) did not (Table 1). Of the 94 sites sampled (multiple samplings were occasionally taken within a geographic zone), MRFV symptoms were noted in 68 (72%) of the sites, while in 26 (28%) symptoms of MRFV were not seen (Table 1).

Of the 255 samples collected, 103 were chosen as representative samples from the different locations for hybridization with the MRFV probe. Seventy-five (73%) were positive and 28 (27%) were negative (Table 2). Figure 1 shows a representative dot blot and how the results were categorized (+++, ++, +, and –). Maize plants infected with MRFV were found in a very wide distribution, from latitude 20 north to latitude 18 south, from longitude 100 west to longitude 63 west, and from sea level to 3,100 meters above sea level (Table 2). MRFV was found in very different zones: coast zone (tropical, basal wet forest in Patalan, Mexico); mountain zone (tropical, high montane forest in Urubamba, Peru); desertic zone (subtropical, basal desert in Chincha, Peru); humid tropics zone (tropical, basal rain forest in Guapiles, Costa Rica); high land savannah (tropical, lower montane dry forest in Bogota, Colombia); and Amazonic zone (tropical, wet forest in Santa Cruz, Bolivia).

Symptomatology. Symptoms of MRFV infection include distinct, conspicuous, small chlorotic spots that develop at the base and along the veins of young leaves in characteristic stippled stripes (7) (Fig. 2A).

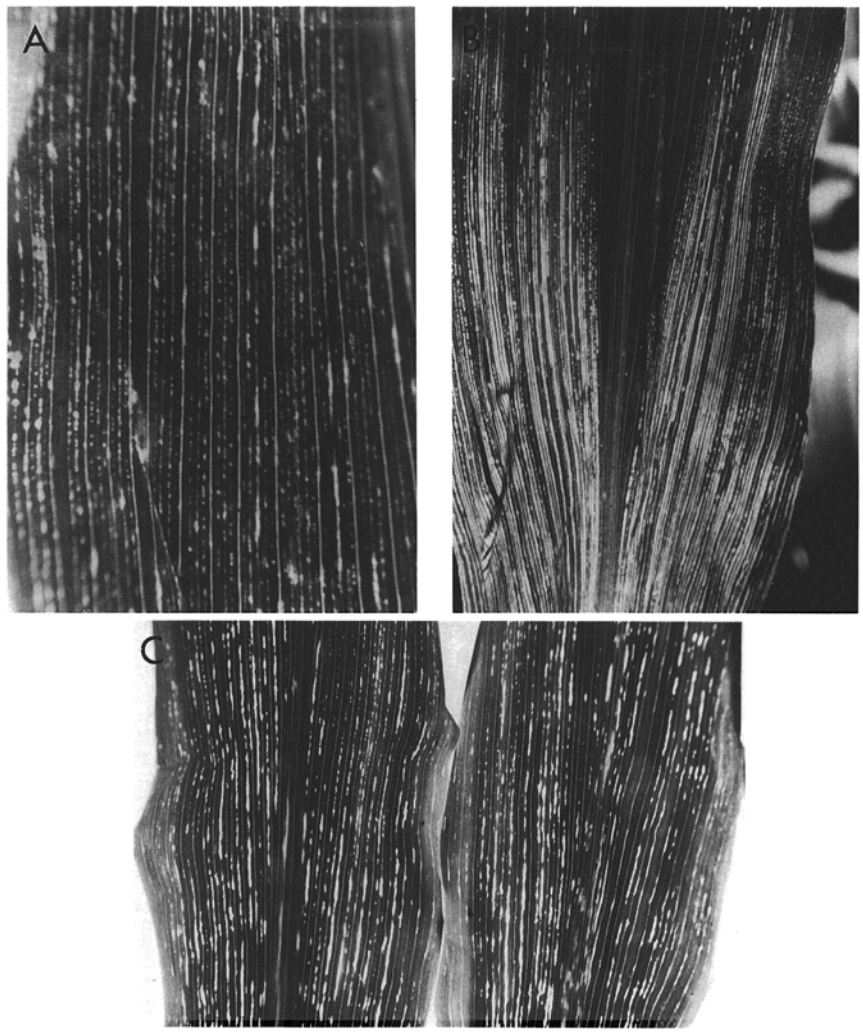

Fig. 2. Variation of maize rayado fino virus (MRFV) symptoms observed in field-infected maize plants. **(A)** Typical moderate to severe symptoms of MRFV infection. **(B)** Atypical symptoms of MRFV infection in maize leaves collected from Cajamarca, Peru. **(C)** Symptoms of MRFV infection in maize from Palmira, Colombia (identified as rayado colombiana virus by F. Varón de Agudelo and G. Martínez-López).

Many variations of the expected symptomatology were found (Fig. 2B,C), including what is considered to be the symptoms of maize rayado colombiano virus (Fig. 2C), which was found in ICA-Mosquera, Bogota, Condinamarca (identified by Martínez-López) (17), and in ICA-Palmira, Valle del Cauca (identified by Francia Varon de Agudelo). In both places, MRFV symptoms were found in the same maize germ plasm next to rayado colombiano virus.

An atypical expression pattern was observed in plants found in Nueva Concepción, Guatemala, and Cochabamba, Bolivia. Virus symptoms in these locations were more pronounced in the older leaves, while symptoms in younger leaves were concentrated at the apex. Symptoms were not apparent in the youngest leaves. All of these samples were positive for MRFV in the hybridization analysis.

DISCUSSION

The method of air drying and preserving the maize leaves in paper bags for further nucleic acid extraction was adequate for the most part, economical, and practical for long collecting field trips. Of the 255 samples collected, less than 10 were lost to decay using the protocol described for sample preservation (all of them from an area in Honduras). The fact that we had negative hybridization results for samples that appeared visually to be infected with MRFV may be explained by either some degradation of the samples during preservation or by the hypothesis that symptoms were produced by another pathogen.

Virus incidence in Mexico and Central and South America typically ranges from 0 to 40% in most sample locations, but up to 100% in others. Yield losses also frequently vary around 40 to 50% of the weight of the mature ear in local genotypes, but reach nearly 100% in recently introduced improved cultivars (7). The susceptibility among maize cultivars to MRFV is variable (4–7,17). Disease symptoms in the more sensitive genotypes are characterized by numerous short and long, chlorotic stripes, wilting of young plants, and general chlorosis and stunting. In the more tolerant genotypes, the characteristic striping gradually becomes milder and may even disappear in the new, youngest, leaves (4,5,17) (Fig. 2). The expression of MRFV symptoms in the plant is concentrated at the base of the leaves, generally with an increase of severity toward the younger leaves.

MRFV is widely distributed throughout Latin America and appears to be present in all regions where maize is abundant in conjunction with the insect vector *D. maidis*, regardless of the latitude or ecological zone. The ecosystems under which the virus and vector are found include nearly 20 different types of life zones, as defined by Holdridge (12), ranging from deserts to savannahs, deciduous and montane forests, and lowland rain forests (9). MRFV occurs in all maize-growing regions of Costa Rica, including 11 different vegetation zones in basal, premontane, lower-montane, and montane altitudinal belts. These four belts differ in altitude, annual mean temperature, amount and seasonal distribution of rainfall, light intensity, soil conditions, and physiography (12). The presence of MRFV in a specific habitat is dependent on the ability of *D. maidis* to survive in those conditions and migrate long distances (21). Wind-assisted migrations may disperse insects from areas where maize is grown throughout the year to areas where the crop is grown only seasonally. The migrations include distances of several hundred kilometers (21) to areas such as the high Peruvian Andes, Mexican Mesa Central, and southern U.S. (9). In these areas, low temperatures and other ecological conditions are unfavorable for the survival of the insect between planting seasons (9). This is consistent with the records and observations made by local farmers that the disease appears only in certain years, or that the disease may be mild at some times and very damaging at others.

Another possible explanation for the wide distribution of MRFV is that corn is a very abundant crop and, although a wide variety of germ plasm is grown, the microhabitat developed within the crop field is similar under many climatic conditions (9). Thus, MRFV and *D. maidis* would not require any exceptional ability to adapt to the different ecological conditions of the diverse zones where they exist (9).

No maize germ plasm is yet known to be resistant to MRFV infection (22). Even though we report here some negative hybridization results for some of the germ plasm, all of these samples appeared to display symptoms of MRFV infection. As these were field samples, no duplicate data exists to confirm these findings.

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