Identification of *Wheat streak mosaic virus* and Its Vector *Aceria tosichella* in Mexico

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ABSTRACT

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The objective of this study was to identify a pathogen observed in wheat in the Texcoco area (Mexico). The symptoms induced by this pathogen are consistent with those caused by a virus. Identification was based on host range, symptoms, mode of transmission, and on serological and morphological characterization. The pathogen was mechanically transmitted to *Triticum aestivum*, *T. durum*, *Secale cereale*, $\times$ *Triticosecale*, *Avena sativa*, and *Hordeum vulgare*. The virus particles were flexuous rods, 700 nm long that produced cylindrical inclusions seen as pinwheel inclusions under the electron microscope. The pathogen reacted with *Wheat streak mosaic virus* (WSMV) antibodies in enzyme-linked immunosorbent assay. An eriophyoid mite associated with this virus was identified as *Aceria tosichella* and found to transmit the virus efficiently. Based on its characteristics, the pathogen was identified as WSMV. During this study, the virus appeared sporadically in test plots without causing significant yield losses.

Wheat is an essential staple food around the world, but its production has been limited by different pathogens, among them viruses. The viruses that have caused most wheat losses are *Barley yellow dwarf virus* (BYDV), *Wheat streak mosaic virus* (WSMV), *Wheat spindle streak mosaic virus* (WSSMV), and *Wheat soil-borne mosaic virus* (WSBMV) (34). In the last few years, symptoms (chlorotic streaks) ranging from weak to severe were observed in wheat fields in the state of Texcoco, Mexico (19°N, 100°W, 2,249 m elevation). Several plants with these symptoms were infested with mites of the family Eriophyidae. The symptoms were similar to the ones caused by WSMV, and for this reason the research was oriented toward this pathogen.

WSMV has caused major economic losses in the U.S. Great Plains (7), in Colorado, Idaho, North and South Dakota, Montana, Texas, Oklahoma, and Washington (6,17,29). This virus has also been reported in Canada, Jordan, Romania (2,3), Germany (25), Bulgaria (23), and Hungary (11). In Mexico, Muñoz-Cárdenas (24)

reported symptoms similar to those caused by WSMV on several wheat-breeding lines in Apodaca, state of Nuevo Leon (26°N, 100°W, 475 m elevation). Apart from this report solely based on symptoms, WSMV has not been identified in Mexico.

The symptoms caused by this virus are fine chlorotic streaks that may turn into severe streaking and mosaic as the disease develops (3). WSMV belongs to the genus *Rymovirus* and is transmitted by mites of the family Eriophyidae (26). It has been suggested, however, that WSMV should be classified in the new genus *Tritimovirus* (family *Potyviridae*) (32). It has a flexible rod particle, 700 nm in length and 13 nm wide. Its nucleic acid is a single-stranded RNA of approximately 8.5 kb, with a molecular weight of 2.8×10^6 (3). WSMV induces amorphous pinwheel inclusions in the cytoplasm of infected plants (20,21). The vector has been reported to be *Aceria tulipae* (30), and even though Keifer (18) described it in wheat as *A. tosichella*, it has still been reported as *A. tulipae* (3,12). Recently, it has been suggested that *A. tulipae* be used only when it is found in Liliaceae and *A. tosichella* when it is found in wheat. Thus *A. tosichella* is considered the vector of WSMV (1,15). This mite acquires the virus during a feeding period lasting 15 to 60 min and maintains its infectivity for more than 6 days (3). The objective of this study was to identify the causal agent of the chlorotic streak symptoms observed in Mexico and its vector.

MATERIALS AND METHODS

Several plants with chlorotic streak and yellow mosaic symptoms were collected in wheat fields in Texcoco. The isolate El Batán 3 was mechanically transmitted to wheat plants and kept in the greenhouse at temperatures between 20 and 25°C.

Host range. The species tested are listed in Table 1. Mechanical transmission was performed using infected sap macerated with 0.1 M phosphate buffer at a dilution of 1:10 wt/vol. Eight plants per variety were inoculated mechanically with infectious sap, and two plants were inoculated with the phosphate buffer only, to serve as negative controls. The inoculated plants were split into two groups and kept in two greenhouses at different temperatures: 28 to 38°C and 20 to 25°C. The presence or absence of the virus in these plants was assessed based on their symptomatology, enzyme-linked immunosorbent assay (ELISA), and the possible reinoculation of the pathogen from these plants to wheat.

Aphid transmission. Transmission by aphids was tested with the species *Metopolophium dirhodum*, *Rhopalosiphum padi*, *R. maidis*, and *Sitobion avenae* at room temperature (18 to 26°C). Twenty aphids from each species were placed on infected wheat leaves, var. Yecora, for a 2-day feeding period in the transmission test for persistent viruses, and for 1 min in the test for nonpersistent viruses. The aphids were later transferred to healthy wheat plants, where they fed 1 day more prior to being eliminated with the insecticide pirimicarb (Pirimor). The plants were checked for symptoms 15 days after inoculation and for the presence of the virus 20 days after inoculation, using ELISA against WSMV.

Mite transmission. Mites found on plants showing symptoms were collected, kept in 70% alcohol, and mounted on slides, by placing 2 to 5 mites on a drop of lactophenol. The preparation was covered with a coverslip and heated for 2 min at 60°C. The mites were identified according to Keifer (18) identification keys.

Mites newly hatched from eggs were placed for feeding on two groups of five plants each, one of uninfected wheat seedlings and the other inoculated with El

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Batán 3. After 2 weeks, the mites fed on diseased or uninfected plants were transferred (20 mites per plant) to 40 or 10 non-infected wheat seedlings, respectively. There, they fed for 7 days before being eliminated with the acaricide propargite (OMITE). Symptoms were evaluated after 15 days, and one leaf per plant was collected after 24 days and maintained for ELISA at -20°C.

Seed transmission. Five hundred seeds harvested from plants mechanically infected with El Batán 3 were sown in pots with sterilized soil. Two months following seed emergence, the plants were checked for the possible appearance of symptoms.

Microscopy. Cell inclusions were observed with a light microscope on epidermic strips of wheat leaves 15 to 20 days after inoculation. The leaves were treated with 5% Triton X-100 for 5 min and washed with distilled water before being stained with methyl-green rhodamine, orange-green, or bromphenol blue.

A copper grid with the Formvar membrane facing down was placed over a drop of infected sap for 1 min, and then immediately onto a drop of phosphotungstic acid (PTA). The grid was left to dry on filter paper and then observed

with an electron microscope (JEOL 100B TEM).

Pieces of tissue of approximately 2 mm², collected 15 days after inoculation, were fixed in glutaraldehyde 6.5%, 0.1 M phosphate buffer, pH 7.2. They were vacuum filtered and kept at 4°C for 1 h. Before subjecting the material to a second fixation period in 1% osmium tetroxide for 1 h, it was washed three times (15 min each time) in 0.1 M, phosphate buffer pH 7.2. Following the second fixation, it was washed three times (15 min each time) with phosphate buffer 0.2 M pH 7.2, and was dehydrated in a graded series of acetone at 25, 50, and 75% for intervals of 15 min and three times (20 min each time) in 100% ethanol. Later, the material was infiltrated with Spur resin for 2 h and placed in a 60°C incubator for 24 h for polymerization. Finally, thin sections (90 to 150 nm) performed with an ultra microtome were stained with uranyl acetate and Reynold's citrate and were placed over a Formvar-coated grid for observation under the electron microscope.

Serology. Antisera against the most important wheat viruses were tested in ELISA: WSMV-ATCC (American-type culture collection) (PVAS-860), WSBMV-

ATCC (PVAS 898), and WSSMV-NY. The plant sap was obtained by macerating the samples in a 1:40 (wt/vol) dilution of 0.1 M ammonium citrate buffer, pH 6.5. To 1 ml of this mixture, 0.5 ml of chloroform was added. The preparation was centrifuged at 12,000 rpm for 2 min. The supernatant was added to the ELISA plates and incubated overnight at 4°C. Plates were washed three times (5 min each time) with PBS-T before adding the antibody diluted 1:2,000 in phosphate-buffered saline with Tween (PBST) + 2% milk + 1/20 non-infected wheat sap (wt/vol). The plates were incubated for 2 h at 37°C and washed 3 times with PBST. Finally, freshly prepared *p*-nitrophenyl phosphate (SIGMA) substrate was added. Optical densities (OD) at 410 nm were measured after 5, 15, and 30 min and after 1 h incubation at room temperature. Samples were considered positive when their OD was three times higher than the OD obtained with non-infected sap.

RESULTS

Host range and symptomatology.

Symptoms were not observed in any of the dicotyledons, *Pennisetum* spp., *Eragrostis* spp., *Echinochloa colona*, *Sorghum bi-*

Table 1. Host range of El Batán 3 through mechanical transmission

Host	20 to 25°C			28 to 38°C		
	Reaction ^a	Incubation ^b	ELISA ^c	Reaction	Incubation	ELISA ^d
<i>Gomphrena globosa</i>	I	-	-	I	-	-
<i>Chenopodium amaranticolor</i>	I	-	-	I	-	-
<i>C. quinoa</i>	I	-	-	I	-	-
<i>Beta vulgaris</i>	I	-	-	I	-	-
<i>Cucurbita pepo</i>	I	-	-	I	-	-
<i>Cucumis sativus</i>	I	-	-	I	-	-
<i>Arachis hypogaea</i>	I	-	-	I	-	-
<i>Vicia faba</i>	I	-	-	I	-	-
<i>Phaseolus vulgaris</i> 'Flor de mayo'	I	-	-	I	-	-
<i>Pisum sativum</i> 'Alaska'	I	-	-	I	-	-
<i>P. sativum</i> 'Early Perfection'	I	-	-	I	-	-
<i>Medicago sativa</i>	I	-	-	I	-	-
<i>Nicotiana tabacum</i> 'Xanthi'	I	-	-	I	-	-
<i>N. rustica</i>	I	-	-	I	-	-
<i>N. glutinosa</i>	I	-	-	I	-	-
<i>N. clevelandii</i>	I	-	-	I	-	-
<i>N. occidentalis</i>	I	-	-	I	-	-
<i>N. multivalis</i>	I	-	-	I	-	-
<i>Physalis floridana</i>	I	-	-	I	-	-
<i>P. ixocarpa</i>	I	-	-	I	-	-
<i>Lycopersicon esculentum</i>	I	-	-	I	-	-
<i>Capsicum annuum</i> 'Morrón'	I	-	-	I	-	-
<i>Triticum aestivum</i> 'Yecora'	M	10-14	0.662	M	7-10	0.957
<i>T. durum</i> 'Altar'	M	10-14	0.468	M	7-10	0.567
× <i>Triticosecale</i> 'Caracal'	St	10-15	0.351	St	10-15	0.403
<i>Avena sativa</i> 'Paramo'	St	10-14	0.941	St	7-10	1.347
<i>Secale cereale</i> 'Elbon'	I	-	0.054	St	10-15	0.224
<i>Hordeum vulgare</i> 'Centinela'	I	-	0.049	St	10-15	0.462
<i>Zea mays</i> (H-522)	I	-	-	I	-	0.055
<i>Zea mays</i> 'Ranchero'	I	-	-	I	-	0.032
<i>Sorghum bicolor</i>	I	-	-	I	-	-
<i>Echinochloa colona</i>	I	-	-	I	-	0.043
<i>Eragrostis</i> spp.	I	-	-	I	-	0.059
<i>Pennisetum</i> spp.	I	-	-	I	-	0.045

^a I = immune; M = mild to severe mosaic; St = fine streaks.

^b Time in days before observing the first symptoms.

^c Enzyme-linked immunosorbent assay (ELISA).

^d Optical density at 410 nm in ELISA with the WSMV-ATCC antibodies.

color, and *Zea mays* var. Ranchero and hybrid H-522 (Table 1). These species were shown to be immune when the virus could not be mechanically re-transmitted to wheat. *Triticum aestivum* and *T. durum* exhibited symptoms that began as chlorotic streaks that later fused to form a yellow mosaic (Fig. 1). In oat, streaks were wider and more diffuse and later turned necrotic. In barley, the symptoms were pronounced chlorotic streaks that later became necrotic. Rye and triticale exhibited very mild symptoms, with fewer streaks of minor size. Symptoms were observed on barley and rye at high temperatures ranging from 28 to 38°C but not between 20 and 25°C.

Mite identification. The full body of the mite is shown in Figure 1B. It presented a dorsal shield with three types of longitudinal lines: median, admedian, and submedian (Fig. 1C). The median line does not reach more than one-half to two-thirds of the shield length. The admedian lines are located on opposite sides of the median line, converging slightly toward the rostrum and curving where the median line ends. The submedian line starts laterally at the level of the tubercle of the dorsal setas. Between the admedian and submedian lines, a line starts on the internal side of the tubercle lobe of the dorsal setas and stops at the level of the lateral curve of the submedian lines. Laterally, there are two incomplete and two complete lines that are located more externally and converge toward the rostrum. The mite also presented an 8-rayed featherclaw on the anterior legs and 7 to 8 rayed featherclaw on the posterior legs (Fig. 1D).

Mode of transmission. Since no symptoms were observed in the plants infected by the aphid species tested in the two types

of transmission, it was concluded that the aphids were not able to transmit El Batán 3. The 40 seedlings inoculated by mites fed on infected plants exhibited the disease after 15 days, while none of the seedlings infected by virus-free mites showed symptoms. The plants showing symptoms reacted positively to ELISA performed with WSMV-ATCC antibodies. No virus was detected in symptomless plants (Table 2). This indicates that *A. tosichella* is an efficient vector of El Batán 3. Seedlings with mite-inoculated virus were more severely damaged than those mechanically inoculated.

None of the 500 seedlings arising from seeds harvested from infected plants showed virus-like symptoms. Fifteen days after seedling emergence, two of the seedlings showed a certain degree of chlorosis and dwarfing; however, these plants tested negative in ELISA against WSMV.

Microscopy. Granular inclusions that aggregated around or close to the nucleus were observed in the stained tissue under light microscopy. They contrasted more when methyl green rhodamine was used (Figure 1E). With blue bromphenol, there were fewer inclusions, but the preparation showed good contrast. With the orange-green, inclusions could be observed only in a few cells. Cytoplasmic cylindrical pinwheel inclusions were observed in electron microscopy. In preparations of infected plant sap, flexuous rod particles approximately 700 nm in length were observed (Fig. 1F). They were found in isolation without forming aggregates.

Serology. El Batán 3 reacted intensively with WSMV-ATCC antiserum, weakly with WSBMV-ATCC antiserum, and did not react at all with WSSMV-NY antiserum (Table 3).

DISCUSSION

The host range of El Batán 3 was similar to the one described for WSMV (3). In some cases, the literature mentions that corn is a susceptible host (14) and in others that it is immune (19). Marcon et al. (22) show genetic variability in corn regarding resistance to WSMV. In our case, we did not succeed in inoculating corn var. Ranchero and hybrid H-522. Mechanical transmission of El Batán 3 to rye and barley only occurred at high temperatures (> 28°C), which suggests that temperature affected mechanical transmission. These findings agree with those of Seifers et al. (28), who found resistance in wheat at temperatures of 20 to 25°C.

Although a complete analysis of the mite's morphology was not conducted, it can be stated that its morphological characteristics are similar to those of *A. tosichella*. This is the first report of this species in Mexico. *A. tosichella* is very similar to *A. tulipae*, which until 1996 was reported to be a WSMV vector (10). The two species differ in their morphology; the median line is more pronounced in *A. tulipae* than in *A. tosichella* (16), and the featherclaw on the anterior legs of *A. tulipae* is always 7-rayed (18). *A. tulipae* does not naturally infest wheat and does not transmit WSMV. Although experimentally this species has colonized wheat and transmitted WSMV at low frequency, it does not tend to reproduce abundantly on wheat, unlike *A. tosichella*, which is an efficient vector of WSMV and severely infests wheat (10). For these reasons, Amrine and Stasny (1) proposed to refer to *A. tulipae* only when the mite is found on Liliaceae and to *A. tosichella* as the main species on cereals.

Fig. 1. A, Symptoms caused by El Batán 3 in wheat. B, *Aceria tosichella*'s entire body ($\times 25$). C, *Aceria tosichella*'s dorsal shield ($\times 100$). D, Featherclaw on the anterior and posterior legs of *A. tosichella* ($\times 100$). E, Cylindrical inclusions induced by El Batán 3 in wheat var. Yecora stained with green methyl-rhodamine ($\times 100$). F, El Batán 3 particle ($\times 50,000$).

The transmission of El Batán 3 by the mite identified as *A. tosichella* indicates that the isolate is a WSMV variant. Although *Wheat spot mosaic virus* (WSPMV) (31) and *High Plains disease virus* (HPV) (27) are transmitted by *A. tosichella*, the possibility that the disease was caused by one of these viruses was discarded because they are not mechanically transmitted.

The virus particles observed have characteristics similar to those of members of the family *Potyviridae* (13). This family comprises two genera that infect cereals: *Bymovirus* and *Rymovirus*. The possibility that El Batán 3 is a *Bymovirus* was discarded because the latter shows a wide range of particles, 300 to 2,000 nm in size (33). El Batán 3 particles coincide with those of *Rymovirus*, which average 700 nm in size (5). The inclusions seen under the light microscope showed great similarity to those reported for WSMV (4,8). Although Christie and Edwardson (8) clearly showed WSMV inclusions in Michigan wheat stained with orange-green, in our study it was difficult to see inclusions with orange-green. This could be because we used a different wheat variety. The pinwheel inclusions observed through electron microscopy are similar to those of other potyviruses. In the case of WSMV, Lee (21) and Langenberg (20) reported pinwheel, tubular and laminated aggregates, the pinwheel type being the most common. In our study, the other types of inclusions were not observed, possibly because the fixation time, the wheat variety, and the virus isolate were different from those reported by Lee (21) and Langenberg (20).

The morphological and serological characteristics, as well as the symptomatology and the transmission pattern of El Batán 3, indicate that it is WSMV. The isolate found in Mexico is not mechanically transmitted to the maize varieties tested (H-522 and Ranchero). In this study, El Batán 3 was not seed transmitted. Using polymerase chain reaction (PCR), French (9) confirmed the virus to be WSMV.

WSMV causes damage to bread and durum wheat under experimental conditions (data not shown), while triticale seems to have a certain level of resistance. This virus has only been found sporadically in Mexico and has not been associated with severe losses. Previous observations based in the 1980s by Muñoz-Cárdenas suggested that it was present in northern Mexico, around 240 km from Texas and 830 km from Texcoco, but this work was purely based on the observations of WSMV-like symptoms on breeding lines and did not include identification or characterization of the pathogen (24).

Therefore, this is the first report of the identification and characterization of WSMV and its vector *A. tosichella* in Mexico and the first time it has been reported so far south on in North America. Choi et al. (unpublished data), through comparing sequences of El Batán 3 (GenBank accession AF285170) and U.S. isolates, showed that the Mexican WSMV diverged from the U.S. isolates (Type [GenBank accession AF285169] and Sidney 81 [GenBank accession AF057533]).

Because of WSMV epidemiology and the spreading mode of *A. tosichella*, we might expect both of them to be present, even sporadically, in other Mexican cereal growing areas. At present, however, WSMV incidence seems to be minimal and not the cause of any significant constraints to cereal production in Mexico.

Further monitoring of its occurrence in Mexico would be useful to measure the importance of this disease, while the study of the relationship between the El Batán 3 isolate and other isolates present in Mexico or United States could give us some knowledge of its epidemiology.

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Table 2. Transmission of El Batán 3 by *Aceria tosichella*

Origin of the mite	Infected/inoculated	Symptoms	ELISA ^a
Infected plants	40/40	+	0.502
Non-infected plants	0/10	—	0.030
Control (without mites)	0/10	—	0.023

^a Optical density at 410 nm in enzyme-linked immunosorbent assay (ELISA), measured after 30 min of substrate incubation (anti-serum *Wheat streak mosaic virus*-American-type culture collection).

Table 3. Reaction of El Batán 3 to three polyclonal antibodies in enzyme-linked immunosorbent assay^a

Sample	Antibody/origin		
	WSMV/ATCC	WSSMV/NY	WSBMV/ATCC
El Batán 3	1.416	0.073	0.161
Non-infected wheat	0.046	0.036	0.048

^a Optical density at 410 nm in enzyme-linked immunosorbent assay, measured after 30 min of substrate incubation.

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