# Squash Mosaic Virus in Morocco

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**ABSTRACT**

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Squash mosaic virus was identified for the first time in Morocco and elsewhere in Africa. The virus was identified on the basis of host range, serological reaction, insect and seed transmissibility, particle morphology, and sedimentation properties. The virus was transmitted experimentally by the coccinellid beetle *Epilachna chrysomelina*. The Moroccan virus isolate belonged to serotype I SqMV and did not differ appreciably in biological or serological properties from two Arizona isolates of SqMV. All three SqMV isolates caused systemic infection in *Chenopodium quinoa*, which was previously reported immune to SqMV. The Moroccan virus was isolated from naturally infected *Chenopodium album* in widely scattered locations in southern Morocco and it also systemically infected *Beta vulgaris*. Although the virus was isolated from wild *C. album*, there was no evidence that *Epilachna* transmitted the virus from this plant to cultivated cucurbits. The virus was identified in 3% of random samples of 150 virus-infected melons, squash, and cucumbers growing in the field or in plastic greenhouses.

Squash mosaic virus (SqMV) is widespread in the Americas (1,5) but has been identified elsewhere only in Israel (2), Australia (4), New Zealand (9), and Japan (10).

Cucurbits grown in the field and in plastic tunnel greenhouses along the Atlantic littoral in Morocco are often severely infected by watermelon mosaic (WMV) and cucumber mosaic (CMV) viruses (3), but SqMV had never been isolated from cucurbits in field surveys (1972–1979). Recent surveys of virus diseases of curcurbits in irrigated areas of the Souss and Massa valleys of southern Morocco revealed widespread infection by several distinct strains of WMV and CMV (Lockhart and Hafidi, *unpublished*). This paper reports the identification of a virus causing ring-mosaic symptoms on greenhouse-grown melons (*Cucumis melo* L.) in two locations in the Massa valley as SqMV.

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## MATERIALS AND METHODS

The virus was isolated from a *C. melo* plant from which neither CMV nor WMV was isolated. The virus was transmitted by mechanical inoculation first to *Chenopodium quinoa* Willd., then from its systemically infected leaves to Small Sugar pumpkin (*Cucurbita pepo* L.), in which it was maintained. Two American isolates of SqMV, one of serotype I and the other of serotype II (6), along with their respective antisera, were supplied by Martha Rosemeyer, University of Arizona, Tucson. These virus isolates were also maintained in Small Sugar pumpkin. All mechanical inoculations consisted of crude extracts obtained by grinding systemically infected leaf tissue in 0.02 M phosphate buffer, pH 7.2, containing 0.2% 2-mercaptoethanol.

Insect-transmission tests were done using nonviruliferous adults of *Epilachna chrysomelina* Fabr. that had been reared on virus-free *Cucurbita pepo* 'Verte d'Alger' for three successive generations. In the first experiment, insects were starved for 4 hr, allowed an acquisition access period of 1 hr on infected *C. pepo*, and then transferred in groups of five to healthy plants of *C. melo* 'Charentais' and *C. pepo* 'Small Sugar,' on which they were allowed to feed for 12 hr. In the second experiment, the acquisition access period was lengthened from 1 to 12 hr, and the inoculation access period was maintained at 12 hr.

Transmission of virus through seed was tested by serologically indexing individual seeds after the testa were removed. Mature fruits of *C. melo* 'Charentais' and 'Honey Dew' were harvested from plants infected at the

seedling stage. One hundred seeds of each cultivar were selected at random and soaked in water overnight. After the testa were removed, individual seeds were rinsed, ground in 0.2 ml of distilled water, and the extract was assayed in immunodiffusion plates against homologous antiserum.

The Moroccan SqMV was purified from systemically infected leaves of *C. pepo* 'Fordhook Zucchini' 10–14 days after inoculation at the cotyledonary stage. The virus was extracted in 0.2 M sodium citrate containing 0.2% 2-mercaptoethanol. After clarification with 8.5% *n*-butanol, the virus was precipitated with 6% polyethylene glycol (MW 6000) and further purified by two cycles of differential centrifugation, followed by rate-zonal density gradient centrifugation in 10–40% sucrose gradients.

An antiserum against the Moroccan SqMV isolate was prepared by injecting a rabbit once intravenously with 2 mg of purified virus, followed by four weekly subcutaneous injections consisting of 10 mg of purified virus emulsified with Freund's incomplete adjuvant. The animal was bled 1 wk after the last injection. Double-diffusion serological tests using undiluted plant sap were done in 0.9% agarose gels containing 0.85% NaCl and 0.05% sodium azide.

## RESULTS

The virus was transmitted mechanically to *Cucumis melo* 'Charentais,' 'Cantor,' 'Honey Dew,' and 'Jaune Canarias'; *Cucurbita pepo* 'Small Sugar' and 'Verte d'Alger'; *Cucumis sativus* L. 'Chicago Pickling' and 'National Pickling'; *Cucurbita moschata* 'Butternut'; and *Cucurbita maxima* 'Buttercup.' No symptoms developed on inoculated leaves of these plants; systemic symptoms on other leaves consisted of mosaic, ring-mosaic, and leaf deformation. A mild systemic mottle was produced on *Colocynthis vulgaris*, and latent systemic infection occurred in *Bryonia dioica* Jacq. Only one of nine plants of *Lagenaria siceraria* (Mol.) Standl. became infected, with systemic symptoms consisting of small chlorotic spots. Because these three cucurbits occur frequently near cultivated fields, they were tested as possible sources of virus. No local or systemic symptoms developed on *Citrullus vulgaris* Schrad. 'Charleston Gray' and 'Sugar Baby.' A few local lesions occurred on inoculated cotyledons and primary leaves of *Cucumis metuliferus* (8), but no systemic symptoms developed.

Systemic infection with no local reaction occurred in *Chenopodium quinoa* and *Pisum sativum* L. 'Lincoln' and 'Doux de Provence.' Systemic symptoms in *C. quinoa* consisted of mottle, chlorotic veinbanding and leaf deformation. Three separate selections of *C. quinoa* from France, Germany, and Minnesota all reacted similarly and all three SqMV isolates produced systemic infection and symptoms in this plant, which was previously reported to be immune to SqMV (10). Mild systemic mottle and chlorosis were produced in *P. sativum*, although fewer than one-quarter of the test plants showed these symptoms. Virus from systemically infected *C. quinoa* and *P. sativum* produced typical ring-mosaic symptoms on Small Sugar pumpkin. No local or systemic symptoms developed on *Beta vulgaris* L. 'Detroit Dark Red,' but the virus was recovered by back-inoculation from systemically infected leaves.

No local or systemic symptoms were observed on *Nicotiana clevelandii* Gray, *N. tabacum* L. 'Samsun,' *N. glutinosa* L., *Datura stramonium* L., *Gomphrena globosa* L., *Phaseolus vulgaris* L. 'Bountiful' and 'Red Kidney,' *Petunia hybrida* Vilm., *Lycopersicon esculentum* Mill., *Vicia faba* L., and *Vigna unguiculata* L. (Walp.) 'California Blackeye.'

Following a 1-hr acquisition test period, *E. chrysomelina* transmitted to three of five test plants of *Cucumis melo* 'Charentais' and to two of five test plants of Small Sugar pumpkin. When the acquisition access period was lengthened to 12 hr, the virus was transmitted to all nine test plants of Small Sugar pumpkin. In a parallel test using *Myzus persicae* Sulzer, no virus transmission occurred when starved aphids were allowed a 5-min acquisition access period on infected Small Sugar pumpkin, then transferred immediately, 10 aphids per plant, to healthy seedlings of the same cultivar and allowed to feed overnight.

In immunodiffusion assays of extracts of seed from infected plants, the virus was detected in 3% of *C. melo* 'Charentais' seeds and in 15% of *C. melo* 'Honey Dew' seeds.

In rate-zonal density gradient centrifugation in 10–40% sucrose gradients, the purified virus sedimented as three components that were recovered separately. All three components consisted of icosahedral particles that reacted with homologous and heterologous SqMV antisera. Purified virus produced typical symptoms on test plants. The three components were not assayed separately for infectivity.

In double-diffusion tests, the virus reacted strongly with antiserum to an American strain of SqMV (formerly American Type Culture Collection PVAS 14). A positive reaction was obtained at an antiserum dilution of 1/256, the highest dilution tested. Identical results were obtained with sap from systemically infected leaves of both *C. pepo* and *Chenopodium quinoa*. No reaction occurred with antisera to CMV (ATCC PVAS 242), WMV-1 (Florida isolate), or alfalfa mosaic (AMV). In comparative serological tests using the two Arizona SqMV serotypes and their homologous antisera, the Moroccan and Arizona SqMV serotype I isolates gave confluent precipitin lines that spurred with those of the Arizona serotype II virus, in all three cases. No differences were found, however, between the homologous and heterologous titers of the three antisera, which were 512 (Arizona SqMV II), 1024 (Arizona SqMV I), and 2048 (Moroccan SqMV), respectively.

SqMV was isolated from *Chenopodium album* plants showing systemic chlorotic mottle and spotting that were collected near cucurbit fields and greenhouses in the Souss-Massa area, as well as in the area of Marrakech, 300 km to the north. Plants with similar symptoms were observed frequently in several other locations, but no tests were done to determine the percentage of plants actually infected with SqMV. Five of the 150 randomly sampled squash, melon and cucumber plants showing virus symptoms were infected with SqMV, as determined serologically.

## DISCUSSION

Based on the properties described, the virus isolated from melon was identified as SqMV belonging to serotype I.

Although the coccinellid beetle, *E. chrysomelina*, was found frequently on cucurbits and is capable of transmitting SqMV, its role in natural transmission of the virus has not been determined. There are other possible natural vectors because SqMV has been transmitted by other phytophagous species, including other coccinellids as well as Chrysomelids (1,4,5). *E. chrysomelina* was also reported to transmit SqMV in Israel (2).

Although *C. album* was found to be a natural reservoir of SqMV, the role of *E. chrysomelina* in transmitting the virus from this species to cultivated cucurbits was not determined. *E. chrysomelina* was reported to feed exclusively on cucurbits in Morocco (7), but adult beetles were occasionally observed feeding on developing seed heads of *Chenopodium* sp. growing near cultivated cucurbits. Attempts to induce *E. chrysomelina* to feed on infected *C. album* and *C. quinoa*, however, were unsuccessful. It is possible that the virus also exists naturally in wild cucurbit species like *Colocynthis* and is transmitted from these plants by *E. chrysomelina* and other phytophagous beetles, or that some other vector is capable of transmitting the virus from *Chenopodium* spp. to cucurbits. Transmission of the virus through commercial seed lots was not tested.

Occurrence of SqMV in Morocco is important because of the close proximity to southern Europe, where the presence of SqMV has not been confirmed, and because SqMV is seedborne in cucurbits and therefore must be monitored in cucurbit seed produced locally for commerical distribution.

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