Infection of Sweet Potato by Cucumber Mosaic Virus Depends on the Presence of Sweet Potato Feathery Mottle Virus

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ABSTRACT

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Cucumber mosaic virus (CMV) severely infects sweet potato fields in Israel causing stunting, chlorosis, and yellowing of plants. Identification of CMV was based on host range, serology, and electron microscopy. All CMV-infected field plants also carried sweet potato feathery mottle virus (SPFMV). Transmission of CMV by mechanical, grafting, and aphid inoculations to healthy sweet potato plants failed, but it was achieved easily if the acceptor plants carried SPFMV. Apparently, for replication of CMV in sweet potato, a factor is required that is provided by the presence of SPFMV. This seems to be the first report that CMV has a helper virus requirement.

Cucumber mosaic virus (CMV) is one of the most widespread plant viruses, recorded in more than 190 species, belonging to 40 families (8). The only report of CMV in sweet potatoes that has come to our attention is by Wellman (16), who succeeded in transmitting the disease from infected to healthy sweet potatoes by aphid inoculation, but not by mechanical inoculation. Cucumber mosaic virus has been isolated from wild *Ipomoea setifera* Poir. (12). Martin (11) succeeded in transmitting CMV by mechanical inoculation to *I. nil* (L.) Roth., *I. purpurea* (L.) Roth., *I. lacunosa* L., and *I. trichocarpa* Ell., but not in transmitting to sweet potato (*I. batatas* (L.) Lam.) 'Puerto Rico'. Furthermore, within the last decade, CMV was not found in a sizable number of surveys of various virus diseases in sweet potatoes (2–4,10,15).

Sweet potato feathery mottle virus (SPFMV), an aphid-transmitted potyvirus with a particle length of 830–850 nm (13,14), has been observed in different regions of the world where sweet potato is grown (2,3,15). In Israel, the virus is widespread and can easily be detected by serological tests (6).

Herein we report on a severe infection of sweet potato by CMV observed first in 1986 in fields in the Sharon Valley of Israel, and the results of studies indicating dependence of CMV transmission to sweet potatoes on the presence of SPFMV.

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MATERIALS AND METHODS

Plants. *Ipomoea batatas* 'Georgia Jet', obtained from meristem cultures (apparently virus-free), were propagated by cuttings. *Ipomoea hederacea* Jacq., *I. hederifolia* L., *I. lacunosa*, *I. nil* 'Scarlet O'Hara', *I. purpurea*, *I. setifera*, *I. setosa* Ker., *I. trichocarpa*, *I. tricolor* Cav. 'Heavenly Blue', *I. trifida* (Kunth) G. Don, *I. wrightii* (Wall.), and *Calonyction aculeatum* (L.) House were propagated from seeds obtained by courtesy of J. W. Moyer, North Carolina State University, C. A. Clark, Louisiana State University, and R. N. Campbell, University of California at Davis. All plants, including standard test plants, were kept in a screened greenhouse.

Transmission. For mechanical inoculations, leaf tissue was ground with the aid of a mortar and pestle in 0.05 M phosphate buffer (pH 7.2) containing 0.01 M sodium diethyldithiocarbamate at a ratio of about 1:5 (w/v).

For aphid transmission, adult apterous *Aphis gossypii* Glover were allowed an acquisition access feeding of 10 min, after fasting for 1 hr. After the access feeding period, aphids were kept on the acceptor plant overnight for inoculation. Each transmission experiment was done on five to 20 sweet potatoes cv. Georgia Jet, *I. nil*, bell pepper (*Capsicum annuum* L.), or cucumber seedlings (*Cucumis sativus* L. 'Bet Alpha'), with 10 aphids per plant.

Graft transmissions to Georgia Jet sweet potatoes were done by side grafts. All experiments were done in a screened greenhouse, and no external aphid-borne transmissions were observed. Cucumber seedlings kept as monitors near the experimental plants remained healthy.

Results of transmission to cucumber seedlings, bell peppers, and *I. nil* were based on symptoms after 4–7 days and on enzyme-linked immunosorbent assay (ELISA), while transmissions to sweet potatoes were evaluated only by ELISA.

ELISA and electron microscopy. ELISA was done according to Clark & Adams (5) three wells/treatment, using antisera prepared against CMV isolates Price's No. 6 (CMV-6) and CMV-T (9) and against SPFMV (6). Immunoglobulins and conjugates were used at dilutions of 1:1,000, and leaf homogenates were diluted 1:10 (v/v) in phosphate-buffered saline (0.02 M of phosphate plus 0.15 M of NaCl and 0.0026 M of KCl at pH 7.4), containing 0.05% Tween 20 and 2% polyvinylpyrrolidone (mol wt 40,000).

Virus samples for electron microscopy were negatively stained with 2% uranyl acetate after partial purification from sweet potato leaves (6). For SPFMV, an antiserum from Moyer and a locally produced antiserum (6) were used.

RESULTS

Field surveys. During 1986, two sweet potato fields (cv. Georgia Jet) in the Sharon Valley with plants severely yellowed and stunted were observed (Fig. 1). About 80–90% of the plants were affected. In 1987, three fields similarly affected were observed.

Transmission. Sap inoculation from such diseased sweet potato plants to *Nicotiana glutinosa* L., bell peppers, and cucumber seedlings was achieved,

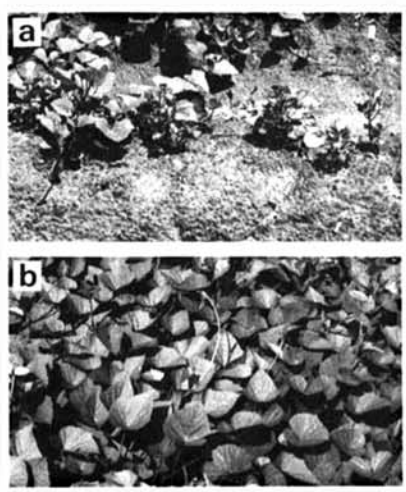

Fig. 1. Sweet potato plants (cv. Georgia Jet) **(A)** severely affected by yellowing and stunting and associated with double infection by cucumber mosaic and sweet potato feathery mottle viruses, compared with **(B)** healthy plants of the same age. (Sharon Valley, Israel).

resulting in systemic mosaic, while inoculation to cowpea (*Vigna unguiculata* Walp. 'Black Eye No. 5') gave reddish local lesions, symptoms typical for CMV. Transmissions to *Chenopodium amaranticolor* Coste & Reyn. and *C. quinoa* Willd. resulted in local lesions. These, however, were not diagnostic for CMV, because SPFMV also incites similar symptoms. Sweet potato feathery mottle virus could not be transmitted mechanically to cowpea, cucumber, or *N. glutinosa*. Sap inoculation from 90 diseased, field-collected plants gave positive reactions for CMV on cowpeas and/or cucumbers in 51 cases (57%).

Aphid inoculations from 20 field-collected Georgia Jet sweet potato plants that previously indexed positive for CMV by inoculation to cowpea resulted in 100 and 33% transmissions to cucumber seedlings and bell peppers, respectively.

Various Convolvulaceae plants were found to be susceptible to CMV. Cucumber mosaic virus was transferred from sweet potatoes by aphids, was cultured in cucumber (immune to SPFMV), and was used for mechanical inoculation. *Ipomoea nil*, *I. purpurea*, *I. lacunosa*, *I. trichocarpa*, *I. hederaceae*, *I. hederifolia*, and *C. aculeatum* were susceptible to CMV, developed symptoms 7–12 days after inoculation, and gave a positive ELISA reaction. *Ipomoea wrightii*, *I. trifida*, and *I. tricolor* were susceptible to CMV, according to ELISA and indexing on cowpeas, but did not develop symptoms themselves when inoculated. Transmissions were not achieved when *I. setosa* was inoculated mechanically, as evaluated by ELISA.

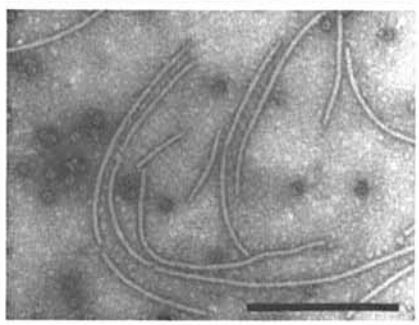

Fig. 2. Electron micrograph of partially purified extracts from sweet potato plants doubly infected with cucumber mosaic virus and sweet potato feathery mottle virus negatively stained with 2% uranyl acetate. Bar = 0.5μ.

ELISA and electron microscopy. Infected field- and greenhouse-grown Georgia Jet sweet potato plants gave positive ELISA reactions with CMV-6 and CMV-T antisera. ELISA values (A_{405}) with CMV-6 antiserum ranged between 0.226 and 2.104, with an average of 1.097 from 60 field-infected plants, compared with a range of 0.000 to 0.047, with an average of 0.015 from 25 control Georgia Jet plants without CMV (infected with SPFMV). ELISA values obtained with extracts from healthy Georgia Jet plants derived from meristem cultures ranged between 0.000 and 0.030. Similar results were obtained with the CMV-T antiserum.

Polyhedral particles, about 28 nm in diameter, were obtained in electron micrographs of sweet potato plants positive for CMV by ELISA (Fig. 2). Typical CMV particles were observed in the electron micrographs from all of 15 field-collected and 10 greenhouse-grown sweet potato plants positive for CMV by ELISA. All these plants gave positive ELISA reactions for SPFMV and all had flexuous particles in their electron micrographs.

Transmission of CMV to sweet potato. A CMV isolate obtained by aphid transmission from sweet potato plants to cucumber seedlings could easily be transmitted mechanically and by aphid inoculation to cucumbers and *I. nil*, but not to healthy sweet potato plants obtained from meristem cultures. However, sweet potato plants were easily infected with CMV by both mechanical and aphid inoculation if the acceptor plant carried SPFMV. Grafting CMV-infected *I. nil* onto sweet potato did not transmit CMV, unless the acceptor plants carried SPFMV (Table 1). The presence of SPFMV in addition to CMV in the sweet potato donors resulted in graft transmission of both CMV and SPFMV to sweet potato, but mechanical transmission of CMV to sweet potato was obtained only when they were already infected with SPFMV. Aphid transmission from doubly infected plants was achieved easily when the acceptor plant was already infected with SPFMV, but only seldom (one out of 35 plants) for healthy sweet potato (Table 1). No transmissions were obtained when nonviruliferous control aphids were placed on cucumber and sweet potato plants (data not shown).

Table 1. Transmission of cucumber mosaic virus (CMV) to healthy sweet potato plants and those infected by sweet potato feathery mottle virus (SPFMV)

CMV inoculum source	Grafting		Mechanical		Aphids[a,b]	
	Healthy	SPFMV	Healthy	SPFMV	Healthy	SPFMV
Cucumber	...	...	0/77[c]	22/22	0/15	9/10
Ipomoea nil	0/11	14/28	0/25	16/40	0/10	9/10
Sweet potato (CMV+SPFMV)	18/31	7/8	0/60	6/18	1/35	17/18

[a] Ten *Aphis gossypii* per plant.
[b] No transmissions were obtained when 10 aphids, without acquisition access feeding, were placed for control on each of 10 cucumber and 10 Georgia Jet sweet potato plants.
[c] Number of plants infected with CMV (as determined by ELISA)/no. of plants used.

DISCUSSION

High rates of infection by CMV were noted in several fields in Israel during the last 2–3 yr. This CMV isolate did not seem to be specific for sweet potatoes, because it could be transmitted easily by aphids to cucumber and bell pepper, and it reacted similarly with two antisera. In CMV-infected sweet potato fields, SPFMV was prevalent, and all plants indexed for CMV also carried SPFMV. These doubly infected plants were severely stunted and yellow. Transmission of CMV to sweet potato either mechanically or by aphids (for the most part) required the presence of SPFMV in the acceptor plant. Grafting of doubly infected (CMV-SPFMV) sweet potato donors to healthy sweet potato plants transmitted both viruses to the acceptors. Apparently, in this case, SPFMV established itself first in the acceptor, thereby providing conditions for CMV replication. In one case, CMV was transmitted by aphids from a doubly infected plant to a healthy sweet potato. It may be that the aphids were able to transmit both viruses simultaneously into the same cell, thereby enabling SPFMV to provide the necessary requirements for CMV replication. It may be that in sweet potato SPFMV acts as a "helper" virus, providing CMV with a necessary factor for replication. Although we cannot rule out that this specificity is unique to the sweet potato cultivar used (Georgia Jet), it does not seem likely, because Martin (11) was unable to transmit CMV mechanically to cv. Puerto Rico or to 20 sweet potato seedlings originating from open-pollinated seed.

A similar case has been reported for the activation of tobacco mosaic virus (TMV) replication in barley plants by a certain strain of barley stripe mosaic virus (BSMV) (7); TMV alone does not replicate in this host. It was suggested that TMV can be regarded as defective in nonhost plants like barley, and that in mixed infected barley cells BSMV compensates for this defectiveness (1).

From the practical point of view, epidemics of CMV in sweet potato can apparently be avoided by using SPFMV-tested propagation material. In sweet potato fields in Israel, where such planting material has been used, CMV has not been observed.

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