Seed Transmission of Squash Mosaic Virus in *Chenopodium* spp.

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ABSTRACT

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Squash mosaic virus (SqMV), which occurs naturally in Morocco in *Chenopodium* spp., was found to be seed-transmitted in *C. murale* and *C. quinoa*. Seed-transmission of SqMV, determined by enzyme-linked immunosorbent assay of germinated seedlings, was 20% in *C. quinoa* and 23% in *C. murale*. *Atriplex glauca*, a widespread, drought-resistant chenopodiacious weed, was infected systemically by SqMV, thereby constituting another potentially important reservoir of the virus.

Squash mosaic virus (SqMV), reported for the first time in Morocco and in Africa in 1982, was found to occur in naturally infected *Chenopodium* spp. in widely scattered locations in Morocco (8). The Moroccan SqMV isolate, like previously described isolates of the virus (1), was found to be seed-transmitted in cucurbits (8). Because of the susceptibility of both wild (e.g., *Chenopodium* spp. and *Atriplex glauca*) and cultivated (e.g., *Beta vulgaris* and sugar beet) chenopodiacious species to infection by SqMV (8), it was considered of interest to determine whether SqMV was seed-transmitted in Chenopodiaceae as well as in Cucurbitaceae. The two chenopodiacious species chosen for study were *C. murale*, which occurs naturally in Morocco (9) and is the most common weed in cultivated cucurbit and other vegetable fields in southern Morocco, and *C. quinoa*, a widely-used virus indicator plant.

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MATERIALS AND METHODS

The virus used in these studies was the Moroccan SqMV isolate described previously (8). The Moroccan SqMV is a serotype I (10) isolate of SqMV. The virus was maintained in *Cucurbita pepo* L. 'Fordhook Zucchini.' The virus isolate was checked by electron microscopy and serology to verify freedom from contamination by watermelon mosaic virus (WMV), since some WMV isolates occurring in southern Morocco, like SqMV, also infect *Chenopodium* spp. systemically (B. E. L. Lockhart, *unpublished*). Healthy seedlings of *C. murale* and *C. quinoa* were inoculated mechanically with SqMV at the four-leaf stage and maintained in the greenhouse until maturity, then the seeds were harvested. Mechanical inoculations were done using crude extracts obtained by grinding infected tissue in 1% K_2HPO_4 containing 0.2% 2-mercaptoethanol.

For seed-transmission tests, seeds collected from infected *C. quinoa* and *C. murale* were rinsed thoroughly with detergent and water and sown in steam-sterilized soil. One hundred seedlings of each species, at the two- to four-leaf stage, were selected randomly and tested by the double-antibody sandwich (DAS) method of enzyme-linked immunosorbent assay (ELISA) (2). The homologous antiserum used in these assays was prepared as described previously (8). Polystyrene plates were coated with gamma globulin at a concentration of 0.1 μg/ml. Plant samples were applied at a dilution of 1/5 (w/v) in phosphate-buffered saline, pH 7.4, containing 0.05% Tween 20 and 2% polyvinylpyrrolidone (PBST-PVP). Alkaline phosphatase was used at a 1/2,000 dilution. Results were determined spectrophotometrically at 405 nm using a Dynatech microplate reader. As a further verification of seed transmissibility of SqMV, several samples that reacted positively in ELISA were inoculated to healthy seedlings of *C. pepo*.

RESULTS AND DISCUSSION

Twenty of 100 seedlings of *C. quinoa* and 23 of 100 seedlings of *C. murale* reacted positively in ELISA with SqMV antiserum. Spectrophotometric readings averaged 0.02 for healthy controls and uninfected seedlings and 0.67 for virus-infected seedlings. Pooled extracts of five positively reacting samples each of *C. quinoa* and *C. murale*, inoculated to healthy seedlings of *C. pepo* 'Fordhook Zucchini,' caused systemic infection in the latter. The symptoms produced were characteristic of SqMV infection, and the identity of the virus was confirmed by serological reaction in agarose immunodiffusion tests.

Although watermelon mosaic virus (WMV-1 and WMV-2) (11) and zucchini yellow mosaic virus (ZYMV) (6) are not known to be seed-transmitted, they occur endemically on cucurbits in Morocco (3,4,7; Lockhart et al, *unpublished*). To verify that the positive ELISA readings obtained with *C. murale* and *C. quinoa* were not due to contamination by WMV-1, WMV-2, or ZYMV, the SqMV gamma globulin and alkaline phosphatase conjugate were tested in DAS ELISA using leaf tissue of *C. pepo* infected with SqMV, WMV-1, WMV-2, and ZYMV. The results, expressed as spectrophotometric readings at 405 nm, blanking with PBST-PVP, were as follows (average of four replicates per treatment): healthy *C. pepo*, 0.05; SqMV, 1.68; WMV-1, 0.04; WMV-2, 0.03; and ZYMV, 0.02. These results clearly demonstrate the absence of any possible contamination by WMV-1, WMV-2, or ZYMV.

The results confirm the seed-transmissibility of SqMV in *Chenopodium* spp. Sugar beet (8), and *Atriplex glauca* (5) were also experimentally infected systemically with SqMV. The latter is a drought-tolerant weed that is distributed widely throughout southern Morocco. SqMV has not been found in nature in *A. glauca*, which remains a potential reservoir of SqMV, and seed-transmissibility of the virus in this widespread chenopodiacious weed would further enhance that potential. Sugar beets are not grown in the cucurbit-growing areas of southern Morocco, and the possible occurrence of SqMV in sugar beets would therefore not need to be considered in the epidemiology of SqMV in cucurbits grown both for export and local consumption in southern Morocco. However, in areas in which both cucurbits and sugar beets are grown, it may be of interest to investigate the possible movement of SqMV between these two crops. The transmission of SqMV from chenopodiacious source plants to cultivated cucurbits remains to be demonstrated. No transmission of SqMV from *C. quinoa* or *C. album* to *C. pepo* could be obtained using *Henosepilachna elaterii* (Rossi) (= *Epilachna chrysomelina* (F.)) (8). Until a suitable vector can be identified, the role of chenopodiacious weeds in the epidemiology of SqMV remains uncertain but of great interest.

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