Evidence for Identity of Plant Rhabdoviruses Causing Vein-Yellowing Diseases of Tomato and *Hibiscus rosa-sinensis*

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ABSTRACT

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A mechanically transmitted plant rhabdovirus associated with vein-yellowing symptoms in hibiscus (*Hibiscus rosa-sinensis*) in Morocco was similar to tomato vein-yellowing virus (TVYV) in particle morphology, dimensions, and symptoms on indicator *Nicotiana* species plants and was indistinguishable from TVYV in agar gel immunodiffusion tests. The virus was readily detected in infected *H. rosa-sinensis* by enzyme immunoassay (EIA) using TVYV immunoglobulin G. These findings suggest that TVYV is more widely distributed than is currently believed and may be spread by infected vegetative stock of *H. rosa-sinensis*.

Tomato vein-yellowing virus (TVYV), a mechanically transmitted plant rhabdovirus, was found in Morocco in 1982 (3) and has not been reported elsewhere. TVYV occurs naturally in tomato (*Lycopersicon esculentum* Mill.), *Solanum sodomaeum* L. (4), eggplant (*S. melongena* L.), and *S. nigrum* L. (B. E. L. Lockhart, *unpublished*). TVYV caused vein yellowing and leaf and stem deformation in naturally infected hosts. It was transmitted mechanically to a narrow range of test plants consisting of solanaceous species and *Gomphrena globosa* L. (4).

Hibiscus (*Hibiscus rosa-sinensis* L.) cultivars are commonly infected with hibiscus chlorotic ringspot (HCRSV) (7) and hibiscus latent ringspot (HLRSV) (1) viruses, both of which have isometric particles.

Rhabdoviruslike particles were observed in ultrathin sections of hibiscus (*H. rosa-sinensis*) with vein-yellowing symptoms in the Canary Islands and in Greece (5). Similar symptoms (Fig. 1) had been observed on *H. rosa-sinensis* throughout Morocco since 1972, but no attempt had been made to isolate the virus from diseased plants. Because of the similarity of symptoms of the vein-yellowing diseases of tomato and of *H. rosa-sinensis* and morphological resemblance of the rhabdovirus particles associated with both diseases, an attempt was made to determine whether any relationship existed between the two viruses.

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MATERIALS AND METHODS

Virus isolates. The TVYV isolate used in this study was isolated from tomato (*L. esculentum* cv. Roma VF) in northern Morocco (4) and propagated in *Nicotiana rustica* L. The hibiscus rhabdovirus was isolated from *H. rosa-sinensis* (cultivar unknown) showing typical vein-yellowing symptoms (Fig. 1). This specimen was collected at Agadir, Morocco. The virus isolate from hibiscus was transmitted mechanically to and propagated in *N. benthamiana* Domin., *N. rustica*, and *N. debneyi* Domin.

Mechanical inoculation. Mechanical inoculations were done by grinding infected leaf tissue of either tobacco or hibiscus in 1% K_2HPO_4 containing 0.2% Na_2SO_3. Carborundum (600 mesh) was added to the crude extract, which was used to inoculate indicator plants.

Electron microscopy. Crude leaf extracts of tobacco and hibiscus were negatively stained with 2% ammonium molybdate, pH 6.8 (AM). Immunoelectron microscopy (IEM) was done as described previously (4). Antisera to HLRSV (1) and HCRSV (7) were supplied by A. A. Brunt, Glasshouse Crops Research Institute, Littlehampton,

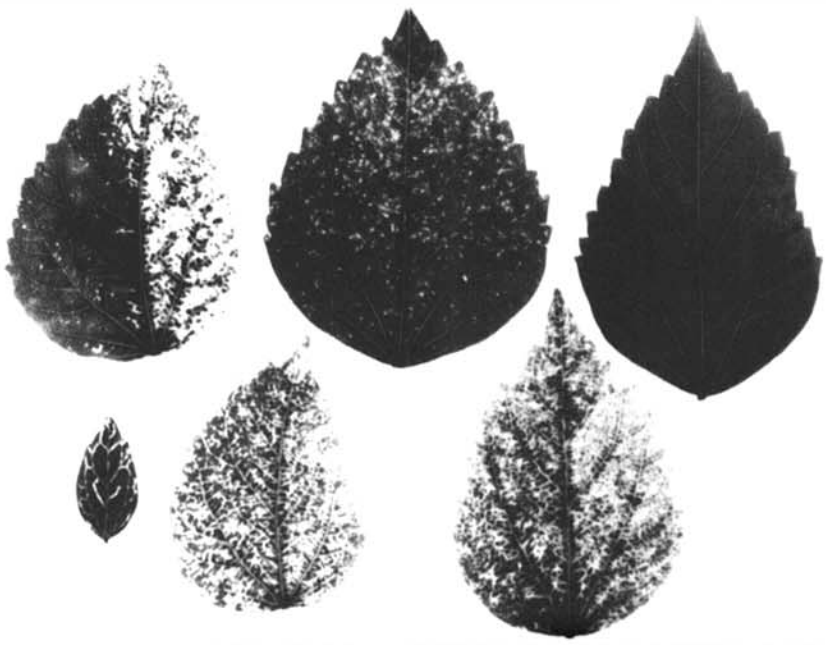

Fig. 1. Vein-yellowing symptoms associated with rhabdovirus infection in *Hibiscus rosa-sinensis*. (Top right) Symptomless leaf. All plants sampled, both with and without vein-yellowing symptoms, were also infected with hibiscus chlorotic ringspot virus as determined by enzyme immunoassay.

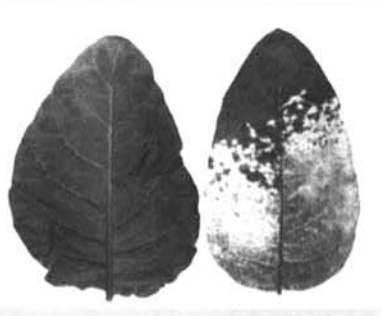

Fig. 2. Systemic symptoms of hibiscus rhabdovirus infection on mechanically inoculated *Nicotiana rustica*. (Left) Healthy leaf and (right) infected leaf.

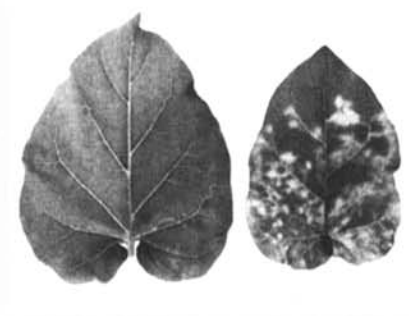

Fig. 3. Systemic symptoms of hibiscus rhabdovirus infection on mechanically inoculated *Nicotiana glutinosa*. (Left) Healthy leaf and (right) infected leaf.

England, and C. J. Gabriel, USDA Plant Introduction Station, Glenn Dale, MD, respectively.

Serology. Preparation of TVYV antiserum has been described previously (4). Immunodiffusion tests were done in 0.8% agarose gels prepared in distilled water containing 0.2% NaN_3. Antigens consisted of undiluted leaf sap of *N. rustica* or *N. benthamiana* infected with either TVYV or the hibiscus rhabdovirus. Undiluted leaf sap from uninfected *N. rustica* or *N. benthamiana* was used as the healthy control. For double-antibody sandwich EIA tests (2), polystyrene plates were coated with immunoglobulin G (IgG) at a concentration of 1 μg/ml. Healthy and diseased hibiscus leaf tissue samples were ground in PBS-Tween (2) containing 1% Na_2SO_3, and the resulting extracts were diluted in the same buffer. Alkaline phosphatase-IgG conjugate was used at a 1/1,000 dilution. Results were determined spectrophotometrically at 405 nm with a Dynatech EIA microplate reader.

RESULTS

Virus transmission, symptoms on test plants. The rhabdovirus associated with vein yellowing in hibiscus in Morocco was readily transmitted mechanically from diseased hibiscus to healthy *N. benthamiana, N. glutinosa, N. rustica,* and *N. debneyi*. Symptoms in these test plants consisted of local and systemic chlorotic spotting and vein yellowing (Figs. 2 and 3). These symptoms were identical to those produced by TVYV on the same test plants (4). *G. globosa*, a host of TVYV (4), was not tested. The hibiscus rhabdovirus was not transmitted by mechanical inoculation to apparently healthy hibiscus. The virus was, however, readily graft-transmitted from diseased to symptomless hibiscus, causing typical vein yellowing. The rhabdovirus infection was confirmed by electron microscopy and EIA. Neither TVYV nor the hibiscus rhabdovirus was transmitted mechanically from infected *N. benthamiana* to healthy hibiscus. Eight months after inoculation, test plants showed no veinclearing symptoms and no virus was detected by EIA.

Electron microscopy. Rhabdovirus particles (Fig. 4) similar in morphology and dimensions to those of TVYV were associated in all cases with vein yellowing in hibiscus. These particles measured 156–176 × 72–75 nm (52 particles measured) in unfixed crude leaf extracts stained in AM. No such particles were ever observed in extracts of leaf tissue of apparently healthy plants or of plants showing symptoms of HCRSV infection (7) only, without vein yellowing. As with those of TVYV (4), the particles of the hibiscus rhabdovirus were disrupted by neutral 2% sodium phosphotungstate (PTA) but were stable in 2% AM. As in the case of TVYV (4), groups of hibiscus rhabdovirus particles occurred in membrane-bound vesicles in negatively stained leaf extracts (Fig. 4).

In IEM tests done to determine whether the rhabdovirus source plant was also infected with HLRSV and/or HCRSV, isometric viruslike particles were trapped by antiserum to HCRSV but not to HLRSV.

Serology. Sap from infected *N. benthamiana, N. rustica, N. debneyi,* or *N. glutinosa* reacted with TVYV antiserum and produced a precipitin line confluent with that of the homologous TVYV antigen (Fig. 5). In intragel absorption tests in which the central depot was initially charged with hibiscus rhabdovirus antigen in *N. benthamiana* leaf sap, before addition of TVYV antiserum, no precipitin lines were observed between the antiserum and peripheral wells charged with either TVYV or hibiscus rhabdovirus antigen.

With EIA, rhabdovirus infection was easily detected by TVYV IgG. Typical A_{405nm} readings (average of six sample wells) for a 1/200 tissue sample dilution were 1.02 for vein-yellowing-infected hibiscus leaf tissue and 0.03 for healthy hibiscus leaf tissue. Similar readings for hibiscus rhabdovirus-infected and healthy *N. glutinosa* leaf extracts were 1.48 and 0.03, respectively. EIA was also used to confirm HCRSV infection in the hibiscus source plant. A_{405nm} readings (average of six sample wells) were 1.13 for infected compared with 0.07 for healthy hibiscus leaf tissue. Readings for both healthy and hibiscus rhabdovirus-infected *N. glutinosa* were 0.09, indicating that HCRSV was not transmitted from hibiscus to *N. glutinosa*.

DISCUSSION

It is likely that the vein-yellowing disease reported in hibiscus in the Canary Islands and in Greece (6) is identical to

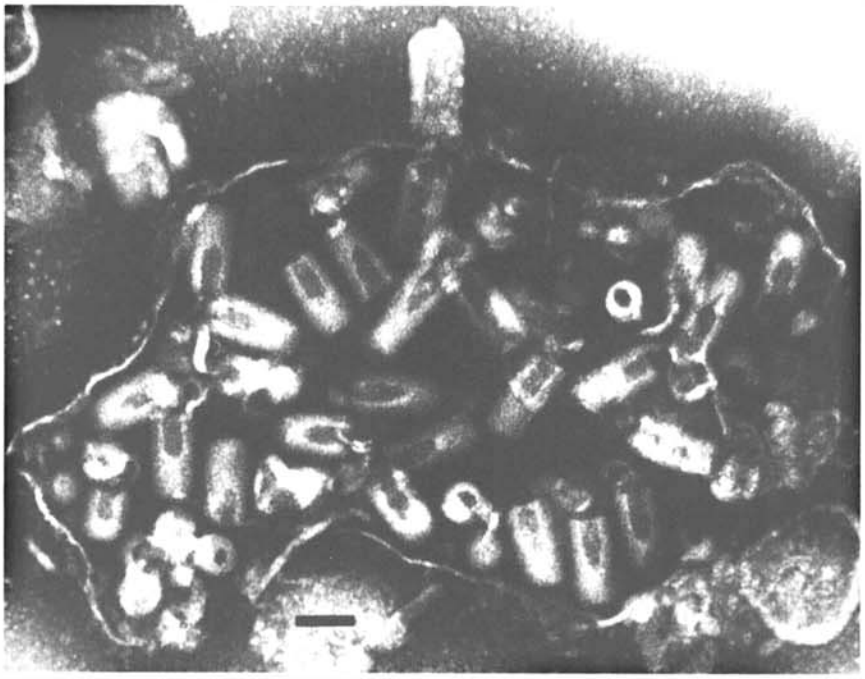

Fig. 4. Rhabdovirus particles, enclosed in a membrane, in crude leaf extract of *Hibiscus rosa-sinensis* leaf with vein-yellowing symptoms (stained with 2% ammonium molybdate, pH 6.8). Scale bar = 100 nm.

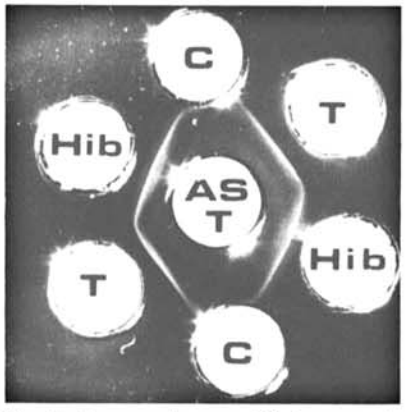

Fig. 5. Agarose immunodiffusion reaction between tomato vein-yellowing virus (TVYV) antiserum (AS-T) and TVYV (T) and *Hibiscus rosa-sinensis* vein-yellowing rhabdovirus (Hib) antigens. Both viral antigens in undiluted infected *Nicotiana benthamiana* leaf sap. Control wells (C) contain healthy *N. benthamiana* leaf sap.

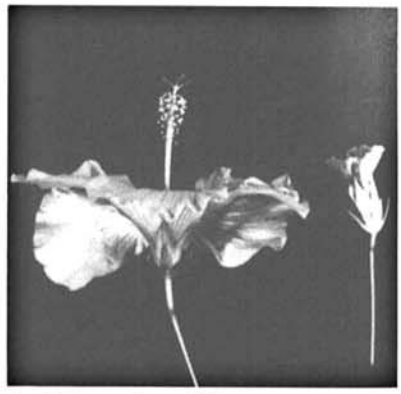

Fig. 6. Flowers from *Hibiscus rosa-sinensis* cv. Empire infected with (left) hibiscus chlorotic ringspot virus (HCRSV) only and (right) with HCRSV plus vein-yellowing rhabdovirus. In mixed HCRSV-rhabdovirus infections, flowers have yellow calices and fall before opening fully.

the one occurring in Morocco and that the same rhabdovirus is associated with the vein-yellowing disease in all three locations. In unfixed extracts stained with AM, the particles of TVYV measured 150 × 75 nm (4) and those of the Moroccan hibiscus rhabdovirus, cited above, measured 156–176 × 72–75 nm. In ultrathin sections, the hibiscus rhabdovirus occurring in the Canary Islands and Greece measured 220–240 × 65–70 nm. No particle dimension of TVYV in ultrathin sections was cited (4), but bullet-shaped particles of TVYV, fixed in glutaraldehyde before negative staining, measured 286 × 86 nm (4). Like TVYV (4), the hibiscus rhabdovirus occurring in the Canary Islands and in Greece accumulates in the perinuclear space (6). Both viruses therefore share this property with members of rhabdovirus subgroup 2 (5).

Based on symptomatology, serological reaction, and particle morphology and size, it is proposed that TVYV and the rhabdovirus associated with hibiscus vein yellowing are either identical or closely related viruses.

Although rhabdovirus infection was invariably associated with vein-yellowing symptoms in *H. rosa-sinensis*, it was not established as the sole cause. To unequivocally attribute hibiscus vein-yellowing symptoms to rhabdovirus infection, it would be necessary to obtain source and/or test plants of *H. rosa-sinensis* free of infection by HCRSV, HLRSV, and other viruses. EIAs determined that typical TVYV-like symptoms, caused by hibiscus rhabdovirus in *Nicotiana* spp., were not associated with simultaneous HCRSV infection.

Because the rhabdovirus associated with hibiscus vein yellowing is closely related to and may be identical to TVYV, there is potential for spread of TVYV by infected hibiscus planting materials. Although no vector has been identified for either virus, spread of TVYV from weed hosts to cultivated tomato and eggplant in Morocco suggests the existence of a natural vector(s).

It should also be noted that although HLRSV and HCRSV, which occur commonly in *H. rosa-sinensis* cultivars, are reported to produce little effect on plant appearance and flower quality (6), vein-yellowing rhabdovirus infection in hibiscus was found to be associated with a pronounced yellowing of the calyx, failure of flower buds to open fully (Fig. 6), and premature flower drop. Whether such flower symptoms are due to infection by vein-yellowing rhabdovirus alone or to mixed virus infection remains to be determined.

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