Bean Yellow Mosaic Virus Isolated from *Gibasis geniculata*

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ABSTRACT

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A potyvirus was isolated from Tahitian bridal veil (*Gibasis geniculata*) plants exhibiting severe stunting and leaf distortion. Infected cells contained laminated aggregates and crystalline inclusions. The virus reacted strongly with bean yellow mosaic virus and clover yellow vein virus antisera but differed in host range from reported bean yellow mosaic or clover yellow vein virus strains. The bridal veil virus isolate has been designated BYMV-BV.

Additional key words: Commelinaceae, greenhouse crops, ornamental crops, *Tradescantia*

Tahitian bridal veil (*Gibasis geniculata* (Jacq.) Rohw.) is a member of the Commelinaceae. It is used as an ornamental house plant and grows as a free-branching creeper with a stringlike stem (3). Slow-growing, compact plants with small, twisted leaves were observed by a commercial grower in a single greenhouse in Virginia. This stunting and leaf distorting disease (Fig. 1) was found to be caused by a potyvirus serologically related to certain bean yellow mosaic virus (BYMV) and clover yellow vein virus (CYVV) strains. Other potyviruses have been described that infect the Commelinaceae, but none of them has been shown to be related to known potyviruses (7–9,11). Data on host range, symptomatology, serology, and electron microscopy of the bridal veil virus are presented herein.

MATERIALS AND METHODS

Diseased Tahitian bridal veil plants (Fig. 1) were obtained from a commercial greenhouse in Roanoke, VA. Leaves and stems from symptomatic plants were homogenized in 0.01 M sodium phosphate (pH 7.0) in chilled mortars and pestles. This preparation was rubbed onto Carborundum-dusted leaves of asymptomatic bridal veil plants propagated from cuttings. Inoculated plants developed leaf distortion and stunting similar to the original plants. The virus was maintained in bridal veil plants by mechanical transmission.

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For host range studies, members of the Commelinaceae, Leguminosae, Chenopodiaceae, Solanaceae, and Cucurbitaceae were grown in a soilless potting mix under greenhouse conditions, inoculated with the bridal veil virus, and observed for 2–4 wk for symptom development. Sap from all plants was back-inoculated to asymptomatic bridal veil plants to confirm presence of the virus.

Particle morphology was determined from leaf-dip preparations of infected bridal veil plants using 1% uranyl acetate as a negative stain. Tissue samples (1–3 mm^2) of infected bridal veil, *Tradescantia fluminensis* Vell., and tobacco (*Nicotiana tabacum* L. 'Burley 21') plants were fixed in Karnovsky's formaldehyde-glutaraldehyde fixative (4), dehydrated in a graded ethanol-acetone series, and embedded in Spurr's resin (16). Thin sections were cut with a diamond knife, stained with uranyl acetate and lead citrate, and examined in a JEM 100-B or a JEM 100-C transmission electron microscope.

Partial purification of the virus was accomplished by using a modification of a method described for soybean mosaic virus (13) and Xanthi tobacco leaves exhibiting local chlorotic lesions 15 days postinoculation (DPI). The virus was concentrated by adding 4% PEG 6000 and was resuspended in 0.005 M sodium citrate, pH 7.0.

Serologically specific electron microscopy using the modified Derrick system (10), with and without decoration, was performed with sap extracted from Alaska pea plants infected with the bridal veil virus. A 1:10 serum dilution was used with all antisera tested except BYMV-OHS antiserum, which was used at 1:8. Ouchterlony double diffusion tests were performed in a medium consisting of 0.6% Ionagar No. 2, 0.2% sodium dodecyl sulfate (SDS), 0.7% sodium chloride, and 0.1% sodium azide (18). Crude sap from Alaska pea plants infected with the bridal veil virus, with BYMV strain 204-1 (designated in this paper as BYMV-KY), or with CYVV was placed in the peripheral wells. A partially purified preparation of the bridal veil virus from tobacco was also used. Antisera used in these tests included those to BYMV-KY, BYMV-OHS, CYVV, soybean mosaic virus (SMV), and tobacco etch virus (TEV). The BYMV strains and their antisera were obtained from R. T. Jones and T. P. Pirone (University of Kentucky) and had been utilized in previous studies (6). The CYVV and its antisera were obtained from O. W. Barnett (Clemson University)

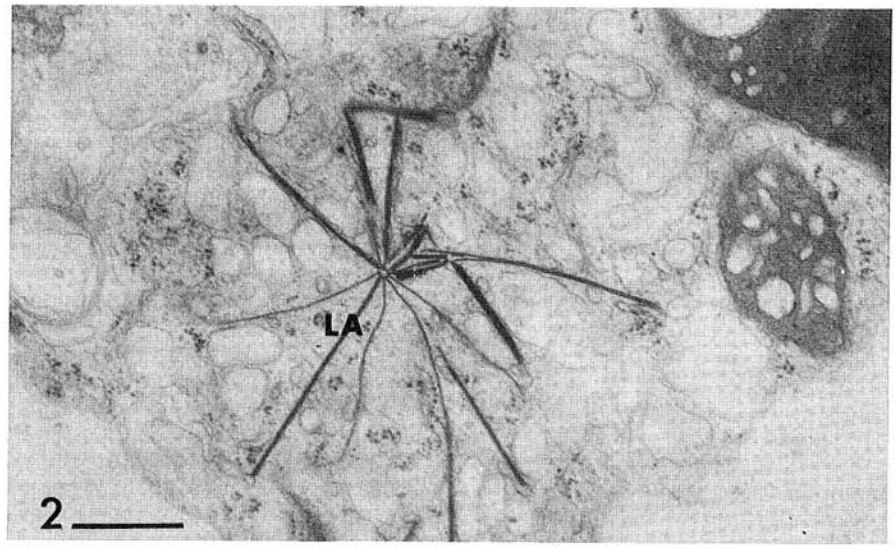
Fig. 1. Tahitian bridal veil plants, *Gibasis geniculata*: **(A)** Stunted plant infected with the bridal veil virus. **(B)** Healthy plant.

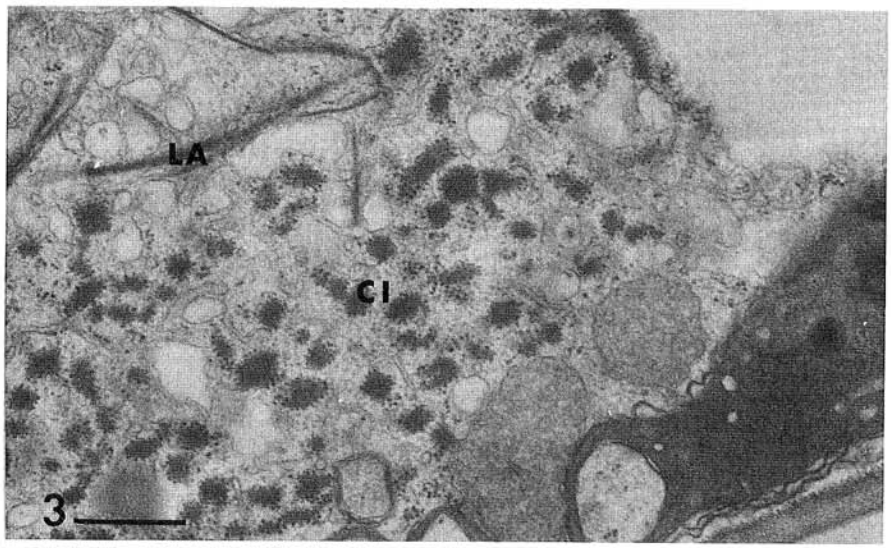
and F. W. Zettler (University of Florida) and were designated CYVV(B) and CYVV(Z), respectively. Antisera to SMV and TEV were prepared in this laboratory to common isolates of the viruses.

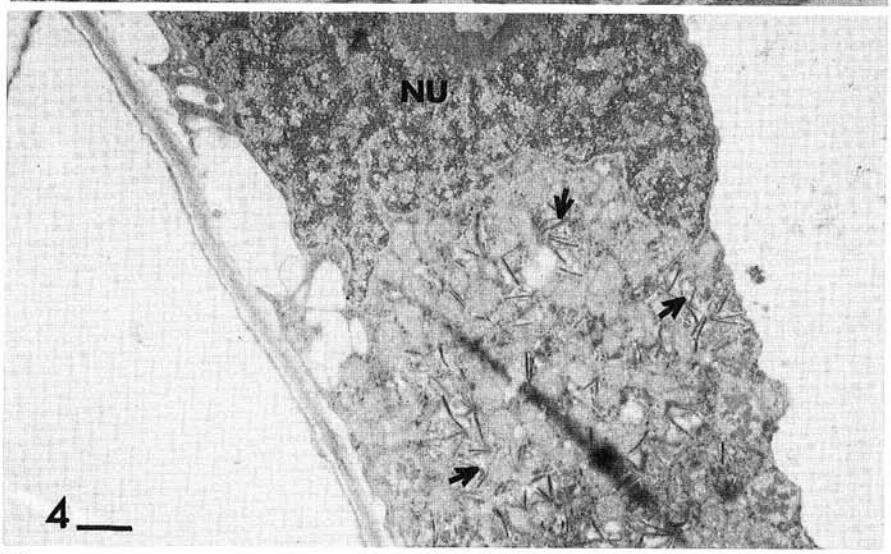
RESULTS

Of the 21 plant species tested in the host range study, nine were susceptible to the bridal veil virus (Table 1). On *T. fluminensis*, the virus induced necrotic lesions and vein-clearing but did not cause stunting. The only symptom observed on *T. albiflora* Kunth., however, was severe stunting of the plants. Local chlorotic lesions developed on three of the four *Nicotiana* varieties tested. The virus induced the most severe symptoms on *Vicia faba* L. 'Long Pod,' causing necrotic lesions on inoculated leaves and a systemic mosaic and upward curling of subsequent leaves. On *Pisum sativum* L. 'Alaska,' vein-clearing appeared on developing leaves 10–12 DPI, with tip necrosis developing within 3–4 wk. *Chenopodium quinoa* Willd. developed necrotic lesions on inoculated leaves 6–7 DPI and subsequently developed systemic symptoms. No symptoms were detected on nor could any virus be recovered from *Phaseolus vulgaris* L. 'Bountiful' or 'Topcrop,' both of which are reported diagnostic hosts for BYMV (1).

Flexuous rod-shaped particles, with a modal length of 808 nm, were observed in leaf dips of infected plants. Laminated aggregates and crystalline inclusions were observed in thin sections of infected bridal veil, *T. fluminensis*, and tobacco leaves (Figs. 2 and 3). The laminated aggregates were seen in the cytoplasm, often in close association with nuclei (Fig.







Figs. 2–4. Electron micrographs of bridal veil leaf cells infected with the bridal veil virus: **(2)** Laminated aggregates (LA) within cytoplasm of mesophyll cell. Bar = 0.5 μm. **(3)** Crystalline inclusions (CI) and laminated aggregates within cytoplasm of mesophyll cell. Bar = 0.5 μm. **(4)** Laminated aggregates and crystalline inclusions (arrows) in close association with the nucleus (NU) of mesophyll cell. Bar = 1 μm.

Table 1. Host range of a virus isolated from Tahitian bridal veil (*Gibasis geniculata*)

Host	Symptoms[a]
Commelinaceae	
Gibasis geniculata (Jacq.) Rohw.	St
Tradescantia fluminensis Vell.	VC, NL
T. albiflora Kunth.	St
Commelina communis aureo-striata L.	NS
Solanaceae	
Nicotiana tabacum L. 'Xanthi'; 'Burley 21'	LCL
N. tabacum 'NC 95'	NS
N. clevelandii Gray × *N. glutinosa* L.	LCL
Datura stramonium L.	NS
Leguminosae	
Phaseolus vulgaris L. 'Topcrop'; 'Bountiful'; 'Kentucky Wonder Wax'	NS
Pisum sativum L. 'Alaska'	VC, TN
P. sativum 'Little Marvel'	NS
Glycine max (L.) Merr. 'Essex'; 'Lee'	NS
Vicia faba L. 'Long Pod'	NL, Mo, ULC
Vigna unguiculata (L.) Walp. 'Blackeye'	NS
Chenopodiaceae	
Chenopodium quinoa Willd.	NL, CS
Cucurbitaceae	
Cucurbita pepo L. 'Caserta'	NS

[a] LCL = local chlorotic lesions on inoculated leaves, NL = local necrotic lesions, Mo = systemic mosaic, St = stunting, VC = vein-clearing, ULC = upward leaf curling, TN = top necrosis, CS = systemic chlorotic spotting, and NS = no symptoms and no recovery of virus in back inoculations to bridal veil.

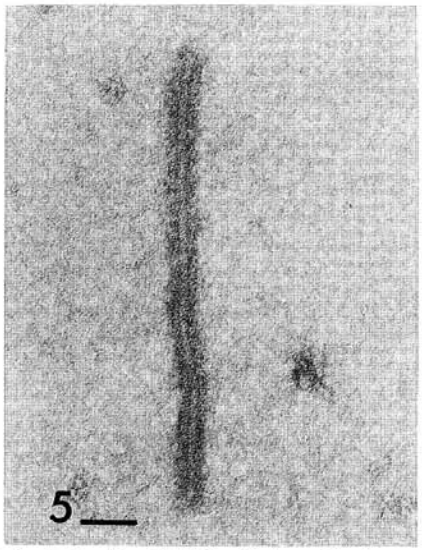


Fig. 5. Particle of bridal veil virus adhering to grid coated with BYMV-OHS antiserum. A halo of virus antibodies can be seen decorating the particle. Bar = 0.1 μm.

4). These inclusions were very similar in appearance to those described by Christie and Edwardson (2) for BYMV.

Flexuous rod-shaped particles from crude sap of plants infected with the bridal veil virus adhered to and were decorated by antiserum to BYMV-OHS (Fig. 5) but not to BYMV-KY, SMV, or TEV. Antisera to CYVV were not used in serologically specific electron microscopy tests.

In Ouchterlony double diffusion tests, the bridal veil virus, either in sap or as partially purified virus, formed strong precipitin bands upon reaction with BYMV-OHS antiserum (Fig. 6A). The bands were similar to those formed by the homologous reaction of this antiserum with BYMV-OHS. Results with BYMV-OHS are not presented because of a limitation in the quantity of the antiserum and problems incurred in maintaining the virus in plants in a sufficiently high concentration to obtain consistent serological reactions. The heterologous reaction of BYMV-OHS antiserum with BYMV-KY formed a band that spurred with the band formed by the bridal veil virus. Antiserum to BYMV-KY, however, did not react with the bridal veil virus (Fig. 6B). A strong homologous reaction, as well as a ring of nonspecific precipitation that occurred in this SDS-gel medium, was observed. No reaction occurred with the TEV or SMV antisera in the SDS gels.

Because the BYMV-OHS may be more closely related to CYVV than to BYMV (5,6), additional tests were made to compare the bridal veil virus to CYVV. Very weak heterologous, as well as homologous, bands were formed with antiserum to CYVV(B). Antiserum to CYVV(Z), however, reacted to form precipitin bands with CYVV(Z) and CYVV(B), as well as with the bridal veil virus (Fig. 7). The bridal veil virus

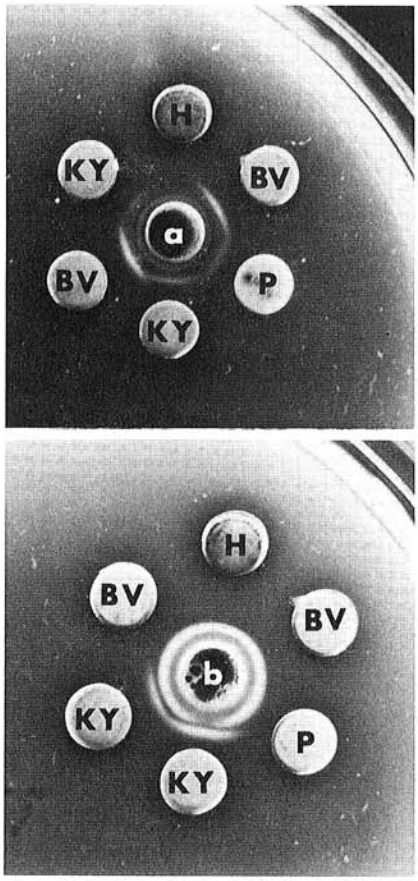


Fig. 6. Reactions of bridal veil virus with bean yellow mosaic virus (BYMV) in double diffusion tests in 0.6% Ionagar No. 2 containing 0.2% sodium dodecyl sulfate, 0.7% sodium chloride, and 0.1% sodium azide. Antisera (center wells) were **(A)** BYMV-OHS and **(B)** BYMV-KY. Antigens (peripheral wells) were: BV = bridal veil, KY = BYMV-KY, P = partially purified bridal veil virus from Xanthi tobacco, and H = healthy sap.

precipitin bands fused with CYVV(B) bands but were spurred over by CYVV(Z) bands.

DISCUSSION

The virus isolated from *G. geniculata* appears to be related to BYMV and CYVV because of serological reactions, particle morphology and length, and the type of inclusions formed within infected cells. The bridal veil virus is the first virus related to either BYMV or CYVV that is reported to infect *Tradescantia* spp., either naturally or experimentally (1,5,6,12,14,15,17,19). The systemic chlorotic lesions induced by the bridal veil virus on *C. quinoa* are similar to those induced by CYVV on this host (5). However, the necrotic lesion production and upward curling of leaves on broadbean are similar to symptoms reported previously for an isolate of BYMV (17). Also, the inability of this isolate to infect Bountiful or Topcrop bean suggests that it may resemble the pea mosaic virus subgroup, or subgroup III, of BYMV (1,14). However, serological results suggest that it is more closely

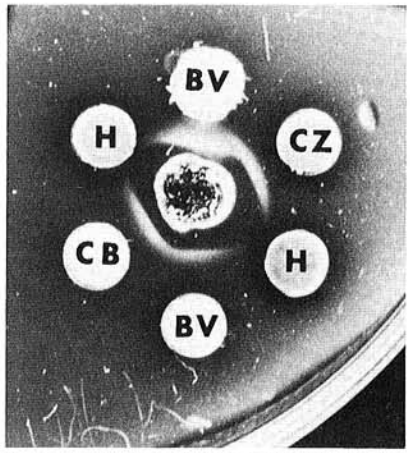


Fig. 7. Reactions of bridal veil virus with clover yellow vein virus (CYVV) in double diffusion tests in 0.6% Ionagar No. 2 containing 0.2% sodium dodecyl sulfate, 0.7% sodium chloride, and 0.1% sodium azide. Antiserum (center well) was CYVV(Z). Antigens (peripheral wells) were: BV = bridal veil, CZ = CYVV(Z), CB = CYVV(B), and H = healthy sap.

related to viruses in subgroup I of BYMV, which includes CYVV (6).

Three potyviruses infecting members of the Commelinaceae have been described previously. Morales and Zettler (11) described a potyvirus infecting *Commelina diffusa* Burm. plants. Their isolate produced laminated aggregates within infected cells, and it did not infect some cultivars of *Phaseolus, Pisum*, or *Nicotiana* spp. Another potyvirus, designated Commelina diffusa virus, was described by Migliori and Lastra (9). It infected only *C. diffusa* and possibly other *Commelina* spp. Like the potyvirus described by Morales and Zettler (11), Commelina diffusa virus failed to react serologically with several potyvirus antisera tested. However, neither BYMV nor CYVV antiserum was included in these tests. A third potyvirus infecting *Tradescantia, Zebrina*, and *Rhoeo* spp. has been described by Lockhart and Betzhold (7) and Lockhart et al (8). It could not be transmitted to *Phaseolus, Pisum, Chenopodium, Nicotiana*, and several other species and did not react serologically to 13 potyvirus antisera, including BYMV.

The bridal veil virus isolate differs from the above viruses by its transmissibility to plant species outside the Commelinaceae and by its reaction with BYMV and CYVV antisera. It is the first potyvirus isolated from a member of the Commelinaceae that has been demonstrated to be related to known members of the potyvirus group. We suggest this isolate be designated BYMV-BV, with the recognition that it is most closely related to viruses in subgroup I, such as CYVV (6).

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