Bearded Iris Mosaic Virus: Transmission, Purification, Inclusions, and its Differentiation from Bulbous Iris Mosaic

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ABSTRACT

A virus from bearded iris in Wisconsin, Scotland, and South Carolina was easily transmitted to *Belamcanda chinensis*. The virus had a restricted host range, and was aphid-transmitted in a nonpersistent manner. In *B. chinensis*, sap infectivity was lost after 36 hours of aging, heating at 50 C for 10 min, or when diluted 1×10^{-4}. Purified particles had a modal length of 753 nm, and inclusions in *B. chinensis* were similar to those observed in plants infected with viruses from the potato virus-Y group. Antiserum conjugated to latex was used to show relations of the purified R58 isolate with several isolates from bearded iris in Scotland and South Carolina. The bearded iris virus was not serologically related to a virus causing mild mosaic in bulbous iris, and the host ranges of the two viruses differed. Phytopathology 61:926-932.

Virus infections of irises are not widely recognized; however, irises are susceptible to at least six viruses. Bulbous iris of the Xiphon group are naturally infected by mild iris mosaic virus (MIMV) and severe iris mosaic virus (SIMV) (5, 7, 8, 12, 13, 17). The Apogon or beardless iris group is infected by beardless iris mosaic virus (BIMV) and *Iris fulva* mosaic virus (FIMV) (6, 15). Tobacco ringspot virus (TRSV), cucumber mosaic virus (CMV), and bearded iris mosaic virus (BIMV) have been isolated from bearded iris (Pogoniris or Eupogon group) (6, 8, 16).

Most of the hybrid bearded irises in a large home-garden collection in Wisconsin were infected with a virus resembling the BIMV described by Brierley & Smith (6). Research was initiated to adequately characterize a virus infecting bearded iris, including the host range and prevalence, physical properties, purification, serology, and inclusion morphology.

MATERIALS AND METHODS.—This research was conducted with a virus isolate (BIMV-R58) from a hybrid bearded iris, Missie Warburton (Fairy Flax × Whitone). The iris was obtained from Mrs. F. W. Warburton of the Median Iris Society, and the virus was maintained in the original rhizomes during the 4 years of research.

Routine mechanical inoculations of BIMV from iris and *Belamcanda chinensis* DC. were made by grinding young leaf tissue in 0.03 M phosphate buffer pH 8 with 0.02 M 2-mercaptoethanol (2-ME) added, and wiping a muslin pad wet with the inoculum over leaves previously dusted with corundum (Bausch & Lomb, Rochester, N.Y.). Occasionally, Celite was used as an abrasive instead of corundum.

An antiserum to BIMV-R58 was made by injecting a rabbit intramuscularly with 2.5 ml purified virus ($A_{260} = 0.47$) emulsified with 2.5 ml Freund's complete adjuvant. Six weeks after the injection, the rabbit was bled. Microprecipitin tests were made with partially purified virus; final readings were made after 4 hr at room temp (22 C). Antiserum was conjugated with latex particles by the procedure of Abu Salih et al. (1, 2). The reactions were performed on a Decaslide (Ortho-Diagnostics, Raritan, N.J.) with one drop antigen and two drops antiserum-latex. The reaction was recorded after 10 to 30 min.

Virus particles were mounted on carbon-coated grids and stained with 2% phosphotungstic acid pH 6.4 (PTA) or alkaline uranyl formate (UrF) (3). Tissue for sectioning was fixed in 5% glutaraldehyde for 2 hr, rinsed several times in 0.08 M cacodylate buffer pH 7.4, postfixed with 2% OsO_4, dehydrated in acetone, and embedded in Araldite 6005. Preparations were stained as described earlier (9). Sections were cut on a Porter-Blum microtome. A JEM 7 or Siemens Elmiskop 1 electron microscope was used for viewing the sections or virus particles. Particle length was measured from prints by the method of Murant et al. (14).

RESULTS.—*Occurrence and symptoms in iris.*—Many bearded irises growing in gardens in Madison, Wis.; Clemson, S.C.; and Dundee, Scotland, showed no obvious symptoms or only a faint, light-green mosaic when examined closely. The flowers from plants with faint symptoms seldom were affected. Bearded iris occasionally displayed a distinct light-green or yellow mosaic on the leaves, especially of hybrid seedlings. This more severe reaction was usually accompanied by flower breaking, often patterned in the form of tear drops. The longevity of flowers showing symptoms was shortened. The hybrid iris (Missie Warburton) with BIMV-R58 bloomed several times in the greenhouse. The standards and falls were broken when fully open, and by the next morning the whole flower was shriveled (Fig. 1-b). In temp-controlled greenhouses leaf symptoms of the bearded iris 'Missie Warburton' were severe at 16 C but were mild at 28 C (Fig. 1-a).

Bearded iris leaves were collected from scattered

sources in Madison, Wis., or vicinity, and inoculated to *B. chinensis*. The leaves were collected without regard for symptoms; some exhibited no symptoms while others showed a faint green mosaic. Twenty-two of the 23 samples collected caused mosaic typical of BIMV on *B. chinensis*. Some of the isolates caused mosaic, stunting, necrosis, and finally death of the *B. chinensis* plants, whereas other isolates caused severe stunting without death. Bearded iris leaves were collected from five sources in Dundee, Scotland. All five sources caused typical BIMV symptoms on *B. chinensis*. Cucumber mosaic virus was also obtained from two of the iris samples. No CMV was detected in bearded iris in Wisconsin or in South Carolina.

Bearded iris mosaic virus-R58, inoculated from *B. chinensis* to hybrid bearded iris, infected one of three seedlings producing a light-green mosaic after several weeks.

Host range.—Bearded iris mosaic virus-R58 was readily transmitted mechanically from iris to *B. chinensis*. With iris or *B. chinensis* as the source plant, BIMV could not be transmitted to any other plant species as determined by symptom development, though over 30 were inoculated, and the plants were kept for 3 to 4 weeks after inoculation. Some of the species inoculated but not infected were: *Amaranthus caudatus* L., *Chenopodium amaranticolor* Coste & Reyn., *C. foetidum* L., *C. quinoa* Willd., *Cucumis sativus* L. 'Lemon', *Freesia* sp. seedlings, *Gladiolus* sp. hybrid seedling, *Iris kampferi* Sieb., *Lycopersicon esculentum* Mill. 'Bonny Best', *Nicotiana megalosiphon* Heurck & Muell., *N. tabacum* L. 'Havana 38', *Petunia axillaris* (Lam.) BSP, *Tetragonia expansa* Murr., *Vigna sinensis* Savi 'Blackeye Cowpea', and *Zea mays* L. 'Golden Bantam'. Purified preparations of BIMV-R58 did not infect *C. amaranticolor, C. quinoa, N. megalosiphon, N. tabacum 'Xanthi nc'*, or *Tetragonia expansa,* but readily infected *B. chinensis,* as determined by back inoculation. Final symptoms were read 3 to 4 weeks after inoculation.

On *B. chinensis,* BIMV caused spreading necrotic local lesions and a light green or yellow systemic mosaic. Leaves which developed after the systemic mosaic appeared were dark green without mosaic but still contained virus (Fig. 1-c). In the few cases where seed pods formed on BIMV-infected *B. chinensis,* there were chlorotic depressions on the pod surface. Seedlings raised from these seeds showed no symptoms of virus infection, but symptoms developed when inoculated with BIMV. In Wisconsin and Scotland, BIMV-R58 could be maintained by serial inoculations to *B. chinensis*. In South Carolina, however, after three or more successive transfers on *B. chinensis,* BIMV-R58 sometimes failed to infect 50% of the plants, whereas BIMV-R58 from iris, inoculated at the same time, infected 100% of the *B. chinensis*.

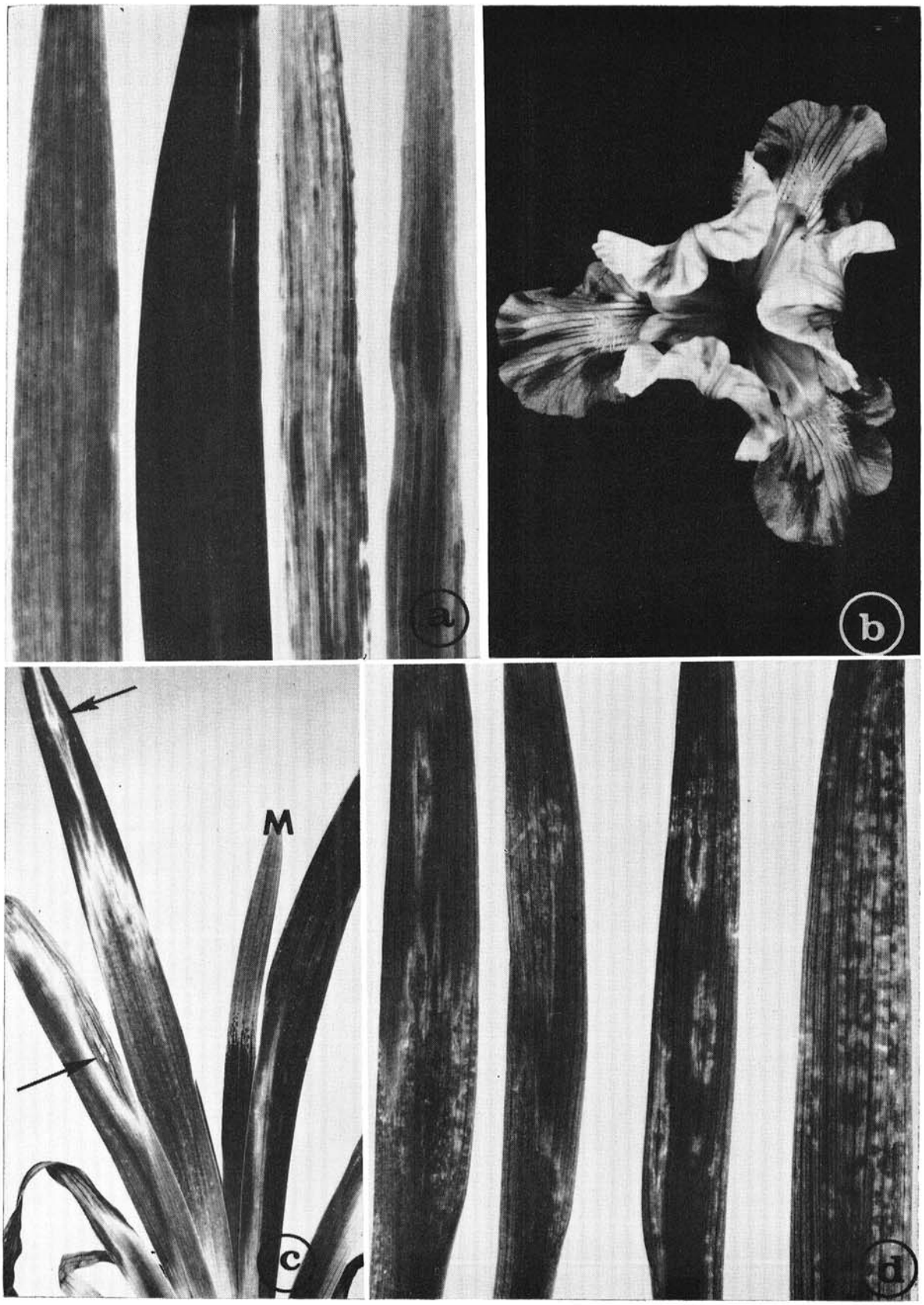
Belamcanda chinensis showed no symptoms, and no virus could be recovered when inoculated with MIMV (an isolate in Prestwick iris obtained from the Glasshouse Crops Research Institute, Littlehampton, England), bean yellow mosaic (BYMV), four strains of CMV (two isolates originating in bearded iris, and one in gladiolus), or two isolates of TRSV (one originating from gladiolus). During this investigation, a virus from bulbous iris cultivars H. C. van Vliet and Wedgwood infected *B. chinensis*. Symptoms on *B. chinensis* developed much slower, and chlorotic-to-necrotic rings and line patterns developed instead of a mosaic (Fig. 1-d). When negatively stained, partially purified preparations from *B. chinensis* were examined in the electron microscope, spherical particles around 30 nm diameter were present. The relationship of this spherical virus from bulbous iris to SIMV is unknown.

Aphid transmission.—Aphids were starved 2 hr and fed on BIMV-R58-infected *B. chinensis* plants for 3 to 5 min, and about 10 aphids transferred to each healthy *B. chinensis* plant. The aphids were killed after remaining on the plants overnight. Bearded iris mosaic virus-R58 was transmitted by *Macrosiphum euphorbiae* (Thos.), but in two trials the virus was not transmitted by *Myzus persicae* (Sulz.).

Properties of BIMV in vitro.—For property tests, *B. chinensis* tissue, inoculated 8 to 14 days earlier, was weighed and 1 g ground in 4 ml 0.03 M phosphate buffer pH 8 for heat inactivation trials and 1 g tissue in 9 ml buffer for aging in vitro and dilution end point trials. The sap was squeezed through cheesecloth prior to treatment, and each treatment was inoculated to at least two *B. chinensis* plants. At least three replications of each physical property test were made. Bearded iris mosaic virus-R58 was infective 24 hr but not 36 hr when stored at greenhouse temp (about 27 C). Virus was infective after heating for 10 min at 45 but not 50 C, and after diluting 1:1,000 but not after diluting 1:10,000.

Purification.—A modified purification procedure used for parsnip mosaic virus (14) worked with BIMV-R58. *Belamcanda chinensis* leaves showing systemic mosaic symptoms were harvested, chilled, and homogenized in 0.1 M borate buffer pH 8.9 with 0.01 M sodium ethylenediaminetetraacetate (EDTA) and 0.02 M 2-ME added (1 g tissue in 2 ml buffer). The homogenized leaf material was squeezed through cheesecloth and the liquid emulsified with chloroform (1:1) by shaking. After centrifuging at 8,000 rpm 10 min, the virus in the aqueous phase was sedimented by centrifuging at 27,000 rpm 2 hr in a Spinco No. 30 rotor. The pellets were resuspended overnight in borate buffer pH 8 with 0.01 M EDTA added. The supernatant from a low-speed centrifugation of the resuspended pellets was adjusted to pH 4.8 with 0.05 M citric acid. The pellets from the subsequent low-speed centrifugation were resuspended in borate buffer with EDTA by gently shaking 5 to 6 hr. The supernatant from a low-speed centrifugation, usually about 4 ml, was applied to a 1.6- × 82.5-cm Sepharose 2B column and eluted with 0.05 M citrate buffer pH 7. Virus was eluted shortly after the void volume. The fractions containing virus were combined and concentrated by ultrafiltration with an Amicon XM-100 Diaflo membrane.

Virus purified by this procedure was aggregated but appeared free of contamination when examined with an electron microscope. The ultraviolet absorption spec-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trum showed a max at 260 nm and a min at 245 nm. The $A_{260/280}$ ratio was 1.24.

Serology.—The antiserum from the one intramuscular injection had a titer of 1:2,048 in microprecipitin tests. In gel-diffusion tests, undiluted serum did not react with healthy *B. chinensis* sap. When conjugated with latex spheres (1, 2), 0.2% globulin proteins gave the optimum reaction. A partially pure BIMV-R58 preparation had a titer of 1:1,028 in latex tests, but only 1:128 in microprecipitin tests. The antiserum conjugated with latex did not react with a partially pure healthy *B. chinensis* preparation nor with crude healthy *B. chinensis* or *C. quinoa* sap.

Bearded iris mosaic virus-R58 in *B. chinensis* reacted readily in the latex test. Bearded iris mosaic virus-R58 in bearded iris seedlings also reacted positively in the latex test, but occasionally the reaction was atypical, possibly because of polysaccharides in the iris sap. When healthy iris seedlings were used as controls, no reaction occurred, so the atypical reaction did not prevent use of the latex technique with iris material as long as a healthy control was included. Bearded iris mosaic virus isolates from Scotland and South Carolina from iris or *B. chinensis* gave positive reactions in the latex test. Bean yellow mosaic virus and clover yellow vein virus, two viruses in the potato virus-Y group, did not react in the latex test. Purified preparations of BIMV-R58 did not react with MIMV antiserum obtained from Glasshouse Crops Research Institute (8).

Electron microscopy.—Filamentous particles of BIMV-R58 were readily found in purified preparations negatively stained with PTA or UrF (Fig. 3-a). The core and helical arrangement of the protein subunits were visible in areas thinly coated with UrF (18). The histogram (Fig. 2) of the particle length distribution (305 particles measured) showed that the purified virus contained many fragmented particles. Fifty or 60% of the particles between 600 and 900 nm were in the 750- to 775-nm range. If the main max of 700-825 nm is used, the normal length (4) is 761 nm. The modal length as calculated by Gibbs et al. (11), is 753 nm. The modal length is probably the more accurate, as it accounts for the skew of the distribution. The few particles seen in dip preparations from infected *B. chinensis* were usually between 750 and 800 nm. Bearded iris mosaic virus is thus morphologically similar to MIMV (8), and belongs to the potato virus-Y group (4).

In thin sections of BIMV-R58-infected *B. chinensis*, all types of inclusions usually associated with viruses belonging to the potato virus-Y group were found (Fig. 3-c, 4-a), except for the circular inclusions and tubes as described by Edwardson et al. (10) for watermelon mosaic virus. Although virus was present (Fig. 3-b), none of the virus-associated inclusions seen in *B. chinensis* occurred in petals of infected iris flowers.

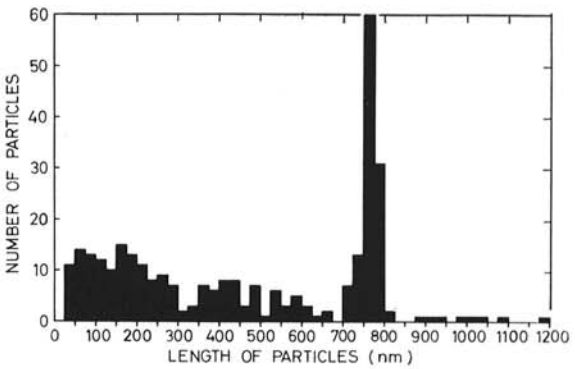

Fig. 2. Length distribution of bearded iris mosaic virus particles from purified virus preparations.

Except for pinwheel inclusions, other views of the virus-induced inclusions (bundles and laminated aggregates) were often associated with either the plasmalemma or the endoplasmic reticulum (Fig. 3-c).

Virus could be observed in *B. chinensis* dispersed in the cytoplasm (Fig. 4-a), and sometimes in bundles of entangled particles (Fig. 3-c) often enclosed in part by a membrane. Virus in infected cells was identified as such because of the lack of similar structures in healthy tissue, the morphology of the structures in the cell indicating a flexuous rod, and the typical virus-staining characteristics of these particles in cross section (Fig. 3-c, inset).

A characteristic of BIMV-infected *B. chinensis* tissue is the occurrence of polyribosomes containing a large number of ribosomes in electron-dense as well as in electron-transparent cytoplasm (Fig. 4-b, c).

DISCUSSION.—Mild iris mosaic virus has a particle length similar to BIMV and other viruses in the potato virus-Y group, but the two viruses are not serologically related. Mild iris mosaic virus also was serologically unrelated to SIMV (12, 13, 17). Although MIMV, SIMV, BIMV, BlIMV, and FIMV were all transmitted by aphids (5, 6, 13, 15), the serological and morphological relationships of BlIMV and FIMV to the other viruses is not known.

The mosaic of bearded iris reported by Brierley & Smith (6) and Travis (15) infected gladiolus, was readily transmitted mechanically to *B. chinensis* without killing the plant, and was transmitted in a nonpersistent manner by *Myzus persicae* but not by *Macrosiphum euphorbiae* or *Aphis fabae*. Gladiolus seedlings were not infected with BIMV-R58, and in limited trials *M. persicae* did not transmit this isolate while *M. euphorbiae* did. The R58 isolate is still considered

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Fig. 1. Symptoms of bearded iris mosaic virus (BIMV) **a)** Leaf symptoms of two isolates of BIMV in iris grown at different temperatures; from left to right: isolate R58 at 16 C; R58 at 28 C; 52B-1 at 16 C; 52B-1 at 28 C. **b)** Iris flower showing symptoms of flower break. **c)** Symptoms on *Belamcanda chinensis*. Note necrotic local lesions (arrow), mosaic symptoms, and young leaf with mosaic (M) at the tip and the recovered dark green younger basal portion. **d)** Symptoms induced by an unidentified icosahedral virus in *B. chinensis* which can be differentiated easily from BIMV in the same host.

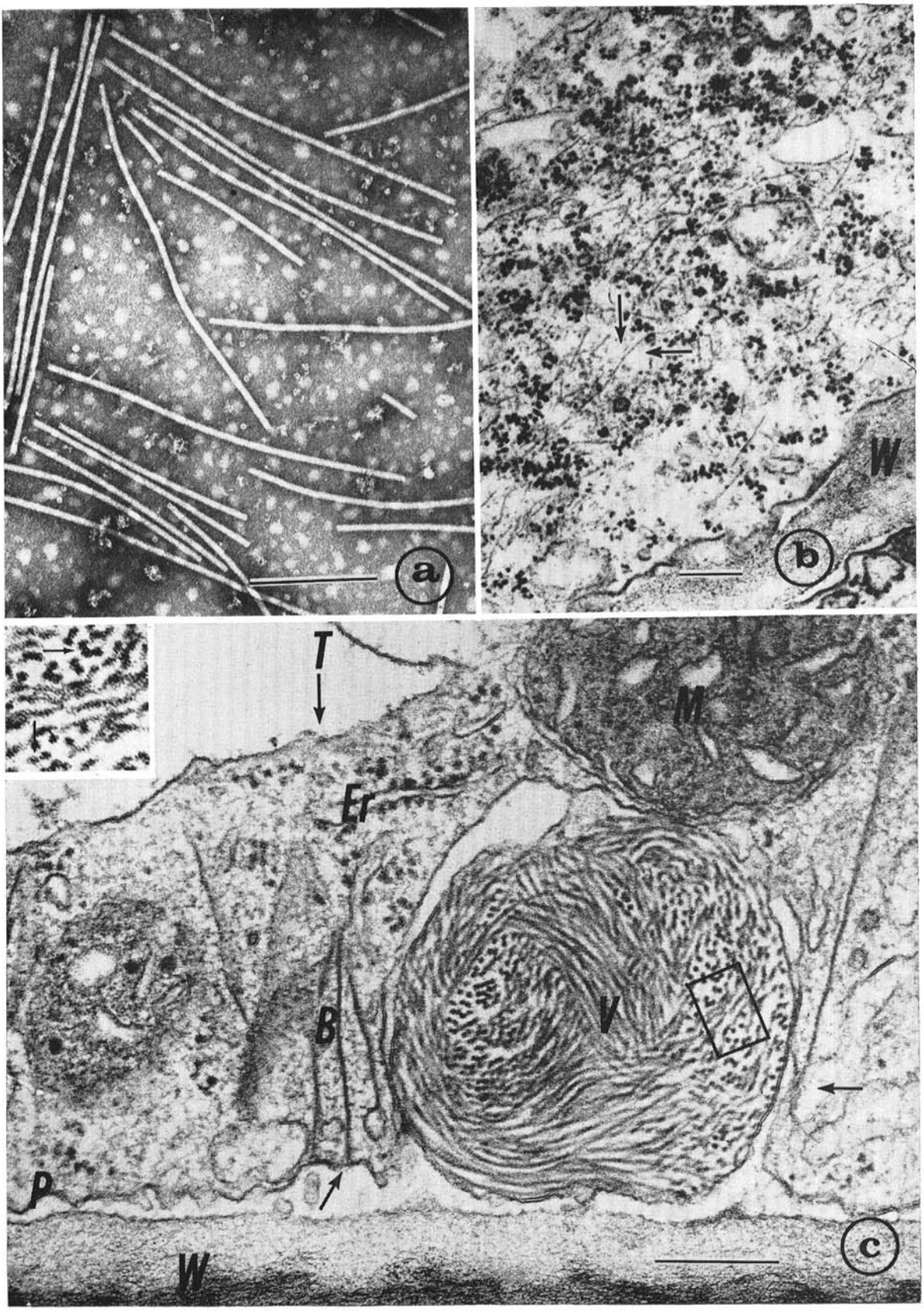
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Fig. 3. **a)** Negatively stained preparation of partially purified, bearded iris mosaic virus (BIMV). (Uranyl acetate ×83000) **b)** Iris flower petal infected with BIMV. Note the virus sectioned longitudinally (arrows) and the absence of inclusions. W = wall (×40000) **c)** Mesophyl cell of *Belamcanda chinensis* showing virus (V) and some membrane-associated inclusions (unmarked arrows). B = bundle; T = tonoplast; M = mitochondrion; Er = endoplasmic reticulum. (×77000) The inset is an enlargement of the area in the rectangle showing the virus nature of particles (arrows). Scale used in all electron micrographs represents 0.25 μ.

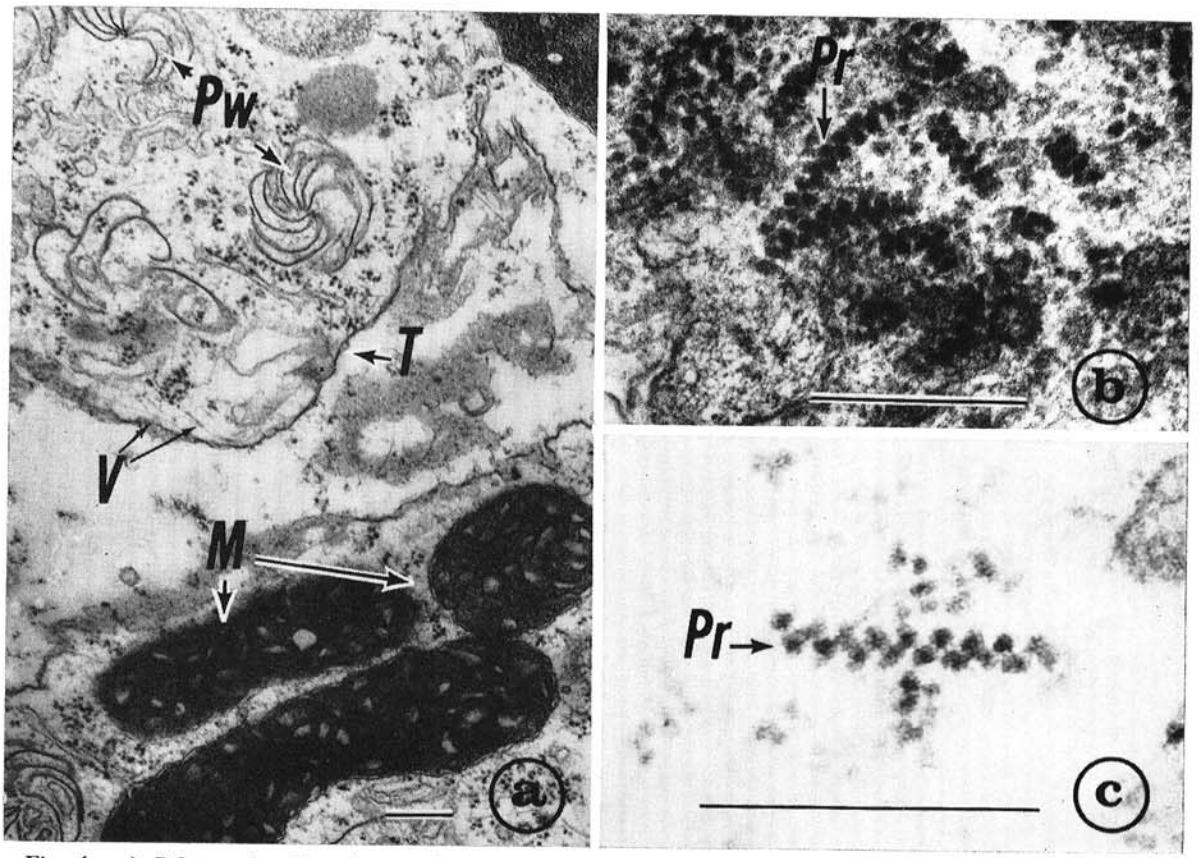

Fig. 4. **a)** *Belamcanda chinensis* mesophyl cell showing the typical inclusion of cells infected with viruses in the potato virus-Y group. PW = pinwheel; T = tonoplast; V = virus; M = mitochondrion. (×33,000) **b)** Part of a young mesophyll cell infected with bearded iris mosaic virus (BIMV). Note the large polyribosomes (Pr). (×110,000) **c)** Part of an old mesophyll cell infected with BIMV. Note the polyribosome (Pr) in the electron-transparent cytoplasm. (×170,000) Scale used in all electron micrographs represents 0.25 μ.

to be an isolate of BIMV, as Brierley & Smith (6) could not infect bearded iris with MIMV or BlIMV and because several virus isolates from bearded iris both in Scotland and South Carolina were serologically related to BIMV-R58.

Balamcanda chinensis is a reliable assay host for BIMV. The virus is easily transmitted from iris to *B. chinensis*, and the other viruses (BlIMV and FIMV) reported to infect *B. chinensis* do not infect bearded iris (6, 15). The reliability of *B. chinensis* as an indicator host for BIMV has not been impaired by the discovery in the latter part of this work of an icosahedral virus from bulbous iris that will infect this host.

In Great Britain, mosaic of rhizomatous iris was associated with CMV (8). Since BIMV was found in all mosaic isolates tested in Scotland and can cause a mosaic in bearded iris, the mosaic or stripe disease of iris is probably caused by BIMV, but in Great Britain the presence of CMV may cause a synergistic reaction.

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