

## ELECTRON MICROSCOPY OF SWEETPEA CELLS INFECTED WITH BROAD BEAN NECROSIS VIRUS

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Broad bean necrosis virus (BNV) is a soil-borne virus, occurring locally in Kyushu district of Japan. BNV particles are rod-shaped,  $25\text{ m}\mu$  in diameter and have two prevalent lengths of  $150\text{ m}\mu$  and  $250\text{ m}\mu$  (Inouye and Asatani, 1968). The authors considered that BNV was a virus distinct from other viruses of rigid rods with a diameter of about  $25\text{ m}\mu$ , such as tobacco rattle virus, pea early browning virus, barley stripe mosaic virus, and wheat soil-borne mosaic virus, on the basis of particle lengths, host range, and other properties of the virus. The paper deals with the appearance of BNV-infected cells of sweetpeas observed under the electron microscope.

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### MATERIALS AND METHODS

Seedlings of sweetpeas (*Lathyrus odoratus* L.) were inoculated with BNV by rubbing. Small pieces of young leaves showing mottled symptoms were fixed in chrom-osmium solution for 30 minutes in ice bath. Following fixation, leaf tissues were dehydrated in a graded series of ethanol and absolute acetone, and embedded in a 4:3:1 mixture of Epon 812, MNA, and DDSA. Pieces of leaf tissue of healthy sweetpeas were similarly fixed and embedded as a control. Thin sections were cut with glass knives using MT-1 (Porter-Blum) ultramicrotome and stained with 50 % ethanol saturated uranyl acetate for 30—45 minutes, followed by lead citrate for 10—20 minutes. The sections were examined with HS-6 (Hitachi) electron microscope.

### RESULTS AND DISCUSSION

The conspicuous features observed in BNV-infected cells of sweetpeas were characteristic membraneous structures and lipid or fat-body like bodies in cytoplasm. The membraneous structures, of which profiles usually appeared to be irregularly shaped network, as shown in Fig. 1, seemed to be composed of closely packed tubules. A large portion of X-bodies, especially of epidermal cells, were occupied with the network membraneous structures (Fig. 2). Unusual development of lipid or fat-body like bodies, many of which were associated with the membraneous structures, was also commonly observed (Fig. 1—3).

Rod-shaped virus particles in cells of young leaf samples were scattered randomly in cytoplasm, in which network membraneous structures and electron

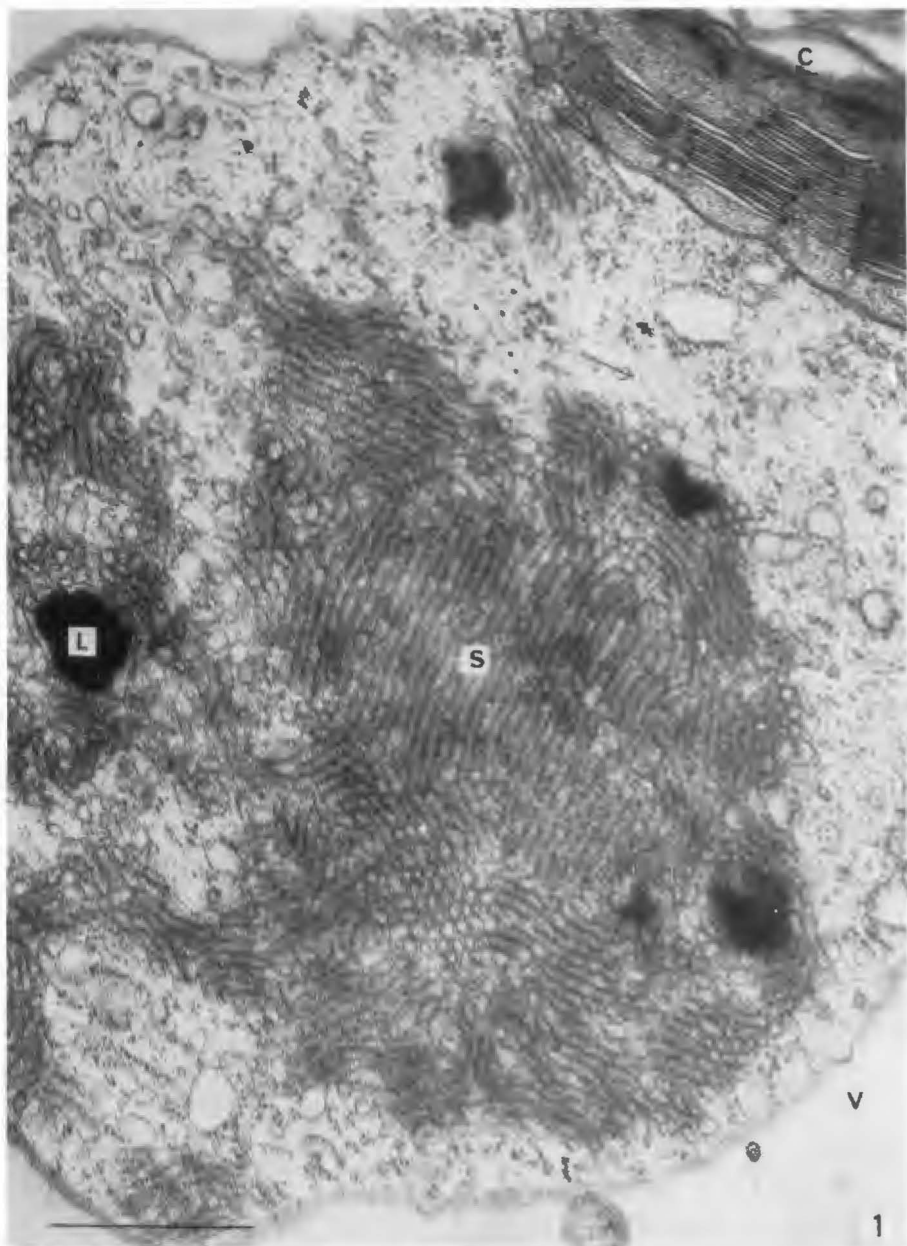


Fig. 1. Network membraneous structures (S) in cytoplasm of a BNV-infected cell of young leaf tissue of sweetpea. The structure is composed of closely packed tubules. Rod-shaped virus particles are scattered randomly in cytoplasm. Electron dense lipid or fat-body like bodies (L) are associated with the structures. C, Chloroplast; V, Vacuole. Scale line is  $1\mu$ .

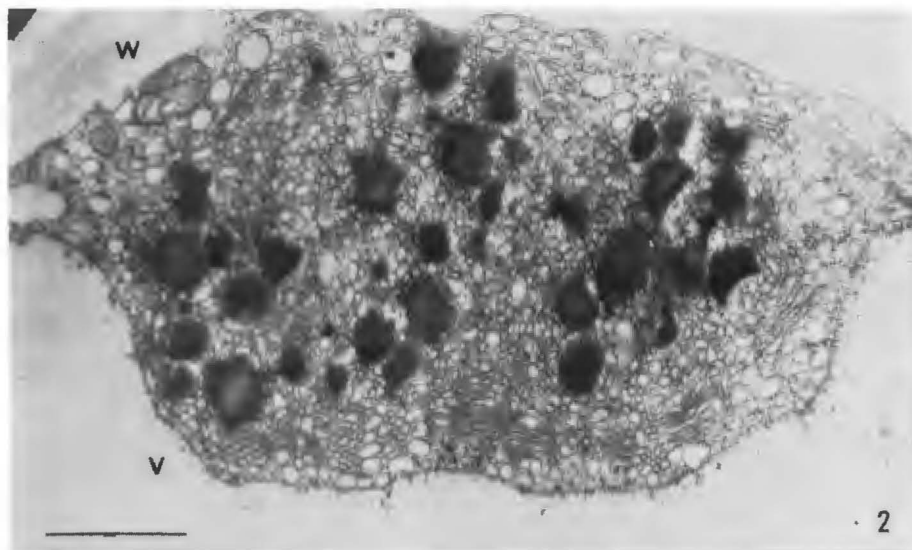


Fig. 2. A X-body in an epidermal cell of BNV-infected sweetpea. A large portion of the X-body is occupied with network membraneous structures. A number of lipid or fat-body like bodies are associated with the structures. W, Cell wall. Scale line is  $1\mu$ .

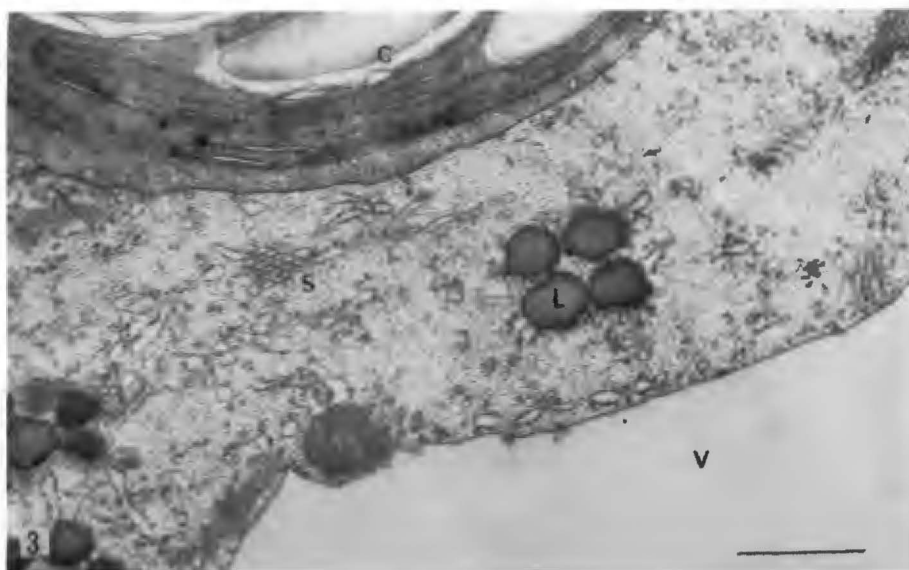


Fig. 3. Randomly scattered virus particles in cytoplasm of an infected cell of young leaf tissue. Scale line is  $1\mu$ .



Fig. 4. Small mass of BNV particles loosely aggregated in cytoplasm of older leaf tissue cell. Mass of lipid or fat-body like bodies associated with membraneous structures are also seen. Mitochondria (M) looks normal in appearance. R., Rod-shaped virus particles. Scale line is  $1\mu$ .

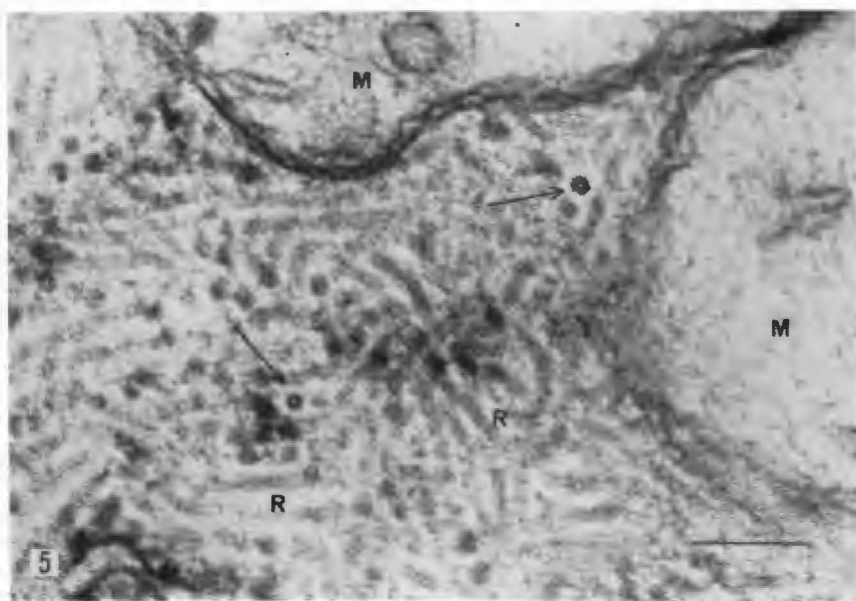


Fig. 5. Mass of rod-shaped virus particles (R) in cytoplasm. Cross sections of virus particles (arrow) in which central hole are seen. Scale line is  $0.2\mu$ .

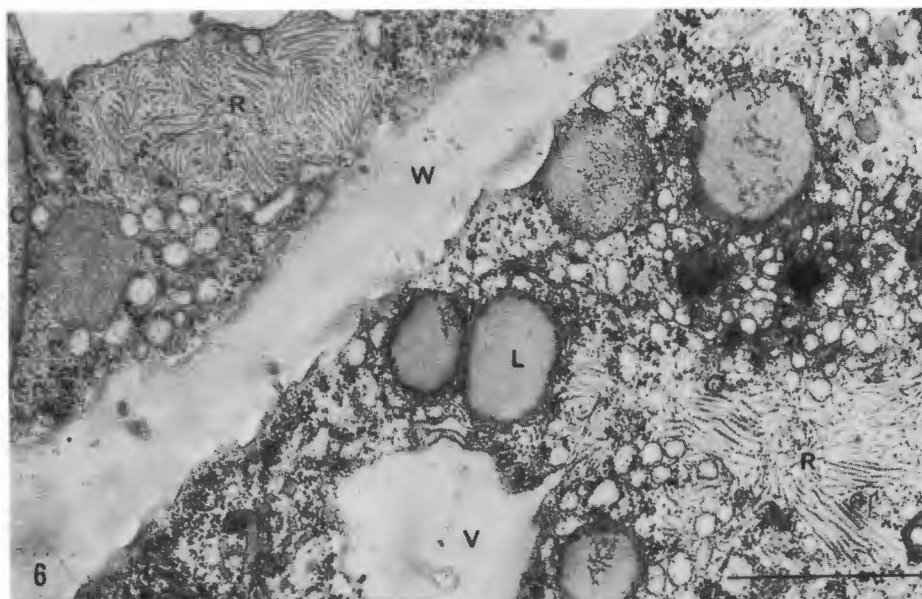


Fig. 6. Mass of virus particles loosely aggregated in irregularly oriented layers found in older leaf cells. Scale line is  $1\mu$ .

dense lipid-like bodies were seen. In older leaf tissues, mass of virus particles loosely aggregated in irregularly oriented layers were frequently seen in cytoplasm (Fig. 4—6). Some of the cross sections showed the central hole of the rod-shaped virus particles (Fig. 5). Mass of fat-body like bodies were also commonly observed in cytoplasm of older leaf tissues, associating with network structure or vesicles.

No virus particles were observed in vacuoles, nuclei, and chloroplasts. No abnormality of mitochondria was observed in BNV-infected cells.

BNV particles are rigid rods, about  $25\text{ m}\mu$  in diameter and having two distinct lengths of  $150\text{ m}\mu$  and  $250\text{ m}\mu$ . It is an interesting subject to enquire which one of the two soil-borne viruses, tobacco rattle virus (TRV) or wheat soil-borne mosaic virus (WSMV), BNV is related with. However, the relationships between BNV and TRV or WSMV are still unknown, because virus vector and serology of BNV have not yet been investigated.

Harrison et al (1970) reported that mitochondria found in cells of TRV-infected plants were abnormal in appearance, and X-bodies were composed of aggregates of these abnormal mitochondria. According to the observations reported by Harrison et al (1968, 1970) and Kitajima et al (1969), many of particles of TRV formed orderly aggregates, arranging vertically on the surface of mitochondria. These ultrastructures of TRV-infected cells were apparently different from those noticed in BNV-infected plant cells appearing in this report.

According to Saito (1970), characteristic tubular structures, which closely resembled those found in the BNV-infected cells of young leaf appearing in this

report, were seen in X-bodies formed in cells infected with some strain of WSMV.

Another type of membranous structures, not quite similar but somewhat resembled those appearing in this report, has been observed in infected cells of barley yellow mosaic virus (BYMV) and wheat yellow mosaic virus (WYMV) (Saito et al, 1966: Inouye, 1969). Both BYMV and WYMV are soil-borne, and their particles are filamentous rods, about 13—15 m $\mu$  in diameter and having two lengths of 250—300 m $\mu$  and 575—600 m $\mu$ . The characteristic structures induced by the infection of BYMV and WYMV were seen rather regular in appearance, compared with those observed in BNV-infected cells. Further, bundle or pinwheel type inclusions, which were never found in BNV-infected cells, were seen in cytoplasm of BYMV and WYMV infected cells.

From the above mentioned comparisons, it is still difficult to draw a conclusion about the similarities between BNV and other soil-borne rod-shaped viruses. However, from the particle morphology and some similarities in ultrastructures of infected cells, it might be considered that BNV resembles WSMV rather than TRV.

#### SUMMARY

Ultrathin sections of infected cells of broad bean necrosis virus (BNV) infected sweetpea leaves were investigated under the electron microscope.

Characteristic membranous structures and unusual development of lipid or fat-body like bodies associated with the structures were commonly observed in BNV infected sweetpea cells. Prominent development of the membranous structures were seen in some sections, especially in X-bodies formed in young leaf cells. The structure seemed to be composed of closely packed tubules. No virus particle was observed within the tubular structures, but in cytoplasm scattering randomly or aggregating loosely in some order. No abnormality of mitochondria was noticed.

The ultrastructures of BNV-infected cells observed here somewhat resembled those of some strains of wheat soil-borne mosaic virus investigated by Saito, but they were quite different from those observed in tobacco rattle virus reported by Harrison et al and Kitajima et al.

#### LITERATURE CITED

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