BIOLOGIA PLANTARUM (PRAHA)

28 (3) : 227–229, 1986

Localization of the Beet Mild Yellowing Virus in *Sinapis alba* L.

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Abstract. Virus particles of isometric shape with a diameter of 26 nm were found in the sieve tubes and accompanying phloem cells in ultrathin sections prepared from the nerves of white mustard (*Sinapis alba* L.) leaves and roots infected with the beet mild yellowing virus (BMYV). BMYV particles were much more frequent in the roots of *Sinapis alba* plants. Isometric particles were not found in the leaves and roots of healthy mustard plants.

The beet mild yellowing virus (BMYV) belongs to the luteoviruses whose localization in infected plants is restricted to phloem tissues. Degeneration of the phloem conductive tissue is a characteristic symptom of the infection with luteoviruses. The properties of the luteoviruses have so far been insufficiently studied because of the difficult preparation of purified luteovirus isolates (ROCHOW and DUFFUS 1981).

MÜLLER *et al.* (1976) found BMYV particles in degenerated phloem when examining ultrathin sections prepared from the cotyledons of *Sinapis alba* L. host plants, whereas the attempts to detect this virus by means of electron microscopy in spontaneously infected beet plants (*Beta vulgaris* L.) and to isolate it were unsuccessful. DUFFUS and RUSSELL (1975) isolated BMYV from *Capsella bursa-pastoris* L. plants and partially purified their isolate. HARTLEB *et al.* (1976) obtained the BMYV from the homogenate of aphids *Myzus persicae* SULZ., and CHEVALLIER and PUTZ (1982), who were the first to prepare an antiserum against this virus, purified the virus from *Claytonia perfoliata* DONN. plants. The same plant was used by KÜHNE *et al.* (1985) who found that *Claytonia perfoliata* DONN. is more suitable than *Crambe abyssinica* HOCHST. During further investigations, CHEVALLIER *et al.* (1983) attempted to determine the structure and physical properties of this virus more precisely. They found that the particles were 26 nm in diameter and icosahedral in shape. Nevertheless, the selection of a suitable host plant from a set of known BMYV hosts (RUSSELL 1965, HARTLEB 1975) with the aim to obtain a higher amount of the purificate is still a topical problem.

The aim of this study was to demonstrate the BMYV by means of electron microscopy in the leaves and roots of *Sinapis alba* plants infected with the virus and simultaneously to observe the frequency of the occurrence of virus

Received February 5, 1985; accepted June 26, 1985

particles in various parts of the plant. Mustard plants were chosen because they respond to the infection with BMYV with well developed symptoms during a relatively short incubation period and can be grown easily. The experiments were carried out with respect to the possibility of effectively solving the problem of BMYV purification.

MATERIAL AND METHODS

Plant Material and the Virus

White mustard (*Sinapis alba* L.) plants grown in pots in the glasshouse were used in the experiments. These plants can easily be grown and grow vigorously and thus much more plant material can be obtained than with other host plants. *S. alba* plants were infected with BMYV at the stage of two developed leaves using aphids of the species *Myzus persicae* SULZ. The BMYV isolate described earlier (POLÁK and CHOD 1975) was used for inoculation. Symptoms appeared three weeks following the inoculation, that is yellowing of the margin of the first (oldest) leaf and appearance of a violet tinge on its lower surface.

Preparation of the Material for Electron Microscopic Scanning

Leaf and root parts were sampled for the preparation of ultrathin sections six weeks after inoculation with the virus. Squares 1 × 1 mm containing the main nerve or the first order nerve were cut from the first leaf showing distinct symptoms of the BMYV. Transverse sections approximately 1 mm thick were prepared from washed main roots. The root material was sampled 5 cm below the root neck. Corresponding parts were simultaneously sampled from healthy plants grown under the same conditions.

Preparation of Ultrathin Sections and Electron Microscopy

Samples of plant material were fixed in 6.25 % glutaraldehyde for 2 h, washed in 0.1 M phosphate buffer, pH 6.9, according to Sörensen, and then fixed again with 1 % osmium oxide for 1 h. The samples were dehydrated with ethanol and processed through the propylene oxide series to the Epon-Durcupan mixture.

Ultrathin sections were prepared with an ultramicrotone Reichert OM-U2, contrasted with uranylacetate and lead citrate and scanned under the electron microscope Tesla BS 500, direct magnification of 10 000—44 000 ×. For determination of BMYV particles tobacco mosaic virus was used as a standard.

RESULTS AND DISCUSSION

Cells of leaf tissues of *Sinapis alba* plants infected with BMYV virus showed cytological changes, compared with the cells from healthy plants in ultrathin sections prepared from the first leaf. The cells of leaf parenchyma often contained chloroplasts with a few abnormally big inclusions, apparently starch grains (Fig. 1), presumably formed as a result of reduced assimilate transport rate in the virus-damaged phloem.

However, the leaf parenchyma cells did not contain particles of the virus. Clusters of the virus particles were found in the sieve tubes and in the phloem parenchyma cells, scattered especially in the proximity of mitochondria

(Figs. 2 and 3). The virus particles were not found in ultrathin sections prepared from the same tissues of healthy plants.

Particles of the BMYV were much more frequent and clustered in ultrathin sections prepared from the roots of *Sinapis alba* plants. The virus particles were exclusively located in the sieve tubes, often in large clusters, sometimes with indications of crystalline-like arrangement (Fig. 4). They were hexagonal in structure (Fig. 5) and their size of 26 nm corresponded to BMYV virions according to CHEVALLIER *et al.* (1983). In the roots of healthy *Sinapis alba* plants virus particles were not found.

The find of BMYV particles in the roots of infected *Sinapis alba* plants may be of practical significance for purification of the virus. Successful purification of the beet yellows virus (BYV) from sugar beet roots and the preparation of a specific antiserum was reported earlier (POLÁK and CHOD 1969). With respect to a relatively easy determination of the BMYV particles in *Sinapis alba* roots, we assume the purification of the virus from root tissues to be perspective.

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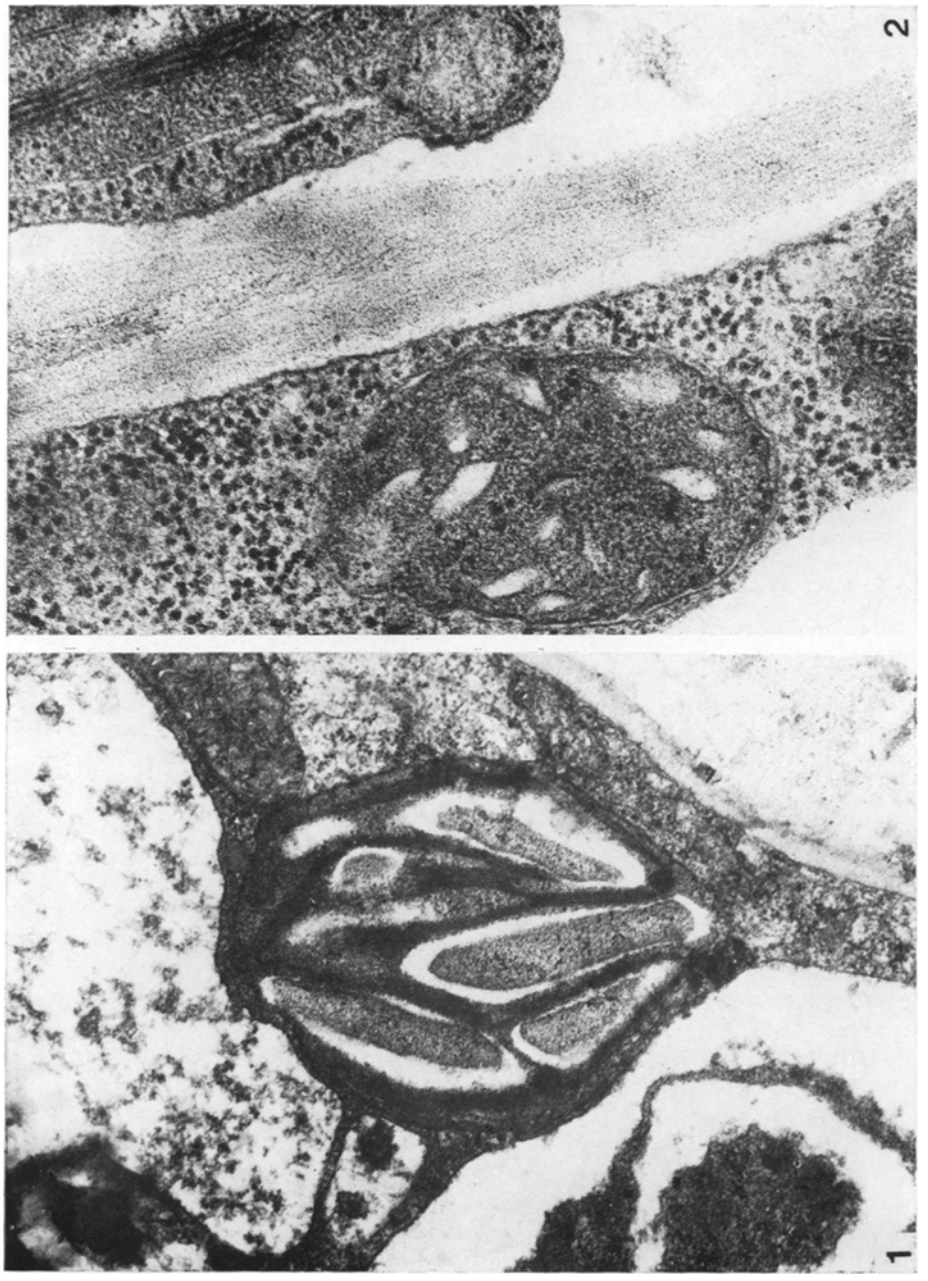
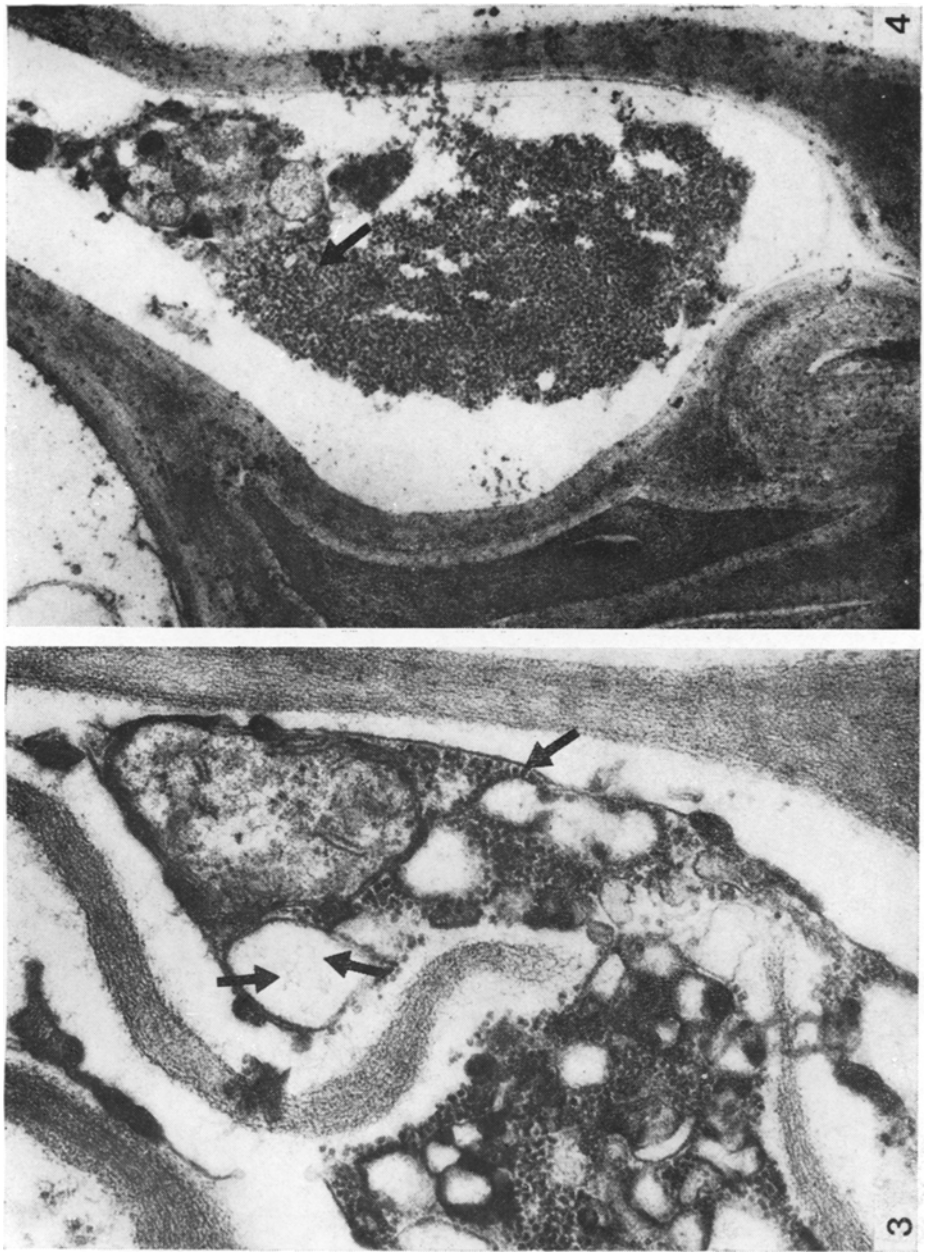
Figures at the end of the issue.



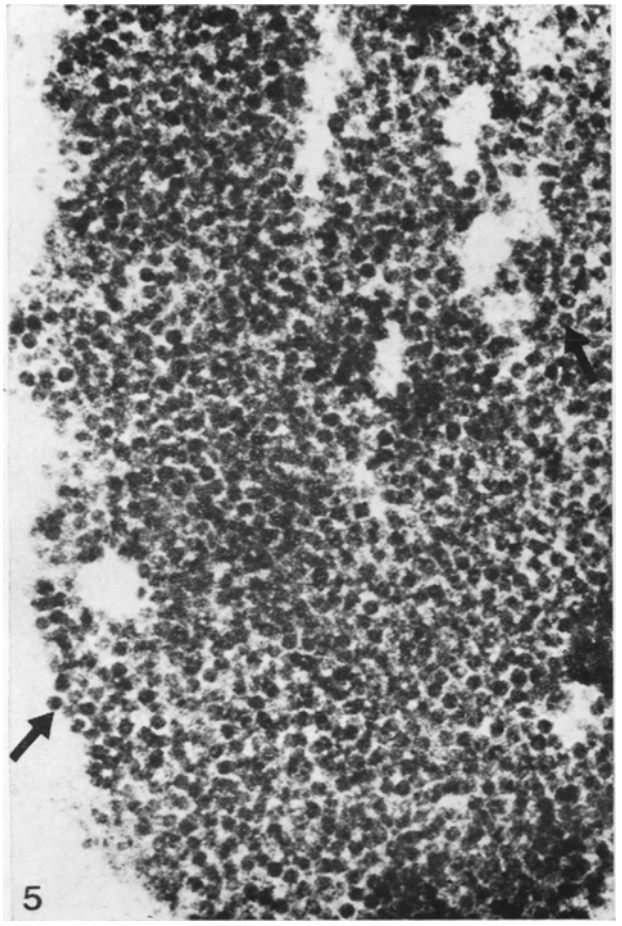
Figs 1 to 5. Ultrathin sections prepared from tissues of *Sinapis alba* L. plants infected with BMYV.

1. Part of a leaf parenchyma cell with chloroplast containing a few abnormally large inclusions, obviously starch grains, formed presumably due to reduced assimilate transport rate in the phloem damaged by the virus (35 000 ×).

2. A mitochondrion surrounded by cytoplasm with numerous particles of the BMYV in a phloem parenchyme cell (84 000 ×).



3. Part of a phloem parenchyma cell with virus-induced vesicles and containing fine network -- arrows; some vesicles are surrounded by endoplasmic reticulum-derived membrane bearing ribosomes (arrows); large clusters of BMYV particles in the cytoplasm (60 000 ×).
4. Section through a root sieve tube with a high accumulation of BMYV particles showing indications of crystalline-like arrangement (arrow) (40 000 ×).



5. A detail of the electronogram shown in Fig. 4; the hexagonal shape of the virions (arrows) is seen (120 000 ×).