Barley Yellow Striate Mosaic Virus and Associated Viroplasms in Barley Cells

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SUMMARY

Barley yellow striate mosaic virus (BYSMV) [cryptogram: */*/*: U*/S,I/Au] was detected in the cytoplasm but not in the nucleus or other organelles of infected barley cells. Stiff bacilliform particles of about 45 × 330 nm, believed to be the mature virus particles, occurred as membrane-bound aggregates while slightly flexuous particles of similar length and 35 to 45 nm wide, possibly representing ‘immature’ virus particles, were seen dispersed within sac-like structures. Such particles had sometimes an electron-transparent end and were associated with spherical vesicles 25 to 60 nm in diam.

Electron-dense intracytoplasmic masses of granular or finely fibrous material, here called viroplasm, were consistently found in connexion with BYSMV particles which were apparently formed by budding from them through membranes limiting the surrounding enclaves. These appeared to have derived from the endoplasmic reticulum and, occasionally, from the separation of the nuclear envelope lamellae. The number and size of BYSMV viroplasms, together with their close connexion with both the endoplasmic reticulum and the outer lamella of the nuclear envelope, seem to be features unique among the described plant rhabdoviruses.

INTRODUCTION

Three plant rhabdoviruses have been found in Italy: the causal agent of clover enation disease (Rubio-Huertos & Bos, 1969), eggplant mottled dwarf virus (EMDV) (Martelli, 1969) and barley yellow striate mosaic virus (BYSMV) (Conti, 1969). Attempts to infect white clover or barley with EMDV or white clover and eggplant with BYSMV have been unsuccessful (unpublished data). This and other features of these viruses (Bos & Grancini, 1968; Conti, 1969, 1972; Martelli & Cirulli, 1969; Martelli & Rana, 1970) suggest that they are probably different.

BYSMV is transmitted propagatively by the planthopper Laodelphax striatellus Fallén but not by sap inoculation. The host range is apparently limited to graminaceous plants, including barley, oats, wheat and some grasses. Some aspects of the appearance and intracellular localization of the virus particles in infected plant tissue have been abstracted by Appiano & Conti (1972), as mentioned by Lovisolo (1971). The present paper is a more detailed study of BYSMV in barley cells.
An isolate of BYSMV originally obtained from *Laodelphax striatellus* planthoppers and maintained in barley through inoculation by glasshouse-reared planthoppers was used. Young instars from healthy colonies, reared on wheat and barley, were exposed to the virus for 5 days on infected barley. After 10 days incubation, the planthoppers were moved to fresh barley seedlings and fed on them for 3 days. Symptoms of striate mosaic virus appeared on the plants after about 1 week and increased in intensity up to 3 weeks after inoculation.

Specimens for electron microscopy were prepared from plants inoculated 3 weeks previously. Small pieces of tissue were excised from the dark-coloured stripes on leaves showing bright mosaic symptoms, fixed in 3% glutaraldehyde in 0.1 M-cacodylate buffer, pH 6.8, post-fixed in buffered 1% osmium tetroxide, dehydrated in series of ethanol and embedded in Araldite. Samples from healthy plants of the same age were collected and prepared in the same way as controls.

Sections, cut on a Reichert OM-U2 ultramicrotome with glass knives, were stained with uranyl acetate and lead citrate and examined in either a Siemens Elmiskop II (at the ‘Centro di Microscopia Elettronica dell’Università’, in Turin) or a Philips EM100 electron microscope.
RESULTS

BYSMV particles were detected in the cytoplasm of parenchyma and phloem cells, including mature sieve elements. The nuclei and other cell organelles were not invaded by the virus and did not appear consistently altered in comparison with those of healthy controls. The general architecture of the cells was, however, affected to various extents by the presence of the virus and related structures.

Membrane-bound aggregates of tightly packed bacilliform particles, generally aligned in parallel, were commonly observed in the tissue examined. The enclosing membranes were probably derived from the endoplasmic reticulum. Single parenchyma and phloem cells usually contained numerous virus aggregates scattered throughout the cytoplasmic area (Fig. 1). No aggregates of this type were found between the lamellae of the nuclear envelope.

The particles in the aggregates were rigid and measured about 45 nm in diam. and 330 nm in length. Transversely sectioned particles, examined at high magnification, revealed a central hollow, which contained an electron-opaque core and was surrounded by an outer coat, apparently consisting of three layers (Fig. 1, inset).
Bacilliform particles of a different appearance were seen in sac-like structures which also seemed to originate from the endoplasmic reticulum (Fig. 2). In contrast with those in the above-mentioned aggregates, such particles were slightly flexuous and dispersed without a definite order though, in some cases, a few of them showed a tendency to cluster in parallel alignments (Fig. 3). Some of the particles had an electron-transparent end (Figs. 2, 3, single arrows). Their length was about the same as that of the stiff particles while their width ranged from 35 to 45 nm. Besides virus particles, the sac-like structures contained spherical vesicles 25 to 60 nm in diam. (Figs. 2, 3, double arrows) and small cytoplasmic masses. The former were also observed as membrane-bound groups apart from virus particles (Fig. 3).

The most striking feature of BYSMV-infected tissue consisted of the presence of intracytoplasmic masses of electron-dense material of granular or finely fibrous appearance (Figs. 4, 6). These structures, named hereafter ‘viroplasm’ by analogy with those observed in association with other viruses (Gerola & Bassi, 1966; Wolanski & Chambers, 1971), were of various sizes and often invaded most of the cell lumen. Occasionally, as in Fig. 5, a large accumulation of viroplasmic material caused flattening of the nucleus. Membrane-bound groups of virus particles were consistently observed at the periphery of the viroplasms and sometimes within them (Fig. 4).
BYSMV and associated viroplasms

Fig. 5. Portion of an infected barley cell invaded by viroplasms (Vp) and associated BYSMV particles. Note the flattening of the nucleus (N). Ch, chloroplast; CW, cell wall.

Particles apparently budding from viroplasms into the surrounding membrane-bound sacs were detected in some cases (Figs. 6, 7). The sacs appeared to derive from the endoplasmic reticulum and, in some instances, from the separation of the inner and outer lamellae of the nuclear envelope (Fig. 7). No signs of abnormality were observed in the adjacent nuclear areas.

DISCUSSION

The BYSMV particles were detected in large numbers in infected barley tissue but the virus did not seem to induce consistent changes in the normal cell constituents. The bacilliform particles tightly packed in the membrane-bound aggregates and measuring approximately $45 \times 330$ nm may be mature BYSMV. Such particles closely resemble those detected in negatively stained crude extracts (Conti, 1972) though the latter are larger in diam. (about 60 nm). Analogous differences in the particle diam. dimensions have been reported for lettuce necrotic yellows virus (LNYV) (Chambers, Crowley & Francki, 1965) and are due to the different preparation techniques (Hummeler, 1971). The slightly flexuous particles observed in the sac-like structures might be immature virus particles. The electron transparent end observed in some of the latter closely resembles that of potato yellow dwarf virus (MacLeod, 1968) and rice transitory yellowing virus (RTYV) (Chen & Shikata, 1971).
Fig. 6. BYSMV particles apparently budding from a viroplasm (Vp) into the surrounding membrane-bound sacs. Ch, chloroplast; N, nucleus.

Fig. 7. Detail of a section analogous to that in Fig. 6, illustrating some BYSMV particles budding from the viroplasm (Vp). The sac containing the virus particles derives from the separation of the nuclear envelope lamellae (see arrows). Cy, cytoplasm; N, nucleus.
BYSMV and associated viroplasms

The spherical vesicles associated with the immature BYSMV particles show some similarities with those described for LNYV which are apparently involved in virus morphogenesis (Wolanski & Chambers, 1971). Their origin in regard to BYSMV is not clear.

In contrast with most of the described plant rhabdoviruses which develop by budding at the inner lamella of the nuclear envelope (Kitajima & Costa, 1966; MacLeod, Black & Moyer, 1966; Richardson & Sylvester, 1968; Rubio-Huertos & Bos, 1969; Martelli & Castellano, 1970), BYSMV does not seem to do so. In the case of *Gomphrena* virus (Kitajima & Costa, 1966), clover enation virus (Rubio-Huertos & Bos, 1969) and *Melilotus* latent virus (Kitajima, Lauritis & Swift, 1969), paracrystalline aggregates consisting of the inner virus component were detected within the nucleus. With the exception of wheat striate mosaic virus (Lee, 1967) and RTYV (Chen & Shikata, 1970), BYSMV and the other rhabdoviruses of graminaceous plants have not been found in intimate association with the nucleus (Razvjaskina & Poljakova, 1967; Shikata & Lu, 1967; Signoret, Giannotti & Alliot, 1972). Occasionally, BYSMV particles did occur inside perinuclear spaces but they appeared to be emerging from viroplasms located in the cytoplasm.

O’Laughlin (1969) and Sylvester & Richardson (1970) described viroplasm-like structures associated with LNYV and sowthistle yellow vein virus, respectively, in the tissue of their aphid vectors. However, LNYV (Wolanski & Chambers, 1971) and BYSMV seem to be the only rhabdoviruses which induce the formation of viroplasms in plant cells though, in the case of BYSMV, the phenomenon is apparently more extensive than with LNYV. Furthermore, BYSMV viroplasms were in close connexion with either the endoplasmic reticulum (see Fig. 4) or the outer lamella of the nuclear envelope (see Fig. 7), both of which appear to be involved in the maturation process of BYSMV particles which bud through them from the viroplasm. This behaviour is unique among plant rhabdoviruses. It is, however, somewhat similar to that of rabies virus (Hummeler, Koprowski & Wiktor, 1967) and other structurally related viruses of vertebrates (Shope et al. 1970; Murphy et al. 1972) which bud through cytoplasmic membranes or the plasmalemma from intracytoplasmic matrices. These structures, known as Negri bodies in the case of rabies virus, resemble BYSMV viroplasms in their location and appearance.

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REFERENCES


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