Occurrence of Clover Yellow Mosaic Virus Aggregates in Transfer Cells of Pisum sativum

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ABSTRACT

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"Spiral" and amorphous aggregates of clover yellow mosaic virus (CYMV) were found in transfer cells and their neighboring parenchyma cells of stems and minor veins of *Pisum sativum* 3 weeks after inoculation. It is suggested that the transfer cells located between sieve elements and tracheary elements may serve as a bridge between a slow cell-to-cell spread and a rapid, long-distance movement of CYMV through the vascular tissues.

Additional key words: electron microscopy, ultrastructure, virus movement.

Transfer cells are morphologically characterized by wall protuberances resembling those previously found in secretory and other kinds of plant cells involved in short-distance translocation of organic solutes (1, 4, 11). The wall ingrowths of the transfer cells extend into the cell lumen and may be branched so as to form a labyrinth. The surface area of plasmalemma is thus greatly enlarged by the development of ingrowths and the enlargement greatly enhances intercellular translocation of organic solutes in the vascular area as it means an increased surface-area-to-volume ratio of the protoplast (2, 3, 4, 5, 6, 10).

At the present time, however, there is little detailed information on the occurrence of virus particles in transfer cells and the role of transfer cells in intercellular virus movement is not yet satisfactorily understood. This paper reports the occurrence of clover yellow mosaic virus (CYMV, cryptogram $*/_{*}: */_{*}: {}^{s}/_{*}$, potexvirus group) aggregates in transfer cells of *Pisum sativum* and discusses its implication in virus movement in plants.

MATERIALS AND METHODS

The virus isolate used in this investigation was originally isolated from vetch (*Vicia sativa* L.) in the Rocky Mountain House area, Alberta. Identification of the isolate as CYMV was based on a series of studies on host range, serology, transmission, virus morphology, and cytology (Rao and Hiruki, *unpublished*). The first leaves of pea (*Pisum sativum* L. 'Alaska') dusted with 22-μm (600-mesh) Carborundum were inoculated with a sap extract obtained by grinding infected leaves with two times their weight of 1% K_2HPO_4, pH 8.5. Both inoculated and noninoculated plants were grown in a greenhouse at about 22 C.

Samples for electron microscopy were obtained 3 weeks after inoculation from stems of a nodal region and minor veins of infected leaves, and from comparable healthy tissues. Sampled tissues (about 2 mm^2) were fixed in 3% formalin-glutaraldehyde mixture (1:1, v/v) in 0.1 M phosphate buffer, pH 7.0, at 4 C for 7 hours. The fixed materials were washed and postfixed in 2% osmium tetroxide for 5 hours, dehydrated in ethanol-propylene oxide series, and embedded in Araldite. Sections were cut on a Reichert ultramicrotome equipped with a diamond knife and stained with 2% aqueous uranyl acetate for 2 hours followed by 2-5 minutes with lead citrate at room temperature (8). A Philips Model 200 electron microscope (60-80 kV) was used for examining the sections.

RESULTS

Ultrathin sections of both stems and minor veins revealed that transfer cells are common in young pea. There was no apparent difference in the extent of development of transfer cells and their wall ingrowths between inoculated and noninoculated plants. Transfer cells occurred in contact with either sieve cells or xylem cells or both. Certain transfer cells also were contiguous with phloem parenchyma cells or other transfer cells (Fig. 1). They contained a large nucleus (Fig. 2, 3), mitochondria (Fig. 2, 3), chloroplasts with grana and starch grains (Fig. 2, 3), and virus aggregates (Fig. 2, 3, 4). The cytoplasm of transfer cells was dense but large vacuoles were seen frequently (Fig. 2, 3). Rough endoplasmic reticulum and ribosomes were present in the cytoplasm. Protuberances, the most outstanding characteristics of transfer cells, were clearly outlined by the close lining with plasmalemma and usually were

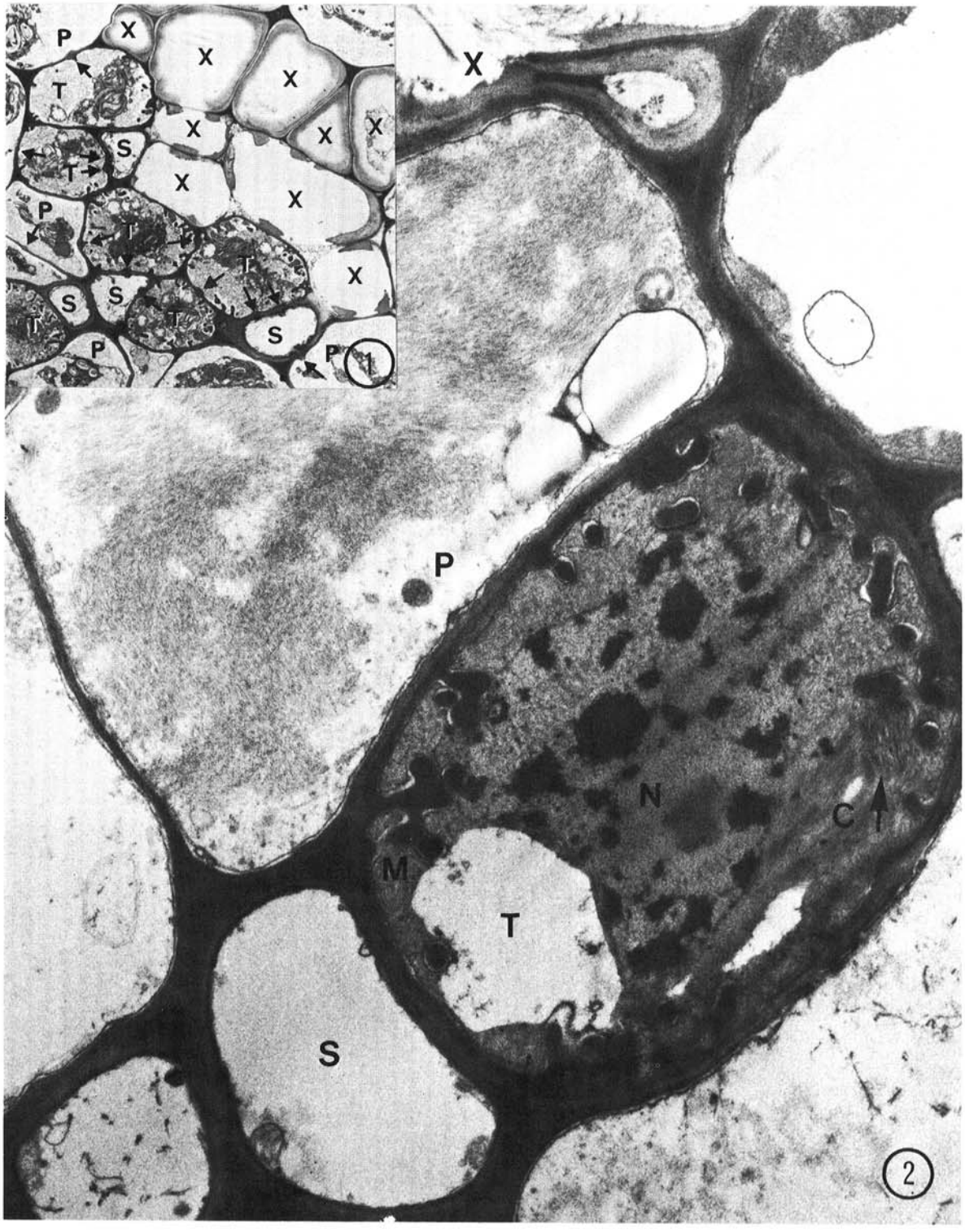
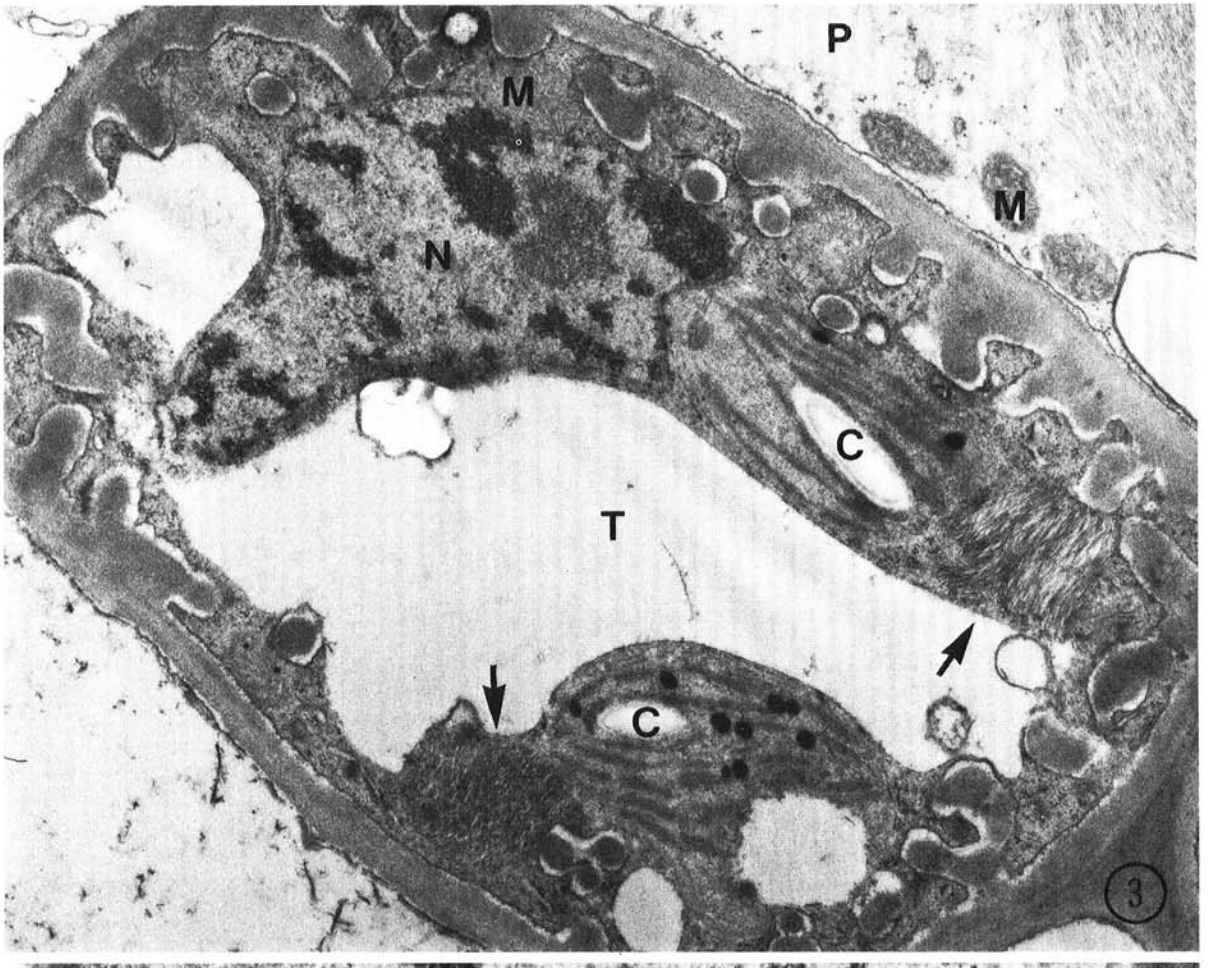
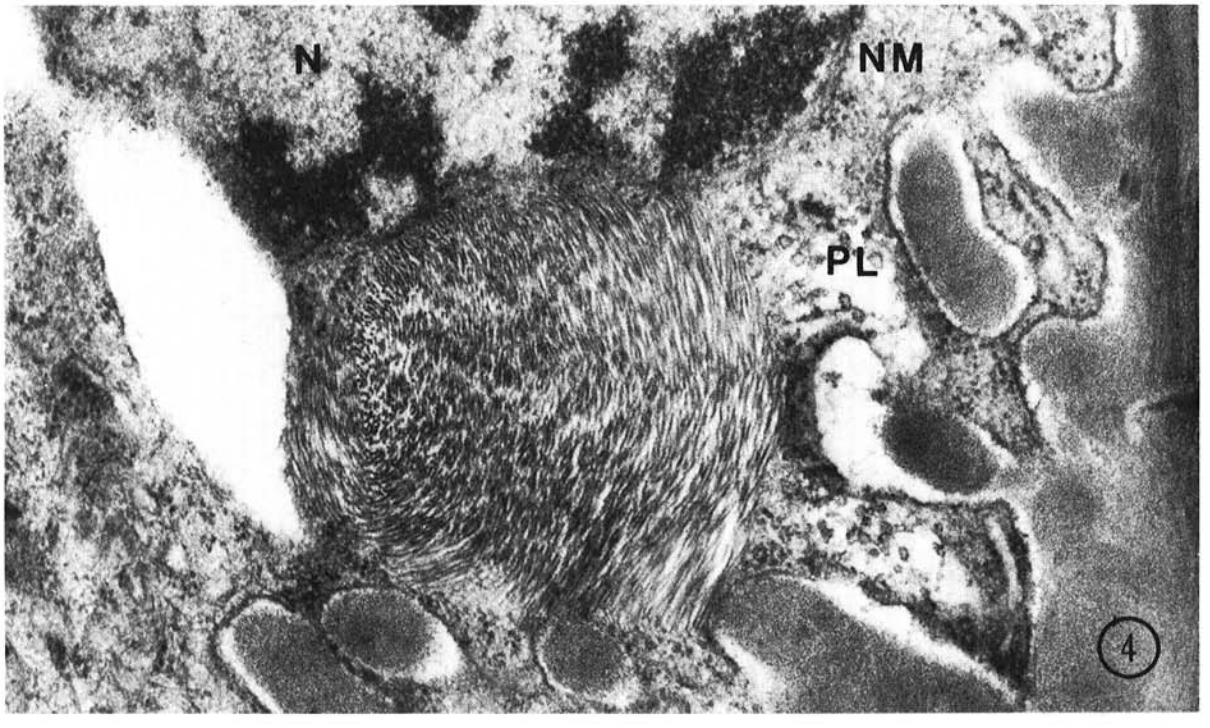


Fig. 1-2. 1) A low magnification electron micrograph of a transverse section of *Pisum sativum* stem. Arrows indicate the positions of plasmodesmata. × 2,200. 2) A typical transfer cell of *P. sativum* stem containing clover yellow mosaic virus aggregates (arrow). Note the occurrence of large amorphous virus aggregates in a paraenchyma cell (P) adjacent to the transfer cell (T). C, chloroplast; M, mitochondrion; N, nucleus; S, sieve element; X, xylem. × 16,300.


P
M
M
N
C
T
C
3


N
NM
PL
4

observed on all walls of a transfer cell in transverse (Fig. 1, 2, 3, 4) and longitudinal sections. Protuberances, however, were sometimes less abundant on the wall contiguous with a sieve cell. Compound plasmodesmata, of the kind described for other companion cells (4), traversed the wall between transfer cells and sieve and parenchyma cells (Fig. 1). Plasmodesmata also interconnected the transfer cells (Fig. 1).

Relatively small virus aggregates were seen in cytoplasm in close proximity to protuberances of transfer cells (Fig. 2, 3, 4). Virus particles in the aggregates were packed in parallel or in "spiral" fashion. A change in particle orientation in relation to the plane of section apparently resulted in these "spirals" (Fig. 4). Parenchyma cells, adjacent to transfer cells, were predominantly occupied by large amorphous virus aggregates in many cases (Fig. 2). The aggregates were not uniform in size or shape or in arrangements of virus particles and were not found in the cellular organelles of transfer cells including nuclei, mitochondria, and chloroplasts.

DISCUSSION

Previous ultrastructural studies were made using samples of leaf tissues of *P. sativum* (7) and *V. faba* (9) infected with CYMV. However, no mention was made of the occurrence of virus aggregates in transfer cells of these host plants. "Spiral" and amorphous virus aggregates found in our sections were identical to those reported previously (7, 9). Morphological and general characteristics of transfer cells were similar to those reported earlier on *P. arvense* and other plants (2, 4).

The existence of a sink-and-source relationship has been implied between individual parenchyma cells to explain the movement of organic solutes from the photosynthesizing cells toward the phloem, or from the phloem toward the cells that utilize the organic solutes (1). Movement of virus particles from one parenchyma cell to another possibly is related by the similar forces that are involved in the movement between parenchyma cells and the sieve elements. Hence, the specialization of parenchyma cells in pea as transfer cells appears to be quite a meaningful one in efficient movement of virus particles. It has been postulated that a general function of nodal transfer cells is to aid in the nutrition of newly formed apices at the nodes (2). Therefore, it can be assumed that the virus particles accumulated in transfer cells are an effective source of virus for infection of young developing shoots. Physiological studies pertaining to the functional role of transfer cells in relation to virus movement should be a productive subject for further investigation.

Whatever the function of transfer cells in virus-infected plants, whether in sequestering organic solutes available for support of virus multiplication or in assisting efficient cross-translocation of organic solutes between neighboring vascular elements, and facilitating the movement of virus particles at the same time, their activities can not be overlooked. Occurrence of virus aggregates in the transfer cells of stems and minor veins and in their neighboring cells strongly indicates a need for more attention to trying to understand the principles and mechanisms of virus movement in plants. It is generally accepted that there are two types of movement of viruses in plants, a slow cell-to-cell spread and a relatively rapid movement over long distances through vascular tissues (1). However, there is no information available as to the mechanism(s) connecting these two types of virus movement in plants. It is tempting to suggest that transfer cells, due to their strategic location, may serve as a bridge between a slow cell-to-cell spread and a rapid, long-distance movement of CYMV through the vascular tissues of plants.

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Fig. 3-4. 3) Transverse section of a minor vein of *Pisum sativum* showing a transfer cell (T) containing clover yellow mosaic virus (CYMV) aggregates (arrows). C, chloroplast; M, mitochondrion; N, nucleus; P, parenchyma cell. × 20,500. **4)** Transverse section of a minor vein of *P. sativum* showing a part of a transfer cell containing a "spiral" aggregate of clover yellow mosaic virus. N, nucleus; NM, nuclear membrane; PL, plasmalemma. × 45,300.