Ultrastructural Localization of Bean Common Mosaic Virus in Dormant and Germinating Seeds of Phaseolus vulgaris

H. C. Hoch and R. Provvidenti

Department of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva 14456.

Accepted for publication 10 October 1977.

ABSTRACT

HOCH, H. C., and R. PROVVIDENTI. 1978. Ultrastructural localization of bean common mosaic virus in dormant and germinating seeds of Phaseolus vulgaris. Phytopathology 68: 327-330.

The ultrastructural localization of bean common mosaic virus (BCMV) was determined for mature dehydrated and germinated *Phaseolus vulgaris* seeds. The BCMV particles appeared only as linear arrays in most cells of mature dehydrated seeds. Inclusions were present only as "scrolls". Other virus-related inclusion material appeared as amorphous or fibrillar areas as well as paracrystalline arrays of undulated filaments. Following 48 hr of water imbibition and growth, meristem cells of the root apex were free of virus particles and virus-related inclusions. Pinwheel inclusions with plates were abundant beyond the 10th cell back from the root meristem initials. Individually dispersed BCMV particles became apparent in older cells of germinated seeds.

In nature, bean common mosaic virus (BCMV) has a limited host range and depends upon aphid and seed transmission for its survival. The virus is known to be seed-borne in common bean (*Phaseolus vulgaris* L.), phasemy bean [*Macroptilium lathyroides* (L.) Urb.], tepary bean (*P. acutifolius* Gray, var. *latifolius* Freeman), mung bean [*Vigna radiata* (L.) Wilczek], and scarlet runner bean (*P. coccineus* L.) (1, 4, 11, 12, 13). For the first three species, there is evidence that BCMV is carried in the embryo (3, 11, 12, 14). The purpose of this investigation was to localize BCMV at the cellular and tissue level in cotyledons and embryonic axes of dormant and germinating seeds of *P. vulgaris,* using electron microscopy.

MATERIALS AND METHODS

Bean seeds were derived from plants of cultivar Michelite 62 that either had been inoculated at the primary leaf stage with the NY 68-95 strain of BCMV (12) or had remained noninoculated. Prior to their use, virus-infected and virus-free seeds were stored at 8 C and 45% relative humidity for about 3 mo.

From each seed, approximately one-fifth of the cotyledons furthest from the embryonic axis (epicotyl, hypocotyl, and radicle) was excised with a sterilized jeweler's saw and indexed for the presence of BCMV. The tissue was triturated in 0.05 M PO_4 (K^+), pH 7.0, and rubbed onto primary leaves of Black Turtle 2 and VC-1822 beans, systemic and local-lesion hosts of BCMV, respectively (12).

Seeds found to be infected with BCMV and those from healthy controls were divided into two groups. The first group was left dehydrated, whereas those of the second group were allowed to imbibe distilled water and germinate for periods of 3 hr to 7 days at 22 C. For electron microscopy of nongerminated seeds, the embryonic axes were excised from the cotyledons, cut into 1-mm lengths, then split longitudinally. Several 1-mm cross sections from the cotyledon midregion also were taken. These specimens were fixed in 4.0% glutaraldehyde, buffered with either 0.1 M PO_4 (K^+), pH 6.8, or 0.1 M cacodylate (Na^+), pH 7.0, for 6 hr. Consecutive 1-mm segments from the first 5 mm of the root tip and 1-mm segments from the midroot, hypocotyl, cotyledon-embryonic axis attachment, primary leaves, and apical bud of germinated seeds were similarly fixed. Next, the tissues were rinsed for 2 hr in corresponding buffer solutions and postfixed with buffered 2.0% OsO_4 for 4 hr. The material was dehydrated via an acetone series and embedded in Spurr's medium (16). Serial thin sections, stained with aqueous uranyl acetate and lead citrate, were examined with a JEOL 100B electron microscope operating at either 60 or 80 KV. Near-median longitudinal sections were made whenever possible. At least six individual dormant seeds or germinated seeds were examined for each stage of development.

Indexing bean seed prior to germination and preparation for electron microscopy provided a means of assuring that the material was either infected with BCMV or free of BCMV. Thus, the cytological aspects of this study dealt only with seeds of known virus infectivity.

Concurrently, some of the adjacent longitudinally-split segments were assayed for BCMV using Black Turtle 2 and VC-1822 beans. Several samples, from corresponding positions on different plants, were pooled to make enough tissue to grind and to inoculate indicator plants. This was necessary, particularly when working with the split terminal 1 mm of root apices.

RESULTS

Several distinct configurations of virus particle arrangement and virus-associated materials were observed in BCMV-infected tissues: (i) typical flexuous

00032-949X/78/000 054$03.00/0

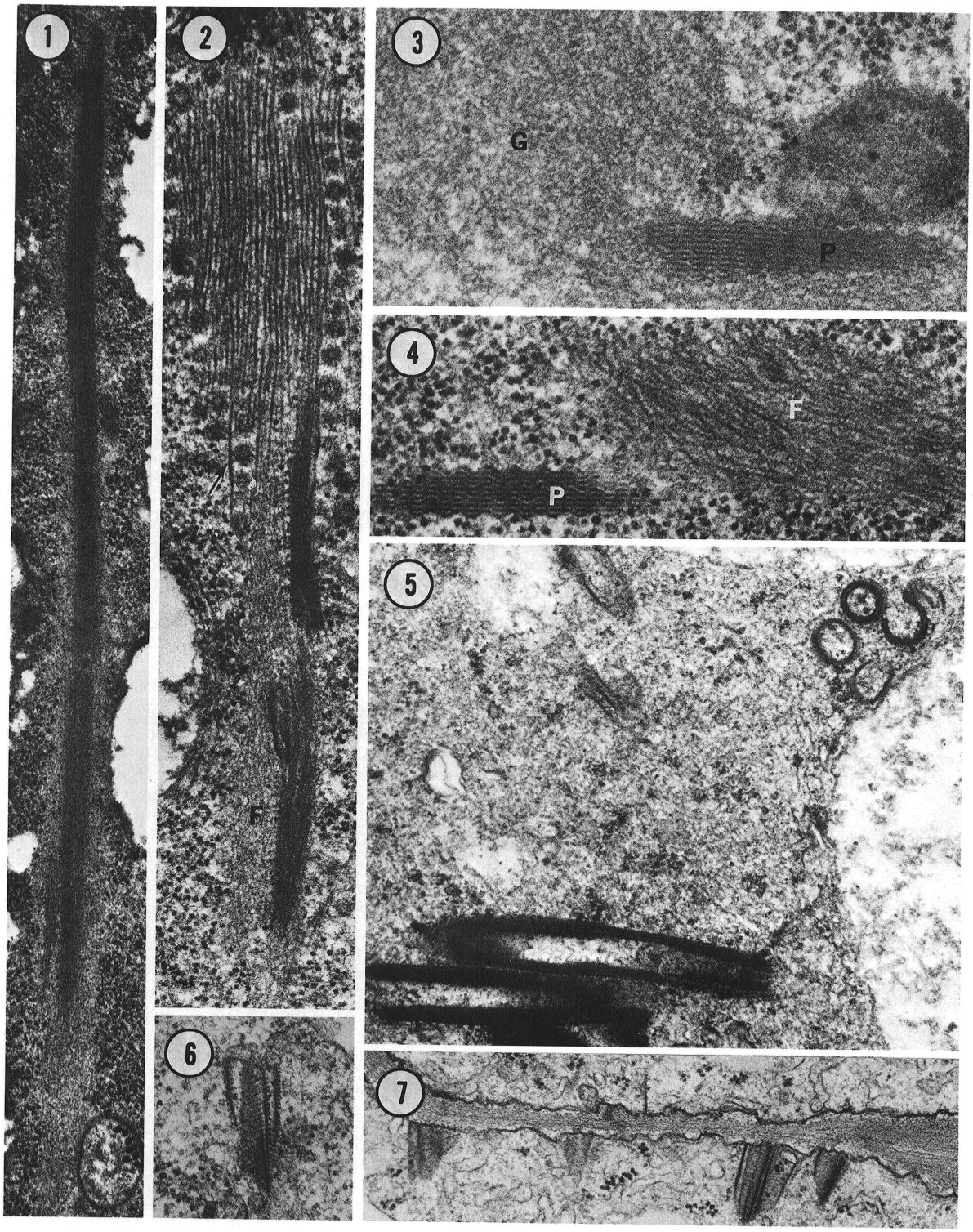

Fig. 1-7. Electron micrographs of bean common mosaic virus-infected *Phaseolus vulgaris* cytoplasm. **1, 2)** Longitudinal sections through virus inclusions in cortical cells approximately 1 mm from the radicle apex of dormant, dehydrated seed. Discrete flexuous virus particles are visible in Fig. 2;F = fibrillar material (×31,400 and ×45,000, respectively). **3, 4)** Virus-related inclusion materials often exhibit paracrystalline (P) structures with an undulating pattern. Other granular (G) and fibrillar (F) areas often are associated closely with the virus-related inclusion material. Fig. 3 is representative of a mature dehydrated radicle; Fig. 4 is of a radicle cell that has imbibed water for 12 hr (both ×65,000). **5)** Longitudinal (lower left) and transverse (upper right) sections through "scrolls" in a cotyledon epidermal cell after 24 hr of water inbibition (×48,500). **6)** Tangential section through a pinwheel inclusion having a striated substructure (×39,300). **7)** Longitudinal sections through pinwheel inclusions attached perpendicular to root plasma membranes. These cells are approximately 10 cells back from the apical meristem initials of the radicle (×34,000).

rod-shaped BCMV particles (14-15 nm wide in thin-sectioned material) dispersed in the ground cytoplasm; (ii) aggregates of virus particles arranged in long arrays, often more than 8 μm long (Fig. 1, 2); (iii) areas usually associated with the virus arrays, exhibiting a granular or fibrillar appearance, depending on the plane of sectioning (Fig. 2-4); (iv) paracrystalline material with an ordered array of elements 10-11 nm wide, spaced approximately 4.5 nm apart, and exhibiting an undulating pattern with a periodicity of 70 nm (Fig. 3, 4); and (v) cylindrical inclusions in the form of either scrolls (Fig. 5) or pinwheels (Fig. 6, 7). None of these structures was observed in any of the noninfected tissues.

In all BCMV-infected mature dehydrated seed, virus aggregates were observed at one time or another in cells of most tissue types of the embryonic axis; e.g., meristem, procambium, and epidermis. They also were observed in the extreme apical meristem initials (cells) of the radicle and in the cells which would ultimately comprise the root cap. In the cotyledon, they were present in nearly all cells of the epidermal layer and in the immediate subjacent cell layer. Virus aggregates were observed less frequently in the next two to three subjacent cell layers, and none was found in cells more than three or four layers from the surface despite extensive searching. It is possible that the cytoplasm was too condensed and electron-opaque for the aggregates to be observed. In nearly every instance, the virus particles were aligned in long arrays (Fig. 1, 2). The virus particles were 14 nm in width in these arrays with certain areas unexplainably wider than 20 nm (arrow, Fig. 2). Virions were seldom seen individually dispersed in the cytoplasm; however, in most cells, the cytoplasm was extremely condensed and electron-opaque and single virus particles could easily have been overlooked.

The ubiquitous cylindrical inclusions common to BCMV, as well as other potato Y-group viruses, were present only in the outer cell layers of the cotyledons (especially in the epidermal cells) and in the primary leaves. They were not observed in the radicle. Furthermore, they were present only as scrolls (Fig. 5) and not as pinwheels as observed in actively growing cells (2).

No changes were evident in the morphological features of virus arrays and associated components, or in their distribution throughout the embryo 3, 12, or 24 hr following water inbibition. At periods beyond 48 hr through 7 days, when the root was in a rapid state of growth, virus arrays and other associated structural components always were absent from the first five to eight cells of the root apical meristem.

Beginning at 72 hr, changes in virus distribution and in the morphology of the related components were noticeable. Pinwheel inclusions, 0.2 to 0.4 μm in length and composed of one to several plates, were first noticed in about the 10th cell back from the apical meristem initials of the radicle and were attached perpendicularly to the plasmalemma (Fig. 7). They were seen in similar situations in the shoot meristem; however, the exact anatomical position of the cells in this region in regard to the apical initials was uncertain. Pinwheels, free in the cytoplasm of both the cortex and central cylinder cells (verified from serial sections), were detected at approximately the 12th cell from the radicle initials. Older portions of 3- to 7-day-old seedlings (i.e., cells in the region of root elongation, hypocotyls, and primary leaves) frequently contained numerous pinwheel inclusions greater than 1 μm in length and were composed of more than 10 plates. Plates of these pinwheels had a striated substructure with individual elements spaced approximately 5 nm apart (Fig. 6). Similar striations have been noted in pinwheel plates for other potato Y-group viruses (2).

In the 3- to 7-day-old seedlings, virus arrays and associated material were noted less frequently, whereas individual virus particles became more noticeably dispersed through the cytoplasm.

All indicator plants, inoculated with sap from virus-infected seed and seedling parts, developed typical BCMV symptoms. These samples included the apical 1 mm of virus-infected embryo radicles and roots of 2-, 5-, and 7-day-old seedlings and corresponded to tissues that were observed ultrastructurally for virus or virus-associated components in these tissues.

DISCUSSION

In this study we have shown that virus or virus-associated components are present in most tissues of the mature dehydrated seed, and that upon germination pinwheel inclusions are observed in shoot and root apicies. The seed coat was not examined for virus particles since its electron opacity made their detection difficult. Immature seed coats have been shown to contain BCMV (3, 14) but the virus eventually was inactivated as the seed coats matured and became desiccated.

Several of the ultrastructural features of BCMV-infected cells noted in this study were heretofore undescribed. These included the long condensed arrays of BCMV particles and the virus-associated paracrystalline arrays of undulated filaments and the granular and fibrillar material. The occurrence of these structures was associated only with seeds and with older tissues of seedlings originally derived from the embryo. Their condensed appearance may be, in part, related to the dehydrated nature of the seed tissues.

The development of pinwheel inclusions observed at different stages of root and shoot histogenesis was similar to that observed by Lawson et al. (6, 7) in *Impomoea setosa* Ker. infected with sweet potato russet crack virus (RCV). In that study, RCV was introduced into healthy cells by *Myzus persicae* Sulz. and studied at specific times thereafter. In *P. vulgaris,* the rapidly growing root and shoot systems provided a continuous supply of cells in which no virus-related components could be seen. Thus, a sequential development of pinwheel inclusions could be observed as the distance increased from the apices.

Smith and Schlegel (15) studied clover yellow mosaic virus in *Vicia faba* by indexing 200-μm-thick transverse sections of the root tips. They found the first 400 μm (which included approximately 300 μm of root cap) to be free of infectious material. Similarly, other workers with other virus-host combinations (5, 9, 10) have observed root tip regions of various lengths (100-1,000 μm) to be free of transmissible virus. Since no virus particles were observed ultrastructurally in the apical five to 10 cells (50-

150 μm, exclusive of the root cap) of actively growing seedling roots in this study, it would be informative, though difficult, to index them for infectivity. Such a study could verify whether or not the cells in this zone were actually free of virus infectivity or simply free of virus particles and associated structural components. It is possible that the initials of the embryo radicle originally were virus infected because rapid and extensive growth did not occur during or immediately after their formation. They apparently became free of virus particles when the plant began to grow and new cells were rapidly formed. Explanations for virus-free zones in root and shoot tips have been postulated (8); however, they remain to be proven.

The results of this study indicate that virtually all cell types of *P. vulgaris* seed systemically infected with BCMV contain BCMV particles or other structures associated with BCMV-infected cells. Heretofore, the presence of BCMV in *P. vulgaris* seeds was known only from embryo and seed coat triturates indexed on appropriate hosts.

LITERATURE CITED

1. CHAMBERLAIN, E. E. 1939. Bean mosaic (Phaseolus virus 1 of Smith, 1937). N. Z. J. Sci. Technol. 20:381A-388A.
2. EDWARDSON, J. R. 1974. Some properties of the potato virus Y-group. Fla. Agric. Exp. Stn. Monogr. Ser. No. 4. 398 p.
3. EKPO, E. J. A., and A. W. SAETTLER. 1974. Distribution pattern of bean common mosaic virus in developing bean seed. Phytopathology 64:269-270.
4. KAISER, W. J., and G. H. MOSSAHEBI. 1974. Natural infection of mungbean by bean common mosaic virus. Phytopathology 64:1209-1214.
5. KASSANIS, B. 1957. The use of tissue cultures to produce virus-free clones from infected potato varieties. Ann. Appl. Biol. 45:422-427.
6. LAWSON, R. H., and S. S. HEARON. 1971. The association of pinwheel inclusions with plasmodesmata. Virology 44:454-456.
7. LAWSON, R. H., S. S. HEARON, and F. F. SMITH. 1971. Development of pinwheel inclusions associated with sweet potato russet cracks virus. Virology 46:453-463.
8. MATTHEWS, R. E. F. 1970. Plant virology. Academic Press, New York. 778 p.
9. MOREL, G., and C. MARTIN. 1955. Guerison de pommes de terre atteints de maladies a virus. C. R. Acad. Agron. 41:472-475.
10. NIELSEN, L. W. 1960. Elimination of the internal cork virus by culturing apical meristems of infected sweet potatoes. Phytopathology 50:840-841.
11. PROVVIDENTI, R., and S. W. BRAVERMAN. 1976. Seed transmission of bean common mosaic virus in phasemy bean. Phytopathology 66:1274-1275.
12. PROVVIDENTI, R., and E. D. COBB. 1975. Seed transmission of bean common mosaic virus in tepary bean. Plant Dis. Rep. 59:966-969.
13. REDDICK, D., and V. B. STEWART. 1919. Transmission of the virus of bean mosaic in seed and observations on thermal death-point of seed and virus. Phytopathology 9:445-450.
14. SCHIPPERS, B. 1963. Transmission of bean common mosaic virus by seed of Phaseolus vulgaris L. cultivar Beka. Acta Bot. Neerl. 12:433-497.
15. SMITH, S. H., and D. E. SCHLEGEL. 1964. The distribution of clover yellow mosaic virus in Vicia faba root tips. Phytopathology 54:1273-1274.
16. SPURR, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26:31-43.