Cytology and Histology

Intracellular Location of Beet Curly Top Virus Antigen as Revealed by Fluorescent Antibody Staining

W. R. Thornley and D. L. Mumford

Immunologist, Beet Sugar Development Foundation, and plant pathologist, Agricultural Research-Science and Education Administration, U.S. Department of Agriculture, Crops Research Laboratory, Utah State University, Logan, 84322.
Cooperative investigations of the Beet Sugar Development Foundation, U.S. Department of Agriculture, and the Utah State Agricultural Experiment Station. Approved as Journal Series Paper 2336, Utah State Agricultural Experiment Station, Logan.
Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply approval to the exclusion of other products that also may be suitable.
Accepted for publication 19 January 1979.

ABSTRACT

W. R. THORNLEY, and D. L. MUMFORD. 1979. Intracellular location of beet curly top virus antigen as revealed by fluorescent antibody staining. Phytopathology 69: 738-740.

The intracellular location of beet curly top virus (BCTV) antigen was studied by using the fluorescent antibody method on paraffin sections and enzyme-separated cells of sugarbeet hypocotyl. Infected cells were examined from tissue collected daily, beginning 1 day after vein-clearing symptoms first were observed. The percentage of infected cells with detectable antigen restricted to the nucleus was high in tissue from plants collected shortly after vein-clearing appeared. The percentage of infected cells with antigen in both the nucleus and cytoplasm increased progressively after initial symptom development. These results indicate that BCTV initially is located in the nucleus but subsequently is distributed throughout the cytoplasm.

Additional key words: Serology, virus multiplication, *Beta vulgaris* L.

In 1977, we reported using fluorescent antibody staining to locate beet curly top virus (BCTV) antigen in several hosts (4). Sections examined at that time were cut from frozen tissue in a cryostat and were not thin enough to determine the intracellular location of BCTV. Paraffin embedding provides sections sufficiently thin so that intracellular location can be determined. Sainte-Marie (7) reported the successful fluorescent antibody

staining of rabbit lymph nodes embedded in paraffin. Nagaraj (5) employed this method and also mesophyll cells separated by enzyme digestion to study the distribution of tobacco mosaic virus in tobacco. We used these methods, with modifications, to determine the intracellular location of BCTV in infected sugarbeet cells. A preliminary report of these results has been made (3).

MATERIALS AND METHODS

Virus. A BCTV isolate collected in 1966 from sugarbeets in northern Utah and designated Logan isolate 66-10 was used throughout this study. This isolate has been maintained by successive transfers to sugarbeet in our greenhouse.

Plants. A susceptible inbred sugarbeet (*Beta vulgaris* L.) designated EL31, obtained from G. Hogaboam, East Lansing, MI, was used for host tissue. Two-week-old seedlings were inoculated by caging two viruliferous beet leafhoppers *Circulifer tenellus* (Baker) on a cotyledon of each seedling for 5 days. Hypocotyl tissue was collected daily for 5 days after folier symptoms (vein clearing) first were observed. Tissue also was collected 14 days after symptoms appeared.

Paraffin sections. Diseased tissue was thoroughly washed in running water, then cut into pieces 4 mm long. Pieces were dehydrated in *t*-butyl alcohol and infiltrated with DMSO-Paraplast (VWR Scientific, Salt Lake City, UT 84110). Dehydration was in a *t*-butyl alcohol series from 40 to 90% at 25 C followed by three changes in 100% at 35C. Dehydration with *t*-butanol resulted in less tissue shrinkage and better preservation of antigen than did dehydration with cold ethanol as used by Sainte-Marie (7). Sections were cut 5 μm thick and mounted on slides precleaned with 70% ethanol on a cotton towel. After drying and before paraffin removal, sections were affixed to slides by gently flooding them with a mixture of xylene and acetone (2:1 v/v). Several sections for examination were prepared from each of 10 inoculated seedlings sampled on successive days after symptom appearance.

Separated cells. Tissue from a pool of 70 seedlings for each collection day was digested in a mixture of seven parts 8% pectinase aqueous solution (containing streptomycin sulfate at 10 μm/ml), two parts sucrose (0.35 M in 0.1 M phosphate buffer, pH 6.0), and one part DMSO. Tissue pieces were split lengthwise, cut to 1-mm lengths, and placed in the digestion mixture at the rate of 0.2 g of tissue in 10 ml of mixture. Tissue and digestion mixture were shaken vigorously for 10 min five times daily for 6 days. We used a higher enzyme concentration and longer digestion period than were used by others so that phloem cells would be separated from the vascular tissue. Undigested tissue was separated from the digestion mixture by gravity settling. Separated cells were washed twice with buffer and concentrated by gravity settling.

Staining. Fluorescein-antibody conjugate was prepared, control procedures were implemented and sections were stained as described previously (4). Separated cells were stained by mixing equal parts of conjugate and cell suspension and incubating the mixture at 5 C for 18 hr. A wet-mount of the cell-conjugate mixture was made for immediate examination by fluorescence microscopy. Paraffin sections and separated cells also were stained with fluorescein conjugated to the globulin fraction from normal serum as a control for nonspecific staining.

Fluorescence microscopy. Sections and separated cells were examined with a Zeiss Universal Fluorescence Microscope with vertical illumination, exciter filters I and II, and barrier filter 50. Photographs were made with photomicrography monochrome films SO-410 and developed with D-19 developer (Eastman Kodak Co., Rochester, NY 14650).

RESULTS

Compared to tissue stained in previous studies (4), there was no apparent loss of antigenicity during the preparation of sugarbeet cells for examination either in paraffin sections or as enzyme-separated cells. The thinner sections obtainable with paraffin-embedded tissue provided more precise location of BCTV antigen within cells than frozen tissue used previously (4).

Examination of tissue from seedlings with severe symptoms revealed that staining was restricted to the nucleus of some cells (Fig. 1A) and distributed throughout the nucleus and cytoplasm of others (Fig. 1B). The percentage of cells with staining in both the nucleus and cytoplasm was higher in tissue from plants that had symptoms for several days. No specific fluorescence was observed when tissue was stained with fluorescein conjugated with normal serum or when healthy tissue was stained with fluorescein-antibody conjugate.

Paraffin sections were examined from tissue collected daily for 5 days after the first observed foliar symptoms and on the 14th day. As indicated in Fig. 2A, 72% of the infected cells observed in tissue collected on the first day after symptoms appeared were stained only in the nucleus, and 28% of the infected cells in tissue collected then were stained in both the nucleus and cytoplasm. The percentage of infected cells stained in both the nucleus and cytoplasm increased rapidly from 28 to 85% in tissue collected on the fifth day. This increase continued more slowly until the 14th day when more than 95% of the infected cells were stained throughout the nucleus and cytoplasm (Fig. 2A).

Results were similar when separated cells were examined. Tissue was collected for study of separated cells on the first, third, and fifth days after symptoms appeared. The first day after symptoms appeared, 60% of the infected cells collected were stained throughout the nucleus and cytoplasm, and by the fifth day more than 95% of the cells were so stained (Fig. 2B). These results indicate that BCTV is located initially in the nucleus but subsequently is distributed throughout the cytoplasm.

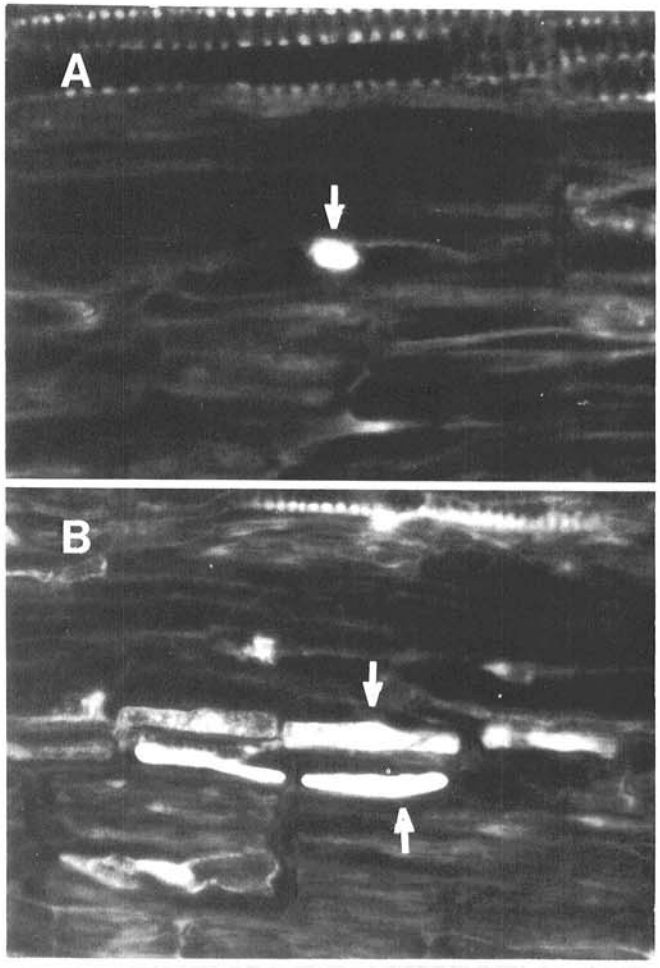

Fig. 1. Photomicrographs of longitudinal sections of sugarbeet phloem infected with beet curly top virus (BCTV). **A,** Infected cell with fluorescence (arrow), indicating BCTV antigen restricted to the nucleus (× 1,000). **B,** Infected cells with fluorescence (arrows) distributed throughout the cytoplasm (×720).

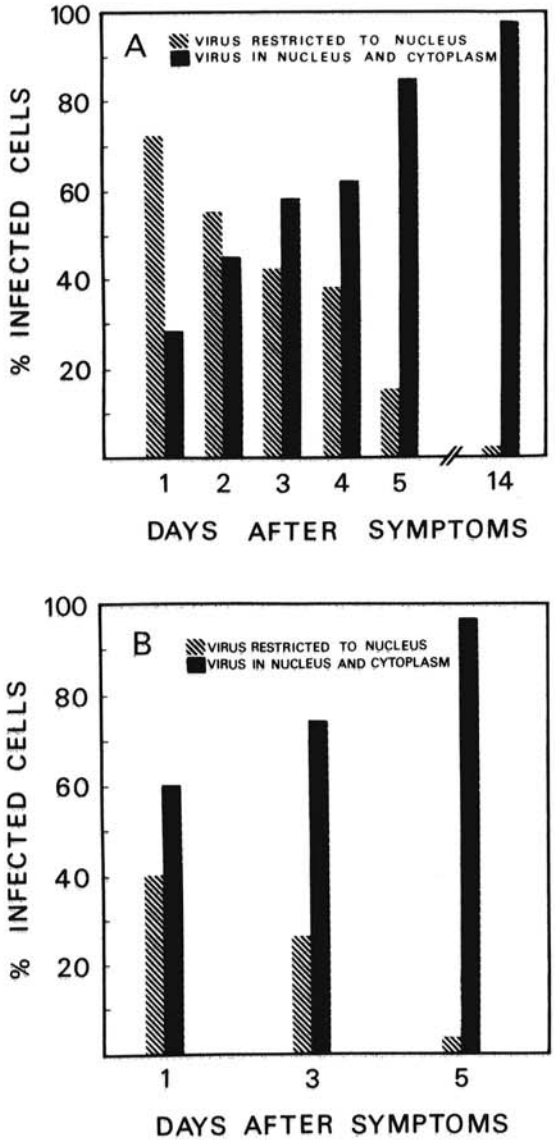

Fig. 2. Increase in percentage of infected sugarbeet phloem cells with curly top virus antigen distributed throughout the cytoplasm, and corresponding decrease in percentage of cells with antigen restricted to the nucleus on successive days after symptoms first appeared. **A**, Counts based on observation of paraffin sections. **B**, Counts based on enzyme-separated cells.

During this study, most of the cells that showed specific fluorescence were phloem parenchyma cells. Occasionally, however, a cell of the cortex adjacent to the phloem and undifferentiated cells adjacent to the cambium fluoresced.

DISCUSSION

Fluorescent antibody staining of thin sections from tissue embedded in paraffin is an excellent method for studying intracellular location of BCTV. Immunological activity was not destroyed by the chemicals used for fixation and dehydration.

These results indicate a sequence of virus appearance similar to that found by Shikata and Maramorosch (8). Their electron microscope observations of pea enation mosaic virus revealed invasion of cell nuclei during the earliest stages of disease; virus was seen in the cytoplasm more frequently during the later stages. This sequence of virus appearance also was observed for carnation etched ring virus (6) and for tobacco mosaic virus injected into tomato hair cells (2).

Using conventional electron microscopy without immunological procedures, Esau and Hoefert (1) found BCTV particles present only in the nuclei of phloem parenchyma cells. The relatively low percentage of cells that show infection (less than 10% of the phloem cells we examined) and the relatively small number of cells that can be examined with the electron microscope might have prevented these researchers from detecting BCTV in the cytoplasm.

LITERATURE CITED

1. ESAU, K., and L. L. HOEFERT. 1973. Particles and associated inclusions in sugarbeet infected with the curly top virus. Virology 56:454-464.
2. HIRAI, T., and A. HIRAI. 1964. Tobacco mosaic virus: Cytological evidence of the synthesis in the nucleus. Science 145:589-591.
3. MUMFORD, D. L., and W. R. THORNLEY. 1977. Intracellular location of curly top virus antigen as revealed by fluorescent antibody staining. (Abstr.) Proc. Am. Phytopathol. Soc. 4:286.
4. MUMFORD, D. L., and W. R. THORNLEY. 1977. Location of curly top virus antigen in bean, sugarbeet, tobacco, and tomato by fluorescent antibody staining. Phytopathology 67:1313-1316.
5. NAGARAJ, A. N. 1965. Immunofluorescence studies on synthesis and distribution of tobacco mosaic virus antigen in tobacco. Virology 25:133-142.
6. RUBIO-HUERTOS, M., S. CASTRO, I. FUJISAWA, and C. MATSUI. 1972. Electron microscopy of the formation of carnation etched ring virus intracellular inclusion bodies. J. Gen. Virol. 15:257-260.
7. SAINTE-MARIE, G. 1962. A paraffin embedding technique for studies employing immunofluorescence. J. Histochem. Cytochem. 10:250-256.
8. SHIKATA, E., and K. MARAMOROSCH. 1966. Electron microscopy of pea enation mosaic virus in plant cell nuclei. Virology 30:439-454.