Light Microscopy of Geminivirus-Induced Nuclear Inclusion Bodies

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ABSTRACT

Christie, R. G., Ko, N.-J., Falk, B. W., Hiebert, E., Lastra, R., Bird, J., and Kim, K. S. 1986. Light microscopy of geminivirus-induced nuclear inclusion bodies. Phytopathology 76:124-126.

Plant tissues infected by five presumed distinct geminiviruses (bean golden mosaic, lima bean golden mosiac, malvaceous chlorosis, Euphorbia mosaic, and Rhynchosia mosaic) were studied by light microscopy. Large, blue-violet nuclear inclusions as well as fibrillar bodies were readily detected in phloem cells in azure-A-stained tissues from infected plants. The cytopathic effects observed in these light microscopic studies were consistent with the ultrastructural changes associated with these viruses. The diagnostic potential of the light microscopic technique was demonstrated with the pseudo-curly top virus infections occurring in Florida. The conspicuous nuclear inclusions and the fibrillar bodies resolved in tissues infected by the pseudo-curly top virus indicate a geminivirus etiology.

ABSTRACT

Christie, R. G., Ko, N.-J., Falk, B. W., Hiebert, E., Lastra, R., Bird, J., and Kim, K. S. 1986. Microscopía de luz de cuerpos de inclusión nuclear inducidos por geminivirus.

Tejidos vegetales infectados por cinco geminivirus presumiblemente distintos (mosaico dorado de la habichuela, mosaico dorado de la haba lima, clorosis de las malvaceas, mosaico de Euphorbia y mosaico de Rhynchosia) fueron estudiados bajo el microscopio de luz. Luego de teñir con azure A tejidos de plantas infectadas, no se requirió gran esfuerzo para detectar inclusiones grandes color azul-violeta y cuerpos fibrilares en células del floema de dichos tejidos. Los efectos citopáticos observados al emplear el microscopio de luz son consistentes con los cambios ultraestructurales asociados con los virus en cuestión. El potencial diagnóstico de la técnica del microscopio de luz fue demostrado al estudiar tejidos infectados por el virus rizado del tope (pseudo-curly top virus) en Florida. Las conspicuas inclusiones nucleares y cuerpos fibrilares detectados en los tejidos infectados por el virus rizado del tope constituyen evidencia de que un geminivirus es el agente etiológico de este mal.

Nuclear changes such as segregated nucleoli, fibrillar bodies, and virus particle aggregates in cells of the vascular region are cytopathic effects consistently associated with the ultrastructure of geminivirus infections (4,5,7–11,13). There is only one reported light microscopic study of geminivirus infections (10). Light microscopy of viral infections has proved to be very useful in viral disease diagnosis, in the selection of tissue for ultrastructural studies, for monitoring host tissues for virus infections, and for monitoring viral inclusion purification (2,3,6). The large field of view, the selective stains, and the speed and ease of tissue preparation and examination are some of the advantages of light microscopy over electron microscopy in studying viral infections.

Here we report the light-microscopic studies of leaf tissues infected with five presumed distinct geminiviruses, bean golden mosaic, lima bean golden mosaic, malvaceous chlorosis, Euphorbia mosaic, and Rhynchosia mosaic. The geminivirus-induced effects observed in these cytological studies were consistent with those reported for these viruses in electron microscopic studies. The information developed in our study was also applied in the examination of plant tissues infected with the pseudo-curly top virus (PCTV) infections occurring in Florida (12,14). The conspicuous nuclear inclusions found in the PCTV-infected leaf tissues indicated a geminivirus etiology of the PCTV disease in Florida.

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MATERIALS AND METHODS

Virus culture. The bean golden mosaic (BGMV), lima bean golden mosaic (LBGMV), Euphorbia mosaic (EMV), malvaceous chlorosis (MCV), and Rhynchosia mosaic (RMV) geminivirus cultures were maintained in *Phaseolus lathyroides* L., *Phaseolus lunatus* L., *Euphorbia prunifolia* Jacq., *Sida carpinifolia* L., and *Rhynchosia minima* DC., respectively. PCTV was maintained in *Nicotiana* × *edwardsonii* Christie and Hall, and *Solanum nigrum* L., and routinely transferred to healthy plants by *Micrutalis malleifera* Fowler (treehoppers). The BGMV and RMV cultures were obtained from R. M. Goodman. The EMV and MCV cultures were maintained in Puerto Rico (J. Bird). Tissue samples from the

LBGMV culture (E. Debrot, R. Lastra, and R. C. de Uzcategui, *unpublished*) were stained and mounted in Venezuela prior to their examination by light microscopy in Florida.

Light microscopy. Generally, young growing tips of systemically infected and noninfected leaf tissues were selected for examination. The tissues were prepared for staining in azure-A (1) by abrading the leaf surface with sandpaper (600-mesh) to remove the cuticle so that the stain could penetrate into the mesophyll and vascular cells (6). The abraded tissues were placed in 2-methoxyethanol for 15–30 min in order to remove the chlorophyll and then in 0.1% azure-A stain for 15–30 min. The excess stain was removed by a 15- to 30-min wash in 95% ethanol followed by a 15- to 30-min wash in 2-methoxyethyl acetate. The tissues were then blotted dry, mounted in a drop of Euparal on a glass slide, and covered with a coverslip before viewing in a light microscope (2,6).

Electron microscopy. Tissues selected on the basis of abundant inclusions as determined by light microscopy were fixed in 5% glutaraldehyde and postfixed in 1–2% osmium tetroxide. The fixed tissue was embedded in Spurr's plastic, sectioned with a diamond knife, stained in uranyl acetate followed by lead citrate, and examined with a Hitachi 600 electron microscope. Areas for thin-sectioning were selected on the basis of light microscopy of thick (1–2 μm) plastic sections stained with toluidine blue (1% in 1% aqueous sodium tetraborate).

RESULTS

Large, blue-violet, nuclear inclusions were readily detected in azure-A-stained tissues infected with BGMV, LBGMV (not shown), EMV, MCV, and RMV (Fig. 1) in the phloem parenchyma cells of immature, expanding leaves prior to and during early stages of symptom development. Many nuclei containing inclusions appeared hypertrophied. In mature, fully expanded leaf tissues, which showed prominent mosaic symptoms, nuclear inclusions were less frequently detected. At a low magnification, the distribution of the nuclear inclusions in MCV-infected tissues is shown (Fig. 2a). The association of the geminivirus-induced nuclear inclusions with the vascular tissues was typical of all geminivirus infections studied. However, such inclusions were not uniformly distributed throughout the leaf vascular system. The resolution of the stained inclusions in the light microscope at this wide field of view was confirmed at a higher magnification of a selected area (Fig. 2b) which revealed the characteristic nuclear inclusions associated with geminivirus infections. These nuclear inclusions were not seen in stained tissues of noninfected host plants.

Light microscopy of host tissues infected with the PCTV revealed conspicuous nuclear inclusions (Fig. 3) similar to those described for the other five geminiviruses (Fig. 1). These nuclear inclusions were particularly obvious in flower tissue (Fig. 3b). Some nuclei also contained inclusions which appeared as ring-shaped fibrillar bodies (Fig. 4) similar to those described for known geminivirus infections (7–10). The cytological effects observed in the light microscope (Fig. 4a) were consistent with the ultrastructural details revealed by electron microscopic examination of thin-sectioned tissues (Fig. 4b). These ring-shaped fibrillar bodies were also observed by light microscopy in all geminiviruses in this study.

DISCUSSION

Light microscopy of azure-A-stained tissues readily resolved the particle aggregate and fibrillar ring inclusions associated with geminivirus infections (7–10). Light microscopy should be useful in the diagnosis and monitoring of plant tissues for geminivirus infections because of the simplicity of the technique and completion of the observations within 2 hr.

The geminivirus-induced inclusions have been detected readily by light microscopy in field as well as greenhouse-grown geminivirus-infected tissues. However, the effects of light and

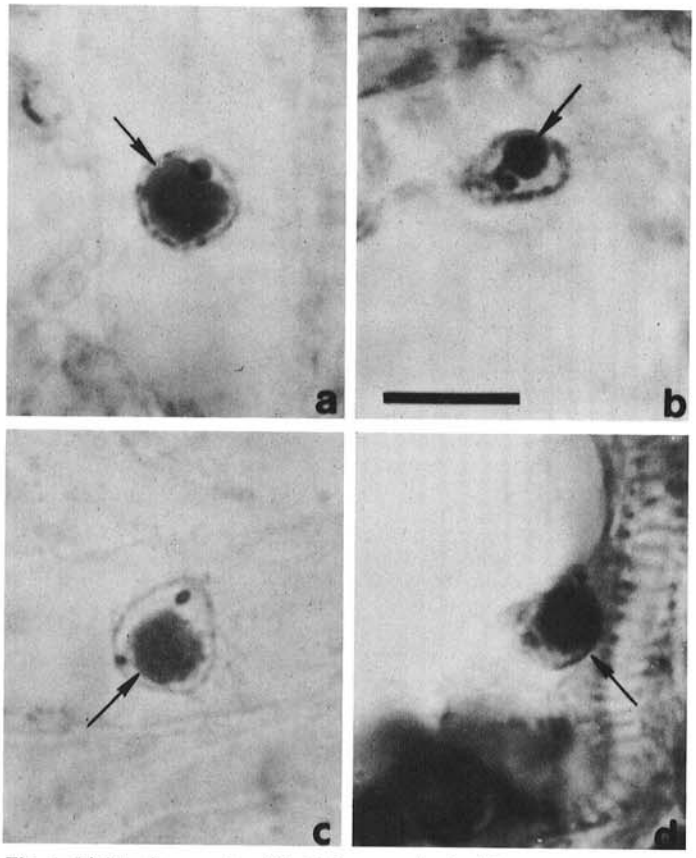

Fig. 1. Light micrographs of leaf tissues stained with azure-A showing nuclear inclusions associated with infections with four geminiviruses. **a,** Malvaceous chlorosis virus; **b,** bean golden mosaic virus; **c,** Euphorbia mosaic virus; and **d,** Rhynchosia mosaic virus. The arrows mark the nuclear inclusions in the nuclei. Bar represents 10 μm.

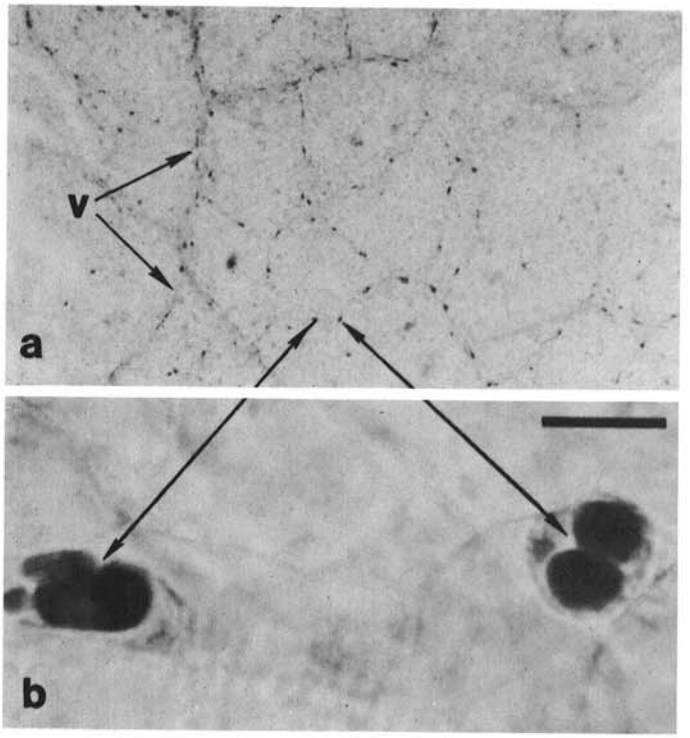

Fig. 2. Light micrographs of leaf tissues stained with azure-A showing nuclear inclusions associated with malvaceous chlorosis virus infections. **a,** Low magnification view showing the distribution of the nuclear inclusions along the vascular tissues (bar = 200 μm). **b,** A higher magnification view of a selected area from (a) showing that the dark bodies barely resolved in (a) are indeed characteristic geminivirus nuclear inclusions (bar = 10 μm). The arrows point to two nuclear inclusions. V, vascular tissues.

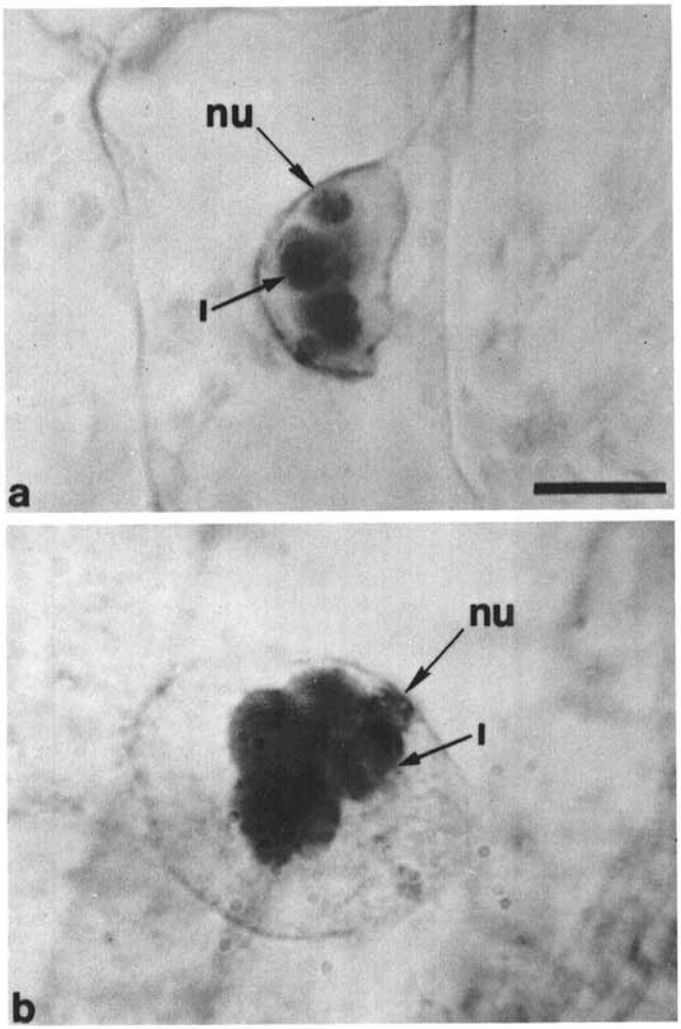

Fig. 3. Light micrograph of leaf tissues stained with azure-A showing nuclear inclusions associated with the pseudo-curly top virus disease in **a,** tobacco leaf and **b,** tobacco flower tissues. I, nuclear inclusion and nu, nucleolus. Bar represents 10 μm.

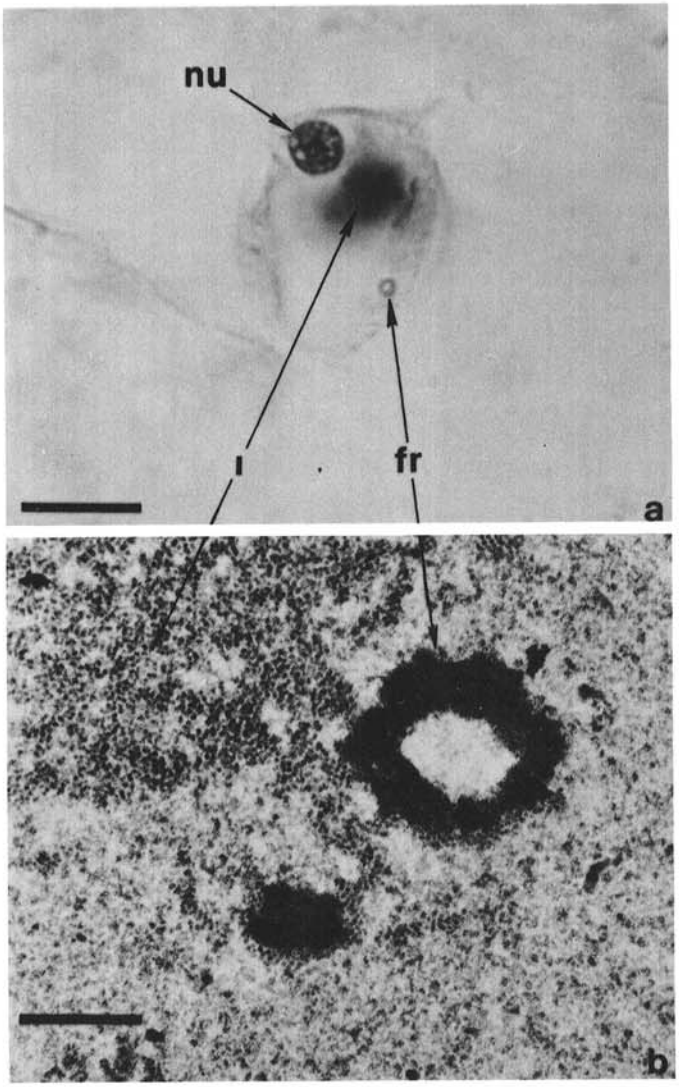

Fig. 4. Light and electron microscopy of pseudo-curly top virus infected tobacco tissue. **a,** Light micrograph of tissues stained with azure-A (bar = 10 μm). **b,** Electron micrograph of an ultrathin section (bar = 0.35 μm). I, nuclear inclusion showing geminiviruslike particles; fr, a ring-shaped fibrillar body; and nu, the nucleolus.

temperature and the effects of different virus-host combinations on geminivirus inclusion development need to be studied and may provide useful information in the culture of these viruses.

The association of cytopathic effects in PCTV-infected tissues similar to those observed in known geminivirus infections is suggestive that the PCTV infection has a geminivirus etiology. PCTV, which was isolated from tomatoes grown in south Florida, induces symptoms in tomatoes like those of beet curly top, a leafhopperborne geminivirus (12,14). If PCTV disease is caused by a geminivirus, it differs from other known geminiviruses in having a treehopper (Membracidae) vector (12). Studies are now underway to determine if the PCTV infection is caused by a geminivirus and if so, its relationship to other known geminiviruses.

LITERATURE CITED

1. Christie, R. G. 1967. Rapid staining procedures for differentiating plant virus inclusions in epidermal strips. Virology 31:268-271.
2. Christie, R. G., and Edwardson, J. R. 1977. Light and electron microscopy of plant virus inclusions. Fla. Agric. Exp. Stn. Monogr. Ser. 9.
3. de Mejia, M. V. G., Hiebert, E., and Purcifull, D. E. 1985. Isolation and partial characterization of amorphous cytoplasmic inclusions associated with infections caused by two potyviruses. Virology 142:24-33.
4. Esau, K., and Magyarosy, A. C. 1979. Nuclear abnormalities and cytoplasmic inclusions in *Amsinckia* infected with curly top virus. J. Ultrastruct. Res. 66:11-21.
5. Goodman, R. M. 1981. Geminiviruses: the single-stranded DNA viruses of plants. Pages 879-910 in: Handbook of Plant Virus Infection. E. Kurstak, ed. North Holland/Elsevier, Amsterdam. 943 pp.
6. Hiebert, E., Purcifull, D. E., and Christie, R. G. 1984. Purification and immunological analyses of plant viral inclusion bodies. Pages 225-280 in: Methods in Virology, Vol. VIII. K. Maramorosch and H. Koprowski, eds. Academic Press, New York.
7. Kim, K. S., Shock, T. L., and Goodman, R. M. 1978. Infection of *Phaseolus vulgaris* by bean golden mosaic virus: Ultrastructural aspects. Virology 89:22-23.
8. Kim, K. S., and Flores, E. M. 1979. Nuclear changes associated with Euphorbia mosaic virus transmitted by the whitefly. Phytopathology 69:980-984.
9. Kim, K. S., and Fulton, R. W. 1984. Ultrastructure of *Datura stramonium* infected with an Euphorbia virus suggestive of a whitefly-transmitted geminivirus. Phytopathology 74:236-241.
10. Lastra, R., and Gil, F. 1981. Ultrastructural host cell changes associated with tomato yellow mosaic. Phytopathology 71:524-528.
11. Matthews, R. E. F. 1982. Classification and nomenclature of viruses. Intervirology 17:4-199.
12. Simons, J. N., and Coe, D. M. 1958. Transmission of pseudo-curly top virus in Florida by a treehopper. Virology 6:43-48.
13. Thongmeearkon, P., Honda, Y., Saito, Y., and Syamananda, R. 1981. Nuclear ultrastructural changes and aggregates of viruslike particles in mungbean cells infected with mungbean yellow mosaic disease. Phytopathology 71:41-44.
14. Zitter, T. A., and Tsai, J. H. 1981. Viruses infecting tomato in southern Florida. Plant Dis. 65:787-791.