

Purification of isometric particles from maize plants infected with rayado fino virus

by

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Abstract: The leafhopper-borne rayado fino virus of maize was purified from infected plant extracts by precipitation with polyethylene glycol and sucrose density gradient centrifugation. Empty protein shells and complete isometric particles of approximately 33 ± 1 nm and 30 ± 1 nm, respectively, separated in the gradient columns. Full particles showed ultraviolet absorbancy typical of nucleoproteins, and were infective when assayed in *Dalbulus maidis*.

Rayado fino disease of maize, *Zea mays* L., is caused by a virus transmitted by the leafhopper *Dalbulus maidis* DeLong & Wolcott in a manner characteristic of viruses that multiply in their vectors. Insects transmit rayado fino virus (RFV) after incubation periods of 8-37 days, and retain infectivity for 1-20 days, but transmission by most insects is intermittent. Inoculativity of *D. maidis* decreases with time, but the virus may be recovered from insects that have lost their transmission ability (Gámez, 1969, 1973; González and Gámez, 1974). Symptoms of the rayado fino disease are characterized by a fine stipple striping of the veins in leaves of infected plants (Fig. 1). The virus appears to be widely distributed in maize growing areas of Central and South America (Gámez, 1977).

MATERIAL AND METHODS

The purification procedure is summarized in Fig. 2. Experimentally infected maize plants, line T-3, served as the source of the virus, and the isolate used was the same described in previous studies (Gámez, 1969, 1973). Approximately 50-100g of leaves were cut into small, 1.0 to 0.5 cm, pieces and homogenized in 300 ml of

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cold 0.01 M phosphate buffer (PB), pH 6.9, in a Sorvall Omnimixer for 5 minutes at 16,000 rpm. The homogenate was filtered through two layers of cheese cloth and clarified in a Sorvall RC2-B centrifuge at 16,400 xg for 10 minutes. The volume of the extract was measured and NaCl and polyethylene glycol 6,000 (PEG) were added to attain a concentration of 0.3 M and 4% respectively. The mixture was stirred for 10 minutes and kept at 4 C for 1 hr. The white precipitate that formed was sedimented at 16,400 xg for 10 minutes at 4 C, resuspended in 30 ml of PB and centrifuged again at 16,400 xg. The preparation was subjected to a second cycle of precipitation with PEG, resuspended in 20 ml of PB, and clarified as described above. Further clarification was attained by centrifuging the preparation against a sucrose cushion. A solution containing 200 g/liter of sucrose in PB was made, and 30 ml of the solution were added to each tube of the RPS-27C rotor of a RP-62 Hitachi Preparative Ultracentrifuge. The clarified virus preparation obtained after resuspension of the PEG precipitate was floated on the surface of the sucrose cushion. After centrifugation at 72,000 xg (20,000 rpm) for 1 hr at 4 C, the virus preparation was removed from the top of the sucrose cushion, and 1 to 5 ml were floated on the surface of 10-40% sucrose quasi-equilibrium density gradient columns, prepared in an automatic Hitachi DGF-U Density Gradient Fractionator, and centrifuged at 72,000 xg for 4 hr at 4 C. The zones appearing in the gradient columns were removed from the top with a needle attached to a hypodermic syringe, and dialyzed overnight against PB at 4 C. Ultraviolet absorbancies were determined in a Beckman DU-2 spectrophotometer.

Infectivity tests were performed by artificially feeding *D. maidis* through Parafilm membranes on the different zones taken directly from the sucrose gradients. Preparations for electron microscopy were made by placing drops of the virus samples onto grids with carbon coated collodion supporting membranes, which had been previously subjected to glow discharge for 15 sec in an Eico IB-3 ion coater. Excess fluid was removed with filter paper, and the preparation negatively stained with 2% uranyl acetate or neutralized 2% sodium silico-tungstic acid. Grids were then examined under a Hitachi HU-12A electron microscope at 100 kV accelerating voltage.

RESULTS

Numerous isometric virus-like particles of 30-33 nm in diameter were observed in the clarified extracts of the infected plants subjected to density gradient centrifugation. These particles were absent in the extracts of healthy plants. Three distinct light-scattering zones were seen in the gradient tubes containing the preparations from infected plants. These zones occurred at 1.3-1.6 (T_1), 1.9-2.6 (T_2), and 3.0-3.5 (T_3) cm from the meniscus (Fig. 3). A single zone at 1.5-3.0 cm was present in the preparations from healthy plants. The three light-scattering zones present in the tubes containing extracts from healthy plants were removed, dialyzed and examined under the electron microscope. No particles were observed in the T_1 zone; numerous empty isometric virus-like particles 33 ± 1 nm in diameter were present in the T_2 zone (Fig. 4); and numerous complete 30 ± 1 nm isometric particles were observed in the T_3 zone (Fig. 5). These particles were not present in the samples taken at the same depths in the tubes containing the extracts from healthy plants.

Samples of the T_2 zone with empty particles showed an absorption spectrum minimum and maximum at approximately 255 and 280 nm, while the preparations containing complete particles (T_3) showed a minimum at 240 and a maximum at 260 nm.

In preliminary tests, the infectivity of the three light-scattering zones was assayed by the membrane feeding technique. Transmission tests with *D. maidis*, carried out as previously described (Gómez, 1973), showed that only insects fed on the T₃ zone became infective.

DISCUSSION

The isometric particles observed only in the preparations from infected plants, but not in the extracts from healthy plants, must represent the RFV virion. The full particles are infective and have absorption spectra typical of nucleoproteins, while the empty particles appear to be noninfective and show ultraviolet absorbancy typical of proteins. The original observations on the morphology of RFV (Gómez & Ramírez, 1975) were thus confirmed in this study. RFV, the Brazilian corn streak virus (Kitajima *et al.*, 1976), and the Colombian maize streak virus (Martínez & Rico de Cujia, 1975), are serologically related and induce similar citopathological effects in cells of infected maize plants (Kitajima *et al.*, 1975; Kitajima & Gómez, 1977, and unpublished data). Isometric particles, 25-30 nm in diameter, were also purified from Brazilian corn streak virus infected plants (Kitajima *et al.*, 1976). The results of this investigation confirm that these viruses share morphological characteristics and may be considered identical or closely related strains.

Isometric particles similar to those associated with RFV have been observed in some insect-borne propagative viruses such as the oat blue dwarf (Banttari and Zeyen, 1969) and the rice stripe (Saito *et al.*, 1964).

Other persistent or circulative leafhopper- or planthopper-transmitted viruses, including rice tungro (Gálvez, 1967), maize chorotic dwarf (Bradfute *et al.*, 1972), maize streak (Bock *et al.*, 1974), maize line and maize stripe (Kulkarni, 1973), have also been shown to possess a similar type of particles. However none of these viruses appear to have more than one centrifugal component as found for RFV in our preliminary work. Both empty and full particles of similar size and shape have been described for the beetle-transmitted turnip yellow mosaic virus (Matthews & Ralph, 1966).

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RESUMEN

El virus del rayado fino del maíz, transmitido por insectos saltahojas, fue purificado de extractos de plantas infectadas por métodos que involucran precipitación con polietileno glicol y centrifugación en gradientes de densidad de sacarosa. Dos componentes centrifugacionales constituidos por cápsulas protéicas vacías y partículas isométricas completas de 33 ± 1 nm y 30 ± 1 nm de diámetro, respectivamente, se separaron en los gradientes de densidad. Las partículas completas mostraron espectros de absorción típicos de nucleoproteínas y fueron infectivas cuando se inocularon en *Dalbulus maidis*.

LITERATURE CITED

Banttari, E. E., & R. J. Zeyen

1969. Chromatographic purification of the oat blue dwarf virus. *Phytopathology*, 59: 183-186.

Bock, K. R., E. J. Guthrie, & R. D. Woods

1974. Purification of maize streak virus and its relationship to viruses associated with streak diseases of sugar cane and *Panicum maximum*. *Ann. Appl. Biol.*, 77: 289-296.

Bradfute, O. E., R. Louie, & J. K. Knoke

1972. Isometric virus-like particles in maize with stunt symptoms. *Phytopathology*, 62: 748.

Gálvez, G.

1967. The purification of virus-like particles from rice tungro virus-infected plants. *Virology*, 33: 357-359.

Gámez, R.

1969. A new leafhopper-borne virus of corn in Central America. *Plant Dis. Repr.*, 53: 929-932.

Gámez, R.

1973. Transmission of maize rayado fino virus by *Dalbulus maidis* DeLong & Wolcott. *Ann. Appl. Biol.*, 73: 285-292.

Gámez, R.

1977. The leafhopper-transmitted rayado fino virus of maize in Central America. In *Proceedings, International Maize Virus Disease Colloquium and Workshop*. Wooster, Ohio (In press).

Gámez, R., & C. Ramírez

1975. *Purificación, serología y microscopía electrónica del virus del rayado fino del maíz*. Reunión Anual, División del Caribe, Sociedad Americana de Fitopatología. CIAT, Cali, Colombia. 4-6 Dec. p. 43. Resúmenes.

Gámez, R., R. Bozarth, T. Fukuoka, E. W. Kitajima, & Y. Kozuka

1976. *Algunas propiedades de un virus de plantas y artrópodos*. VI Congreso Latinoamericano de Parasitología. San José, Costa Rica. 7-11 Dec. p. 243. Resúmenes de Trabajos Libres.

González, V., & R. Gámez

1974. Algunos factores que afectan la transmisión del virus del rayado del maíz por *Dalbulus maidis* (DeLong & Wolcott). *Turrialba*, 24:51-57.

Kitajima, E.W., & R. Gámez

1977. Histologic observations on maize leaf tissues infected with rayado fino virus. *Turrialba*, 27 (in press).

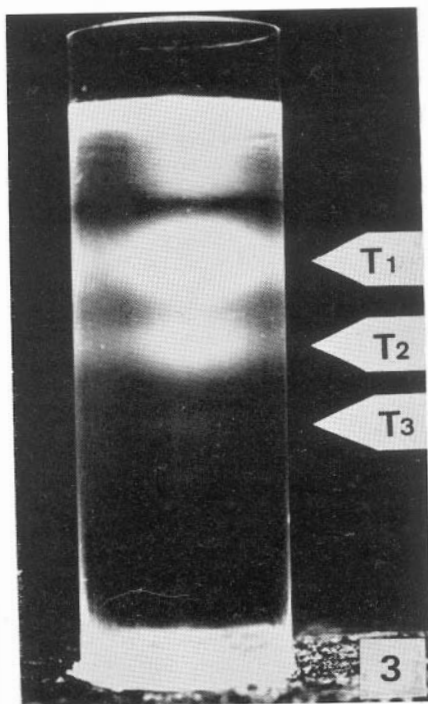
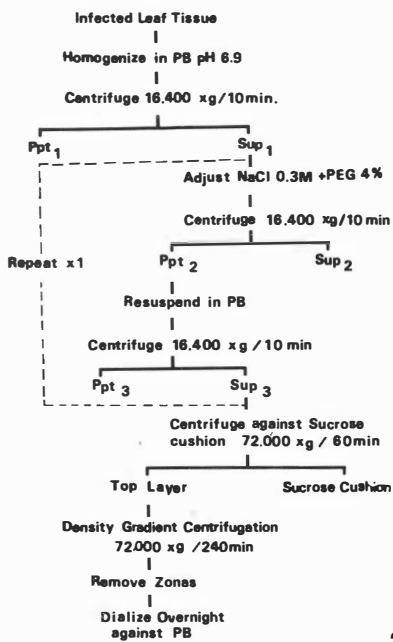
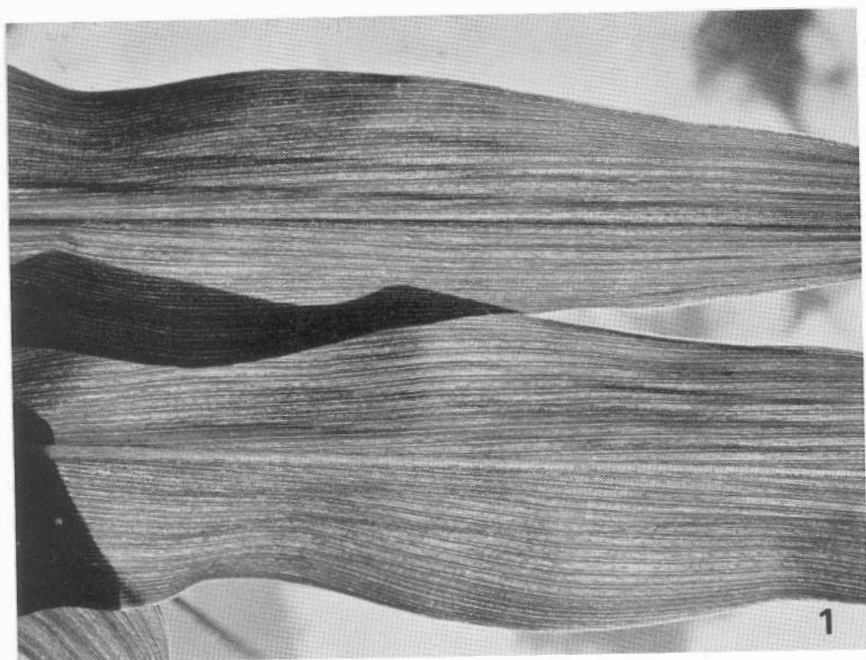
Kitajima, E. W., R. Gámez, & M. T. Lin

1975. *Comparación serológica e histológica del virus del rayado fino del maíz de Costa Rica y el virus del rayado del maíz brasileño*. Reunión Anual, División del Caribe, Sociedad Americana de Fitopatología. CIAT, Cali, Colombia. 4-6 Dec. p. 51. Resúmenes.

Fig. 1. Stipple stripe symptoms of maize rayado fino disease.

Fig. 2. Purification procedure of maize rayado fino virus.

Fig. 3. Sucrose density gradient column after 4 hr at 72,000 xg.



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Kitajima, E. W., T. Yano, & A. S. Costa

1976. Purification and intracellular location of isometric particles associated with Brazilian corn streak virus. *Ciênc. e Cult.*, 28: 427-430.

Kulkarni, H. Y.

1973. Comparison and characterization of maize stripe and maize line viruses. *Ann. Appl. Biol.*, 75: 205-216.

Matthews, R. E. F., & R. K. Ralph

1966. Turnip yellow mosaic virus. *Adv. Virus Res.*, 12: 273-328.

Martínez-López, G., & Luz M. Rico de Cujia

1975. El virus del rayado colombiano del maíz. *Noticias Fitopatológicas*, 4:27-32.

Saito, Y., T. Inaba, & K. Takanashi

1964. Purification and morphology of rice stripe virus. *Ann. Phytopathol. Soc. Japan*, 29: 286.

Fig. 4. Empty isometric particles of maize rayado fino virus, 33 ± 1 nm in diameter.

Fig. 5. Complete isometric particles of maize rayado fino virus, 30 ± 1 nm in diameter.

