Comunicaciones

Purification of the whitefly-transmitted Bean Golden Mosaic Virus

Sumario. El virus del mosaico dorado del frijol es transmitido por la mosca blanca (**Bemisia tabaci** Genn) y es un virus no transportado por la semilla. En este estudio se encontró que su punto de inactivación termal fue de 55°C, su punto final de dilución de 1:100, y su longevidad **in vitro** de 48 horas, la máxima estudiada. El virus fue estable en fosfato, 0.1M, desde pH 4.5 hasta 9; con la adición de reductores y quelatos la estabilidad del virus aumentó levemente

Para su aislamiento, 30 g de hojas infectadas con BGMV se homogenizaron en 30 ml de fosfato, 0,1M, pH 7,5 más 1 por ciento de 2-mercaptoethanol. Este supernadante se clarificó con 7 por ciento de n-Butanol con agitación constante por 1 hr. La fase acuosa se separó a 10 000 rpm, por 15 min para luego someterla a 2 ciclos de 6 por ciento PEG-6000 más 1 por ciento NaCl con agitación constante por 1 hr cada ciclo. El precipitado se resuspendió en 2,3 ml del tampón, los cuales se centrifugaron a 24.000 rpm por 240 min en columnas de gradientes de sucrosa, las cuales se prepararon flotando 4, 7, 7, y 7 ml de una solución preparada con 100, 200, 300 y 400 mg de sucrosa por ml de tampón, respectivamente. El densitómetro ISCO, utilizando su mayor sensibilidad, registró a 254 nm un componente diferente en el extracto enfermo, el cual contenía partículas polihédricas unidas en dimeros de 30 x 19 nm de diámetro, y, fue infectivo únicamente cuando las partículas estaban unidas en esta forma. Cuando se separaron en partículas individuales con EDTA, 0,1M, pH 7,5, perdieron su poder infectivo. Por lo tanto, se postula que las partículas en dímeros corresponden al virus del BGMV. La transmisibilidad de este virus purificado por las moscas blancas queda por demostrarse

Bean Golden Mosaic Virus (BGMV) causes a serious disease on beans (*Phaseolus vulgaris* L.), which limits their production in the tropics (2, 7, 8). Although 5,000 bean cultivars have been tested for resistance to the disease, only 'Turrialba 1' and 'Porrillo 1' have shown partial tolerance to it (6, 8). The disease is transmitted by the whitefly, *Bemisia tabaci* Genn (2, 7, 8); it is not seed-borne (8, 10). Recent attempts to transmit the causal agent mechanically from one bean plant to another were partially successful under special temperature conditions (3, 9).

Several attempts have been made to isolate whitefly-transmitted viruses. Tobacco leaf curl virus was first reported by Sharp and Wolf (11) as rod shaped, but later the same authors described it as a soft, spherical particle about 39 nm in diameter (12). Recently, Bird *et al.* (1) found spherical particles (30 nm) in ultrathin sections and in the low-speed (5,000 rpm) supernatant of crude *Datura stramonium* L. sap affected with Euphorbia mosaic. These particles were not present in healthy plants. Bock (personal communication) has been able to isolate a rod-shaped virus from a whitefly-transmitted disease of sweet potatoes in East Africa.

This report describes the purification of BGMV and some of its biological properties. Two BGMV isolates were used: one obtained from El Espinal, Colombia and the other from Santa Tecla, El Salvador. Mechanical transmission in the greenhouse (av. 25°C) was low at the beginning. The susceptible cultivars 'ICA-Gualí' and 'Red Kidney' were used as test plants, as well as sources of the virus.

Inoculation and purification studies used sodium or potassium phosphate buffer, 0.1 M, pH 7.5 plus 1 per cent 2-mercaptoethanol. The plants were inoculated in both primary leaves, which had been previously dusted with 600-mesh carborundum. The inoculum was obtained from infected leaves of plants that had been inoculated 15 - 20 days before.

Among phosphate, EDTA, Tris. HCl, borate, and hepes buffers at 0.1 M, pH 7.5 with 1 per cent 2-mercaptoethanol, the best were phosphate and hepes, respectively. The virus was highly stable in phosphate buffer, 0.1 M, at pH's ranging from 4.5 up to 9.0; 25 per cent and 90 per cent infectivity, respectively were obtained. The molarity of the buffer was most critical at 0.1 M in a range tested between 1 M and 0.01 M. The use of reducing or chelating agents had little effect on the stability of the virus.

PURIFICATION OF BEAN GOLDEN MOSAIC VIRUS

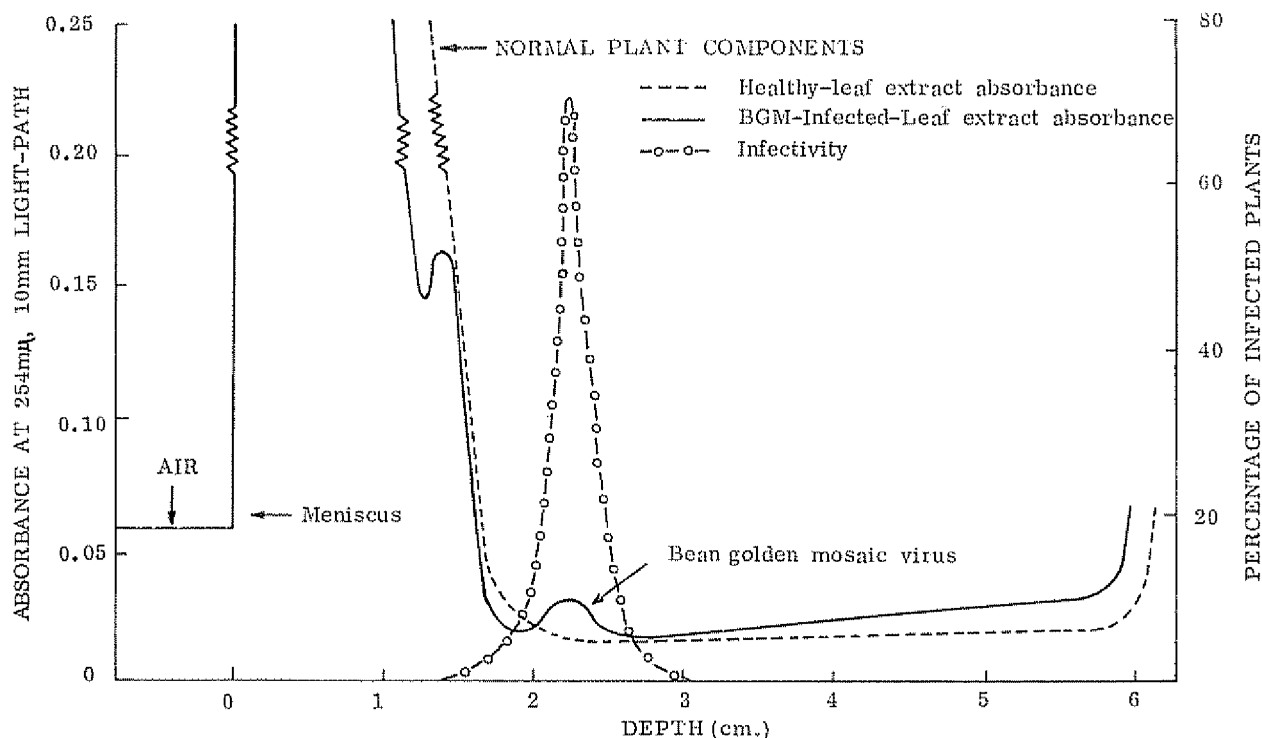

Fig. 1 —*The virus was purified by clarification with 7 per cent n-butanol, and PEG-NaCl precipitation, at 6 per cent and 1 per cent, respectively. The pellet was resuspended in the buffer phosphate, 0.1 M, pH 7.5 plus 1 per cent 2-mercaptoethanol; and after a low speed centrifugation (10,000 g for 15 min), 2 ml were layered on sucrose density-gradient columns. They were centrifuged at 60,000 g for 4 h in a Spinco 25 1 rotor, and analyzed in an ISCO densitometer, using its highest sensitive range (0.1 - 0.25), and a 10-mm light path cell. The peaks were collected and used to determine infectivity and particle structure. Note that there was not a peak present when healthy material was used as well as infectivity was not obtained when aliquots taken at the same depth of the peak were inoculated.*

The highest virus concentration was detected 15 - 20 days after inoculation and dropped dramatically 40 days after inoculation.

Some biological properties were determined using 15 - 20 day inoculum. The inoculum was extracted 1:1 (w/v) in the buffer and then treated. A thermal death point of 55°C, a dilution end point of 10^{-1} (in only one instance 10^{-2}) were obtained. The aging *in vitro* at room temperature was as long as 48 h, the maximum time tested, when 3 - 20 per cent of the plants showed symptoms.

A Sorvall omnimixer was used to homogenize 60 g of infected leaves at 6,000 rpm at 4°C for 45 - 60 s in 60-ml potassium phosphate, 0.1 M, pH 7.5, 1 per cent 2-mercaptoethanol. The supernatant after 10,000 *g* for 15 min was treated with 7 per cent n-butanol (v/v) and stirred continuously for 1 h. The phase containing the virus was separated by centrifugation at 10,000 *g* for 15 min. Baker polyethylene glycol 6000 (PEG) and NaCl were added with stirring to the clarified extract to give a concentration of 6 and 1 per cent, respectively. The mixture was stirred for 1 h, and the virus was precipitated by low-speed centrifugation (10,000 *g* for 15 min). The pellet was resuspended in the buffer and recentrifuged at a low speed; the supernatant was given another cycle of PEG-NaCl treatment. The pellet was resuspended and homogenized in 2.3 ml of buffer; and after a low-speed clarification, 2 ml were layered on sucrose density-gradient columns, which were prepared by layering 4, 7, 7 and 7 ml of 100, 200, 300 and 400 mg of sucrose per ml of buffer, respectively. The columns were used after standing overnight at 4°C; they were centrifugated at 60,000 *g* for 4 h in a Spinco SW 25.1 rotor. The gradients were then analyzed and fractionated with an ISCO density-gradient fractionator attached to an ultraviolet analyzer (254 nm), using its highest sensitive range (0.1 - 0.25) and a 10-mm light path cell. The peaks were collected and used to determine infectivity and particle characteristics.

The presence of a component not found in healthy extracts is shown in Fig. 1. The peak was at 2.3 cm from the meniscus and contained the causal agent of the Bean Golden Mosaic disease as shown by repeated infectivity assays. Samples from healthy extracts taken at this same depth did not shown any infectivity (Fig. 1). A sample of this component was concentrated by centrifugation at 250,900 *g* for 1 h. The pellet was fixed for 30 min with neutral 2 per cent formaldehyde and then

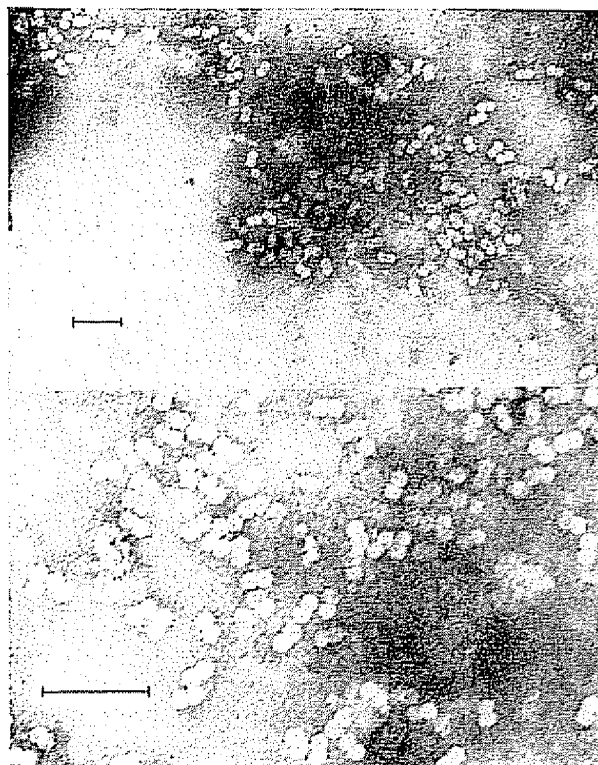

Fig 2.—*Electron micrographs of BGMV stained with 3 per cent uranyl acetate plus 0.05 per cent bovine serum albumen. Both represent virus that were banded in sucrose density-gradient columns, and then concentrated by centrifugation at 250,900 g for 1 h. The pellet was fixed for 30 min with neutral 2 per cent formaldehyde and then resuspended in 1 per cent Formvar-coated copper grids. Note the numerous paired particles (a relation of 10 to 0 1 were counted). The bonded side of these paired particles had a flattened appearance. They are about 32 x 19 nm, and, this bonded particle corresponds actually to the Bean Golden Mosaic Virus structure. The bar in both pictures represents 100 nm*

resuspended in 1 per cent ammonium acetate. Droplets of this suspension were placed on Formvar-coated copper grids and stained with 3 per cent uranyl acetate, 2 per cent PTA, pH 6.8, or 3 per cent ammonium molybdate. The preparation were then examined in a JEM 7A electron microscope. Numerous paired particles were observed (Fig 2). The bonded side of these paired particles had a flattened appearance. Almost no single particles were seen (100:1). The bonded particle measured about 32 x 19 nm and the single one 15 - 20 nm. The bonded particle is easily dissociated by EDTA, 0.1 M, pH 7.5, losing its infectivity almost completely. It is postulated that the bonded particle is the Bean Golden Mosaic Virus. Its transmissibility by the white flies remains to be demonstrated.

Similar structures are found in maize streak virus, (4, 5) as well as cassava brown streak (5), beet curly top (5) and bean summer death (5). These viruses are leafhopper-borne except for cassava brown streak, whose vector is still unknown. A virus affecting the fungus *Neurospora crassa* also has a similar morphological structure (Bozarth, personal communication). This unique morphological structure would suggest that these viruses might form a new group. The interesting dimer structure of the particles raises the question of whether each segment carries the complete genetic information for replication and infectivity or whether each segment of the bonded structure contains only part of the complete genetic code. If the paired particle is not the virus, can the single particle, which would be one of the smallest virus particle known (15 - 18 nm), contain all this genetic information in itself?

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