Isolation and Characterization of Mottle Virus from Wild Peanut

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ABSTRACT

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Wild species of peanut are being used in breeding programs to transfer disease resistance into common cultivars. One such peanut accession, PI 276235 ([GK10602] *Arachis chacoense*) had mild mottling and was suspected of carrying peanut mottle virus (PMV). A long flexuous rod-shaped virus 600–900 nm long, serologically and symptomatically related to PMV, was isolated from PI 276235. The virus was mechanically and graft-transmitted to two cultivars, producing severe mottling, chlorosis, leaf rolling, and stunting. The virus, as expressed by the mild symptoms on PI 276235, seems to have little effect on the growth of that plant under greenhouse and field conditions. From our studies, we have shown that *A. chacoense* plants may serve as reservoirs for PMV.

Peanut mottle virus (PMV) causes a disease on peanut (*Arachis hypogaea* L.) in the major peanut-producing areas of the world, including southeastern United States (3–5,11,12). Demski et al (5) reported PMV infection on peanut in New Mexico, Oklahoma, and Texas. Resistance to PMV in seven wild rhizomatous peanut introductions was reported by Demski and Sowell (6). Wild species of peanut are being used at the USDA, ARS, at Oklahoma State University in breeding programs for transferring disease resistance into common cultivars. All plants of one wild peanut accession, PI 276235 ([GK 10602] *A. chacoense* Krap. et Greg. nom. nud, section Arachis), had mild mottling under greenhouse and field conditions and were suspected of carrying PMV. Because of the nature of the suspect virus (1,8) and the proposed use of wild peanut, it was necessary to determine if the plants were reservoirs for PMV. We report on the isolation of PMV from one such wild species, PI 276235.

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MATERIALS AND METHODS

The virus was isolated from a wild peanut, PI 276235, and was successfully propagated in *Pisum sativum* L. 'Alaska' and 'Little Marvel' snow peas. Inoculum was made by grinding infected tissue in 0.01 M phosphate buffer (pH 7.2) containing 0.01 M diethyldithiocarbamate (1:2, w/v). Host plants were inoculated by rubbing leaves with the inoculum plus Carborundum (400 mesh).

Purification of the virus generally followed the methods of Paguio and Kuhn (9) with modification. Infected pea tissue was comminuted in a blender with inoculation buffer (1:2, w/v) and the homogenate was passed through four layers of cheesecloth. The filtrate was mixed with chloroform at 10% (v/v), then the mixture was shaken for 5 min and the emulsion broken by centrifugation at 12,000 *g* for 10 min at 4 C. Polyethylene glycol (PEG) (mol wt 6,000) and KCl were added to the supernatant, giving a final concentration of 4% and 0.2 M, respectively. The mixture was held at 4 C for 1 hr, then centrifuged at 12,000 *g* for 10 min at 4 C. Pellets were resuspended overnight in 0.05 M phosphate buffer containing 0.5 M urea. A second PEG-KCl precipitation was then performed. The resultant pellets were resuspended in 10 ml of the same buffer and layered over discontinuous sucrose gradients of the following composition: 10, 10, 10, and 5 ml of 10, 20, 30, and 40% sucrose (w/w) in 0.01 M phosphate buffer, pH 7.2.

Centrifugation at 20,000 rpm for 2 hr in a Beckman SW 28 rotor resulted in opalescent bands at the 20–30 and 30–40% interfaces. The band at the 20–30% interface consisted mainly of fragmented particles with few whole viral particles when observed by electron microscopy (EM). The band at the 30–40% interface contained almost completely intact virus particles when observed by EM and showed optimum infectivity. Therefore, the band at the 30–40% interface was further purified with a second density gradient centrifugation. The resultant band was dialyzed against 0.01 M phosphate-buffered saline (0.15 M) pH 7.2 containing 0.01 M sodium diethyldithiocarbamate and stored at 4 C for the remaining experiments. Particle morphology was determined with uranyl acetate negative-stained or shadow-cast preparations of the purified virus. Leaf tissue was fixed and embedded for transmission electron microscopy as described by Al-Mousawi et al (2).

Local-lesion assays were performed by rubbing the appropriate sample mixed with Carborundum onto upper surfaces of leaves of *Phaseolus vulgaris* L. 'Topcrop' beans.

Longevity in vitro was determined for the virus by incubating homogenate of leaves of Tamnut 74 in inoculating buffer at 4 C and performing daily local-lesion assays on Topcrop beans. Similar homogenates were used to determine the thermal inactivation point for the virus. Samples of the homogenate were kept on ice prior to a 10-min incubation in a water bath at the prescribed temperature. The range of temperatures was from 25 to 65 C at five-degree intervals. After the thermal treatment, the homogenate was assayed on Topcrop beans. To determine the dilution end point, infected leaves of Tamnut 74 were homogenized in inoculation buffer (1:2, w/v) and serial dilutions were inoculated onto Topcrop beans. To determine if the virus could be mechanically transmitted to standard cultivars, Tamnut 74 and Comet were inoculated with infectious sap as outlined before. To determine if the virus could be graft-transmitted, five shoots of Tamnut 74 were grafted onto infected PI 276235. To determine if the virus was pollen-transmitted, pollen was collected from the flowers of PI 276235, ground in inoculating buffer, and inoculated onto Topcrop beans.

Purified virus from sap of infected pea

leaves or sap from healthy pea leaves was mixed in a ratio of 1:1 with a solution containing 0.2% sodium dodecyl sulfate and 0.85% sodium chloride. The resulting solutions were reacted with PMV-specific antiserum in capillary ring-interfacial tests. Known positive antisera to PMV were provided by S. A. Tolin, Virginia Polytechnic Institute and State University, and T. T. Herbert, North Carolina State University. In addition, the same reactions were run with antisera prepared in our laboratory to the purified viral isolate.

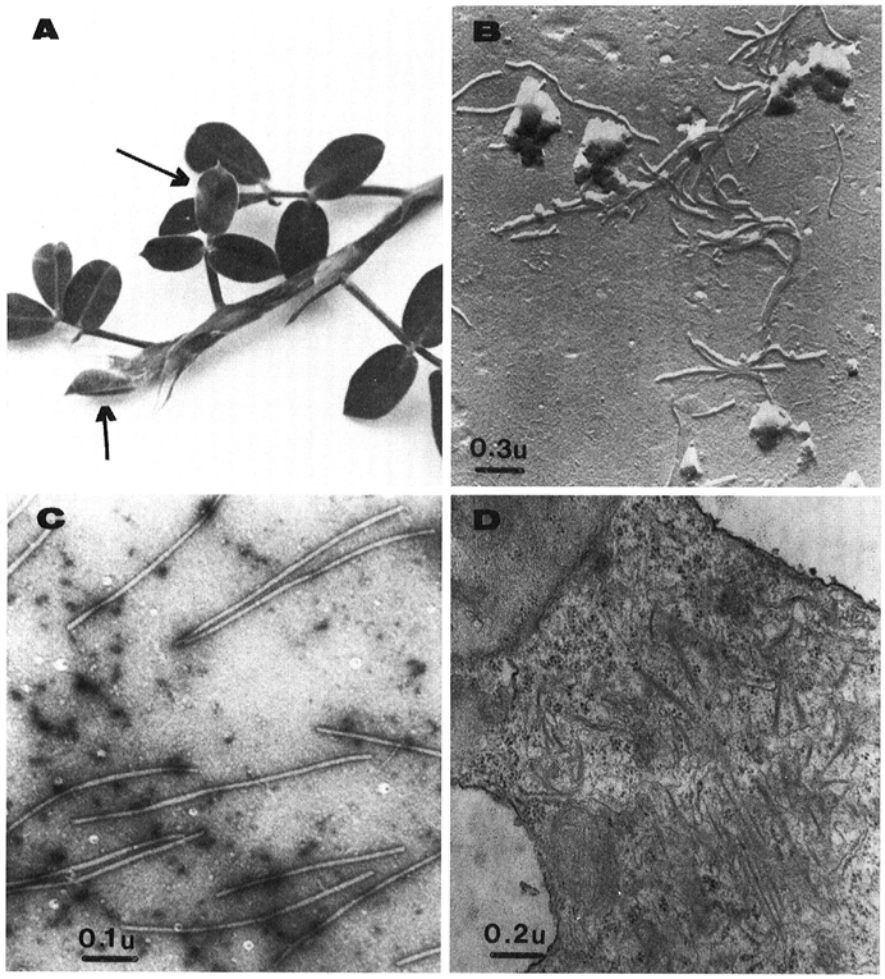

Fig. 1. (A) Virus symptoms on wild PI 276235 appear as a mild mottle (arrows) with little leaf curling and no necrosis. **(B)** Shadow-cast preparation of the isolated virus in the absence of urea. The particles are clumped and found with some cellular debris. **(C)** Negative-stained preparation of the virus isolated in the presence of urea, demonstrating the good separation provided by this method. **(D)** Thin section of a Tamnut 74 peanut leaf with viral inclusions in the cytoplasm of the cell.

Fig. 2. Virus symptoms on Tamnut 74 peanut, demonstrating the more severe results of virus infection, including mottling, leaf curling, and chlorosis.

RESULTS AND DISCUSSION

The virus was successfully isolated from wild peanut PI 276235, and titer increased in Little Marvel and Alaska snow peas. In either host, the virus produced a netlike vein chlorosis. On Topcrop beans, dark brown local lesions were produced. These symptoms are similar to those reported for PMV by Paguio and Kuhn (7,10).

The characteristic clumping of the virus during isolation and purification observed by others (9) was reduced in our isolation and purification procedures by the inclusion of 0.5 M urea without reducing infectivity.

The symptomology of the virus on PI 276235 and the long flexuous rodlike nature of the viral isolate are presented in Figure 1. The clumping characteristic of the particles in the absence of urea is shown in the shadow-cast preparation (Fig. 1B). Addition of urea reduced clumping of particles (Fig. 1C). Viral inclusions were found in the cytoplasm of a Tamnut 74 peanut leaf thin section (Fig. 1D).

The length of the virus particles ranged from 600 to 900 nm. The dilution end point in peanut sap was between 10^{-3} and 10^{-4}. Thermal inactivation of the virus was between 55 and 60 C. Longevity in vitro at 4 C was between 3 and 4 days.

The virus was mechanically transmitted to the cultivars Tamnut 74 and Comet and graft-transmitted from PI 276235 to Tamnut 74. Attempts to transmit the virus by pollen and seed (36 seeds) were negative.

Serological tests were positive for all three antisera with the purified virus and infected sap, whereas only one antiserum sample showed slight cross-reactivity with sap from healthy plants.

The symptomology of the virus in this wild peanut closely resembles the mild strain of PMV reported by others (7,10). After transmission to either Tamnut 74 or Comet cultivars, however, the symptoms were different and approximated those seen for the severe or necrotic strains of PMV (10). In Tamnut 74 and Comet, we found the new leaves severely mottled to chlorotic, with leaf rolling and stunting. Necrosis was rarely seen on peanut cultivars and never observed on PI 276235. This divergence of symptomology is shown by comparing Figure 1A with Figure 2. Figure 1A represents the mild symptoms found on naturally infected PI 276235, whereas Figure 2 shows results of the virus transferred to Tamnut 74. The Tamnut 74 plant shows much more severe symptoms. Because Tamnut 74 and Comet cultivars are Spanish botanical types and related to the Argentine peanut used by Paguio and Kuhn (10) for strain differentiation, we believe the isolated virus is probably a severe strain of PMV.

In summary, the virus isolated from our wild peanut closely resembles PMV.

Symptomology on Tamnut 74 and Comet cultivars of peanut indicates the virus may be a severe strain of PMV. From these studies, we found that *A. chacoense* can serve as a potential reservoir for the virus. The mild symptoms on the wild peanut seem to have little effect on their growth under greenhouse and field conditions.

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