

Isolation of Cowpea Mild Mottle Virus from Diseased Soybeans in the Ivory Coast

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ABSTRACT

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A virus isolated from soybeans showing severe mosaic was identified as an isolate of cowpea mild mottle virus.

Soybean (*Glycine max* L. Merr.) is a recent introduction in the Ivory Coast. Great development of this crop is planned and numerous cultivars have been tested since 1972. During summer 1978, plants showing mosaic and crinkling of leaves were observed in experimental plots of the Ecole Nationale Supérieure Agronomique near Abidjan (southern Ivory Coast); similar symptoms were observed in fields near Bouake (central Ivory Coast). Characterization of an associated virus that appears to be an isolate of cowpea mild mottle virus (2,3) is reported here.

MATERIALS AND METHODS

Inoculum for mechanical transmission tests was sap from leaves of diseased soybean that were ground in 0.1 M phosphate buffer, pH 7.1, containing 0.02 M cysteine hydrochloride. Test plants were grown in insect-proof glasshouses under local climatic conditions (mean temperature of 28 C, average relative humidity 90%).

The aphids *Aphis craccivora* Koch and *A. spiraecola* Patch were used in transmission tests. Insects were starved for 2-4 hr, then allowed an acquisition access of about 15 min on diseased leaves of soybean and an inoculation access period of 1-3 days on healthy seedlings of soybean (10 insects per plant).

Thermal inactivation point, dilution end point, and aging in vitro were determined according to the methods of Bos et al (1) using crude sap from diseased soybeans and soybean seedlings as test plants.

For purification of the virus, leaves of infected soybean were ground in 0.2 M potassium phosphate buffer, pH 7.8, containing 1% thioglycolic acid (1 g/2 ml); chloroform was added during the

grinding (1/2 vol). The moisture was centrifuged for 10 min at 10,000 g and the supernatant was centrifuged at 78,000 g for 120 min. Pellets were resuspended in 0.05 M borate buffer, pH 8, containing 0.2% Triton X 100 (1/10 of the initial volume). After removal of the insoluble material by centrifugation for 10 min at 10,000 g, the virus was sedimented through a 2-cm-deep layer of 20% sucrose in the SW 27 rotor (95,000 g for 150 min). The pellets were resuspended in the same borate buffer (2% of the initial volume) and further purified by sucrose density gradient centrifugation (10-40% in 0.1 M phosphate buffer, pH 7.5). An opalescent virus band was collected, diluted with water, and centrifuged 150 min at 78,000 g before being resuspended in borate buffer, pH 8.

For electron microscopy, virus preparations were stained with 2% aqueous uranyl acetate and observed with a Siemens Elmiskop 102 at the Groupe d'Etude et de Recherches en Microscopie Electronique at Adiopodoumé.

The molecular weight of coat protein was determined by electrophoresis in 7.5, 10, and 12.5% polyacrylamide-sodium dodecyl sulfate gels using the procedure of Weber and Osborne (6). Phosphoglycerate kinase (mol wt 47,000), alcohol dehydrogenase (35,000), carbonic anhydrase (31,000), trypsin inhibitor from soybean (21,000), and lysozyme (14,300) were used as standards.

Antiserum was prepared in rabbits by six weekly intravenous injections of about 1 mg of purified virus. The microprecipitin reaction under paraffin oil in petri dishes was used for serologic tests (5).

RESULTS

Symptomatology and host range. Naturally infected soybeans were stunted and showed a light green mosaic. On inoculated soybean seedlings, vein-clearing evolving into yellow mosaic with occasional crinkling developed on youngest leaves 9-12 days after inoculation (Fig. 1); inoculated plants were reduced in size compared with healthy plants.

The virus was readily transmitted by mechanical inoculation as follows: Amaranthaceae: *Gomphrena globosa* L. (mottle); Chenopodiaceae: *Beta vulgaris* L., *Chenopodium amaranticolor* Coste & Reyn., *C. quinoa* Willd., and *C. foetidum* Schrad. (chlorotic local lesions); Leguminosae: *Arachis hypogaea* L. (mild crinkling), *Cajanus cajan* Millsp. (chlorosis), *Canavalia ensiformis* DC. (mosaic and crinkling), *Crotalaria usaramoensis* Bak. (chlorosis), *Glycine max* (mosaic), *Phaseolus lathyroides* L. (mosaic), *P. lunatus* L. (chlorosis), *P. mungo* L. (chlorosis), *P. vulgaris* L. (chlorotic spots), *Pisum sativum* L. (chlorosis), *Psophocarpus tetragonolobus* DC. (mottle), *Vigna cylindrica* Skeels (mosaic), *V. sinensis* Savi (mosaic), *V. unguiculata* Walp. (mosaic), and *Voandzeia subterranea* Thouars (light mosaic); Pedaliaceae: *Sesamum indicum* L. (chlorotic spots); Scrophulariaceae: *Penstemon hirsutus* Willd. (chlorosis); Solanaceae: *Browallia demissa* L. (chlorosis), *B. speciosa* Hook. (chlorosis), *Nicotiana clevelandii* Gray (chlorosis), *N. megalosiphon* Arg. (mottle), and *Solanum carolinense* L. (chlorosis).

Transmission. The virus was not transmitted by *A. craccivora* (0/100) nor by *A. spiraecola* (0/100) from soybean to soybean.

The virus was seedborne in *G. max* cvs. Jupiter (1/200) and Santa Rosa (5/60).

In vitro properties. The dilution end point of the virus was between 10^{-4} and 10^{-5} ; the thermal inactivation point was between 65 and 70 C; and infectivity was retained for 2 days at 25 C, 28 days at 4 C, and more than 2 mo at -20 C.

Purification and virus properties. Based on the extinction coefficient $E_{260}^{0.1\%}$



Fig. 1. Symptom
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= 2.1 for cowpea mild mottle virus (2), average yields of purified virus were 15–20 mg/kg of leaf tissue. When the previously described procedure (2) was used, yields were less than 5 mg/kg of leaf tissue. Ultraviolet absorption spectrum of purified virus showed a maximum at 260 nm and a minimum at 247 nm, with an A_{260}/A_{280} ratio of 1.15 (without correction for light scattering).

Electron micrographs of purified virus preparations showed rod-shaped particles 635 ± 20 nm in length (based on 260 particles). Preparations of virus protein gave a single component with estimated molecular weight of $33,000 \pm 1,000$.

Serology. Antiserum against the virus reacted with purified virus to a titre of 1,024. Purified virus reacted with two antisera to cowpea mild mottle virus (homologous titres 1/2,048 and 1/4,096) at dilutions to 1/1,024 and 1/2,048 (respectively) and with an antiserum to

groundnut crinkle virus (titre unknown) (4) to 1/128.

DISCUSSION

Based on similarities in host range, symptomatology, virus properties, and serologic relationships, the virus isolated from soybean in the Ivory Coast appeared to be an isolate of the cowpea mild mottle virus described in Ghana by Brunt and Kenten (2,3).

The soybean isolate infected *Nicotiana megalosiphon*, which was not infected by the cowpea isolate from Ghana (2). Other new hosts were *Browallia demissa*, *B. speciosa*, *Solanum carolinense*, *Sesamum indicum*, and *Penstemon hirsutus*.

The method of purification developed for the soybean isolate yielded 15–20 mg of virus, which is considerably higher than yields obtained using previously described methods (2).

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