SORGHUM ARUNDINACEUM, A NATURAL HOST OF PEANUT CLUMP VIRUS IN UPPER-VOLTA

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A part of this work was supported by IRHO (Institute de Recherche sur les Huiles et Oléagineux). Our thanks are expressed to M. Djigma for field assistance in Saria (Upper-Volta), Claire Fauquet for translation, and Professor L. Hirth (Director of IBMC - Institut de Biologie Moléculaire et Cellulaire -, Strasbourg, France) for helpful discussion and manuscript corrections.

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ABSTRACT

Sorghum arundinaceum (great millet) was found as a symptomless natural host of peanut clump virus (PCV) in Upper-Volta. The PCV was characterized by mechanical transmission on Chenopodium amaranticolor and peanut, physical properties, electron microscope observations, and serology after purification from great millet leaves.

Plant Dis. Reptr. 60: 1076-1080.

Peanut clump disease has been observed in two countries only, Upper-Volta and Senegal (3, 6), which are 2,000 km apart. A study of peanut clump virus (PCV), discovered in Upper-Volta, enabled us to determine some of its physico-chemical characters and its serological properties, and to establish that it was a new two-components rod-shaped virus (5). PCV is soilborne (1), and the disease is recurrent on peanut in spite of a rotation of 6 years without that crop plant (M. Djigma, personal communication). We made a study to find which weeds or cultivated plants might be PCV hosts in contaminated fields.

The present paper reports the results obtained and describes great millet (Sorghum arundinaceum) as a new natural host of PVC.

MATERIALS AND METHODS

Virus and plant sources: Samples of various plants, consisting mainly of whole plants with roots, were collected from fields regularly contaminated by PCV, put into small plastic bags that were kept in an ice-box with ice, and taken to the laboratory as soon as possible.

Test plants and inoculations: To test the possible presence of PCV in the samples, we inoculated from leaves and roots to Chenopodium amaranticolor, a specific host for PCV. Test plants were kept in optimal conditions for PCV multiplication, that is, in a glasshouse where the temperature is 30-35° C and humidity reaches 90%. For inoculations, samples were ground in pH 7.1, 0.1 M potassium phosphate buffer (5 ml/g of tissue) containing 0.02 M cysteine hydrochloride and 2.5 mg/ml bentonite. The sap obtained was rubbed manually on carborundum-dusted leaves and, eventually in case of great millet, on carborundum-dusted roots of uprooted seedlings (2 weeks) which were planted again after inoculation.

Transmission by soil: Some great millet plants (with roots and soil) picked out from the contaminated fields of Upper-Volta were dug up and transplanted to big containers full of sterilized earth. To test the soil transmission, peanut and great millet seeds were sown around the plants of great millet, drawing two circles with a diameter of 20 and 40 cm, respectively. Then, all of the seedlings were tested 1 month later on C. amaranticolor.

Purification: Virus was purified from great millet leaves, frozen for 3 weeks. The purification method was one previously described for the PCV (5). The main steps of the purification were grinding leaves in a meat grinder, clarification of the extract with butanol-chloroform, precipitation with 5% polyethylene glycol (mol weight 20,000), and centrifugations alternatively at low or high speed. A small translucent pellet of purified virus remained, which was resuspended in 0.05 M borate buffer (pH 8) containing 0.001 M EDTA.

Spectrophotometric properties: On a dilution of the resuspended virus, an ultra-violet absorption spectrum was made with spectrophotometer Zeiss PMQ II.

Electron microscopy: One drop of the purified suspension was diluted and put on a formvar-carbon coated grid, rinsed with distilled water, then stained with 0.5% uranyl acetate in 0.05% ammonium acetate buffer pH 7, containing 0.2% EDTA. Micrographs were taken with a Siemens Elmiskop 1 A, at a magnification of 40,000. Length measurements were made on prints with a final magnification of 120,000.

Serology: Serological tests were made, by using the microprecipitin technique, under paraffin oil in petri dishes (4). The antiserum used was prepared in the laboratory with PCV collected from peanut and propagated on C. amaranticolor (5), with a titer 1/2,048.

RESULTS

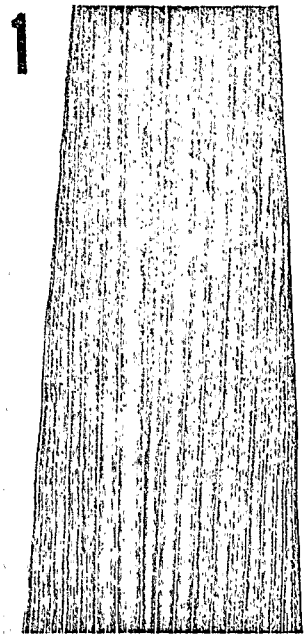
Results of inoculations: Fragments of leaves and roots of the following plants were tested: Gossypium hirsutum (cotton), Pennisetum typhoides (pearl-millet), Sorghum arundinaceum (great millet), Vigna unguiculata (cowpea), and Zea mays (corn).

Cowpea showed symptoms of mottle and mosaic. Great millet plants were chosen from plants with a very slight yellow stripe, nearly symptomless (Fig. 1). The other plants showed no specific symptom.

We also tested weeds; among the most frequent were: Cyperus sp., Digitaria sp., and unidentified Leguminosae. They showed no particular symptom.

Only C. amaranticolor inoculated from great millet leaves developed ringspots and line-patterns specific of PCV (Fig. 2) 6 days after inoculation. Extracts of great millet roots did not infect C. amaranticolor.

Transmission by soil: All peanuts grown in the containers were affected. All great millet seedlings appeared without symptom after 1 month, but when tested on C. amaranticolor they reacted positively.



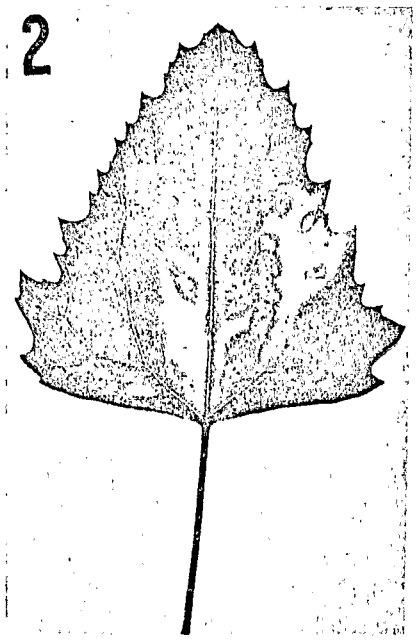


FIGURE 1. Light yellow stripes, symptoms of virus disease on leaf of great millet.

FIGURE 2. Symptoms produced by virus which was isolated from great millet, on Chenopodium amaranticolor leaf.

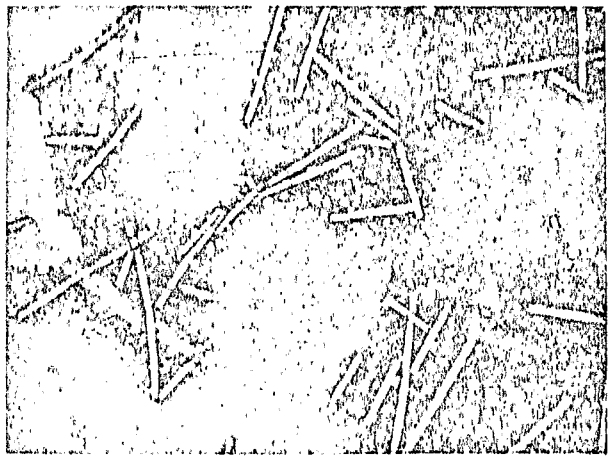


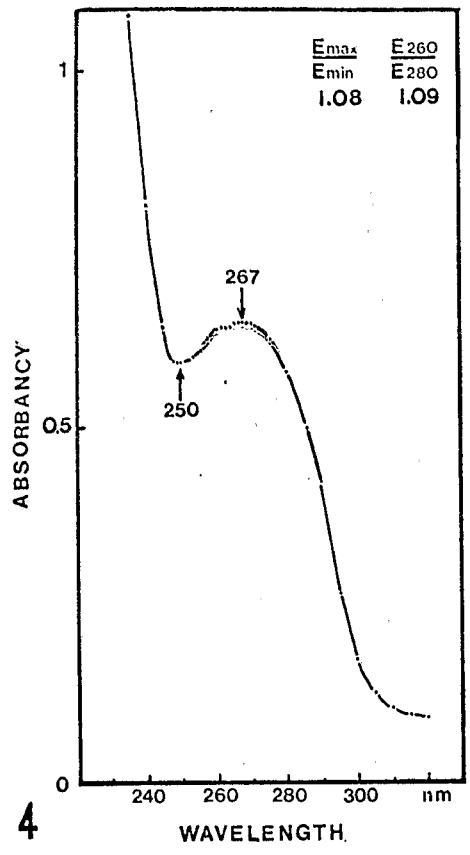
FIGURE 3. Electron microscopy of viral particles isolated from great millet, after staining with uranyl acetate 0.5% (G = approximately 30,000).

Characteristics of virus purified from S. arundinaceum: Leaves of great millet seedlings grown in the containers were collected and used for purification. The yield was about 1 mg/kg of great millet leaves.

Purified virus inoculated on C. amaranticolor leaves caused the specific ringspots of PCV; when it was inoculated on peanut seedlings, it caused symptoms of clump disease. The purified virus was inoculated on the leaves of 100 great millet seedlings and on the roots of 36 great millet seedlings. One month later, all of the seedlings were tested on C. amaranticolor. None of the 100 millet plants that were leaf-inoculated became infected, whereas 5 of 36 root-inoculated plants became infected.

The absorption spectrum of virus purified from Sorghum arundinaceum reached a maximum at 267 nm and a minimum at 250 nm, with a ratio maxium/minimum = 1.08, and E_{260}/E_{280} = 1.09 (values not corrected for light scattering). Those values correspond exactly to values found for PCV (Fig. 4).

Rod-shaped particles appear with an electron microscope observation of the virus suspension purified from great millet (Fig. 3). Particles are of two different lengths: 190 ± 20 nm and 250 ± 20 nm. Their width is about 20 nm (Fig. 5). Those dimensions are similar to those of PCV (5).



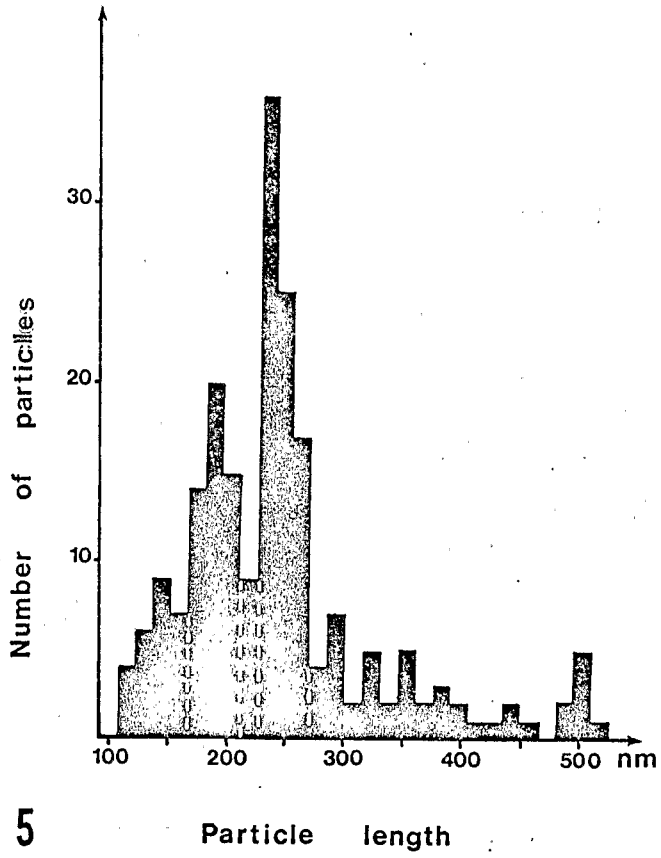


FIGURE 4. The ultra-violet absorption spectrum of virus purified from great millet.
FIGURE 5. Length distribution of particles from purified viral suspension, observed by electron microscopy.

This purified suspension reacts with antiserum to PCV up to a dilution of 1/2,048, just as does the PCV strain isolated from peanut.

DISCUSSION

Among weeds and cultivated plants grown in contaminated fields in Upper-Volta, only the great millet contained a virus that reacted positively on C. amaranticolor and developed the characteristic symptoms of PCV. It was possible to infect great millet and peanut seedlings naturally by propagating them in contaminated soil.

The quantity of virus purified from these newly infected great millet is very small (1 mg/kg), but nevertheless indicates that there is a low multiplication in the great millet. Although difficult, it is possible to infect great millet mechanically, when roots are inoculated with purified virus.

The symptoms on C. amaranticolor or on peanuts inoculated with virus purified from great millet were characteristic of PCV. Moreover, all of the properties of this virus purified from great millet, namely spectrophotometric values and existence of two particle lengths, correspond to the properties of PCV (5).

Finally, the positive reaction to PCV antiserum proves that the virus isolated from great millet is PCV.

These results confirm observations made by Delassus (2), who noticed an increase in peanut clump disease after great millet culture. We can report that great millet was grown every 2 years on sites where peanut clump disease remained, although no peanuts had been grown for 6 years at the same sites.

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