Sonderdruck aus „Phytopathologische Zeitschrift“
[Phytopath. Z. 100, 182—185, 1981]

Verlag Paul Parey, Berlin und Hamburg

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Phytopath Z., 100, 182—185 (1981)

ISSN 0031-9481 / InterCode: PHYZA3

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Received May 28, 1980

Groundnut crinkle disease has been observed in the southern region of the Ivory Coast (DUBERN and DOLLET 1979). A study of groundnut crinkle virus (GCV) enabled to determine some of its physico-chemical and serological properties, and to establish that it was a new filamentous virus, belonging to the carlavirus group (DUBERN and DOLLET 1980). *Centrosema pubescens* was easily infected with GCV by mechanical transmission and, in spite of failure of natural transmission (DUBERN and DOLLET 1980), this plant which was one of the most common weeds in contaminated fields, was supposed to be naturally-infected by GCV.

The present paper reports the results obtained and describes *Centrosema pubescens* as a new natural host plant of GCV.

Materials and Methods

Samples of *Centrosema pubescens*, consisting of whole plants with roots, were collected from fields contaminated by GCV and kept on ice.

To test the possible presence of GCV in the samples, *Arachis hypogaea* L. cv. Te3 was inoculated with crude sap extract from symptomatic leaves. This groundnut variety develops very typical symptoms when inoculated with GCV. Test plants were kept in optimal conditions for GCV multiplication, that is in a glasshouse where the temperature is 30—35 °C and humidity reaches 90 %. For inoculations, samples were ground in pH 7.3, 0.05 M potas-

sium phosphate buffer (4 ml/g of tissue) containing 0.01 M sodium diethyldithiocarbamate and 12.5 mg/ml magnesium bentonite (FRANKEL-CONRAT *et al.* 1961). The extract obtained was rubbed manually on Carborundum-dusted leaves.

Aphid transmission experiments were done in the laboratory using *Aphis craccivora*, *A. citricola* reared on groundnut plants, and *A. gossypii* reared on *Eupatorium* sp. Adults and late instar apterous aphids were starved for 1—3 h, then allowed an acquisition access time of 24 h on diseased samples, and an inoculation access period of 24 h, 48 h or 72 h, on healthy groundnut seedlings. They were then killed by applying an insecticide (systoate: dimethyldithiophosphorylacetic monomethyl amide). Ten aphids per plant were used in each experiment. Aphids used in the tests were virus free; this was established by placing control lots of insects on healthy groundnut seedlings serving as negative controls.

The standard procedures described by BOS, HAGEDORN and QUANTZ (1960) were used with crude sap extracts from young leaves of groundnut plants infected at least five weeks by inoculation of *Centrosema pubescens* extracts, as the source of the virus. For all the studies, extracts were prepared as inocula for mechanical transmission. The dilution end point was determined by serial dilutions in 0.05 M potassium phosphate buffer, at pH 7.3. The thermal inactivation point was tested by immersing 2 ml quantities of extracts, contained in 5 ml tubes, in a water bath at various temperatures for 10 min, and placing the tubes in an ice bath and inoculating immediately. To test aging *in vitro*, the infectivity of extracts kept at 25 °C was checked daily. Resistance to air-drying was determined by testing daily the infectivity from *Arachis hypogaea* leaves dried in boxes dehydrated by Silica gel.

Serological tests were made, by using the microprecipitin technique, under paraffin oil in Petri dishes (VAN SLOGTEREN 1954). The antisera used were gifted by Dr. WETTER; no antiserum against GCV was produced in the laboratory.

Results

Mechanical transmission

Samples of *C. pubescens* collected in GCV-contaminated fields were symptomless plants and plants showing mosaic and distortion. Only some symptomless plant extracts produced crinkling and stippling on inoculated groundnut seedlings, symptoms that are typical of groundnut crinkle virus (DUBERN and DOLLET 1979). Symptoms were developed 4—6 weeks after inoculation. *Centrosema pubescens*, inoculated mechanically with these symptomless samples or with the first infected groundnut seedlings, developed necrotic local lesions on inoculated cotyledonary leaves 8 days after inoculation; a very light mottling appeared 3—5 weeks after inoculation on the new-emitted leaves, so light that plants seemed nearly symptomless. Necrotic local lesions were developed on the cotyledonary leaves of *Soja max*, *Vigna unguiculata*, *Dolichos jacquinii*, *Phaseolus lathyroïdes*, *Desmodium polycarpum*, *Cassia occidentalis* and *C. obtusifolius*. Mottling was developed 3—6 weeks after inoculation by *Soja max*, *Vigna unguiculata*, *Canavalia ensiformis*, *Psophocarpus tetragonolobus*, *Cassia occidentalis* and *C. obtusifolius*. Only these species in this family were infected. Other plant species belonging to Compositae, Chenopodiaceae, Aizoaceae, Scrophulariaceae, Malvaceae, Solanaceae and Passifloraceae were inoculated; none was infected (back inoculation to groundnut seedlings gave negative transmission).

Aphid transmission assay

All the attempts to transmit the disease by this way failed.

In vitro properties

Dilution end point was about 10^{-3}; no infection was obtained with sap diluted to 10^{-4}. Thermal inactivation point was about 65 °C; infectivity was abolished after 10 min at 70 °C. Dried groundnut leaves lost slowly infectivity, but they kept still infectivity two months after air-drying. Infectivity of groundnut extracts was kept three days at 4 °C but no longer.

Serology

Clarified sap extracts of diseased groundnut plants, inoculated with collected symptomless samples, reacted with antisera to passiflora latent virus (up to a dilution of 528, homologous titre 1024), potato virus S (up to a dilution of 1024, homologous titre 1024), and carnation latent virus (up to a dilution of 16 384, homologous titre 16 384). These antiserum were previously absorbed with clarified sap extract of healthy groundnut plants.

Discussion

From symptomless *Centrosema pubescens* samples collected in GCV contaminated fields, it was possible to recover GCV. Groundnut seedlings, inoculated mechanically, developed typical crinkling and stippling. Host range obtained with the sample extracts was identical with GCV host range. Aphid transmission attempts failed, that is to compare with failure of GCV aphid transmission. *In vitro* properties were the same and clarified extracts reacted with four virus of the carlavirus group. So it may be considered that *Centrosema pubescens* is a natural host plant of groundnut crinkle virus.

The author is much indebted to Dr. REMAUDIERES (Institut Pasteur) for identifying the aphids, M. J.-L. RENARD (I.R.H.O.) for providing Te3 cultivar seeds, Dr. C. WETTER for providing antisera and, M. AHO KOUAKOU for technical assistance.

Summary

Centrosema pubescens was found as a natural host plant of groundnut crinkle virus in Ivory Coast. The virus from *C. pubescens* was characterized by mechanical transmission, host range, *in vitro* properties, lack of aphid transmission and, serological relationship with groundnut crinkle virus.

Résumé

***Centrosema pubescens*, hôte naturel du virus de la frisolée de l'Arachide**

Le virus de la frisolée de l'Arachide infecte naturellement *Centrosema pubescens*. Cette observation a été effectuée en champ dans le sud de la Côte

d'Ivoire. Le virus isolé de *C. pubescens* a été caractérisé par sa transmission mécanique, sa rangée d'hôtes, l'absence de transmission par puceron, ses propiétés physico-chimiques et ses relations sérologiques avec le virus de la frisolée de l'Arachide.

Zusammenfassung

Centrosema pubescens, ein natürlicher Wirt des Erdnuß-Kräuselvirus an der Elfenbeinküste

Als natürlicher Wirt des Kräuselvirus der Erdnuß wurde *Centrosoma pubescens* an der Elfenbeinküste nachgewiesen. Das aus *Centrosoma* isolierte Virus wurde durch mechanische Übertragung, durch seinen Wirtspflanzenkreis, seine Eigenschaften *in vitro* und seine serologische Verwandtschaft mit dem Erdnuß-Kräusel-Virus identifiziert.

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