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Identification of a Potyvirus Infecting Groundnut, Arachis hypogeae L., in Malaysia

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Key words: Legume virus; potyvirus; electron microscopy.

RINGKASAN

Sejenis penyakit virus yang kelihatan bertompok-tompok pada daun kacang tanah, Arachis hypogeae L. cv. V 13 telah dilihat dipetak-petak penyelidikan di Universiti Pertanian Malaysia, Serdang, Malaysia, dalam tahun 1982. Virus tersebut boleh ditransmitkan melalui cairan dan juga ditransmitkan oleh kutu daun, Aphis craccivora Koch di dalam keadaan tidak berkekalan. Bidang perumahnya adalah sempit. Ia mengeluarkan luka kekuningan setempat di Chenopodium amaranticolor Coste & Reyn dan luka kekuningan sistemik di Vigna sinensis Sav. Pemeriksaan ke atas daun-daun kacang tanah di mikroskop elektron yang dijangkiti menunjukkan adanya zarah-zarah virus yang melengkur dan panjangnya lebih kurang 800 nm dan 'pinwheel inclusions' dihirisan tisu; oleh itu virus ini diletakkan di bawah kumpulan potivirus.

SUMMARY

A virus disease showing leaf mottling in groundnuts, Arachis hypogeae cv. V 13, was observed in experimental plots at the Universiti Pertanian Malaysia, Serdang, West Malaysia, in 1982. The virus is sap-transmissible and can also be transmitted by the aphid, Aphis craccivora Koch, in a non-persistent manner. The host range is narrow. It produced chlorotic local lesions in Chenopodium amaranticolar Coste & Reyn and systemic chlorotic lesions in Vigna sinensis Sav. Electron microscopic examination of virus infected groundnut leaves revealed 'the presence of long flexuous particles about 800 nm in length and pinwheel inclusions in tissue sections; therefore, the virus belongs to the potyvirus group.

INTRODUCTION

The occurrence of a virus disease on groundnuts, Arachis hypogeae L., showing leaf mottling has been reported in several states in Malaysia since 1969 (Ting et al., 1972). However, detailed studies on the properties of the virus have not been carried out.

This paper presents studies on host range, transmission and ultrastructure of the virus found in groundnut in the experimental plots at Universiti Pertanian Malaysia, Serdang, Selangor.

MATERIALS AND METHODS

Infected groundnut plants showing mottle symptoms were collected at the Universiti Pertanian Malaysia experimental plots. The virus in the plants was maintained in the glasshouse through periodic transfers of groundnuts. For host range studies, test plants were grown in a 25°C constant temperature room under artificial lighting. Inoculations were made by grinding pieces of infected leaf material with a pestle and mortar in 0.01M phosphate buffer, pH 7.0. Leaves of plants were first dusted with 500-mesh carborundum and then rubbed with a finger moistened with the inoculum. After inoculation, the leaves were washed with water.

Thin sections were prepared from young, mottled leaves for electron microscopy. Small pieces of tissue, about 2 x 1 mm, were excised and fixed in 3% glutaraldehyde post-fixed in 1% osmium tetroxide, dehydrated and embedded in Epon (Hatta and Francki, 1977). Thin sections were cut with glass knives, stained with lead citrate and examined in a Philips HMG 400 electron microscope.

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Leaf-dip preparations were used for detecting virus particles. Small pieces of leaf material were crushed in drops of 2% phosphotungstic acid adjusted to pH 6.8 with KOH. A small drop of the extract was then placed on carbon-strengthened, Formvar coated grids. Excess liquid was removed with a piece of filter paper and air-dried specimens were examined in the electron microscope. For transmission experiments, Aphis craccivora Koch, collected from healthy colonies, were reared on groundnuts. They were starved for 3 hr before being allowed to feed on infected plants for 1 hr and then transferred to healthy groundnut plants in groups of 5 aphids per plant. To investigate the manner of transmission, the aphids were also allowed to feed for 60-90 sec on infected leaves and were individually transferred to healthy groundnut plants.



- Fig. 1. Systemic symptoms in A. hypogeae cv. V 13.
- Fig. 2. Local chlorotic lesions: in Chenopodium amaranticolor.
- Fig. 3. Negatively stained leaf-dip preparation of infected A. hypogeae showing the presence of virus particles (arrows). (Bar represents 450 nm).
- Fig. 4. Leaf cells of infected A. hypogeae showing pinwheel inclusions (pw) in cross-section (Bar represents 250 nm).
- Fig. 5. The inclusions are seen longitudinally (arrows). (Bar represents 250 nm).

RESULTS

Disease Sysmptoms on Groundnut Plants.

A distinct type of disease symptom was recognized in the groundnut growing area during the 1982 season. The symptoms could be described as mottling which ranged from a diffuse indistinct mottle to a pronounced dark green blotch mottle in the groundnut cultivar V 13 (Fig. 1).

Host range

The virus had a narrow host range. It induced chlorotic local lesions on *Chenopodium amaranti*color Coste & Reyn on the inoculated leaves 6-7 days after inoculation (Fig. 2). This would serve as a useful assay-host. Systemic chlorotic spots were observed in Vigna sinensis Sav. No symptoms were observed on the following test plants and no virus were detected by back inoculations : Cassia occidentalis L., Glycine max (L.) Merr. Nicotiana clevelandii Gray, Phaseolus vulgaris L. (Local cultivar), N. glutinosa L., N. tabacum L. cv. White Burley, Pisum sativum L. cv. Greenfeast and Capsicum annum L.

Properties of the Virus.

In negatively stained leaf-dip preparations from infected groundnut leaves, the virus particles were flexous with an average length of 800 nm (Fig. 3). In ultrathin sections, numerous pinwheel inclusions were observed in the cytoplasm (Fig. 4 and 5). The virus was transmitted in an non-persistent manner by A. craccivora from infected groundnuts to healthy groundnuts.

DISCUSSION

The consistent association of flexous virus particles with mottling symptoms in groundnut indicates that this virus is the causal agent of the disease. A similar disease has been observed since 1969 (Ting *et al*, 1972) but the pathogen was not identified. It is possible that the virus isolate described in this paper is similar to the one described by Ting *et al*. (1972) based on its host range and transmission properties.

In particle morphology, cylindrical inclusion formation and non-persistent aphid-transmissibility, the virus causing mottle symptoms in groundnuts resemble other members of the potyvirus group (Edwardson, 1974 a, b). Based on these properties as well as host range and symptomatology, the virus resembled the isolates of peanut mottle virus which had been reported in Indonesia (Roechan *et al.*, 1978) and in Thailand (Choopanya, 1983). In view of known variability within the potyvirus group (Bos, 1970) there seems to be no reason for considering this virus isolate as a new virus.

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