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A Serious Disease of Groundnut Caused by Cowpea Mild Mottle Virus in the Sudan

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With 4 figures

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Abstract

A serious disease of groundnut (*Arachis hypogaea* L.) characterized by stunting of plants, downward rolling, mottling, general chlorosis and reduced size of the leaflets was observed in the Sudan. Surveys conducted from 1992 to 1994 showed that this disease was restricted to irrigated groundnut crops grown between the two Niles. The virus had slightly flexuous filamentous particles (626 nm long) and was transmitted by whiteflies. It was identified serologically as cowpea mild mottle virus (CPMMV). This appears to be the first record of natural occurrence of CPMMV on groundnut in the Sudan and the first evidence that it causes a disease of major economic importance.

Zusammenfassung

Das Cowpea mild mottle virus als Erreger einer schweren Krankheit der Erdnuß im Sudan

Im Sudan wurde eine schwere Krankheit der Erdnuß (*Arachis hypogaea* L.) beobachtet. Sie äußerte sich durch folgende Symptome: kümmernde Pflanzen sowie nach unten eingerollte, gescheckte, chlorotische, auffällig kleine Blättchen. Von 1992 bis 1994 durchgeführte Untersuchungen zeigten, daß die Krankheit nur auf bewässerten Erdnußfeldern zwischen dem Weißen und dem Blauen Nil auftrat. Das Virus war leicht gebogen, fadenförmig und 626 nm lang. Es wurde durch Weiße Fliegen übertragen und serologisch als Cowpea mild mottle virus (CPMMV) identifiziert. Dies ist offenbar der erste Bericht über ein natürliches Vorkommen des CPMMV bei Erdnuß im Sudan und der erste Nachweis, daß dieses Virus eine wirtschaftlich sehr wichtige Krankheit verursacht.

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Introduction

Groundnut production in Eastern Africa is dominated by Sudan, which accounts for 71% of the region's groundnut area and 66% of its production. Groundnut is an important cash and food crop in Sudan and is produced about 60% under rainfed and 40% under irrigated conditions (Mahmoud et al., 1992). The low yields of groundnuts have been attributed to several abiotic and biotic factors, including viral diseases. Disease surveys conducted in major groundnut-producing areas from 1992 to 1994 revealed the wide distribution of a disease different from the virus diseases previously reported from the Sudan (Ahmed and Idris, 1981; Ahmed, 1991). We present evidence that this disease is caused by a virus serologically related to cowpea mild mottle virus (CPMMV). This is the first report of CPMMV causing a disease of major economic importance.

Materials and Methods

Disease surveys were conducted mostly during August to September when the crop was about 2–3 months old. Infected plants were tested for peanut stripe virus (PStV, now considered as a strain of bean common mosaic virus, Barnett et al., 1995), peanut mottle virus (PeMoV), peanut stunt virus (PSV) and CPMMV by using the direct antigen coating form of enzyme-linked immunosorbent assay (DAC-ELISA) as described by Hobbs et al. (1987). Antisera used in this study were from the following sources: ICRISAT, India (PStV, PeMoV and CPMMV), Dr A.A. Brunt, Horticulture Research International, UK (CPMMV) and Dr S.A. Ghabrial, University of Kentucky, USA (PSV).

Leaves sent to the Scottish Crop Research Institute were tested for groundnut rosette virus (GRV) by mechanical inoculation. Infected leaflets were triturated in 0.01 M phosphate buffer, pH 7.2, containing 0.02 M 2-

mercaptoethanol and inoculated to 2-week-old healthy groundnut seedlings, and to the following test plants: *Chenopodium amaranticolor*, *C. quinoa*, *Nicotiana benthamiana* and *N. clevelandii*.

The disease was established in a glasshouse (25–30 °C) by graft-inoculating healthy groundnut plants. Vector transmission tests were done with aphids (*Aphis craccivora*, *A. gossypii*) and whiteflies (*Bemisia tabaci*). They were allowed to feed on young leaflets showing clear symptoms for an acquisition access period of 1–24 h and then transferred to 10-day-old healthy groundnut seedlings for an inoculation access period of 24–48 h.

Leaf-dip preparations were made by triturating leaflets in 0.01 M phosphate buffer, pH 7.2, containing 0.04 M Na₂SO₃. A droplet of the extract was transferred to 300-mesh carbon-coated copper grids, stained with 1% uranyl acetate and examined in a Philips Model CM 10 electron microscope. Immunosorbent electron microscopy analyses of leaf samples were done with antisera to PSTV, PeMoV, PSV and CPMMV. Virus particle morphology and modal length were determined by trapping the virus particles in crude extracts with CPMMV antiserum at 1:1000 dilution. Particles were stained and observed by electron microscopy as described above.

Results and Discussion

Under field conditions, infected plants appeared stunted with reduced internodal length and leaf size. A range of symptoms consisting of leaf mottling, general chlorosis, leaf size reduction and downward rolling were observed, depending on the cultivar (Figs 1, 2 and 3). Plants infected early showed severe stunting and proliferation of axillary buds. Under greenhouse conditions, initial symptoms consisting of downward rolling and chlorosis along the veins appeared on newly developing leaflets 10–12 days after inoculation (Fig. 2). Leaves developing subsequently showed mottling symptoms similar to those observed under field conditions. The disease was observed in groundnut grown under irrigated conditions between the two Niles but not in rainfed groundnuts in Western Sudan. The disease incidence was greater in late sown crops (early July) than in early sown crops (late

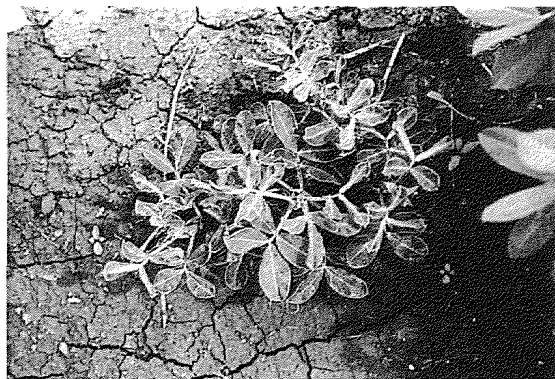


Fig. 1 An early infected groundnut plant showing stunting, downward curling of leaflets and mottling symptoms



Fig. 2 A groundnut plant showing initial symptoms consisting of downward rolling and veinal chlorosis symptoms



Fig. 3 Close up view of a groundnut leaflet showing mottling symptoms

May to early June). Over a period of 3 years, incidence usually ranged between 10 and 50%, but in some fields amounted to 100%. Selected fields were visited at regular intervals (biweekly) and plants with early (before flowering) and late infection (after flowering) tagged for yield estimates. Data showed that early infection gave no pods whereas infection after flowering caused yield losses up to 60%.

In tests for GRV, the symptoms induced following mechanical inoculation of herbaceous test plants were unlike those of GRV (Reddy et al., 1985) but resembled those described for CPMMV (Brunt and Kenten, 1973), viz: large necrotic lesions of 2–3 mm in diameter in *C. amaranticolor* and *C. quinoa*, and a faint systemic chlorosis in *N. clevelandii*; no obvious symptoms were seen in *N. benthamiana*. In DAC-ELISA, samples collected from the field or greenhouse gave positive reactions with antiserum prepared against a CPMMV isolate infecting groundnut in India (Iizuka et al., 1984) and a cowpea isolate from Ghana (Brunt and Kenten, 1973), but not with antisera to other groundnut viruses tested. These results were further confirmed in the immunosorbent electron microscopy tests in which the virus particles were trapped only with CPMMV antiserum. Electron microscopy of leaf-dip preparations revealed slightly

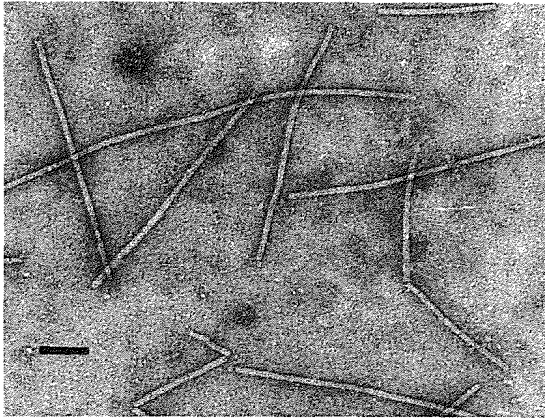


Fig. 4 An electron micrograph of filamentous virus particles. Bar represents 100 nm

flexuous filamentous virus particles (Fig. 4). These particles (100 measured) were 626 ± 16 nm long and 16 ± 2 nm in wide. The virus was transmitted by *B. tabaci* but not by aphids (*Aphis craccivora* and *A. gossypii*) that transmit known groundnut viruses. The symptoms induced in plants inoculated mechanically or by whiteflies were the same as those observed in the field, and extracts from these plants reacted with CPMMV antiserum in ELISA.

Transmission of the Sudanese groundnut virus by *B. tabaci* further confirmed its identity as CPMMV which, though tentatively placed in the genus *Carlavirus* (Brunt, 1995), differs from other Carlaviruses in being transmitted by this whitefly, and not by aphids. This is the first record of CPMMV from the Sudan, and of its association there with a serious disease of groundnut. CPMMV was first described from Ghana, in cowpea (Brunt and Kenten, 1973), and was shown to infect groundnut experimentally, but the first reports of the virus occurring naturally in groundnut were from Kenya and Tanzania by Bock et al. (1975, 1977), who showed that it was related to the Ngomeni mottle virus of Storey and Ryland (1957). Natural occurrence of CPMMV in groundnut has also been reported from India (Iizuka et al., 1984), Malaysia and Indonesia (Iwaki et al., 1986), Solomon Islands (Brunt, 1987) and Yemen Arab Republic (Walkey et al., 1990). A *B. tabaci*-transmitted virus with c.650 nm filamentous particles occurring in Ivory Coast was called groundnut crinkle virus (GCV, Dubern and Dollet, 1981). Jeyanandarajah and Brunt (1993) stated that GCV is serologically related to CPMMV and is probably a strain of that virus. The extent of the relationship is unclear but there seem to be differences in host range: GCV is restricted to Leguminosae whereas both the Ghanaian (type) and the Sudanese isolates of CPMMV also infect such hosts as *C. amaranticolor*, *C. quinoa* and *N. clevelandii*. Moreover, CPMMV and GCV induce different symptoms in groundnut.

Bock and Wangai (1984) considered that CPMMV was responsible for the most serious disease of groundnut in eastern coastal regions of Kenya and our observations

showed that CPMMV is responsible for a serious disease of groundnut in irrigated areas in the Sudan. Moreover, it is known to infect other leguminous crops that are important in the same area such as beans, cowpea, alfalfa and faba beans. Indeed, continuous growth of leguminous crops under irrigation would seem to provide ideal conditions for survival and spread of the virus. Thus, there is clearly a need for study of the economic importance, ecology and epidemiology of whitefly transmitted CPMMV and CPMMV-like viruses in food legumes in the semi-arid tropical countries of Africa. This is especially true in the context of increasing recognition of the importance of whitefly borne plant virus diseases in the tropics.

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