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Laboratoire de Phytovirologie des Régions Chaudes, CIRAD-ORSTOM, Montpellier, France

Characterization of Peanut Mottle Virus in Cote d'Ivoire

ABDUL SAMAD, J.-C. THOUVENEL and J. DUBERN

Authors' addresses: ABDUL SAMAD, Plant Virus Laboratory, Department of Botany, A.M.U. Aligarh, 202 002 India. J.-C. THOUVENEL, ORSTOM, Virology Laboratory, Faculty of Agriculture, Cairo University, Giza, Egypt. J. DUBERN, ORSTOM, L.P.R.C.-C.I.R.A.D., BP 5035, 34032 Montpellier, France.

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Abstract

A virus, hitherto unreported in Cote d'Ivoire, was detected in groundnut plant (*Arachis hypogaea*) from the northern parts of the country. Infected plants showed mottling, interveinal depression, and very prominent light and dark green patches on leaves. The causal agent was characterized as a strain of peanut mottle virus, a member of the potyvirus group, on the basis of its host range, mode of transmission, physical properties, particle morphology and on the basis of its serological properties studied in comparison with these of other groundnut viruses.

Résumé

Caractérisation du virus de la Marbrure de l'Arachide en Côte d'Ivoire

Un virus a été récemment isolé de l'Arachide dans la région septentrionale de la Côte d'Ivoire, pays où il n'avait pas encore été diagnostiqué. Il provoque, sur les feuilles, des marbrures, des dépressions internervaires ainsi que des taches très typées vert clair et vert foncé. Ce virus a été caractérisé: il s'agit d'une souche du virus de la Marbrure de l'Arachide (Peanut Mottle Virus), virus membre du groupe des Potyvirus; les études ont porté sur la gamme des plantes hôtes, le mode de transmission, la morphologie des particules, les propriétés physico-chimiques et sérologiques, en comparaison avec d'autres virus de l'Arachide.

Zusammenfassung

Charakteristik des Virus der Marmorierung der Erdnuß in Côte d'Ivoire

An der Elfenbeinküste wurde auf der Erdnuß ein neues Virus nachgewiesen. Es verursacht Marmorierungen, Depressionen zwischen den Rippen und sehr typische hellgrüne und dunkelgrüne Flecken auf den Blättern. Das Virus wurde charakterisiert: es handelt sich um einen Stamm des Virus der Marmorierung der Erdnuß (Peanut Mottle Virus, Potyvirusgruppe). Die Untersuchungen umfaßten andere Wirtpflanzen, Übertragungsart, Morphologie der Partikel sowie physikalisch/chemische und serologische Eigenschaften im Vergleich mit anderen Viren der Erdnuß.

Peanut mottle virus (PMoV) was first identified in U.S.A. (KUHN 1965). It has also been reported from different parts of the world such as Australia (BEHNCKEN 1970), China (XU *et al.* 1983), East Africa (BOCK 1973), Egypt

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(ABDELSALAM *et al.* 1986), Europe (SCHMIDT and SCHMELZER 1966), India (REDDY *et al.* 1978), Israel (MARCO 1986), Japan (INOUYE 1969), Philippines (BENIGNO and FAVALI-HEDAYAT 1977), South America (HEROLD and MUNZ 1969), Sudan (AHMED 1981) and West Malaysia (GEH and TING 1973).

Recently, mottling of the youngest leaves, light and dark green areas, curling of the interveinal tissues were observed on an unnamed cultivar of groundnut in the northern part of Cote d'Ivoire. Infected plants were generally not stunted and did not show other symptoms.

This paper describes the transmission, host range, particle morphology, and some properties of the peanut mottle virus isolated from these groundnut plants.

Materials and Methods

Virus source and maintenance

Leaves were collected from a naturally infected unnamed local groundnut cultivar (*Arachis hypogaea*) showing prominent symptoms. They were ground in 0.1 M potassium phosphate buffer containing 0.02 M cystein hydrochloride, at pH 7.5, and inoculated into groundnut plants and *Nicotiana benthamiana.*

Host range and transmission studies

Several plant species from different families were evaluated for their susceptibility to the virus by mechanical inoculation in an insect-proof greenhouse (climatic conditions were: mean temperature 28 °C, average relative humidity 90 %). Inoculated plants were observed up to 30 days after inoculation for the development and severity of symptoms. Plants with no symptoms were further checked by back inoculations to *N. benthamiana.*

Aphid transmission was studied using conventional methods with *Aphis craccivora* Koch. Aphids were first starved for 2—3 h and were placed on virus-infected groundnut leaves for 2—5 min. Aphids were then transferred to healthy *N. benthamiana* seedlings for 24 h, and were later killed with an insecticide. Similar experiments were conducted using infected *N. benthamiana* leaves instead of groundnut plants, followed by transfer of aphids to healthy *N. benthamiana* plants. Ten aphids were used in each test. Aphids placed on healthy groundnut leaves were also transferred to healthy plants to serve as control.

Biological properties

Infected *N. benthamiana* leaves were used as a source of inoculum to determine dilution-end-point (DEP), thermal inactivation point (TIP) and longevity *in vitro* (LIV), according to the method described by NOORDAM (1973).

Purification

The virus was purified from *N. benthamiana* grown in a temperature-controlled room maintained at 20 °C. The leaves were collected 16 days after inoculation.

The purification procedure involved extraction of sap from infected *N. benthamiana* leaves in 0.1 M potassium phosphate buffer containing 1 % mercaptoethanol at pH 7.5, clarification with 50 % chilled chloroform, centrifugation at 7,000 rpm for 10 min and then high-speed centrifugation of the aqueous phase at 40,000 rpm for 2 h. Following this second centrifugation, the pellet was resuspended in 0.05 M potassium phosphate buffer containing 1 % Triton X-100, at pH 7.0, and was centrifuged at 7,000 rpm for 10 min. Then the new aqueous phase was loaded on a 2 cm thick layer of 20 % sucrose and was centrifuged at 40,000 rpm for 3 h in a Beckman Ti50 rotor. The resultant pellet was resuspended in 0.1 M phosphate buffer at pH 7.5. An opalescent virus band was collected, which was later diluted with water and centrifuged for 4 h at 40,000 rpm. Finally, the pellet was resuspended in 1 ml distilled water.

Electron microscopy

The viruses from leaf dip and purified preparations were stained with 2 % aqueous uranyl acetate. The grids were examined using an electron microscope (Jeol CX 100).

Gel electrophoresis of Coat Protein (SDS-PAGE)

The molecular weight of virus coat protein was determined by electrophoresis in 7.5 % polyacrylamide-sodium dodecylsulphate gels using the procedure of WEBER and OSBORNE (1969). Standard proteins of various molecular weights were run simultaneously with the samples.

Serology

A purified virus preparation was used for rabbit immunization. Antiserum to PMoV was produced by injecting rabbits intravenously with 1 ml of purified virus suspension, on eight occasions at weekly intervals. The blood was collected two weeks after the last injection. The microprecipitin reaction test, under paraffin oil in Petri dishes, was mainly used (VAN SLOGTEREN 1954) for checking IgG production.

IgG were prepared from antisera in order to detect the presence of virus in host plants using the direct ELISA tests (VAN REGENMORTEL 1982).

To compare serological properties of some common and economically-important groundnut viruses, the microprecipitin reaction test was first used (VAN SLOGTEREN 1954). To classify more accurately the ivorian strain, indirect ELISA tests were then used (CLARK and ENGVALL 1980).

Results

Host range

The virus was transmitted by both grafting and sap-inoculation. For host range studies, the virus isolate was found to be easily mechanically transmissible from *N. benthamiana* to other plant species. Of 46 tested plant species, belonging to 9 families, 18 species from 4 families were found to be susceptible, while the remaining 28 species from 8 families did not become infected. Most of the susceptible hosts were members of the *Leguminosae* and *Solanaceae*. No host with suitable local lesions was found for quantitative studies. Back transmission from symptomless plants was performed on *N. benthamiana* in order to test for latent infections and further verified using serological tests. The susceptible host-range is reported in Table 1.

The virus could not be involved from symptomless plants of the following species: *Brassica napus*, *Capsicum annuum*, *Chenopodium amaranticolor*, *C. quinoa*, *Crotalaria retusa*, *Cucurbita maxima*, *C. pepo*, *Hibiscus esculentus* cv. Gombo 25, *Lycopersicum esculentum*, *Nicotiana glutinosa*, *N. hybrida*, *N. tabacum* cv. Samsun, *N. tabacum* cv. Xanthi, *Petunia hybrida*, *Physalis floridana*, *P. peruviana*, *Solanum nigrum*, *Torenia fournieri*, *Vicia faba*, *Vinca rosea*.

Vector transmission

The virus was efficiently transmitted by *Aphis craccivora* in a non-persistent manner.

After an acquisition access time of c. 2—5 min on an infected groundnut plant, the virus was transmitted by the aphids in three experiments to 12/15, 11/12

Table 1
Host range and symptoms of PMV Ivorian isolate

Family	Host-plants	Local symptoms	Systemic symptoms
Amaranthaceae	*Gomphrena globosa* (*)	—	severe curling, mosaic
Cucurbitaceae	*Cucurbita pepo* (*)	—	severe mottling, deformation
Leguminosae	*Arachis hypogaea*	—	green areas, deformation, mottling
	Canavalia ensiformis	—	mild mosaic
	Cassia occidentalis	—	systemic ringspot, mosaic
	Centrosema pubescens	—	mosaic
	Glycine max	—	chlorotic patches, mosaic, deformation, vein banding
	Phaseolus vulgaris	nLL	—
	P. vulgaris cv. *Mange-tout*	nLL	—
	Pisum sativum	—	vein clearing, chlorotic patches
	Vigna radiata (*)	—	mottling, mosaic
	Vigna unguculata	—	vein clearing, mosaic
	V. unguiculata cv. 87	nLL	yellowing, mosaic
	V. unguiculata cv. Calif. Black	nLL	vein clearing, mosaic
Solanaceae	*Nicotiana benthamiana*	—	vein clearing, mottling
	N. megalosiphon	—	systemic necrotic lesion, deformation
	N. clevelandii	—	symptomless
	N. tabacum cv. White Burley (*)	—	vein clearing

Abbreviation of symptoms. nLL: necrotic local lesions; — no symptom.
* = prominent symptoms but not detected by back inoculation and ELISA tests.

and 8/12 *N. benthamiana* plants, respectively. In other experiments, instead of an infected groundnut plant, an infected *N. benthamiana* plant was used: 8/10 and 9/15 *N. benthamiana* were successfully infected.

In retention studies, aphids did not transmit the virus after an acquisition access time of c. 2—5 min, and after being kept on *Petunia hybrida* for 12 h, then transferred on 20 healthy *N. benthamiana.*

In vitro properties

The dilution-end-point of the virus was observed between 10^{-3} and 10^{-4}. The thermal inactivation point was about 60—62 °C. Infectivity was retained for 3 days at 10 °C.

Purification

Usual clarification methods, using butanol, percipitation with PEG, 0.2 M citrate buffer at pH 6.5 and/or 1 % 2-mercaptoethanol, were found to be unsuitable. However, Triton X-100 prevented aggregation of the particles in the resuspension buffer.

The ultraviolet absorption spectrum of the virus was typical for a potyvirus; maximum absorption was at 260 nm, minimum at 246 nm, and the $A_{260/280}$ ratio was 1.3. as previously reported for a typical PMoV strain (PAGUIO and KUHN 1973; SUN and HEBERT (1972). The yield was about 0.34 mg nucleoprotein/100 g of fresh leaves. The coat protein of the virus migrated in SDS-PAGE as a single band corresponding to a molecular weight of 33.000 ± 500 d.

Electron microscopy

Flexuous, filamentous particles were observed in infectious crude sap and purified preparations. Mean length and width of 80 particles were 700 ± 40 nm and 13 ± 1 nm, respectively.

Serology

The homologous antiserum, following absorption with clarified sap of healthy plants, reacted up to dilutions of 1/512 using microprecipitin tests.

In microprecipitin tests the virus isolate (PMoV-Ci) did not react with antisera to Bean Common Mosaic, Bean Yellow Mosaic, Cowpea Aphid-borne Mosaic, Groundnut Eyespot (GEV-Ci), Passionfruit Ringspot, Pepper Veinal Mottle (PVMV-Ci), Soybean Mosaic (SMV), Yam Mosaic, and Watermelon Mosaic viruses. Nevertheless the isolate did react with antisera to different strains of PMoV (Overseas Development Administration strain, groundnut strain, *Cassia* strain, *Vondzeia* strain, *Phaseolus lunatus* strain, USA-Kuhn strain).

Table 2
Serological properties of the PMoV ivorian strain

Virus \ Serum	PStV-d	PStV-j	PStV-t	PMoV-in	GEV	SMV-T	PVMV
Healthy plant	–	–	–	–	–	–	–
PStV-W	+	(+)	+	+	+	(–)	(+)
PMoV-Ci	–	–	(–)	(+)	–	–	–
GEV-Bu	+	(–)	(+)	(+)	(+)	(–)	(+)
PVMV-S	+	(+)	+	+	+	(–)	(+)

Serological reactions in ELISA tests of four viruses, PMoV-Ci, PStV-W, PVMV-S, and GEV-Bu. against antisera to seven different viruses, PStV-d, PStV-j, PStV-t, PVMV-in, GEV-Ci, PMoV-in and SMV-T.
The average value of the optical density of the healthy plants was considered as the negative threshold. and twice this value was considered as the positive threshold; (–) was near and above the negative threshold, considered also as negative; (+) was near and under the positive threshold and considered as distantly positive reaction.

In indirect ELISA tests, the ivorian isolate (PMoV-Ci) reacted distantly with the antiserum to PMoV-in, an indian strain, while it dit not react with antisera to Soybean Mosaic Virus (SMV-T), Peanut Stripe Virus (PStV-d, PStV-j, PStV-t, three asiatic strains), Groundnut Eyespot Virus (GEV-Ci, an ivorian strain), Pepper Veinal Mottle Virus (PVMV-Ci, an ivorian strain). Reactions of the

ivorian strain PMoV-Ci were compared to the reactions of three other potyviruses: Peanut Stripe Virus (PStV-W), Groundnut Eyespot Virus (GEV-Bu, a strain of Burkina Faso) and Pepper Veinal Mottle Virus (PVMV-S, a senegalese strain). Contrary to PMoV-Ci, these three last viruses reacted strongly against an antiserum to PStV-d; however, only PStV-W and PVMV-Ci reacted strongly against antisera to PStV-t, PMoV-Ci, GEV-Ci.

Discussion

A lot of groundnut virus diseases have been reported in Cote d'Ivoire (DOLLET *et al.* 1985) and many are economically important. On groundnut plants, symptoms similar to these produced by the isolate under study had previously been observed (DUBERN 1979), but the causal agent has not been characterized.

The symptoms produced on groundnut plant and other hosts were similar to those observed for PMoV from other countries (BEHNCKEN 1970, BOCK 1973, HEROLD and MUNZ 1969, REDDY *et al.* 1978, AHMED 1981). Studies on the host range, mode of transmission, particle morphology, *in vitro* and serological properties indicated that the described virus is an isolate of the peanut mottle virus.

The serological analysis by ELISA confirmed this identification, but showed an antigenic difference between the ivorian isolate and the indian strain of PMoV.

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