Ann. appl. Biol. (1988), 112, 127–132
Printed in Great Britain

Identification and characterisation of a newly described potyvirus in West Africa: Asystasia mottle virus

BY J.-C. THOUVENEL, C. FAUQUET AND D. FARGETTE

Laboratoire de Phytovirologie d'Adiopodoumé, ORSTOM, B.P. V51, Abidjan, Ivory Coast, West Africa

(*Accepted 17 July 1987*)

SUMMARY

Asystasia mottle virus (AsMV) was detected serologically in samples of *Asystasia gangetica* with mottle symptoms, from several areas of tropical West Africa. It infected 12 species systemically and induced local lesions in a further four.

The virus lost infectivity after dilution to 10^{-4}, after 10 min at 75 °C and after 3 days at 27 °C. Purified virus had an A_{260}/A_{280} ratio of *c.* 1·2 and a protein subunit mol. wt of *c.* 33 000. Particles were *c.* 750 nm long and cytoplasmic inclusions typical of potyviruses were seen in ultrathin sections of infected leaves. The antiserum prepared had a titre of 1/1024 in microprecipitin tests but purified virus failed to react with 31 antisera to known potyviruses.

The virus was transmitted in the non-persistent manner by *Aphis spiraecola* but only very infrequently. On the basis of these properties, AsMV is considered to be a new member of the potyvirus group.

INTRODUCTION

A yellow mottle and line-pattern (Plate, fig. 1) on a ruderal roadside plant *Asystasia gangetica* (L.) T. Anders (Acanthaceae), was observed near Abidjan in 1980 and the causal virus identified as a possible member of the potyvirus group due to its particle morphology and to the typical inclusions induced in host plants; however insect transmission tests failed (Thouvenel, Fauquet & Monsarrat, 1982). We have since found this virus on numerous surveys made in western Africa. It is the first potyvirus to be described from a member of the Acanthaceae in the whole of tropical West Africa. This paper adds new information on the transmission and characterisation of the virus which we have named Asystasia mottle virus (AsMV).

MATERIALS AND METHODS

Virus. Asystasia gangetica leaves, harvested from a single plant with severe symptoms, and collected near Abidjan (Ivory Coast) were used as the original inoculum.

Mechanical transmission and plant growth conditions. The virus was maintained in *Nicotiana benthamiana* and transmitted by mechanical inoculation of sap obtained by grinding leaves in 0·1 M phosphate buffer, pH 7·1, containing 0·02 M cysteine hydrochloride. Test plants were grown in insect-proof screenhouses under local climatic conditions (mean temperature 28 °C, average r.h. 90%). Inoculated plants were checked for infection by back inoculation to *N. benthamiana* and *Chenopodium amaranticolor* or by serology.

Stability in sap. The thermal inactivation point, dilution end point, and period of ageing *in vitro* in crude sap of *N. benthamiana* were determined according to the method of Bos, Hagedorn & Quantz (1960) using *C. amaranticolor* as test plants.

Purification and spectrophotometry. Leaves of infected *N. benthamiana* were ground in 0·2 M potassium phosphate buffer, pH 7·8, containing 1% thioglycollic acid (1 g/3 ml); chloroform was added during the grinding (1/2 vol).

The mixture was centrifuged for 10 min at 10 000 *g* and the supernatant centrifuged for 3 h at 78 000 *g*. The pellets were re-suspended in 0·05 M borate buffer, pH 8 and, after removal of the insoluble material by centrifugation for 10 min at 10 000 *g*, the virus was sedimented through a 20 mm cushion of 20% sucrose in the SW 27 rotor (90 000 *g* for 3 h). The pellets were re-suspended in the same borate buffer and further purified by density gradient centrifugation (10 – 40% sucrose in 0·1 M borate buffer, pH 8). The opalescent virus band was collected, diluted with water, centrifuged for 3 h at 78 000 g and the resulting pellets re-suspended in borate buffer, pH 8.

The u.v. absorption spectrum of the virus preparation was determined using a Beckman 5230 Spectrophotometer.

Determination of mol. wt and amino-acid composition of the coat protein. The mol. wt of the viral protein was determined by electrophoresis on poly-acrylamide gel in the presence of SDS. The standard proteins used were (mol. wt in parentheses): ovalbumin (43 000), aldolase (40 000), carbonic anhydrase (30 000), trypsin inhibitor (20 100), ribonuclease a (13 700). The amino-acid composition was estimated after hydrolysing purified viral protein in a 6 N solution of hydrochloric acid at 108 °C for 24, 48 or 72 h, then separating and estimating the relative amounts of the resulting amino acids with a Durrum D-500 amino-acid analyser.

Electron microscopy. For electron microscopy, virus preparations were stained with 2% aqueous uranyl acetate and observed with a Siemens Elmiskop 102 at the Groupe d'Etude et de Recherches en Microscopie Electronique at Adiopodoumé.

Serology. Antiserum was prepared in rabbits by six intravenous weekly injections of about 1 mg of purified virus. Microprecipitin reactions under paraffin oil in Petri dishes (Van Slogteren, 1955) were used for serological tests, using crude sap or purified virus preparations.

Aphid transmission. The aphids, *Aphis craccivora* Koch collected from *Arachis hypogea* and *A. spiraecola* Patch collected from *Eupatorium odoratum*, were used in transmission tests. Insects reared on healthy plants were transferred by brush to a Petri dish and starved for 2 – 4 h, then allowed an acquisition access period, which varied from about 15 min to 24 h, on diseased leaves of *Nicotiana megalosiphon* and an inoculation access period of 1 day on healthy seedlings of *N. megalosiphon* (10 insects per plant). Plants were sprayed with an insecticide at the end of the inoculation access period.

RESULTS

Symptomatology, host range and distribution

The virus was transmitted easily by mechanical inoculation.

The following plants developed symptoms and virus was recovered by back-inoculation to *N. benthamiana*:

Acanthaceae: *A. gangetica* (veinal mottle and yellow line-pattern, Plate, fig. 2), *Crossandra flava* (slight mottling); Aizoaceae: *Tetragonia expansa* (necrotic local lesions); Chenopodiaceae: *Chenopodium amaranticolor* (necrotic local lesions); Leguminosae: *Phaseolus mungo* (chlorosis), *Phaseolus vulgaris* (chlorosis); Pedaliaceae: *Sesamum indicum* (chlorotic spotting); Scrophulariaceae: *Antirrhinum majus* (chlorosis), *Penstemon hirsutus* (chlorosis); Solanaceae: *Browallia speciosa* (chlorosis), *Nicotiana benthamiana* (mosaic and crinkling, Plate, fig. 3), *N.*

clevelandii (mottle), *N. megalosiphon* (mottle), *N. tabacum* Samsun NN and *N. tabacum* Xanthi nc (local lesions, concentric necrotic spots), *Solanum nigrum* (chlorotic local lesions).

The virus was identified serologically in samples of *A. gangetica* showing typical symptoms, from Kindia (Guinea), Kpalimé (Togo), Buea (Cameroun) and Loudima (Congo).

Stability in sap

In crude sap of *N. benthamiana*, AsMV was infective at a dilution of 10^{-3} but not 10^{-4}, after 10 min at 70 °C but not 75 °C and lost infectivity after 3 days at about 27 °C. Crude sap was still infective after 4 months and purified virus after 6 months at 4 °C.

Infectivity was retained during more than 2 yr in *N. benthamiana* leaves dried over silica gel.

Purification and properties of particles

Purified virus was highly infectious and induced characteristic symptoms of AsMV when inoculated to AsMV hosts. The u.v. absorption spectrum had a maximum at 260 nm and a minimum at 247 nm with a A_{260}/A_{280} ratio of 1·20 ± 0·05 and a A_{260}/A_{247} ratio of 1·10 ± 0·05 (without correction for light scattering). Based on the extinction coefficient $E^{0\cdot1\%}_{1\text{ cm},260}$ commonly used for potyviruses (Hollings & Brunt, 1981), yields of purified virus averaged 15 mg/kg leaves.

Electron microscopy

Electron micrographs of purified virus preparations showed filamentous particles 750 ± 15 nm in length and 13 ± 1 nm in width (Plate, fig. 4).

Ultrathin sections of araldite-embedded leaf tissue of *N. benthamiana* systemically infected by AsMV showed cytoplasmic inclusions typical of potyviruses; these were pinwheels with numerous associated laminated aggregates (Plate, fig. 5).

Mol. wt and composition of protein subunit

The mol. wt of the protein subunit was found to be 33 000 ± 1000 daltons.

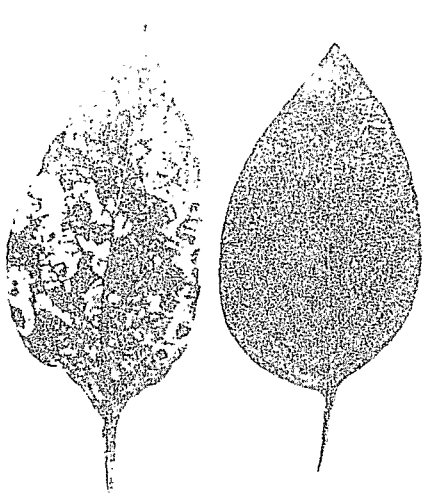
The amino-acid composition was : Asp 35, Thr 17, Ser 38, Glu 40, Pro 11, Gly 41, Ala 25, Cys 2, Val 17, Met 6, Ile 12, Leu 17, Tyr 7, Phe 9, His 8, Lys 11, Arg 15, Trp 1, total 312.

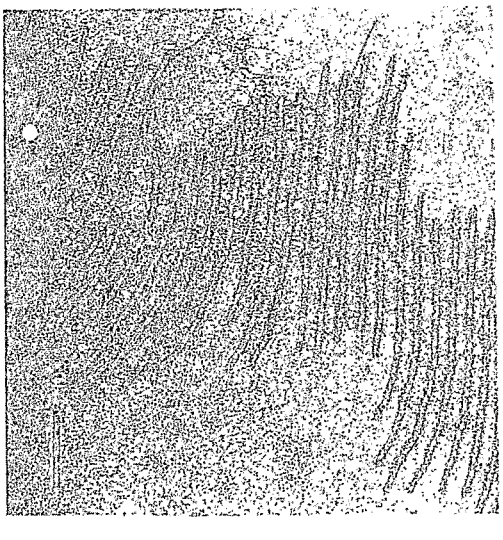
Serology

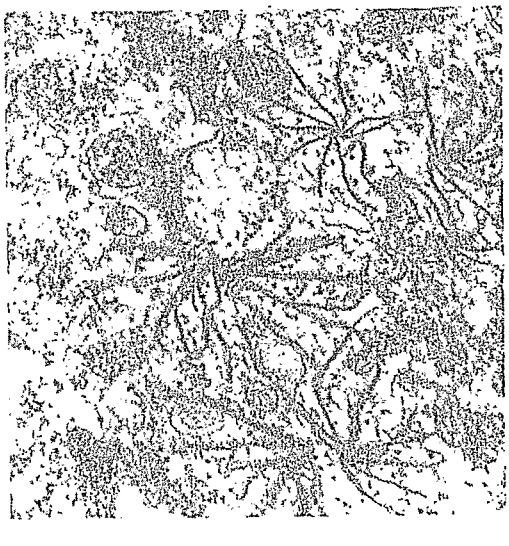
The antiserum prepared reacted with purified AsMV (Ivory Coast isolate) in micro-precipitin tests to an antiserum dilution of 1/1024.

Purified AsMV failed to react with antisera prepared against the following viruses (homologous titre and donors in parentheses) : Aneilema mosaic (1/8192; Dr K. Bock), bean common mosaic (1/256; Dr L. Bos), bean yellow mosaic (1/1024; Dr L. Bos), clover yellow vein (1/1024; Dr O. W. Barnett), Columbia Datura virus (?; Dr R. Bartels), cowpea aphid-borne mosaic (1/4000; Dr K. Bock), henbane mosaic (1/8000; Dr R. Bartels), Hippeastrum mosaic (1/1024; Dr A. A. Brunt), Iris fulva mosaic (1/256; Dr O. W. Barnett), parsnip mosaic (1/64; Dr A. F. Murant), passionfruit woodiness (?; Dr P. R. Smith), pea seed-borne mosaic (1/400; Dr G. I. Mink), peanut mottle (1/4000; Dr K. Bock), peanut mottle strain M 2 (1/256; Dr K. Bock), potato A (1/160; Dr R. Bartels), potato Y (1/512; Dr R. Bartels), soybean mosaic (1/128; Dr O. W. Barnett), sugarcane mosaic (?; Dr A. G. Gillaspie), tobacco etch (?; Dr R. Bartels), tobacco 3 (1/16 000; Dr E. J. Guthrie), turnip mosaic (1/64; Dr J. A. Tomlinson), and Wisteria vein mosaic (1/1024; Dr E. Luisoni).

Moreover, no reaction occurred with antisera prepared against the following potyviruses found in the Ivory Coast (Fauquet & Thouvenel, 1980): Canavalia mosaic (1/2000), groundnut eyespot strain A (1/2048), groundnut eyespot strain B (1/256), guinea-grass mosaic strain A







(1/1024), guinea-grass mosaic strain B (1/2048), passionfruit ringspot virus (1/2048), pepper veinal mottle (1/4096), Voandzeia distortion mosaic (1/1000), and yam mosaic (1/2048).

Transmission

Transmission by aphids was very infrequent. In tests using an acquisition access period of 15 min, *Aphis spiraecola* infected only 2/60 *N. benthamiana* test plants and 1/60 *N. megalosiphon* plants. No transmission occurred with acquisition access periods of 3 h (0/120) or 24 h (0/120) using *N. benthamiana* as test plant or using *A. gangetica* as test plant with an acquisition access period of 15 min (0/70). Similar tests using *N. megalosiphon* as test plant and *Aphis craccivora* as vector, with an acquisition access period of 15 min, all failed (0/80).

DISCUSSION

The morphology, non-persistent aphid-transmission and the characteristic type of inclusions produced in infected plants, indicate that the causal agent of the mottle of Asystasia is a virus belonging to the potyvirus group. The presence of laminar aggregates associated with the pinwheels indicates that AsMV should be placed in the second subdivision of potyviruses, following the classification of Edwardson (1974).

This is the first report of a member of the potyvirus group infecting a plant of the Acanthaceae family, and the host range of the virus includes such crop plants as bean (*Phaseolus vulgaris*) and sesamum (*Sesamum indicum*).

No serological relationship was found with any of more than 30 antisera prepared against different members of this group, so we conclude that Asystasia mottle virus is a new member of the potyvirus group.

ACKNOWLEDGEMENTS

We thank L. D. C. Fishpool for revising the English and donors cited in the text for supplying the antisera.

REFERENCES

BOS, L., HAGEDORN, D. J. & QUANTZ, L. (1960). Suggested procedures for international identification of Legume viruses. *Tijdschrift over Plantenziekten* 66, 328–343.

EDWARDSON, J. R. (1974). *Host range of viruses in the PVY group.* Florida Agricultural Experiment Station, Monograph Series No. 5, 225 pp.

FAUQUET, C. & THOUVENEL, J.-C. (1980). *Viral diseases of crop plants in Ivory Coast.* Initiations-Documentations Techniques, No. 46, ORSTOM, Paris, 128 pp.

HOLLINGS, M. & BRUNT, A. A. (1981). Potyvirus group. *CMI/AAB Descriptions of Plant Viruses* No. 245, 7 pp.

EXPLANATION OF PLATE

Fig. 1. Systemic symptoms of Asystasia mottle virus on a naturally infected plant of *Asystasia gangetica*.

Fig. 2. Systemic symptoms of AsMV on *A. gangetica* leaf (left), after mechanical inoculation of a young seedling, and healthy leaf.

Fig. 3. Systemic crinkling and mosaic induced by AsMV on *N. benthamiana*.

Fig. 4. Electron micrograph of a purified preparation of AsMV, magnification 50000 (bar represents 200 nm).

Fig. 5. Electron micrograph of pinwheel inclusions induced by AsMV in a parenchyma cell from a systemically infected leaf of *N. benthamiana* (magnification 35000).

THOUVENEL, J.-C., FAUQUET C. & MONSARRAT, A. (1982). Un nouveau potyvirus : le virus de la marbrure d'*Asystasia gangetica*. *Comptes-rendus de l'Académie des Sciences, Paris* 295, 213–218.

VAN SLOGTEREN, D. H. M. (1955). Serological micro-reactions with plant viruses under paraffin oil. *Proceedings of the 2nd Conference on Potato Virus Disease*, Lisse-Wageningen, 1954, pp. 51–54.

(*Received 28 April 1987*)