Identification of Peanut Stunt Virus in the Sudan

A. H. AHMED, Department of Crop Protection, Faculty of Agriculture, Shambat, Sudan, and P. R. MILLS, Plant Pathology Research Division, Department of Agriculture for Northern Ireland, Belfast, UK

ABSTRACT

Ahmed, A. H., and Mills, P. R. 1985. Identification of peanut virus in the Sudan. Plant Disease 69:173-174.

A spherical virus isolated from mottled lucerne (*Medicago sativa*) was identified by host range, physical properties, electron microscopy, and serology as peanut stunt virus (PSV). The virus was also found in naturally infected *Phaseolus vulgaris, Vigna unguiculata, Dolichos lablab, Clitoria ternata*, and *P. trilobus*. This is the first report of PSV in the Sudan.

Legumes such as lucerne, peanut, beans, clitoria, cowpea, lubia, phillipesara, and soybeans are important forage and cash crops in the Sudan (9). Peanut is grown on 2.5 million feddans (1 feddan = 0.42 ha) mainly as an oil crop, and lucerne is the most important forage legume. Many plants in legume crops show yellowing, mottling, and mosaic pattern symptoms indicative of virus infection, and several viruses isolated from lucerne crops in Sudan have been identified (1,10). A survey of lucerne crops around Shambat, Khartoum North, showed a high incidence of a yellow mottle symptom. Sap from these plants inoculated onto indicator plants produced symptoms distinct from those of known viruses of legumes in the Sudan. The unknown virus was identified as peanut stunt virus (PSV).

MATERIALS AND METHODS

Lucerne plants from the University Farm in the Sudan showing yellow mottle were ground in 0.05 M phosphate buffer, pH 7.0, and the expressed sap was used to inoculate Carborundum-dusted *Phaseolus vulgaris* 'Topcrop' and *Vigna sinensis*. Single-lesion isolates were taken from *V. sinensis* and maintained in *Nicotiana tabacum* 'Harrow Velvet.' Systemically infected *N. tabacum* leaves were used as inoculum for determining host range, dilution end point, and thermal inactivation point (5).

Accepted for publication 24 April 1984.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

Aphid transmission tests were done with nonviruliferous, apterous adults of *Myzus persicae*. Aphids were starved for 2 hr, given an acquisition period of 2–3 min on infected *N. bigelovii*, and transferred to healthy *N. tabacum* for 30 min. Five aphids were transferred to each plant.

Purification was done by a modification of the method described for cucumber mosaic virus (11). Inoculated and systemically infected leaves of *N. tabacum* were homogenized in 0.5 M citrate buffer (pH 6.5, containing 0.1% thioglycollic acid). The homogenate was filtered through two layers of muslin and an equal volume of *n*-butanol was added and stirred for 30 min. The mixture was centrifuged at 10,000 *g* for 20 min and the aqueous layer removed. Polyethylene glycol 6000 and NaCl were added to a final concentration of 8% and 0.2 M, respectively. The resulting precipitate was removed by centrifuging at 10,000 *g* for 15 min and resuspended in borate buffer (pH 9.0, 0.005 M). Preparations were further purified by two cycles of high- and low-speed centrifugation, sucrose density gradient centrifugation for 2 hr at 80,000 *g* followed by fractionation with an ISCO density gradient fractionator.

Purified virus and crude sap from infected plants were used in agarose gel double-diffusion tests (0.7% agarose, 0.6% NaCl) with antisera to PSV (PSV-E, PSV-W, PSV-V, and PSV-P), cucumber mosaic virus (CMV-PY and CMV-G), and broad bean wilt virus (BBWV). An antiserum was prepared by injecting 1.8 mg of purified virus intramuscularly into the hind leg of a rabbit. This was followed 8 days later with an intravenous injection. Both injections were 1.5 ml without adjuvant.

For electron microscopy, virus preparations from light-scattering density gradient zones were negatively stained with 2% uranyl acetate and viewed in a GEC-AEI EM801A electron microscope.

RESULTS

Natural host range. PSV symptoms on naturally infected lucerne plants ranged from bright leaf mottling to green-yellow mottle (Fig. 1A). Symptoms on naturally infected peanut (*Arachis hypogaea*) included pronounced mottling, leaf deformation, and stunting (Fig. 1B).

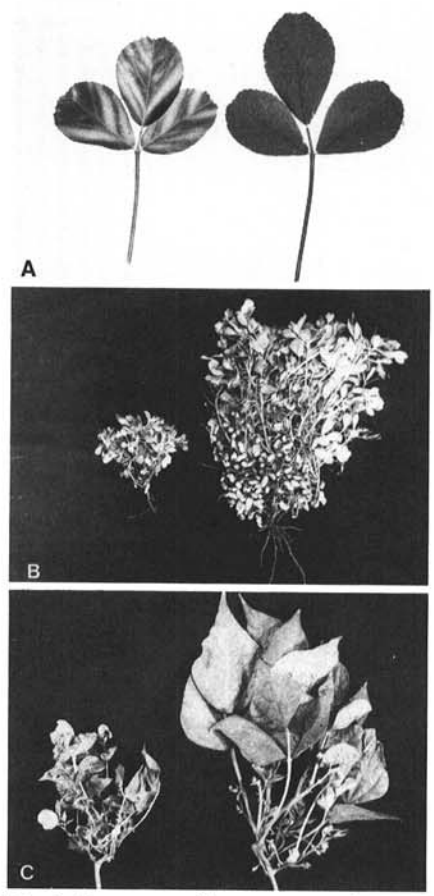

Fig. 1. Symptoms of peanut stunt virus on naturally infected **(A)** lucerne, **(B)** peanut, and **(C)** french bean.

Similar severe symptoms were observed on infected beans (*P. vulgaris*) (Fig. 1C) and cowpea (*V. unguiculata*). Symptoms on naturally infected lubia (*Dolichos lablab*) were characterized by a bright yellow mottle, and those on clitoria (*Clitoria ternata*) and phillipesara (*P. trilobus*), by green-yellow mottle.

Experimental host range. The virus produced chlorotic or necrotic local lesions and systemic symptoms on *A. hypogaea*; *Capsicum annuum*; *Chenopodium amaranticolor*; *C. quinoa*; *Datura stramonium*; *Dolichos lablab*; *N. bigelovii*; *N. debneyi*; *N. glutinosa*; *N. rustica*; *N. tabacum* 'Harrow Velvet,' 'White Burley,' and 'Xanthi'; *N. virginiae*; *Petunia hybrida*; *Phaseolus vulgaris* 'The Prince'; *Vicia faba*; and *Vigna sinensis*. Local lesions with symptomless systemic infection occurred in *Gomphrena globosa*, and local lesions without systemic infection occurred in *Cucumis sativus* 'Telegraph Improved.' This is essentially similar to the host range described for PSV (4,6).

General physical properties. Dilution end point was between 10^{-2} and 10^{-3}, thermal inactivation point was 50 C, and longevity in vitro was between 1 and 2 days.

Purification. Highly infectious preparations were obtained from the light-scattering zones produced in sucrose density gradient tubes. Yield was about 130 mg/kg of infected tissue.

Aphid transmission. In a single experiment, the virus was transmitted by *M. persicae* to one of five healthy *N. tabacum* plants.

Electron microscopy. Particles obtained from the light-scattering density gradient zones were spherical and about 30 nm in diameter.

Serology. Crude sap from infected leaves and purified virus preparations reacted with antisera to PSV. Homologous precipitation lines were continuous with PSV-E and PSV-V and formed a spur with PSV-W. The virus did not react with antisera to PSV-P, CMV-PY, CMV-G, or BBWV.

DISCUSSION

The green-yellow mottle disease of lucerne from the Shambat region in the Sudan is caused by a spherical virus identified as PSV on the basis of host range, aphid transmissibility, serology, physical properties, and electron microscopy. This isolate appears serologically similar to PSV-E and PSV-V. The virus failed to produce systemic symptoms in cucumber but systemically infected *C. amaranticolor, C. quinoa, V. sinensis*, and *P. vulgaris*, distinguishing it from many isolates of CMV. The Sudanese PSV isolate differs from tomato aspermy virus in that it also infects *D. stramonium* systemically. Natural hosts of PSV in the Sudan include peanut, beans, cowpea, clitoria, lubia, and phillipesara. Transmission in the field is probably by aphids. In this work, more emphasis has been attached to the disease in lucerne because it is widespread in this crop; since lucerne is usually left on the ground for 3–4 yr, it is likely to act as a reservoir for PSV.

PSV has been reported in the United States (8), Europe (2,3), Morocco (4), and Japan (7). This is the first report of PSV in the Sudan.

ACKNOWLEDGMENTS

We wish to thank Jennifer O'Brien and Nancy Stevens for technical help. We also thank A. C. Bell for the aphid transmission test and S. A. Tolin, G. I. Mink, O. M. Stone, and J. A. Tomlinson for gifts of antisera.

LITERATURE CITED

1. Ahmed, A. H., and Idris, M. O. 1981. Peanut mottle virus in the Sudan. Plant Dis. 65:692-693.
2. Diaz-Ruiz, J. R., Kaper, J. M., Waterworth, H. E., and Devergne, J. C. 1979. Isolation and characterization of peanut stunt virus from alfalfa in Spain. Phytopathology 69:504-509.
3. Douine, L., and Devergne, V. C. 1978. Isolement en France du virus du Rabourgrissenent de l'Arachide (peanut stunt virus, PSV). Ann. Phytopathol. 10:79-92.
4. Fischer, H. V., and Lockhart, B. E. L. 1978. Host range and properties of peanut stunt virus from Morocco. Phytopathology 68:289-294.
5. Gibbs, A., and Harrison, B. 1976. Plant Virology: The Principles. Edward Arnold, London.
6. Hampton, R., Beczner, L., Hagedorn, D., Bos, L., Inouye, T., Barnett, O., Musil, M., and Meiners, J. 1978. Host reactions of mechanically transmissible legume viruses of the Northern temperate zone. Phytopathology 68:989-997.
7. Iizuka, N., and Yunoki, T. 1974. Peanut stunt virus isolated from soybeans, *Glycine max* Merr. Tokuhu Nat. Agric. Exp. Stn. Bull. 47:1-10.
8. Mink, G. I. 1972. Peanut stunt virus. Descriptions of Plant Viruses. No. 92. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England.
9. Musa, M. M., and Burhan, H. O. 1974. The relative performance of forage legumes as rotational crops in the Sudan. Exp. Agric. 10:131-140.
10. Nour, M. A., and Nour, J. J. 1962. A mosaic disease of *Dolichos lablab* and diseases of other crops caused by alfalfa mosaic virus in the Sudan. Phytopathology 52:427-432.
11. Scott, H. 1963. Purification of cucumber mosaic virus. Virology 20:103-106.