Some Properties of an Isolate of Pea Early-Browning Virus Occurring in Morocco

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ABSTRACT

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A virus occurring naturally on pea *(Pisum sativum)* and broad bean *(Vicia faba)* in Morocco was identified as pea early-browning virus (PEBV). This is the first report of the occurrence of PEBV outside western Europe. The Moroccan PEBV isolate had particles of two predominant lengths, 90 and 190 nm, respectively. In undiluted pea sap the virus had a thermal inactivation point (TIP) of 75-80 C and a longevity in vitro (LIV) of more than 6 months at 20-24 C. The virus reacted with antisera to both PEBV and tobacco rattle virus (TRV) and was present in seeds from infected pea plants. No information on nematode transmission was obtained. The Moroccan virus differed somewhat from a British and a Dutch strain in test plant reaction.

In mid-1974, a disease of peas (*Pisum sativum* L.) was observed in the field along the Atlantic coastal plain of Morocco. Symptoms consisted of severe stunting, systemic mottle, and occasional necrosis on leaves. The disease was observed on the pea cultivars Lincoln and Doux de Provence, the two cultivars most widely grown in Morocco. The only viruslike particles observed during electron microscopic examination of negatively stained leaf-dip preparations from such plants were tubular particles typical of the tobravirus group. In initial host-range studies no evidence was obtained to indicate the presence of any other viruses commonly reported to infect peas. In late 1974, a virus of identical particle morphology and host range was isolated from broad beans in the Tadla region of central Morocco. During the 1975-76 growing season, the virus again was isolated from peas in the northwestern quadrant of the country, both from fields along the Atlantic coast and in the inland areas around Merchouch, Tiflet, and Khemisset. Additional symptoms observed in the field consisted of necrotic streaking of stems, necrotic rings on terminal leaves of younger plants, and rings, pits, distortion, and discoloration of pods (Fig. 1). In host-range and particle morphology the virus resembled PEBV, which has been reported only from western Europe (4, 10). Further tests were then carried out to establish more precisely the relationship of the Moroccan virus to previously described members of the tobravirus group, which comprises all known isolates of PEBV and tobacco rattle virus (TRV).

MATERIALS AND METHODS

Virus culture and mechanical transmission.—The virus used in these studies was isolated from a plant of Lincoln pea and maintained in the pea cultivar Mammoth Melting Sugar. Test plants were inoculated with crude sap prepared by grinding systemically infected (12 days post-inoculated) leaves in 0.02 M phosphate buffer pH 7.2. Test plants were kept under artificial illumination at 24-27 C and observed for up to 8 weeks after inoculation.

Properties in crude sap.—Undiluted sap from systemically infected pea leaves 12 days after inoculation of the basal leaves was used for tests of thermal inactivation point (TIP) and longevity in vitro (LIV). Groups of 10 plants of *Lupinus albus* L. were used as assay plants for each treatment in both series of determinations.

Presence of virus in seed.—Seeds from five infected Mammoth Melting Sugar plants were soaked overnight in distilled water, ground individually in separate mortars in a small amount of phosphate buffer, and used to inoculate *L. albus*.

Virus purification and electron microscopy.—Leaf-dip or purified virus preparations were stained in 2% neutralized phosphotungstic acid (PTA) and examined in a Zeiss EM9S electron microscope. The virus was readily

purified by any of several methods tried. The method of Lister and Bracker for TRV (6) was, however, found to be the most satisfactory.

Serology.—Serological tests were done using the double-diffusion method in 0.5% agarose (Bio-Rad Laboratories) dissolved in 0.01 M borate buffer pH 8.2. Antisera were placed in the center well and purified virus at 1 mg/ml, assuming $E_{260\ nm}^{0.001} = 3.0$, placed in the peripheral wells.

RESULTS AND DISCUSSION

Host range.—The host range of the Moroccan virus essentially was similar to those reported for other PEBV strains (4, 7, 10). The virus infected all 26 cultivars of pea tested, although symptoms varied from pronounced mosaic and browning to a very faint mottle. Local lesions without systemic invasion (as determined by back-inoculation) were produced in *Chenopodium amaranticolor* Coste & Reyn., *C. quinoa* Willd., *Cucumis sativus* L. 'National Pickling', 'Ohio MR-17' and 'Lemon', *Lupinus albus* (Fig. 2-b), *Beta vulgaris* L., *Cyamopsis tetragonoloba* (L.) Taub. (guar), *Gomphrena globosa* L., *Nicotiana tabacum* L. 'Xanthi', and 11 cultivars of bean (*Phaseolus vulgaris* L.). The lesions produced on the bean cultivars were the large (2-3 mm in diameter) lesions typical of PEBV (4), and differing from the typical pin-point lesions produced by TRV (3). A pronounced systemic mosaic was produced in the bean cultivars 'Beka' (Fig. 2-a) and 'Black Turtle Soup' (Fig. 2-c). No symptoms were produced on *N. glutinosa* L. or *Petunia hybrida* Vilm., but the virus was recoverable from the inoculated, though not from the new, leaves. Occasional necrotic local lesions were produced on *N. clevelandii* Gray, in which systemic symptoms were very mild or lacking altogether. *Medicago sativa* L. (lucerne), *Zinnia elegans* Jacq., and *Vicia faba* L. similarly were infected systemically, but showed only a very mild mosaic. *Capsicum annuum* L. 'California Wonder' (pepper) was not infected, but a few of the plants of the cultivar Marconi that had been inoculated at a very young stage, developed chlorotic and necrotic rings on inoculated leaves and a mild systemic vein-clearing. No symptoms were produced on, and no virus recovered from, *Solanum nigrum* L., *S. tuberosum* L. 'King Edward' (potato), *Lycopersicon esculentum* Mill. 'Marmande' and 'Tropi-gro' (tomato), *N. tabacum* 'White Burley NN' and 'Samsun', *Glycine max* L. 'Bansei' (soybean), and *Phaseolus lunatus* L. (lima bean).

In comparative host reaction tests, a Dutch (7) and a British (2) PEBV isolate differed from the Moroccan virus in failing to infect the bean cultivars Beka and Black Turtle Soup systemically, and by consistently producing a pronounced systemic mosaic in plants of pepper cultivar Marconi. Symptoms in *N. clevelandii,* and the pea cultivars Mammoth Melting Sugar, Dwarf Gray Sugar, and Alaska also differed noticeably. The Dutch PEBV isolate differed from the British and Moroccan isolates in that it produced a black interveinal necrosis instead of mild mottle in systemically infected leaves of *N. clevelandii,* and apical necrosis instead of mild mosaic in Dwarf Gray Sugar pea. The British isolate produced, in the pea cultivars Alaska, Dwarf Gray Sugar, and Mammoth Melting Sugar, a characteristic rolling of the top leaflets that was not observed in case of infection by the two other viruses.

All three PEBV isolates gave local necrotic lesions on guar and lupin. In contrast, none of the four TRV isolates

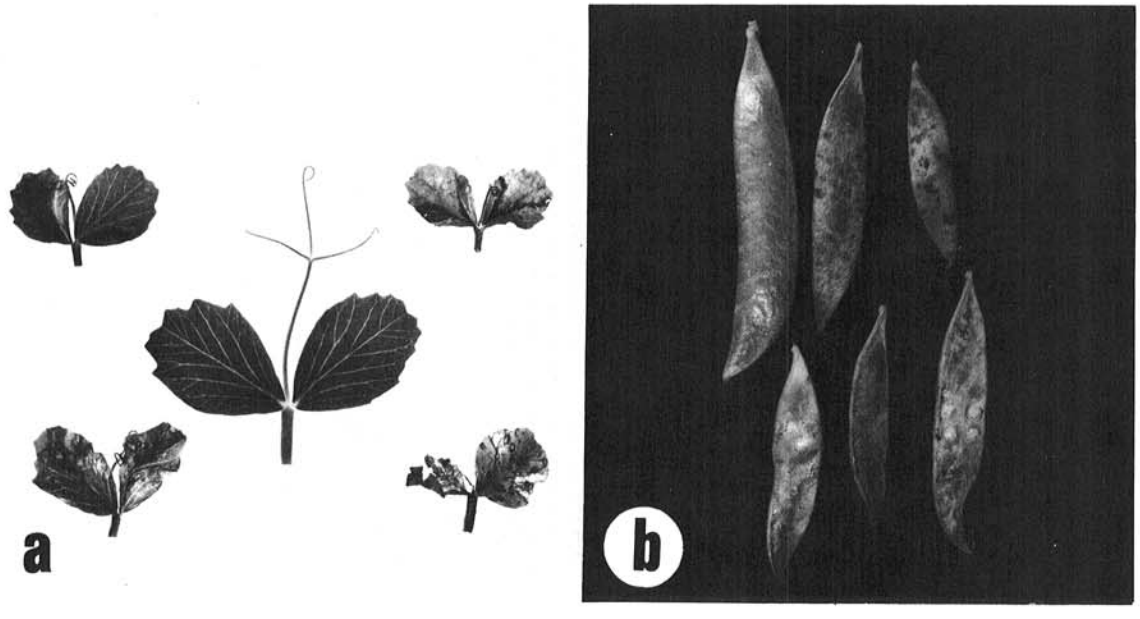

Fig. 1-(a, b). Symptoms produced by the Moroccan isolate of pea early-browning virus (PEBV) on naturally infected leaves and pods of pea cultivar Lincoln: **a)** leaf symptoms showing typical veinal and interveinal necrosis. Necrotic rings can be seen on leaflet at lower right. Healthy leaf in center; and **b)** pod symptoms showing discoloration, pits, and rings. Healthy pod at upper left.

tested [PRN (1), CAM (5), a Dutch isolate (from D. Z. Maat), and a California pepper isolate (8)] infected these two plants. All the TRV isolates gave local lesions on Mammoth Melting Sugar pea, but none could be recovered systemically by back-inoculation to *N. clevelandii*.

Properties in crude sap.—The TIP of the virus in pea sap was found to be 75-80 C. The LIV exceeded 6 months, the last period at which the infected sap was assayed. These values for the physical properties correspond to those previously reported for other PEBV isolates (4).

Presence of virus in seed.—By testing a sample of 66 seeds from infected Mammoth Melting Sugar pea it was found that the virus could be recovered from 21 of these, or approximately 30%, by inoculation to lupin. After seeds had been soaked overnight, the testae were removed and assayed separately. No virus was recovered from any of the testae, indicating that virus transmission does not take place upon or within this tissue. Seed transmission of Dutch and British strains in pea have been reported at 37% and 1-2%, respectively (4).

Virus purification and electron microscopy.—From measurements of 990 particles in negatively stained leaf dip preparations, it was determined that the virus has particles of two predominant lengths, 90 nm and 190 nm. In rate-zonal density gradient centrifugation in 10-40% sucrose gradients, purified virus sedimented as two bands corresponding to the two particle lengths. Infectivity was associated with the longer (190 nm) particles. These particle dimensions fall within the range reported for some other PEBV isolates (9).

Serology.—In double-diffusion plates the Moroccan PEBV reacted positively with antisera to four strains of TRV: ATCC PVAS 73, ATCC PVAS 74, ATCC PVAS 75, and a California isolate (Fig. 3), and a Dutch PEBV isolate. With ATCC PVAS 74 (Fig. 3-b) and ATCC PVAS 75 (Fig. 3-c) the precipitin lines of the Moroccan PEBV and the PRN isolate of TRV fused, both sets spurring with the precipitin lines of the CAM isolate of TRV. Against the California TRV antiserum (Fig. 3-d), the precipitin lines of all three viruses spurred with each other. These results indicate clearly the serological relationship between the Moroccan PEBV isolate and TRV, and suggest that the Moroccan virus may be more closely related serologically to the PRN than to the CAM isolate of TRV. The serological relationship between TRV and PEBV isolates has been described previously (7).

Both TRV and PEBV have been shown to be transmitted by soil-inhabiting nematodes of the genus *Trichodorus* (3, 4). An unidentified species of *Trichodorus* was isolated from soil in one of the areas in which the virus was found (D. MacDonald, University of Minnesota, *personal communication*) but no experiments on nematode transmission of the virus have been conducted.

On the basis of host range, particle morphology, and serological reaction the Moroccan virus was identified as an isolate of PEBV. The differences in test plant reactions were sufficiently clear to distinguish the Moroccan isolate from the British and Dutch strains with which it was compared.

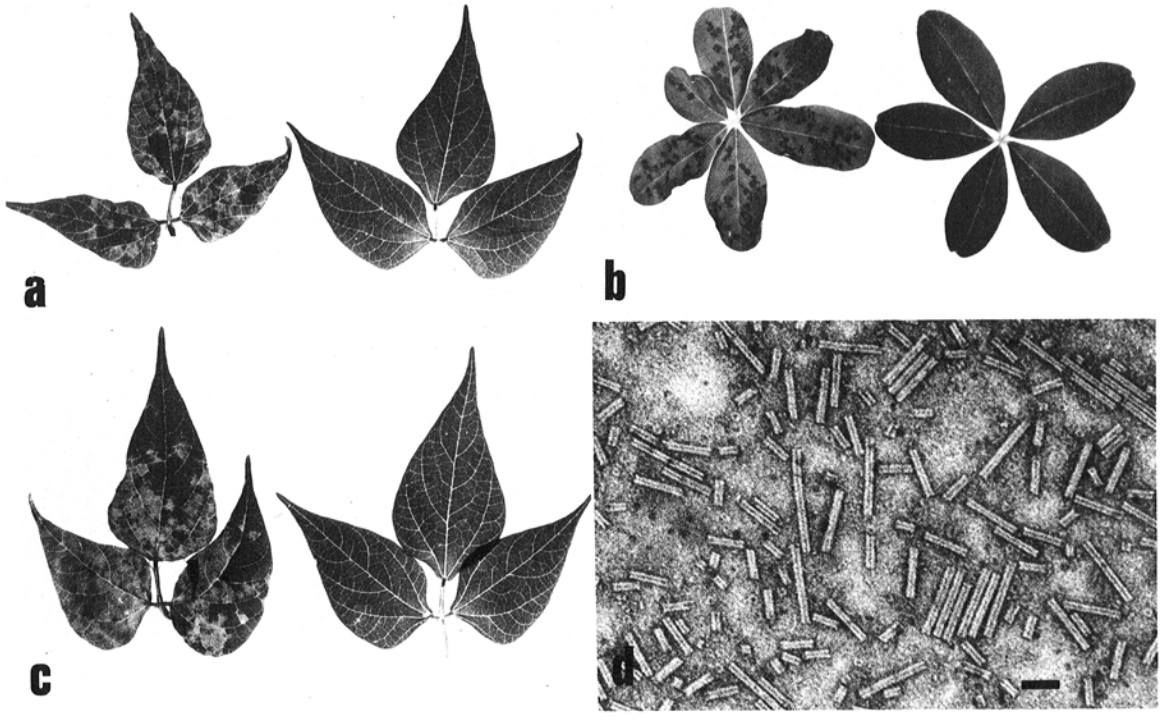

Fig. 2-(a to d). Symptoms produced by the Moroccan isolate of pea early-browning virus (PEBV) isolate on three test plants: **a)** systemic symptoms in bean cultivar Beka; **b)** lesions on inoculated leaf of lupin; **c)** systemic symptoms in bean cultivar Black Turtle Soup. Healthy leaves on right in all pictures; and **d)** negatively stained unfractionated purified preparation of the Moroccan PEBV. Bar represents 100 nm.

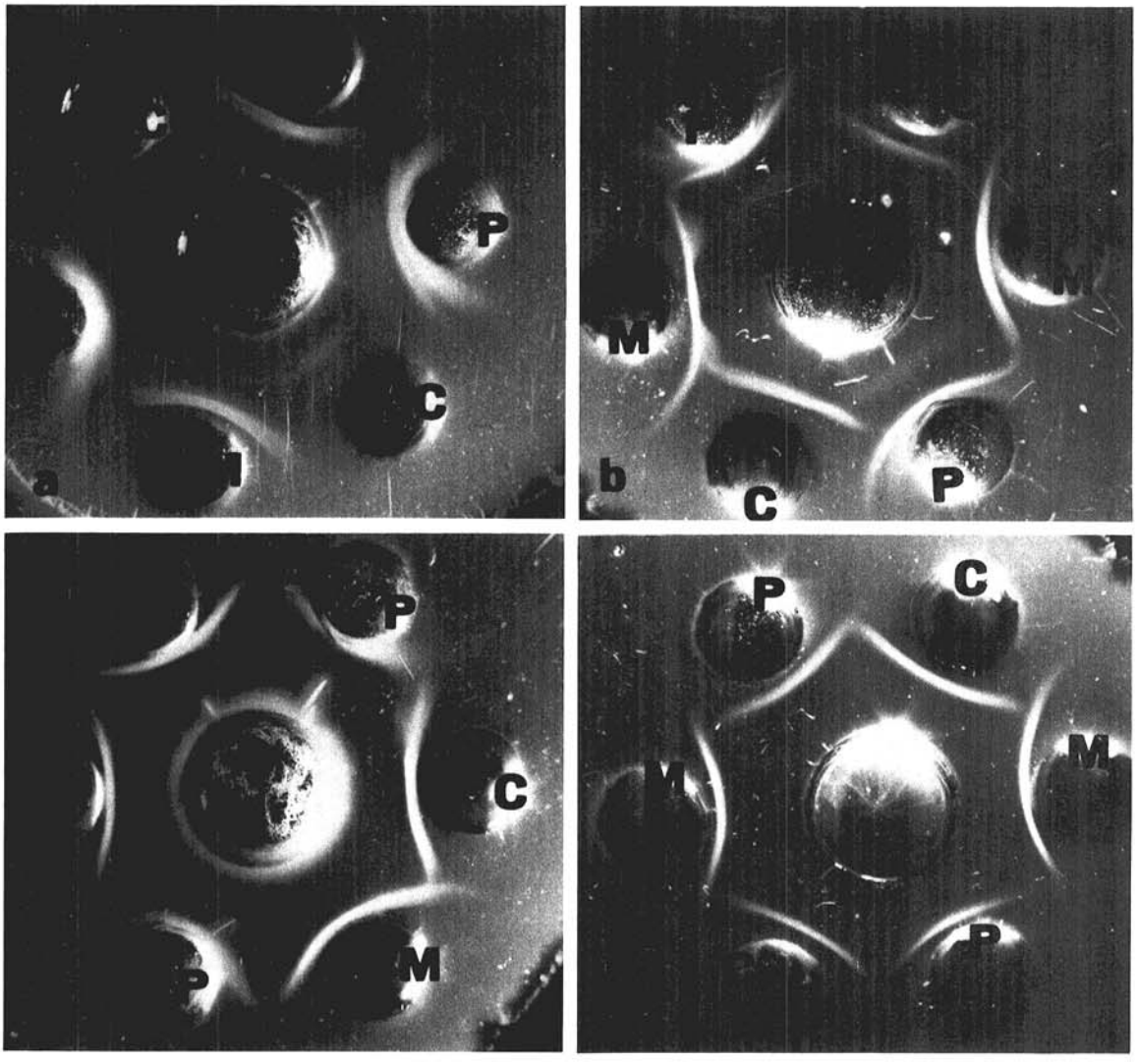

Fig. 3-(a to d). Serological reaction of the Moroccan pea early-browning virus (PEBV) isolate (M), and the PRN (P) and CAM (C) isolates of tobacco rattle virus (TRV) with antisera to four TRV isolates: **a)** ATCC PVAS 73; **b)** ATCC PVAS 74; **c)** ATCC PVAS 75; and **d)** a California pepper isolate.

LITERATURE CITED

1. CADMAN, C. H., and B. D. HARRISON. 1959. Studies on the properties of the soil-borne viruses of the tobacco-rattle type occurring in Scotland. Ann. Appl. Biol. 47:542-556.
2. GIBBS, A. J., and B. D. HARRISON. 1964. A form of early-browning virus found in Britain. Ann. Appl. Biol. 54:1-11.
3. HARRISON, B. D. 1973. Tobacco rattle virus. No. 12 *in* Descriptions of plant viruses. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England. 4 p.
4. HARRISON, B. D. 1973. Pea early-browning virus. No. 120 *in* Descriptions of plant viruses. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England. 4 p.
5. HARRISON, B. D., and R. D. WOODS. 1966. Serotypes and particle dimensions of tobacco rattle viruses from Europe and America. Virology 28:610-620.
6. LISTER, R. M., and C. E. BRACKER. 1969. Defectiveness and dependence in three related strains of tobacco rattle virus. Virology 37:262-275.
7. MAAT, D. Z. 1963. Pea early-browning virus and tobacco rattle virus–two different but serologically related viruses. Neth. J. Plant Pathol. 69:287-293.
8. SEMANCIK, J. S. 1966. Purification and properties of two isolates of tobacco rattle virus from pepper in California. Phytopathology 56:1190-1193.
9. VAN HOOF, H. A. 1969. Some properties of Dutch pea early-browning virus isolates. Meded. Rijksfac. Landbwetensch. Gent. 34:888-894.
10. VERHOYEN, M., and M. GOETHALS. 1967. Présence en Belgique du virus du brunissement précoce du pois. Parasitica 23:128-131.