

Characterization of a Potexvirus Isolated from Night-Blooming Cactus

A. E.-S. A. FUDL-ALLAH, L. G. WEATHERS, and F. C. GREER, JR., Department of Plant Pathology, University of California, Riverside 92521

ABSTRACT

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Plants of night-blooming cactus *Hylocereus undatus* as well as scions of several species of cactus grafted to it were stunted, malformed, and systemically mottled. A flexuous virus averaging 515 nm long was isolated from the affected plants. The virus was sap-transmitted to a limited number of hosts. Symptoms in *Chenopodium quinoa* consisted of chlorotic primary lesions and systemic mottle and necrosis. *Gomphrena globosa* and *Amaranthus caudatus* showed primary local lesions. Plants of *Saponaria vaccaria* showed only systemic mottling. Crystalline inclusions were found by light microscopy in epidermal cells of infected *H. undatus*. The virus had a thermal inactivation point above 95 C and a dilution end point of 10^6 - 10^7 in sap from infected *C. quinoa*. The virus reacted with antisera prepared against California barrel cactus and Chessin's isolate of cactus virus X.

Night-blooming cactus *Hylocereus undatus*, sometimes called night-blooming cereus or Honolulu queen cactus, is frequently clonally propagated as an ornamental cactus as well as being vegetatively propagated as a rootstock for grafting to other species of cactus. Stunted, slightly malformed, and blotchy plants of *Mammillaria* sp. grown on *H. undatus* were observed recently in

nursery-grown plants in California. In a limited survey of several nurseries, all *H. undatus* plants were found infected with a virus as were scions of cactus plants grafted on *H. undatus*. This paper reports the identification and partial characterization of an isolate of cactus virus X isolated from these plants.

MATERIALS AND METHODS

Virus. The virus isolate was obtained from a naturally infected *H. undatus* plant. The virus was transmitted mechanically from infected cactus plants to *Chenopodium quinoa* Willd. seedlings. Infected *C. quinoa* plants served as donors for further inoculations and virus purification. Inocula were prepared

by triturating leaves from systemically infected *C. quinoa* in 0.03 M phosphate buffer, pH 7.2.

Host range and symptomatology. Carborundum-dusted leaves of 10 plant species were rub-inoculated with sap from infected *C. quinoa* leaves. All test plants were kept in a greenhouse for observation at least 1 mo after inoculation.

Distribution in *H. undatus* tissues. Small pieces of epidermal and succulent green tissues of *H. undatus* were homogenized separately in cold distilled water in a glass homogenizer. Each extract was mechanically inoculated onto leaves of *Gomphrena globosa* L. seedlings.

Purification. Fresh or frozen leaves from systemically infected *C. quinoa* plants were homogenized with a Waring Blendor in cold distilled water (3 ml/g tissue) containing 0.2% sodium sulfite and 0.2% ascorbic acid. The homogenate was expressed through cheesecloth and the extract mixed with an equal volume of cold ether. The mixture was shaken vigorously for 5 min and the emulsion broken by low-speed centrifugation (2,000 rpm for 5 min). The aqueous phase was mixed with an equal volume of CCl_4 and shaken and centrifuged as before. The aqueous phase was collected and centrifuged in a Beckman type 30 rotor at 23,000 rpm for 1.5 hr. Pellets were

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resuspended in either distilled water or 0.03 M phosphate buffer, pH 7.2, centrifuged at 2,000 rpm for 5 min, and the supernatant fluid collected and frozen.

Electron microscopy. Extracts from infected *C. quinoa* leaves and from epidermal tissue of *H. undatus* were examined by electron microscopy. Preparations were either shadowed with palladium or negatively stained with 2% phosphotungstic acid, pH 7.2, and examined using an Hitachi H-600 electron microscope.

Serology. Serological studies were done by the ring-interface test as described by Ball (4). Purified virus preparations were tested against antisera

to tobacco mosaic virus, tobacco etch virus, potato virus X, potato virus Y, Chessin's isolate of cactus virus X, California barrel cactus virus, and d-protein of potato virus X.

RESULTS

Host range and symptomatology. *C. quinoa*, *G. globosa*, *Amaranthus caudatus* L., and *Saponaria vaccaria* L. were susceptible to the virus. *C. quinoa* seedlings developed chlorotic local lesions on inoculated leaves (Fig. 1A) 10 days after inoculation with sap from infected cactus plants. When *C. quinoa* plants were inoculated with sap from infected *C. quinoa* leaves, symptoms appeared 3–5 days after inoculation.

Systemic symptoms of chlorosis and mottling of expanded leaves appeared after about 2–3 wk. Immature leaves usually showed no symptoms. *G. globosa* plants exhibited necrotic local lesions with red margins 5 days after inoculation. *A. caudatus* seedlings showed only local lesions. *S. vaccaria* plants produced no visible symptoms in inoculated leaves but systemic, faintly striated mottling of leaves appeared 3–4 wk after inoculations.

Distribution in *H. undatus* tissues. Inoculum prepared from the epidermal layer of *H. undatus* produced more local lesions on *G. globosa* leaves than inoculum from succulent green tissues (Table 1). Examination of the infected epidermal layer with light microscopy

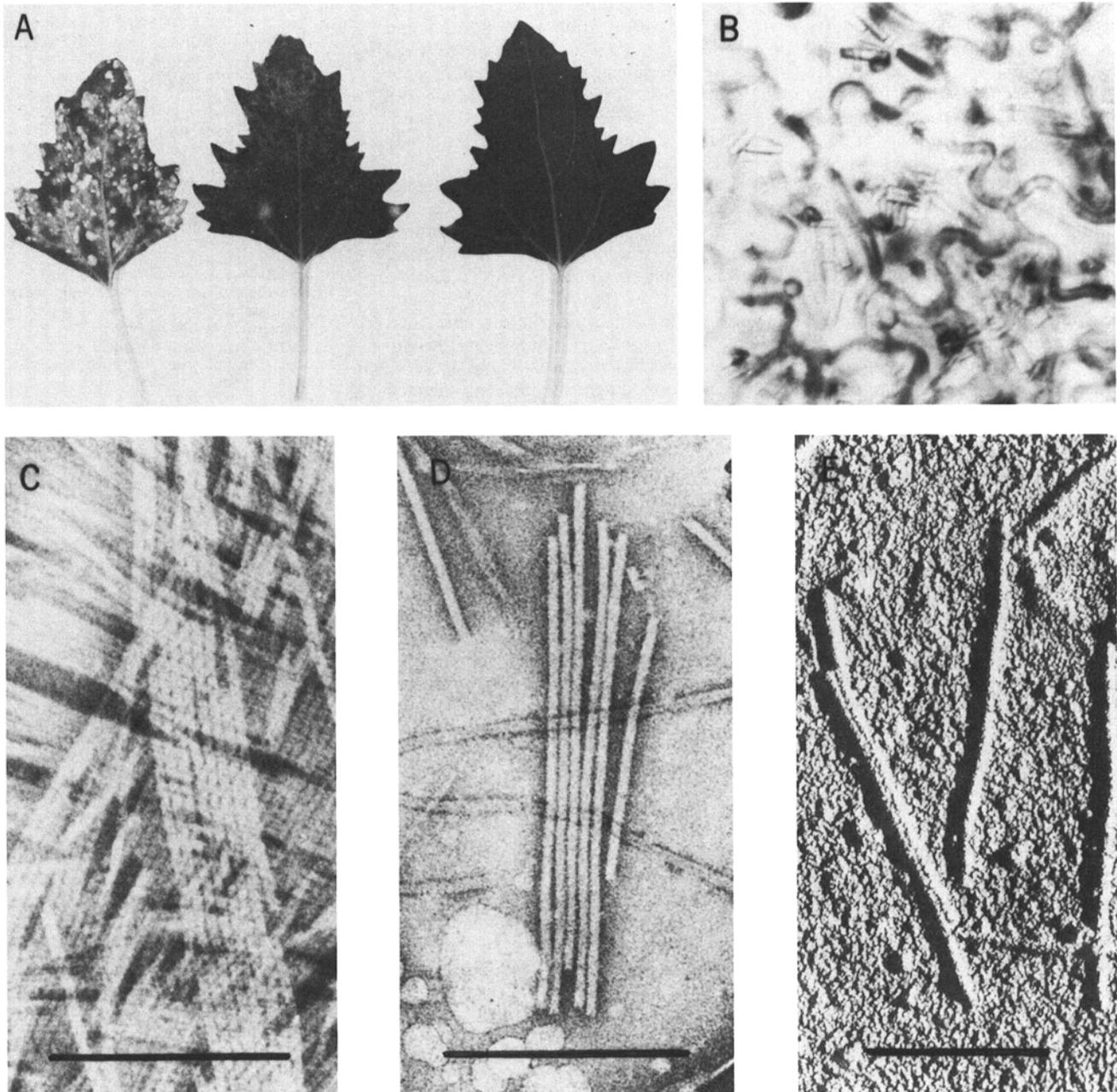


Fig. 1. (A) Leaves of *Chenopodium quinoa* (left) inoculated with extract from *Hylocereus undatus* showing necrotic and chlorotic local lesions, (middle) systemically infected showing chlorotic lesions, and (right) healthy. (B) Light microscopy of crystalline and spindle-shaped inclusions in the epidermal cells of infected *H. undatus*. (C) Electron micrograph of partially purified preparations of the virus from *C. quinoa*. Note tendency of virus particles to aggregate. Bar = 250 nm. (D) Electron micrograph of a preparation from epidermal cells of virus-infected *H. undatus*. (E) Electron micrograph of shadowed preparations of the virus from *C. quinoa*.

Table 1. Numbers of local lesions on leaves of *Gomphrena globosa* mechanically inoculated with sap from virus-infected *Hylocereus undatus* plants

Plant	No. local lesions ^a	
	Inoculum from epidermal layer	Inoculum from succulent green tissue
1	49	1
2	34	6
3	36	6
4	38	1

^aData are totals from four leaves.

revealed many crystalline and spindle-shaped inclusion bodies (Fig. 1B). Electron microscopy showed these crystals to be aggregated virus particles. No similar inclusions were found in healthy tissues.

Physical properties. The virus remained infective in crude extract of systemically infected leaves of *C. quinoa* for 13 wk at room temperature. Crude extract was infectious after diluting to at least 10^{-6} – 10^{-7} . Infectivity was retained after heating at 96 C for 10 min but not at 98 C.

Purification. The procedure described previously provided good yields of relatively pure virus, but it did not prevent the aggregation of virus particles. Cleaner virus preparations with little aggregation were obtained only when epidermal layers of *H. undatus* were homogenized in cold distilled water (Fig. 1C–E).

Electron microscopy. Negatively stained and shadowed preparations contained flexuous rod-shaped particles (Fig. 1D,E). The normal length of the particles determined in preparations from cactus and *C. quinoa* was about 515 nm.

Serology. The virus reacted with antisera against Chessin's isolate of cactus virus X, California barrel cactus, and d-protein of potato virus X but not against antisera to tobacco mosaic virus,

tobacco etch virus, potato virus X, and potato virus Y.

DISCUSSION

Reactions of *C. quinoa*, *G. globosa*, *A. caudatus*, and *S. vaccaria* to the virus obtained from the night-blooming cactus *H. undatus* were similar to those to cactus virus X and California barrel cactus virus (3,5). Time required for symptom development in *C. quinoa* was very similar to that reported for cactus virus X (10) and California barrel cactus virus (3). Our virus differed in host reactions in that it did not infect sugar beet, *Beta vulgaris* L., which cactus virus X does (10), and it produced systemic mottling in *S. vaccaria*, whereas California barrel cactus virus produces symptomless systemic infections in this host (3). The thermal inactivation and dilution end points for night-blooming cactus virus also were higher than those reported for both cactus virus X and California barrel cactus virus (3,5). Serological studies indicated, however, that our virus was related to both viruses.

The virus produced striated spindle-shaped inclusions in epidermal cells of infected plants similar to those produced by some strains of cactus virus X and California barrel cactus virus (1–3,5,10). In addition, the virus produced many crystalline inclusions in the epidermal cells of *H. undatus*. These inclusions are specific for cactus virus X and could be useful for diagnosis of virus-infected plants in which external symptoms are not pronounced.

The virus reached high concentrations in *Chenopodium* plants but tended to aggregate when purified from them, as do cactus virus X and California barrel cactus virus (3,8). Nonaggregated virus, however, was readily purified from the epidermal layer of infected *H. undatus* after homogenization in cold phosphate buffer or distilled water. Electron microscopy, infectivity tests, and presence of crystalline inclusions in the epidermal cells indicate that the virus was present in

much higher concentrations in the epidermal layer of the infected cactus *H. undatus*.

Serology, particle morphology, and crystalline aggregation of virus particles in infected cells are typical of potexviruses (2,6,7,9,11,12). The virus in night-blooming cactus is considered to be a strain of cactus virus X and responsible for the disease associated with *H. undatus*. The continued propagation of these plants by clonal cuttings seemingly perpetuates the virus from one generation of plants to another.

LITERATURE CITED

- Amelunxen, F. 1958. Die Virus-EiweiBspindeln der Kakteen. Darstellung, elektronenmikroskopische und biochemische Analyse des Virus. *Protoplasma* 49:140-178.
- Amelunxen, F., and Thaler, I. 1967. Die Feinstruktur der EiweiBspindeln von *Zygoacactus truncatus*. *Z. Pflanzenphysiol.* 57:269-279.
- Attathom, S., Weathers, L. G., and Gumpf, D. J. 1978. Identification and characterization of a potexvirus from California barrel cactus. *Phytopathology* 68:1401-1406.
- Ball, E. M. 1974. Ring interface precipitin test. Pages 7-9 in: Tests for the Identification of Plant Viruses. E. M. Ball, ed. American Phytopathological Society, St. Paul, MN, 31 pp.
- Bercks, R. 1971. Cactus virus X. Descriptions of Plant Viruses. No. 58. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 4 pp.
- Brandes J. 1964. Identifizierung von gestreckten pflanzenpathogenen Viren auf morphologischer Grundlage. *Mitt. Biol. Bundanst. Land. Forstwirtschaft. Berlin-Dahlem* 110:26.
- Doraiswamy, S., and Lesemann, D. 1974. Comparison of cytological effects induced by three members of the Potexvirus group. *Phytopathol. Z.* 81:314-319.
- Koenig, R. 1975. Serological relations of narcissus and papaya mosaic viruses to established members of the Potexvirus group. *Phytopathol. Z.* 84:193-200.
- McWhorter, F. P. 1965. Plant virus inclusions. *Annu. Rev. Phytopathol.* 3:287-312.
- Plese, N., and Milličić, D. 1966. Vergleichende Untersuchungen an isolaten des Kakteen-X-Virus mit Testpflanzen. *Phytopathol. Z.* 55:197-210.
- Purcifull, D. E., Edwardson, J. R., and Christie, R. G. 1966. Electron microscopy of intracellular aggregates in pea (*Pisum sativum*) infected with clover yellow mosaic virus. *Virology* 29:276-284.
- Turner, R. H. 1971. Electron microscopy of cells infected with narcissus mosaic virus. *J. Gen. Virol.* 13:177-179.