A Seedborne Potyvirus Causing Mosaic of Cowpea in India

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ABSTRACT

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A seedborne virus isolated from CM-11 cowpea was identified as a member of the potyvirus group. The isolate resembled blackeye cowpea mosaic more than it resembled bean yellow mosaic virus, based on transmission studies, host range, physical properties, serology, cytology, and electron microscopy. The virus was readily transmitted mechanically and by *Aphis gossypii* in a nonpersistent manner. It was also transmitted through CM-11 cowpea seeds at a level of 41.6%. The virus was inactivated between 60 and 65 C and at dilutions of 10^{-4}. The virus had a restricted host range and infected 10 of 42 hosts tested in the families of Amaranthaceae, Chenopodiaceae, and Leguminosae.

Additional key words: aphid transmission, cowpea seedborne virus

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important food legume (pulse) crop grown in many tropical and subtropical countries; it provides a major source of protein for vegetarians in India. Of the virus diseases that affect cowpeas, seedborne viruses are most important since they inflict heavy losses by providing primary inoculum and secondary spread (7,13,15). Of 23 cowpea cultivars from the All India Coordinated Improvement Project on Pulses at Badnapur, Maharashtra, CM-11 cowpea plants grown in the glasshouse were infected through seeds. The symptoms on primary leaves were mild mosaic, followed by irregular mosaic or yellow mottle, puckering, slight distortion and arching of trifoliolate leaves (Fig. 1). Slight stunting of the plants was also observed.

Studies of transmission, host range, physical properties, serology, cytology, and electron microscopy were done to identify the virus involved in CM-11 cowpea seedborne mosaic disease.

MATERIALS AND METHODS

For mechanical transmission, sap was extracted by triturating symptomatic leaves of cowpea with a mortar and pestle in a cold 0.1 M Tris-buffer, pH 7.0. Test plants were inoculated by a conventional leaf rub method with a cotton swab. Carborundum (800 mesh) was used as an abrasive. The primary leaves of 10 healthy CM-11 cowpea plants were used in the sap inoculation studies. The plants that did not show any symptoms after 4–6 wk were backindexed on *Chenopodium amaranticolor* for the recovery of virus. The test was repeated twice.

For aphid transmission, about 20 nonviruliferous apterous *Aphis gossypii* were given a 50–60 sec acquisition feeding on virus-infected CM-11 cowpea leaves after a 30-min fast and then were transferred to 10 cowpea plants for a 4-hr inoculation feeding. Aphids were later killed by a spray of 0.02% dimethoate insecticide, and plants were maintained in an insectproof glasshouse. The test was repeated twice.

Seeds from infected CM-11 cowpea plants were grown in earthen pots containing steam-sterilized soil, sand, and compost (2:1:1) in an insectproof glasshouse to determine the percentage of seed transmission. Seedborne infection was determined by symptoms on the primary and first trifoliolate leaves. The virus identity was checked by inoculating *C. amaranticolor* plants and by serology.

For host range studies, five plants of each host species (Table 1) were inoculated by the leaf rub method. All leguminous plants were inoculated on primary leaves before the first trifoliolate leaf emerged. All other plants were inoculated on the first leaf or fully expanded leaves. After 6–8 wk, symptomless plants were backindexed on *C. amaranticolor* to detect latent infection.

Thermal inactivation point, dilution end point, and longevity in vitro were studied by the procedures outlined by Bos et al (5). Virus-infected cowpea plants were the sources of crude sap, and *C. amaranticolor* was the assay host. The thermal inactivation point was tested at 5° intervals between 40 and 75 C. The dilution end point was tested between 10^{-1} and 10^{-8} and longevity in vitro between 8 and 64 hr with an interval of 8 hr at 27–30 C. Each study was replicated five times by inoculating five uniform-sized leaves per plant of an assay host. The leaves of an assay host similarly inoculated with untreated or standard extract served as controls.

The virus was serologically tested in drop precipitin tests on slides (3) with antisera of alfalfa mosaic (AMV), bean common mosaic (BCMV), bean yellow mosaic (BYMV), cucumber mosaic (CMV), tobacco ringspot (TRSV), and tobacco mosaic (TMV) viruses. Sap from virus-infected cowpea plants filtered through two layers of cheesecloth or clarified by low-speed centrifugation was used in the serologic reactions. Parallel controls with healthy cowpea sap, normal rabbit serum, and 0.01 M neutral phosphate buffer and 0.15 M NaCl (PBS) as diluents were also included.

For electron microscopy, leaf dip preparations (6) of virus-infected and healthy cowpea and *C. amaranticolor* leaves were made using negative staining (1% uranyl acetate) on carbon-coated grids. The grids were examined in a Philips EM 201C transmission electron microscope.

For cytological studies, epidermal strips from healthy and virus-infected leaves of CM-11 cowpea prepared for light microscopy were either stained with 1% solution of phloxine in water (22) or with phloxine and methylene blue (1:20) as described by Christie (9). In the latter method, the epidermal strips were treated with Triton X-100 for solubilizing plastid bodies (10) before staining.

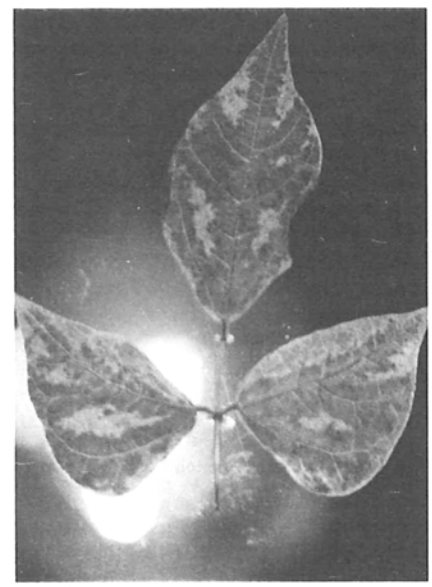

Fig. 1. Trifoliolate leaves of CM-11 cowpea with virus symptoms.

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Table 1. Host range of a potyvirus causing seedborne mosaic in cowpea

Family Species Cultivar	Reactions[a] Local	Systemic
Amaranthaceae		
Beta vulgaris		
M 1001 × 606	–	–
Gomphrena globosa	NLL	–
Aizoaceae		
Teragonia expansa	–	–
Chenopodiaceae		
Chenopodium album	–	–
C. amaranticolor	CLL/NLL (discrete)	–
C. folilosum	–	–
C. murale	RLL	Mt
C. quinoa	CLL/NLL	–
Compositae		
Carthamus tinctorius	–	–
Helianthus annuus		
EC-68414	–	–
Zinnia elegans	–	–
Cucurbitaceae		
Cucumis sativus		
Delicates	–	–
Bangalore Special	–	–
Gramineae		
Zea mays		
Deccan Double Hybrid	–	–
Labiatae		
Ocimum basilicum	–	–
Leguminosae		
Arachis hypogaea		
SB-XI	–	–
Canavalia ensiformis	–	–
Cicer arietinum		
Chaffa	–	–
Cyamopsis tetragonoloba		
Pusa Naubahar	–	–
Dolichos biflorus	NLL (not discrete)	–
D. lablab		
Dharwar-38	–	–
Glycine max		
Monetta	CLL	M
Bragg	–	Mt
Lens esculenta	–	–
Phaseolus lunatus	–	Sev M
P. vulgaris		
Biela Kockova	CLL	M
Perlicka	CLL	–
Pinto	–	Mt
Prince	NLL	M
Processor	–	–
Tender Green	–	–
Top Crop	–	–
Pisum sativum		
B-520	–	–
Bonneville	–	–
Khaperkheda	–	–
Raman	–	–
SL-420	–	–
Trigonella foenum graecum	–	–
Vicia faba		
Slovak Local	–	–
Vigna mungo		
Sindkheda-1-1	–	–
V. radiata		
Jalgaon-781	NLL	Vc, Mt
V. vexillata	–	–
V. unguiculata		
Blackeye	–	Mt
C-152	–	Mt
CG-28	–	Mt
CM-11	–	M
Co. Pusa-4	–	Vb, M
Early Ramshorn	–	Vb, M
K-11	–	–
No. 2-1	–	–
P. 1476	–	–
Solanaceae		
Capsicum annuum		
NP. 46A	–	–
Datura inermis	–	–
D. metel	–	–
Lycopersicon esculentum		
Pusa Ruby	–	–
Nicotiana clevelandii	–	–
N. glutinosa	–	–
N. tabacum		
Samsun	–	–
Xanthi	–	–
White Burley	–	–
Petunia axillaris	–	–
Solanum melongena		
American Black Beauty	–	–
Pedaliaceae		
Sesamum indicum	–	–

[a] CLL = chlorotic local lesions, M = mosaic, Mt = mottle, NLL = necrotic local lesions, RLL = ring local lesions, Sev = severe, Vb = vein banding, Vc = vein clearing, – = host not infected.

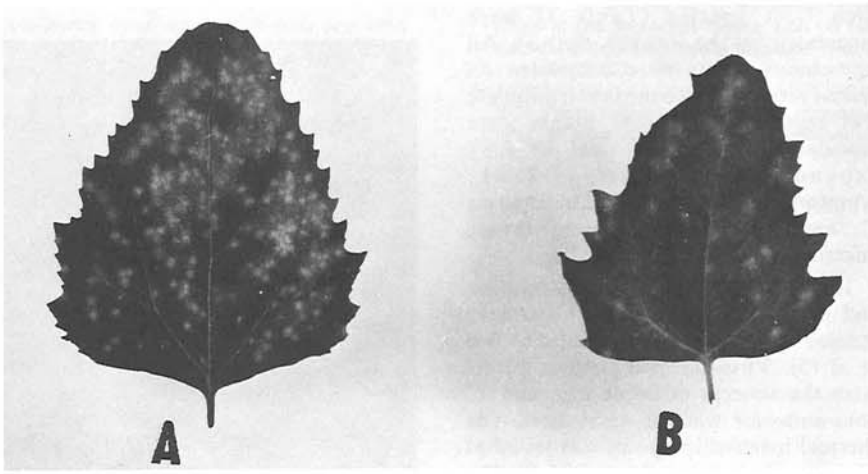

Fig. 2. Local lesions elicited by the virus on **(A)** *Chenopodium amaranticolor* and **(B)** *C. quinoa.*

RESULTS AND DISCUSSION

The virus was readily transmitted from cowpea to cowpea and *C. amaranticolor* plants. All 10 inoculated CM-11 cowpea plants developed systemic symptoms; none became infected locally. However, all 10 *C. amaranticolor* plants developed local lesions (Fig. 2A), and none became infected systemically. The CM-11 virus was also transmitted mechanically to other cowpea cultivars, namely Blackeye, C-152, CG-28, Co. Pusa 4, and Early Ramshorn. All these cultivars reacted systemically, and none became infected locally. K-11, No. 2-1, and P-1476 were not infected by the virus, however, and virus was also not recovered when back-indexed on *C. amaranticolor* (Table 1). The experiment was repeated twice with the same results.

The virus was also transmitted by the cotton aphid, *Aphis gossypii*, in a nonpersistent manner. All 10 CM-11 cowpea plants used in the aphid transmission tests developed characteristic virus symptoms after 4–6 wk. The virus was also transmitted through the seeds of CM-11 cowpea. Of the 206 seedlings grown from seeds derived from CM-11 virus-infected plants, 85 were infected through seeds. The level of seed transmission in CM-11 cowpea was 41.6%.

The virus was inactivated between 60 and 65 C and at dilutions of 10^{-4}. All the inoculated leaves of the assay host became infected at temperatures to 60 C but not at 65 C; however, local lesions decreased progressively as the temperature was increased. All inoculated leaves of the assay host became infected at dilutions to 10^{-3} but not at 10^{-4}; local lesions decreased progressively as the dilutions were increased. The virus was viable for 56 hr but not 64 hr at room temperature. All of the inoculated leaves of the assay host became infected, but lesions decreased progressively as aging of the sap was increased at room temperature.

In filtered or clarified sap from cowpea, the virus did not react with AMV, BCMV, CMV, TMV, or TRSV antisera and was not related serologically to these viruses. However, the virus reacted strongly with the antiserum of BYMV of red clover (TpM3) and broadbean (FvM1) isolates (20).

Antiserum gave precipitin end points of 1:256 with the broadbean isolate (titer: 1,024) and red clover isolate (titer: 1,024), 1:64 with the CM-11 cowpea virus. The antiserum end points with CM-11 cowpea virus antigen demonstrated a closer immunological relationship with broadbean than with red clover isolates of BYMV. There were no serologic reactions between healthy sap from cowpea, normal rabbit serum, or PBS and BYMV antiserum (TpM3 and FvM1).

Electron micrographs of negatively stained leaf dip preparations of CM-11 cowpea and *C. amaranticolor* infected with the virus revealed flexuous rods about 750 nm long (Fig. 3). Similar rod-shaped virus particles were also seen in electron micrographs of CM-11 cowpea leaves infected with the virus from seed but not in preparations from healthy

leaves of cowpea and *C. amaranticolor*. The concentration of virus particles in virus-infected samples was usually low.

The results of cytological studies indicated that in the epidermal strips from virus-infected CM-11 cowpea leaves stained with 1% phloxine, several cytoplasmic granular inclusions were seen, usually in contact with the cell nucleus. The inclusions were deeply stained and smaller than the nucleus. No intranuclear inclusions were observed in any of the test samples. Similarly, cytoplasmic or intranuclear inclusions were not observed in stained epidermal strips.

The results on host range and reactions are shown in Table 1. The virus infected 10 of 42 hosts tested in the families Amaranthaceae, Chenopodiaceae, and Leguminosae but none in Aizoaceae, Compositae, Cucurbitaceae, Labiatae, Solanaceae, and Pedaliaceae. Sugarbeet, New Zealand spinach, *Chenopodium album*, *C. folilosum*, safflower, sunflower, zinnia, cucumber, maize, sweet basil, peanut, pigeonpea, swordbean, chickpea, sunnhemp, guar, hyacinth bean, pea, fenugreek, broadbean, urdbean, *Vigna vexillata*, pepper, jimson weed, *D. inermis*, tomato, tobacco, *Nicotiana clevelandii*, *N. glutinosa*, petunia, brinjal, and sesamum were not hosts of the virus.

By particle morphology, insect transmission, and physical properties, the virus involved in CM-11 seedborne mosaic disease seems to be a member of potyvirus group (14). The present virus is close to bean yellow mosaic virus (BYMV: */*:*/*:E;E:S/Ap.) in serologic relationship (4). BYMV strains from French bean (19,26), broadbean, sweet clover (21), and mungbean (25) have infected cowpeas. Natural infection of cowpeas by BYMV has also been reported from Alabama (12), Florida (1,2), and Georgia (16,17). This virus from cowpea is referred to as the cowpea strain of BYMV (4,17) based on serologic relationship. However, there has been considerable confusion regarding natural infection of cowpea by BYMV.

Recent investigations with virus-induced inclusion bodies have demonstrated that BYMV was incorrectly identified as the cause of a virus disease of cowpea in Georgia and that the virus was an isolate of blackeye cowpea mosaic virus (BICMV) (22). BYMV and BICMV have been reported to be cytologically different and distinct members of the potyvirus group (10). The CM-11 seedborne virus induced cytoplasmic inclusion bodies in cowpea, but the inclusion bodies induced by BYMV and BICMV (10) were not seen in CM-11 cowpea.

Notwithstanding the critical cytological studies, the virus involved in CM-11 seedborne mosaic disease is more like BICMV than BYMV for host range and seed transmissibility in cowpea (27).

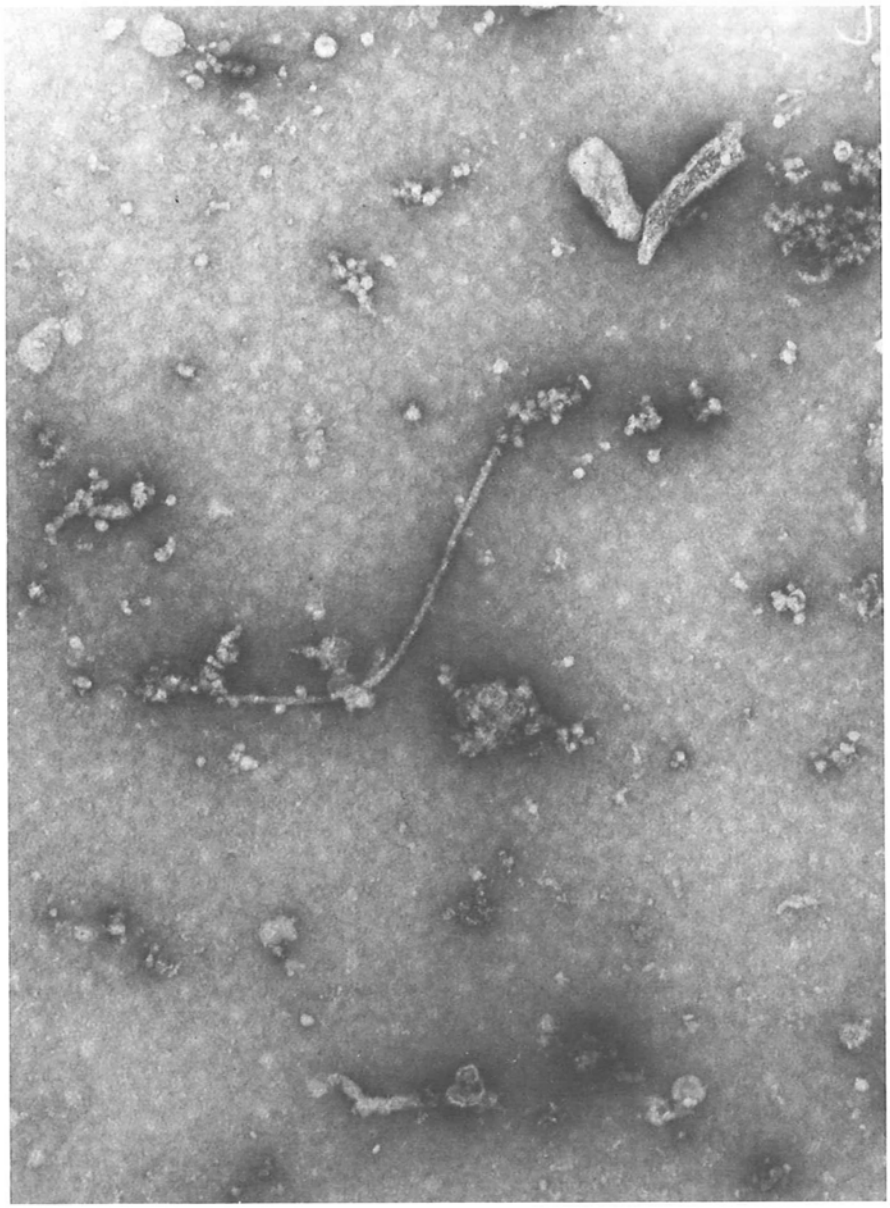

Fig. 3. Electron micrograph of the virus from CM-11 isolate (×50,000). Uranyl acetate-forming aggregates around the particle may be related to the use of too high a beam current. Electron optical magnification is within the range of ± 5%.

However, the level of seed transmission in CM-11 cowpea (41%) was higher than those reported for Kunckle Purple Hull (13–30%) and Ramshorn Blackeye (22%) cowpeas from Florida and Georgia (11,27) but lower than the level of seed transmission in White Acre pea (0–55%) cowpea (2). This might be due to varietal differences.

In India, BCMV, CMV, and TMV (15,23,24) cause seedborne diseases of cowpea, but this is the first report of BICMV causing a seedborne mosaic disease of cowpea in India.

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