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NARROW LEAF DISEASE OF CHICKPEA (CICER ARIETINUM L.) CAUSED BY BEAN YELLOW MOSAIC VIRUS

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ABSTRACT : A virus isolated from *Cicer arietinum* showing narrow leaf disease symptoms was identified as a strain of bean yellow mosaic virus. The virus had a very restricted host range confining to the family leguminosae. Crude sap of infected chickpea was infective after heating for 10 min to 55° but not to 60°C, after dilution to 10^{-3} but not to 10^{-4} and after storing at room temperature (25–28°C) for one but not two days. The virus was transmitted by two aphid species in a non-persistent manner. Electron microscopy of leaf dip preparations and purified virus revealed the presence of long flexuous rods. Pinwheels were observed in ultrathin sections of infected chickpea leaves. The virus reacted with an antiserum to bean yellow mosaic virus but not with that of bean common mosaic virus.

Keywords : Narrow leaf disease, Bean yellow mosaic virus, Chickpea, Aphid transmission, Elec-

Chickpea is an important grain legume of dryland agriculture in Asia, Africa and Central and South America. Six viruses have been reported to naturally infect chickpea in different parts of the world (Nene, 1980). These include alfalfa mosaic virus (AMV) (Erwin and Snyder, 1958; Kaiser and Danesh, 1971; Nene *et al.*, 1978), bean yellow mosaic virus (BYMV) (Kaiser *et al.*, 1968; Kaiser and Danesh, 1971), cucumber mosaic virus (CMV) (Dhingra *et al.*, 1979; Kaiser *et al.*, 1968; Kaiser and Danesh, 1971); lettuce necrotic yellows virus (LNYV) (Corbin, 1975), pea leaf roll virus (PLRV) (Kaiser and Danesh, 1971; Nene and Reddy, 1976) and pea enation mosaic virus (PEMV) (Erwin and Snyder, 1958). Of these, only AMV (Nene *et al.*, 1978), PLRV (Nene and Reddy, 1976) and CMV (Dhingra *et al.*, 1979) occur naturally on chickpea in India. The present paper describes some properties of a sap transmissible virus isolated from chickpea plants which was identified as a strain of BYMV.

MATERIALS AND METHODS : Mechanically infected chickpea leaves were ground with a pre-chilled pestle and mortar in 0.05 M potassium phosphate buffer, *p*H 7.5 containing 0.01 M sodium sulphite and 0.01M sodium diethyldithiocarbamate (DIECA) (1 : 4, w/v). Celite (1 per cent w/v) was added to the inoculum which was rubbed with the broad end of the pestle onto the leaves of test plants. *In vitro* properties of the virus were determined using the procedure described by Bos *et al.* (1960). Since no local lesion host was found, 10 kabuli chickpea (ICC 162) seedlings were inoculated with each treatment in the assay.

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Fig. 1. Symptoms produced by bean yellow mosaic virus on chickpea : A. An infected plant with filiform leaves; B. Showing proliferation of a branch with small pods (right), healthy branch on left; C. Deformed and shrivelled chickpea seeds from an infected plant (right), seeds from a healthy plant on left.

Fig. 2. Electron micrograph of purified bean yellow mosaic virus (BYMV) stained with 2 per cent potassium phosphotungstate (A) and of ultrathin section of BYMV-infected chickpea leaf (B) showing pinwheels (PW) Bar in both cases represents 180 nm.

Two aphid species; viz., *Aphis craccivora* Koch and *Myzus persicae* (Sulzer), were used for transmission studies. They were fasted for an hour before allowing an acquisition feeding period of 1 min on virus-infected chickpea and then transferred to healthy test plants for inoculation feeding periods of 12–14 hrs. Ten to 15 aphids were used per test plant.

For purification of the virus, infected chickpea tissue was homogenized in 0.1 M Tris-phosphate buffer, *p*H 8.5, containing 0.1 per cent thioglycolic acid and 0.01 M Ethylene diamine tetra acetic acid (EDTA). The sap was treated with 25 per cent carbon tetrachloride and centrifuged at 10,000 g for 5 min The aqueous phase was separated from which the virus was precipitated with 4 per cent polyethylene glycol (PEG, 6000). The precipitated virus was separated by differential centrifugation (low speed at 10,000 g for 5 min in Sorvall centrifuge and high speed at 100,000 g for 2 hr in SW-27 rotor in a Beckman L5-50 ultracentrifuge). Pellets from high speed centrifugation were suspended in 0.01 M Tris-phosphate buffer containing 0.2 M urea (TPU). During high speed centrifugation, the virus was pelleted through a sucrose pad (30 per cent sucrose in TPU, containing 4 per cent PEG). Further purification of the virus was achieved through 10-40 per cent sucrose density gradients prepared in TPU. After centrifuging at 100,000 g for 90 min, the light scattering zone was collected with a bent needle attached to a syringe, diluted with TPU and the virus was pelleted by centrifuging at 100,000 g for 90 min. The pellet was dissolved in a small amount of resuspending medium without urea.

For electron microscopy, leaf dip or purified preparations were stained in 2 per cent potassium phosphotungstate and examined in a Philips 201C electron microscope. The normal length of virus particles was determined in leaf dip preparations, following the procedure described by Noordam (1973).

For cytological studies, pieces of infected chickpea leaves were fixed in 3 per cent glutaraldehyde post-fixed in 2 per cent osmium tetroxide, dehydrated with acetone and embedded in epoxy resin. Sections were cut (50-60 nm thick) with a Reichert-Jung ultra-cut microtome, stained for 3 min each in saturated uranyl acetate and 5 per cent lead citrate and observed in the electron microscope.

Serological studies were done using the agar double-diffusion test (Ball, 1974) after treating the antigen (Crude infected sap or purified virus) with 0.3 per cent sodium dodecyl sulphate (Purcifull and Batchelor, 1977).

RESULTS AND DISCUSSION : The virus had a very restricted host range as it infected only the plant species belonging to the family leguminosae. On chickpea, the virus caused drooping of the terminal bud 6-7 days after inoculation. This was followed by proliferation of branches bearing very narrow leaves (Fig. 1A). Infected plants were stunted and bore very small pods (Fig. 1B). The seeds from infected plants were black, small and very much shrivelled (Fig. 1C). The virus produced mosaic in *Canavalia ensiformis* D.C., *Phaseolus vulgaris* L. 'Ababa', and veinal necrosis and mosaic in *P. vulgaris* 'Red kidney' 'Dwarf' and 'Pinto'. The virus did not infect *Tetragonia expansa* Murt., *Gomphrena globosa* L., *Chenopodium amaranticolor* Coste et Ryne, *C. murale* L., *Chrysanthemum* sp., *Cucumis sativus* L., *Cucurbita pepo* L., *Arachis hypogaea* L.; *Cajanus cajan* (L.) Millsp., *Lablab purpureus* (L.) Sweet, *Phaseolus vulgaris*, 'Porrilo', 'Munroe' and 'Lavica', *Pisum sativum* 'Local', *Vicia faba* L., 'Early longpod,' *Vigna unguiculata* (L.) Walp 'Early ramshorn', 'Barsati mutant', and 'Pusa dophasli', *Capsicum annuum* L., *Datura stramonium* L., *Locopersicon esculentum* Mill. 'Pusa ruby', *Nicotiana tabacum* L. 'Xanthi-nc', *N. rustica* L., 'White pathar' and *Petunia hybrida* Vilm.

Crude sap of infected chickpea was infective after heating for 10 min at 55° but not at 60°C, after dilution to 10^{-2} but not to 10^{-3} and after storing at room temperature (25-28°C) for one but not two days. The virus was transmitted by both the

aphid species tested in a non-persistent manner. Purified virus preparations werecomposed of unaggregated virus particles, though there was some degree of breakage (Fig. 2A). Measurement of virus particles from crude sap showed a modal length of 750 nm. Ultrathin sections of infected chickpea leaves (Fig. 2B) showed pinwheel inclusions in the cytoplasm which are typical of potyviruses (Martelli and Russo, 1977). In agar double-diffusion tests, both purified virus and crude sap from infected chickpea reacted with BYMV antiserum obtained from Dr. N. Conti, Laboratorio Fitovirologia Torino, Italy. No reaction was noticed with either the healthy sap or buffer. The virus did not react with antiserum to bean common mosaic virus.

The results indicate that the virus isolated from chickpea is a strain of BYMV. Bean yellow mosaic virus has been reported on chickpea from Iran and U.S.A. (Erwin and Snyder, 1958; Kaiser *et al.*, 1968; Kaiser and Danesh, 1971). This is the first report of its natural occurrence in chickpea in India. Presently the natural incidenec of narrow leaf disease of chickpea in India, as shown by our surveys, is less than 1 per cent. However, a possibility of BYMV becoming widespread in chickpea in future cannot be ruled out considering the inclusion of several leguminous hosts in the current cropping patterns. This warrants identification of the sources of resistance to this virus in chickpea, and we have initiated a screening programme in our Institute.

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