Etiology of Virus-Induced Wilt of Cicer arietinum

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ABSTRACT

In Iran, chickpeas (*Cicer arietinum*) are naturally affected by four viruses: alfalfa mosaic (AMV); bean yellow mosaic (BYMV); cucumber mosaic (CMV); and pea leaf roll (PLRV); which cause wilting symptoms in this host. Symptomatology, host range studies, physical property tests, vector-virus relationships, serology, and electron microscopy were utilized in virus identification. In addition to wilting, virus diseases caused stunting, yellowing, abundant formation of secondary shoots, leaf deformation, dwarfing, and phloem discoloration. Microorganisms were seldom isolated from the vascular elements of stems of diseased chickpeas, nor did they reproduce the disease symptoms in greenhouse inoculation studies. However, inoculation with the different viruses produced disease symptoms similar or identical to those observed in nature. All viruses, except PLRV, were juice-transmissible and stylet-borne by *Aphis craccivora*. PLRV was transmitted only by aphids in a circulative manner or by grafting. Phytopathology 61:453-457.

Additional key words: virus disease, aphid transmission of viruses, food legume.

Chickpea (*Cicer arietinum* L.), also called garbanzo bean or Bengal gram, is the most important high-protein pulse crop (food legume) cultivated in the Middle East and Southeast Asia. In 1966, over 26 million acres (10.4 million hectares) of *Cicer* were grown in this region (7). In Iran, chickpeas are an important staple grown on approx 75,000 hectares as a rain-fed or irrigated crop (9).

Diseased chickpeas with wilting and yellowing symptoms have been reported from California (4, 5, 6, 16), India (10, 12, 19), and Iran (9). Although usually attributed to fungi, investigators in California (6, 16) and Iran (9) reported wilting to be caused by different viruses.

We have collected wilted plants in various regions of Iran since 1966 (9). The results of a study to determine the etiology of wilting are reported here.

MATERIALS AND METHODS.—Diseased and healthy chickpeas were collected from various areas and cultured for fungi or assayed for virus infection. At times, nonviruliferous aphids (*Aphis craccivora* Koch) were fed in leaf cages for 48-72 hr on diseased plants before being transferred to healthy indicator plants in the greenhouse for a 72-hr inoculation feeding period.

Plant tissues were ground in 1% K_2HPO_4, pH 7.0, or 0.1 M phosphate buffer, pH 7.0, with a mortar and pestle and rubbed with the thumb and forefinger on Carborundum-dusted leaves of several indicator plants. Differential hosts included species in the Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Leguminosae, and Solanaceae (Table 1). Plants were inoculated when young, but the age varied with the species.

Virus isolates, with the original host, were as follows: alfalfa mosaic virus (AMV), chickpea; bean yellow mosaic virus (BYMV), chickpea; cucumber mosaic virus (CMV), chickpea; and pea leaf roll virus (PLRV), broadbean (*Vicia faba* L.). Virus isolates were maintained in the following hosts: AMV, chickpea and tobacco (*N. tabacum* L. 'Xanthi-nc'); BYMV, broadbean and white sweetclover (*Melilotus alba* Desr.); CMV, cucumber (*Cucumis sativus* L.) and tobacco (*N. glutinosa* L.); and PLRV, broadbean.

Physical property studies were carried out with one isolate of AMV and BYMV, following the procedures outlined by Bos et al. (2). Infectivity assays were conducted on cowpea (*Vigna sinensis* [Torner] Savi 'Early Ramshorn') and broadbean (Algerian) for AMV and BYMV, respectively. Serology tests utilizing the Ouchterlony agar double-diffusion technique (1) were used in the identification of greenhouse and naturally infected plant specimens of AMV and CMV.

Fungus isolations were made by plating surface-sterilized pieces of root and stem tissue on water agar (WA), potato-dextrose agar (PDA), and acidified potato-dextrose agar, pH 4.0-4.5 (APDA). Cultures were maintained on slants of PDA. Pathogenicity tests were

TABLE 1. Host range, aphid transmission, and physical properties of four viruses affecting chickpeas (*Cicer arietinum*) in Iran

Host, vector or physical property[a]	Virus[b]			
	AMV	BYMV	CMV	PLRV
Amaranthaceae				
Gomphrena globosa L.	LL[c]	—	LL	—
Chenopodiaceae				
Chenopodium amaranticolor Coste & Reyn.	LL	LL	LL	—
Cucurbitaceae				
Cucumis sativus L. 'National Pickling'	S	—	S	—
Leguminosae				
Cicer arietinum L. 'Ghazvin'	S	S	S	S
Dolichos lablab L.	S	—	—	—
Glycine max (L.) Merr. 'Chippewa'	S	—	—	S
Lens esculenta Moench 'Ghazvin'	S	S	S	S
Medicago sativa L. 'Yazdi'	—	—	—	S
Melilotus alba Desr.	—	S	S	—
Phaseolus lunatus L. 'Jackson Wonder'	—	—	S	—
Phaseolus vulgaris L. 'Bountiful'	LL, VN	S	—	S
P. vulgaris 'Great Northern U.I. 123'	LL, VN	—	—	S
P. vulgaris 'Pinto U.I. 111'	LL, VN	S	—	—
P. vulgaris 'Stringless Green Refugee'	LL, VN, S	S	—	S
P. vulgaris 'Tendercrop'	LL, VN	S	—	S
P. vulgaris 'Topcrop'	LL, VN	S	—	—
P. vulgaris 'U.S. 5 Refugee'	LL, VN, S	S	—	S
Pisum sativum L. 'Dwarf Telephone'	S	S	LL, S	S
Vicia faba L. 'Algerian'	S	S	LL	S
Vigna sinensis (Torner) Savi 'Early Ramshorn'	LL	—	S	—
Solanaceae				
Datura stramonium L.	—	—	S	—
Nicotiana glutinosa L.	—	—	S	—
N. tabacum L. 'Xanthi-nc'	S	—	S	—
Insect vector: *Aphis craccivora*	+, SB[d]	+, SB	+, SB	+, C
Thermal inactivation point	55-60 C	55-60 C		
Dilution end point	10^{-3}-10^{-4}	10^{-4}-10^{-5}		
Longevity in vitro	2-4 d	8-16 d		

[a] Differential hosts are listed alphabetically by family and genus.
[b] Viruses are: AMV (alfalfa mosaic); BYMV (bean yellow mosaic); CMV (cucumber mosaic); and PLRV (pea leaf roll).
[c] LL = local lesions; S = systemic; VN = vein necrosis; — = not susceptible; d = days.
[d] + = positive virus transmission; C = circulative manner; SB = stylet-borne manner.

conducted by incorporating macerated PDA cultures or cornmeal:sand (5-95%, v/v) inoculum of each fungus in pasteurized soil, and planting surface-sterilized chickpea seed in the infested soil. Soil was also collected from beneath diseased chickpeas in the field, and planted to chickpea seed in the greenhouse.

RESULTS.—*Symptoms of disease.*—Symptoms commonly observed were wilting (Fig. 1-C), yellowing and mottling of the foliage, stunting, leaf deformation (Fig. 1-E, F), stimulation of the axillary buds (Fig. 1-A, B, D), shortening of internodes (Fig. 1-D), and discoloration of phloem. Roots of diseased plants generally showed no necrosis or discoloration. Wilting usually began in the shoot and progressed downward, eventually resulting in severe stunting or death of the affected plant. The varied sequence and types of symptoms exhibited by diseased plants suggested that a complex of pathogens and/or other factors were involved in the chickpea wilting disease prevalent in Iran.

The relationship of microorganisms to wilting of chickpeas.—Numerous isolations were made from the foliage and vascular elements of stem and roots of plants exhibiting wilting, stunting, yellowing, and phloem discoloration. Microorganisms were seldom isolated from foliar parts of wilted plants. From roots of these plants, *Fusarium oxysporum* Schlecht. and *F. solani* (Mart.) Appel & Wr. were most frequently isolated. No fungi or bacteria isolated produced symptoms in controlled greenhouse pathogenicity tests, nor were they produced in chickpea planted in field soil collected from beneath wilted chickpea plants. However, four viruses were isolated from diseased chickpea. In the greenhouse and field we reproduced symptoms of wilting, stunting, yellowing, and phloem discoloration in

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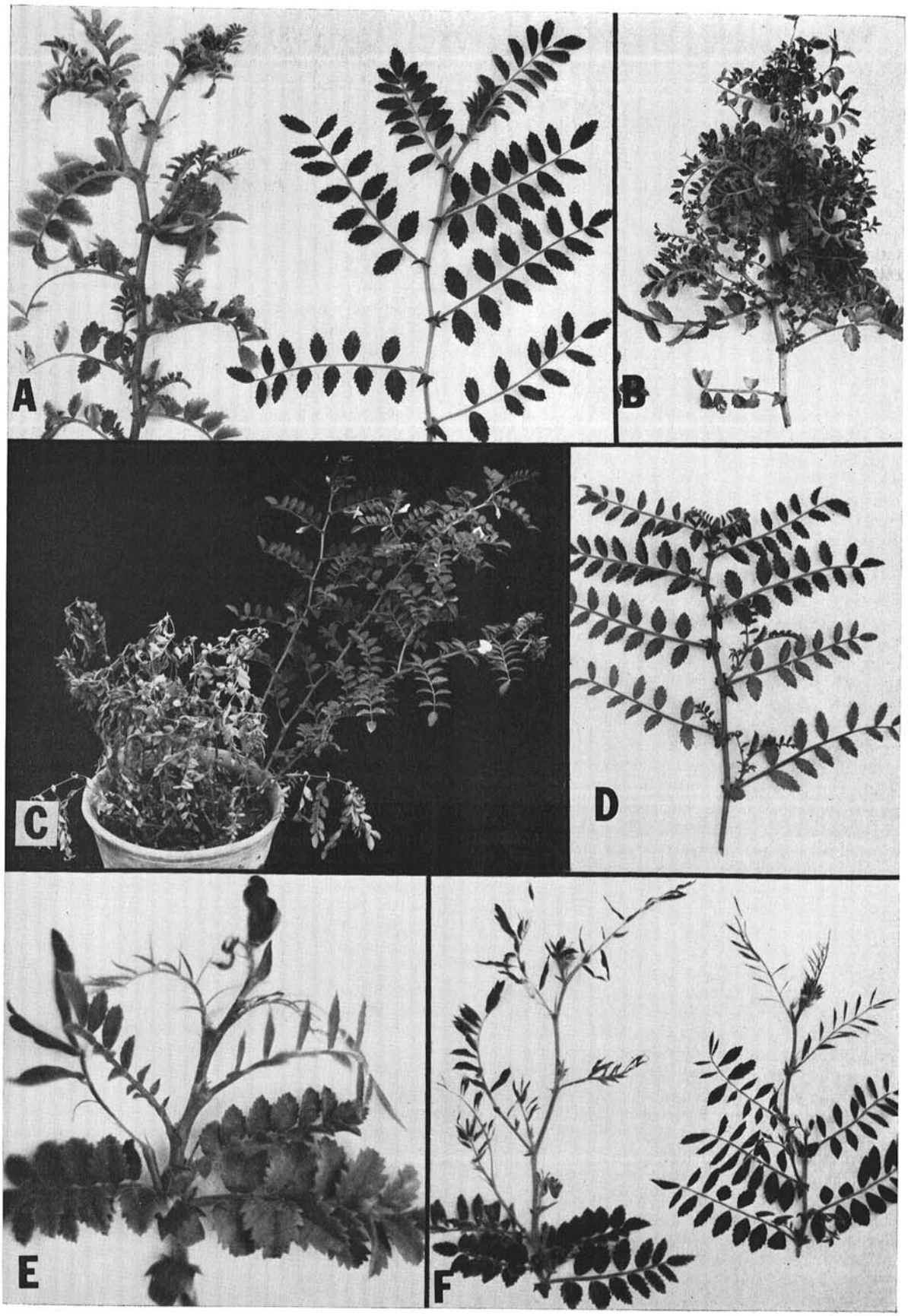
Fig. 1. Symptoms produced by alfalfa mosaic (AMV), bean yellow mosaic (BYMV), cucumber mosaic (CMV), and pea leaf roll (PLRV) viruses in chickpea. **A)** Yellowing and branching caused by AMV (left); healthy branch (right). **B)** Bushiness and shortening of internodes with CMV. **C)** Wilting and yellowing symptoms produced by a virulent strain of BYMV; healthy plant in background. **D)** Axillary bud development and leaf-curling with PLRV. **E)** Feathery, deformed leaves caused by a less virulent strain of BYMV. **F)** Similar symptoms produced by strains of BYMV (left) and CMV (right).


A
B
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F

chickpeas by inoculating with different isolates of AMV, BYMV, CMV, and PLRV, singly or in combination.

Identification of chickpea viruses.—AMV.—The host range of AMV included plants in the following families: Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Leguminosae, and Solanaceae (Table 1). In Ouchterlony agar double-diffusion tests, crude juice of virus-infected chickpea, tobacco, and cucumber formed strong precipitation bands when reacted with AMV antiserum, indicating a serological relationship to AMV.

BYMV.—With the exception of *Chenodopium amaranticolor* Coste & Reyn., all hosts of BYMV were of the Leguminosae (Table 1). One Iranian and two German isolates of BYMV reacted with BYMV antiserum (titer 1/8,000) at antiserum dilutions up to 1/320. Electron microscopy of leaf dip preparations showed that infection was associated with flexuous, rod-shaped virus particles about 750 mμ in length.

CMV.—Several strains of CMV isolated from different pulse crops in Iran varied greatly in host range and symptomatology. Five CMV isolates from chickpea had host ranges similar to the CMV isolate shown in Table 1. Serological tests indicated that this isolate was related to CMV.

PLRV.—Repeated attempts to transmit the virus by sap inoculation to different test plants failed. The virus was transmitted only with aphids and by grafting. Iranian isolates of PLRV belong to the group of circulative aphid-borne viruses which are not sap-transmitted (13, 17, 18). The host range of different PLRV isolates was confined to the Leguminosae (Table 1).

No virus particles were observed in electron micrographs of leaf dip preparations. Serology tests with antisera of several legume viruses were negative. There have been no reports in the literature concerning the morphological or serological properties of PLRV.

Symptoms of virus-infected chickpeas.—Wilting, stunting, yellowing, proliferation of axillary buds, leaf deformation, shortening of internodes, and phloem discoloration were symptoms most commonly produced in chickpea infected with AMV, BYMV, CMV and PLRV. The sequence and/or severity of these symptoms varied among and between isolates of the four viruses. Virus symptoms often observed in naturally infected or greenhouse-inoculated chickpea are shown in Fig. 1-A-F.

Discussion.—The four viruses AMV, BYMV, CMV, and PLRV are widely distributed in most chickpea-growing regions, and contribute annually to the low and erratic yields produced in some areas of Iran. Several viruses have been reported as infecting chickpeas in nature (6, 9, 16), but this is apparently the first known report of natural infection of *Cicer* by PLRV. With the exception of BYMV (6, 16), the host range of *Cicer* viruses has not been explored. Symptoms in chickpeas infected with some isolates of BYMV from Iran differ from those of isolates from California (6, 16), suggesting strain differences.

Other viruses may later be shown capable of inducing wilting symptoms in this host. Snyder et al. (16) reported that chickpeas were infected by several viruses in California, including pea enation mosaic (PEMV). PEMV was isolated recently from diseased chickpeas and peas (K. Izadpanah, *personal communication*) in Fars Province, Iran. Also, Severin & Henderson (14) established the susceptibility of chickpeas to curly top virus (CTV) in greenhouse trials. In 1966, Gibson (8) reported the possible incidence of CTV in plantings of sugarbeet (*Beta vulgaris* L.) in Southern Iran (Fars Province), and Kheyri et al. (11) isolated the virus from naturally infected sugarbeets growing in this area. Iranian isolates of CTV infected Bountiful and Tendercrop beans in greenhouse studies (W. J. Kaiser, *unpublished data*), and will probably infect other pulses, including *Cicer,* when plantings of these crops are located near CTV-infected sugarbeets.

Diseased chickpeas observed by the senior author in Jordan, Turkey, and India appeared to be virus-infected. Although no virus identifications were made, the symptoms of yellowing, stunting, and phloem discoloration were similar to those observed in chickpeas naturally infected by viruses in Iran.

Viruses affecting chickpeas are usually difficult to identify with certainty from symptoms observed in the field. Some strains of BYMV and CMV produce similar symptoms in chickpea. Virus-induced wilting and yellowing symptoms in *Cicer* can easily be confused with diseases caused by species of *Fusarium, Operculella,* and *Verticillium* (4, 5, 6, 10, 12, 19). Therefore, other criteria, in addition to symptomatology, should be used to establish the identity of viruses affecting chickpeas in nature.

Symptoms on indicator plants, physical properties and aphid-transmissibility generally agreed with those compiled by Smith (15) and others (6, 16, 20) for AMV, BYMV, and CMV. Serological tests were utilized to confirm the identity of AMV, BYMV, and CMV from diseased chickpeas after tentative identifications had been made by other methods. Electron microscopy indicated that chickpea viruses with long, flexuous rods about 750 mμ in length belonged to the potato virus Y group of rod-shaped viruses, a group to which BYMV belongs (3). The mechanically transmissible chickpea viruses were all transmitted by *A. craccivora* in a stylet-borne manner. Since little published information was available concerning the physiochemical and serological properties, or morphology of PLRV, a circulative virus, isolates of this virus were identified by symptomatology, host range, and insect transmission (15, 16, 18, 20).

Fungi isolated from the roots and stems of diseased chickpea failed to reproduce symptoms of stunting, wilting, yellowing, or phloem discoloration in this host. However, similar or identical symptoms were reproduced consistently in field and greenhouse inoculations with several viruses isolated from diseased chickpeas in nature. All strains of AMV, BYMV, and PLRV isolated from different reservoir hosts, especially alfalfa (*Medicago sativa* L.) and white sweetclover, were

pathogenic to chickpea in greenhouse inoculation studies, whereas some strains of CMV encountered in nature failed to infect this host.

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