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Biology of Four Viruses Affecting Cicer arietinum in Iran

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ABSTRACT

The biology of alfalfa mosaic (AMV), bean yellow mosaic (BYMV), cucumber mosaic (CMV), and pea leaf roll (PLRV) viruses, all infectious to chickpea (*Cicer arietinum*), was studied under field conditions in pulse-growing regions of Iran. Annual and perennial forage legumes, weeds, and cultivated crops were found to be important reservoir and overwintering hosts of viruses affecting chickpeas. Three aphid species, *Aphis craccivora*, *Acyrthosiphon pisum*, and *Acyrthosiphon sesbaniae*, were primarily responsible for the spread of viruses from alternate hosts into and, subsequently, within chickpea plantings. All viruses except PLRV were transmitted by aphids in a stylet-borne manner, but aphid vectors transmitted PLRV in a circulative manner. Natural spread and incidence of viruses in chickpea plantings varied from 3 to 13%, and were usually related to the proximity of chickpeas to virus-infected reservoir hosts. Virus infection reduced yields and increased plant mortality. Although there were differences among viruses and isolates, yield reductions (92-100%) and mortality (14-99%) were greatest in field inoculation studies when plants were infected before flowering. PLRV caused the largest yield reductions and the highest plant mortality. Chickpea viruses were not seed-borne in this host; however, seeds from diseased plants were often discolored, deformed, and shrivelled, and the germination was adversely affected. Phytopathology 61: 372-375.

Additional key words: food legume, aphid transmission.

Chickpeas (*Cicer arietinum* L.) are the most important pulse crop grown in the Middle East and in Southeast Asia (3). Diseases are an important factor contributing to the low and erratic yields obtained in many areas of the world (1, 2, 4, 5, 6, 9).

Of the several diseases attacking chickpea in Iran, those caused by viruses are more widely distributed and of greater importance in adversely affecting annual chickpea production (5). Virus diseases of *Cicer* were commonly observed in chickpea plantings, but virus infection varied with the season and locality. With the exception of brief reports from California (1, 6) and Iran (4, 5), very little has been published concerning the identity of viruses affecting chickpeas in nature. Four viruses identified as alfalfa mosaic (AMV), bean yellow mosaic (BYMV), cucumber mosaic (CMV), and pea leaf roll (PLRV) (4, 5) were isolated from naturally infected chickpeas in Iran, but knowledge of spread, vectors, alternate and reservoir hosts, or effects of virus infection on growth, yields, and mortality of *C. arietinum* is very limited. Therefore, we sought to elucidate various aspects of the field biology and epidemiology of the viruses which affect this food crop in Iran.

MATERIALS AND METHODS.—Isolates of four chickpea viruses from different hosts and localities in Iran used in greenhouse and field studies were: AMV 1, *Trifolium* sp; AMV 2, cowpea (*Vigna sinensis* [Torner] Savi 'Early Ramshorn'); AMV 3, chickpea; BYMV 4, common vetch (*Vicia villosa* Roth); BYMV 5, white sweetclover (*Melilotus alba* Desr.); BYMV 6, chickpea; CMV 7, chickpea; CMV 8, chickpea; PLRV 9, broadbean (*Vicia faba* L. 'Algerian').

Inoculations with isolates of four viruses were conducted in replicated field trials at Karaj, Iran, with a local white-seeded chickpea variety in plots which were two rows wide and 5 m long with four replications of each virus isolate per inoculation date. Inoculum of each isolate was prepared by grinding virus-infected plant tissue of each sap transmissible virus (AMV, BYMV, CMV) with a mortar and pestle in 0.01 M phosphate buffer, pH 7.0, and 320-mesh Carborundum. Immediately after trituration, the homogenates were rubbed on leaflets of chickpea plants with the thumb and forefinger. Plants were inoculated at two stages of growth, prebloom (about 4 weeks after planting) and full bloom (about 9 weeks after planting). With PLRV, which is not sap-transmitted (4, 8), aphids (*Aphis craccivora* Koch) were given a 6-10 day acquisition feeding period on PLRV-infected broadbeans before being transferred in groups of 15-20 to chickpeas in small leaf cages. After a 3-day inoculation feeding, aphids were killed with an application of a dimethoate spray. All plots in the field trial were sprayed at 15-20 day intervals with liquid formulations of dimethoate or diazinon. Prior to each inoculation, plots were rogued of all unhealthy appearing plants. Twenty to 25 days after inoculation, plants exhibiting virus symptoms

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were tagged, and dead plants were counted in each plot 11-12 weeks after seeding. At harvest time, seed yields were determined from 25 tagged plants in each plot.

Transmission of chickpea viruses by insect vectors was studied in the greenhouse and field. Colonies of aphid species were reared on healthy host plants in the greenhouse. Aphids collected from diseased chickpeas and alternate hosts in the field were transferred in groups of 1-20 to healthy indicator plants in leaf cages for 18-72 hr. In greenhouse tests, aphids were starved for 1-3 hr before being given acquisition feeding periods of less than 1 min (duration of a single probe) to 72 hr on virus-infected source plants. They were then transferred to healthy test plants in groups of 1 to 20 for an 18- to 72-hr inoculation feeding. At the termination of each test, plants were sprayed with liquid formulations of malathion or diazinon. Aphids collected from virus-infected chickpeas and alternate hosts, and used in the vector studies, were identified as *Aphis craccivora*, *Acyrthosiphon pisum* Harris, and *Acyrthosiphon sesbaniae* David.

Chickpea plantings in the Karaj area were surveyed at periodic intervals for virus infection and spread. The effect of infection on plant growth, seed yields, and mortality were observed. The importance of weeds, forage legumes, and cultivated plants as alternate and reservoir hosts of the aphid vectors and viruses in different areas of Iran was determined. Viruses were identified by symptomatology, host range, insect transmission, electron microscopy, and serology (5).

RESULTS.—*Effect of virus infection on yields and mortality of chickpea.*—Virus infection always resulted in decreased seed yield and usually in increased mortality (Table 1, Fig. 1, above). Seed yields from chickpea plants infected at prebloom by each virus isolate were reduced by 92-100%, while those infected at full bloom were decreased by 83-94%. Largest yield reductions occurred from plants infected with PLRV. Seeds from virus-infected plants were often deformed, discolored, and shrivelled (Fig. 1, below), and the percentage of germination was reduced.

Host mortality varied between virus isolates, and was influenced by the stage of plant development at time of infection. Mortality was higher (14-99%), except for BYMV, isolate 6, in plants infected prior to flowering. Mortality of plants inoculated at the prebloom and full bloom stages of growth varied from 14-99% to 0-71%, respectively. PLRV was the most lethal virus included in these studies.

Insect transmission studies.—Three aphid species, *Aphis craccivora*, *Acyrthosiphon pisum*, and *Acyrthosiphon sesbaniae*, were implicated on the basis of field observation as principal vectors of viruses infecting chickpeas in Iran included in the transmission studies. The aphids acquired AMV, BYMV, and CMV from virus-infected test plants in a stylet-borne manner (7) with brief probes of less than 2 min; however, transmission of these viruses by groups of 1-5 aphids was usually less than 20% when virus-infected chickpeas were used as source plants or young chickpeas as assay plants. Aphid transmission was invariably higher when annual or perennial hosts of these viruses, e.g., white sweetclover and BYMV, or cucumber (*Cucumis sativus* L.) and CMV, were used instead of chickpea as source and assay plants

The vector-virus relationships of PLRV differed markedly from those of the other stylet-borne chickpea viruses, but were similar to those of circulative viruses (4, 7, 8). Aphids did not acquire PLRV in brief probes. Transmission occurred only when aphids were given acquisition feeding periods of 3-6 hr on diseased plants. The probability of acquiring the virus increased with longer feeding periods on infected plants up to 48 hr. Once PLRV was acquired, vectors remained infective for several days, even after molting (ecdyse), indicating that the virus had entered the vector's body cavity.

Virus symptoms in chickpea resulting from aphid transmission under controlled conditions were similar or identical to those generally observed in naturally infected (also aphid-transmitted) or mechanically inoculated chickpeas.

Annual and perennial hosts of chickpea viruses.—Alfalfa (*Medicago sativa* L.) was the main reservoir

TABLE 1. Effect of infection by four viruses at two stages of plant growth on seed yield and mortality of chickpea in field inoculation trials at Karaj, Iran

		Prebloom[a]			Full bloom		
Virus[b]	Isolate	Yield, g[c]	% Decrease in yield	% Mortality	Yield, g	% Decrease in yield	% Mortality
Healthy control		2,015	0	0	2,015	0	0
AMV	1	13	99.4	65.3	272	86.5	0
AMV	2	18	99.1	64.0	344	82.9	1.3
AMV	3	81	96.0	82.5	322	84.0	71.2
BYMV	4	8	99.6	45.0	302	85.0	19.7
BYMV	5	0	100.0	78.7	298	85.2	9.2
BYMV	6	161	92.0	13.9	322	84.0	35.8
CMV	7	7	99.7	59.8	257	87.2	2.9
CMV	8	45	97.8	36.8	273	86.5	16.5
PLRV	9	1	99.9	99.0	117	94.2	

[a] Plants were inoculated 28-34 days (prebloom) and 64-70 (full bloom) after planting, respectively.
[b] Viruses used in field trials were: alfalfa mosaic (AMV); bean yellow mosaic (BYMV); cucumber mosaic (CMV); pea leaf roll (PLRV).
[c] Seed yield from 100 plants.

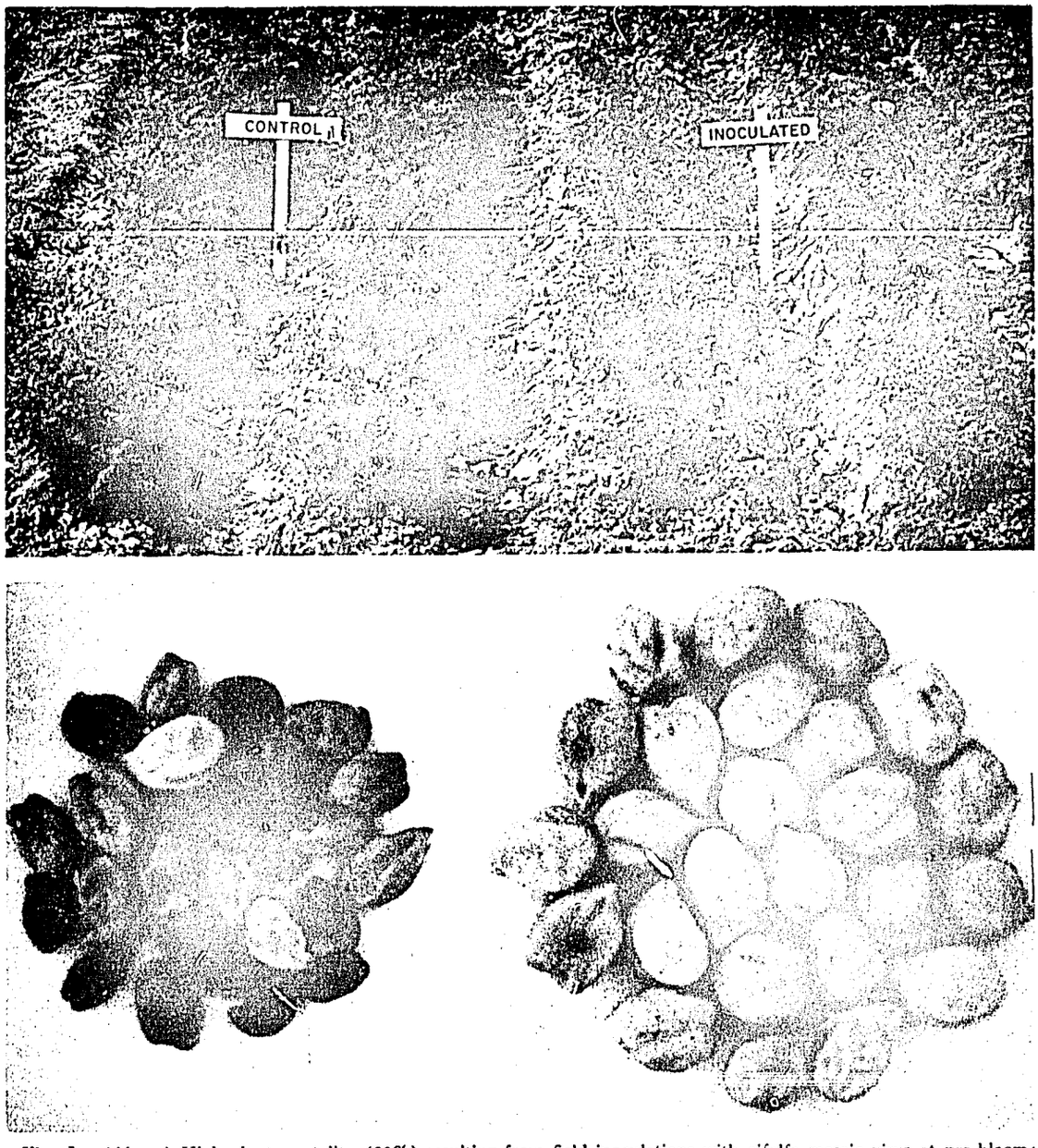

Fig. 1. (Above) High plant mortality (82%) resulting from field inoculations with alfalfa mosaic virus at pre-bloom; surviving plants were stunted and chlorotic when compared to the healthy plants, left. (Below) Discolored, deformed, and shrivelled chickpea seeds (left) from plants infected with bean yellow mosaic virus compared with seeds from healthy plants (right).

and overwintering host of AMV and PLRV in Iran. Most plants naturally infected by BYMV, with the exception of *Gladiolus* sp., were legumes. White sweetclover was the major reservoir of BYMV and its aphid vectors. Important reservoir hosts of CMV were cucumber, melon (*Cucumis melo* L.), eggplant (*Solanum melongena* L.), and other vegetables, but not all strains infect chickpea. Similarly, all natural hosts of PLRV were legumes. Previously unrecorded hosts of PLRV were *Lens esculenta* Moench, *Medicago hispida* Gaertn., *Medicago lupulina* L., *Melilotus indica* L., *Trifolium resupinatum* L., and *Vigna sinensis*.

Seed transmission studies.—To determine whether the viruses were seed-borne in chickpea, seeds from virus-infected plants were sown in pasteurized soil in the greenhouse. Abnormal plants were tagged at periodic intervals, and after 4-6 weeks virus transmission from these and normal seedlings to healthy indicator test plants was attempted. None was observed in 300-1,000 seeds obtained from chickpea plants infected

TABLE 2. Natural spread of alfalfa mosaic, bean yellow mosaic, and pea leaf roll viruses in a planting of chickpea (*Cicer arietinum*) at Karaj, Iran, and effect of virus infection on seed yield and plant mortality

Weeks after planting	% Total virus infection[a]	% Decrease in yield[b]	% Decrease in 100 seeds	% Mortality[c]
8	3.8	99	33.3	76
10	5.7	98	36.4	75
12	9.1	92	31.8	82

[a] All viruses are grouped together in the totals.
[b] Seed yields were from 144 plants.
[c] Plant mortality was recorded 14 weeks after planting.

with AMV, BYMV, CMV, or PLRV. Germination of shrivelled and discolored seeds from plants infected before flowering was usually erratic and reduced.

In the Karaj area, white sweetclover was the primary overwintering host of BYMV, but no seed transmission was observed in more than 1,000 seedlings raised from seed of diseased plants.

Natural spread and/or incidence of viruses in chickpea plantings.—Observations on the natural spread of viruses in chickpea plantings near alfalfa at Karaj, Iran, showed that incidence of virus-infected plants increased from 3.8% to 9.1%, 8 and 12 weeks after seeding, respectively (Table 2). Yields from diseased plants were reduced 92-99% below the control, and plant mortality varied from 75-82%. From virus-infected plants, the wt of 100 seeds, many of which were shrivelled and discolored, was reduced by 31.8-36.4% (Table 2).

Percentage of infection of BYMV in a planting of six chickpea selections (12 weeks after seeding) surrounded on three sides by irrigation ditches containing virus-infected white sweetclover ranged from 5 to 13 (Table 3). Infected plants were stunted and devoid of pods, as reflected by yield reductions of 98-100%. Incidence of virus infection seemed to be related to the presence of virus-infected alternate hosts, and appeared to increase as the distance between these reservoirs and chickpea plantings decreased.

TABLE 3. Incidence of bean yellow mosaic virus in a planting of six chickpea selections at Karaj, Iran, and the effect of virus infection on seed yield

Selection	Virus infection	Seed yield from 25 plants	
		Healthy	Diseased
	%	g	g
W 1[a]	7	407	3
W 2	6	389	6
W 3	13	372	6
B 1	5	421	1
B 2	10	287	2
B 3	6	281	2

[a] W refers to white-seeded and B to black-seeded chickpea types.

DISCUSSION.—Virus diseases of chickpea may have escaped detection in many countries where this crop is cultivated, since the macroscopic symptoms produced in virus-infected chickpeas are often very similar to those caused by wilt or root-rot fungi (1, 4), and could be overlooked unless transmission studies were performed to detect virus infection.

The development of resistant varieties would provide the most efficient means of control, and we have conducted disease surveys attempting to locate sources of resistance. Promising lines are also being screened for resistance in greenhouse trials. Preliminary results indicate that most white-seeded selections of chickpea tested have little or no resistance to virus infection, although the black-seeded types appear to be more resistant to most pathogens, including viruses. It will be necessary to develop varieties resistant to virus isolates in different chickpea-growing regions of the country, since most viruses infecting *Cicer* are composed of a multiplicity of strains which vary greatly in their reaction and virulence to chickpea and other hosts (4). In the meantime, chickpea plantings should not be established near important reservoir hosts of viruses infectious to this crop.

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