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NATURAL INFECTION OF COWPEA AND MUNG BEAN BY ALFALFA MOSAIC VIRUS IN IRAN

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ABSTRACT

Field plantings of cowpea (Vigna unguiculata) and mung bean (Vigna radiata) in Iran were naturally infected by alfalfa mosaic virus (AMV) in 1966-1971. Cowpea and mung bean plants typically were stunted, and their foliage exhibited yellow mosaic (calico) symptoms, as did the pods of some infected cowpea accessions. The incidence of AMV in cowpea and mung bean plantings was usually less than 10%. Inoculation of cowpea in the field with AMV strains from cowpea or alfalfa at the full bloom and prebloom stages reduced seed yields about 15% and 50%, respectively. No mortality of infected plants was observed in these studies. The virus was transmitted by the pea aphid (Acyrthosiphon pisum) in a stylet-borne (noncirculative) manner from AMV-infected alfalfa and cowpea to healthy cowpea. Seeds harvested from naturally or artificially infected cowpeas apparently did not transmit the virus. Identification of AMV strains isolated from naturally infected food and forage legumes was based on symptomatology, host range, host reactions, serology, and vector transmission mode.

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Cowpea (Vigna unguiculata) and mung bean (Vigna radiata) are two of several food legumes grown in Iran for human consumption. At least four aphid-transmitted viruses infect both crops in various areas of the country (4, 5, 6, 7). Cowpea aphid-borne mosaic virus (CAMV) was the most important and widely distributed virus affecting cowpeas (7), whereas a strain of bean common mosaic virus caused the most important disease of mung bean in Iran (6). Alfalfa mosaic virus (AMV) was also frequently encountered as a pathogen of cowpea and mung bean in that country. The primary reservoir of AMV in Iran is alfalfa (author, unpublished data), which is also the most widely cultivated forage legume. The present study was initiated to describe the diseases of cowpea and mung bean caused by AMV and to investigate various properties of the virus.

MATERIALS AND METHODS

Tissue from cowpea, mung bean and other food and forage legumes were collected in various agricultural regions of Iran from 1966-1971. Diagnostic studies of the tissue were done at Karaj, Iran using standard methods for mechanically transmitted viruses. Test plants were grown in pasteurized soil at 15-30°C in the greenhouse. Insecticides were applied to prevent the buildup of insects.

Isolates of AMV used were: cowpea yellowing isolates from Karaj (AMV-K) and Shiraz (AMV-S), a mung bean yellowing isolate from Karaj (AMV-M), and an alfalfa (Medicago sativa) isolate from Karaj (AMV-A). Isolates of AMV were separated from other viruses by serial transfers of single local lesions from inoculated primary leaves of bean (Phaseolus vulgaris) or cowpea cultivars. Isolates of AMV were maintained in alfalfa, cowpea, and/or tobacco (Nicotiana tabacum 'Samsun NN' or 'White Burley'). At the end of each host range inoculation trial, plants were assayed on 'Bountiful' bean or 'Early Ramshorn' cowpea, or on both, to detect

systemic infection. Indexing for AMV or cucumber mosaic virus (CMV), or for both, also was done by serology in agar double-diffusion tests.

Insect transmission studies were carried out with the pea aphid, Acyrthosiphon pisum, by using standard procedures. Virus-free aphids were maintained on healthy broadbean (Vicia faba) 'Algerian'. Seeds were collected from cowpea plants naturally and artificially infected with AMV-K and were tested for virus transmission in the greenhouse.

Cowpea lines were tested for resistance to AMV-K in greenhouse inoculation trials. Ten to twenty plants of each line were inoculated 12-15 days after seeding and again after 25-30 days. Symptomless cowpea plants were assayed on Bountiful bean after 60-70 days.

Between 1966 and 1971, cowpea and mung bean variety trials at Karaj were surveyed for AMV infection 8-9 weeks after planting. Percentage of infection was evaluated on a scale of 0-9, where 0 is free from infection and 9 equals 100% infected plants. Early Ramshorn cowpeas at various stages of growth in the field and greenhouse were observed for the effects of infection with AMV on yield and survival. At Karaj, replicated plots (4 rows wide x 5 m long) of Early Ramshorn cowpea were manually inoculated with Karaj isolates of AMV from cowpea (AMV-K) and alfalfa (AMV-A) at the prebloom (about 3-4 weeks after planting) and full bloom (about 8-9 weeks after planting) stages of plant growth. At the same time, a similar trial was carried out with CAMV.

In the greenhouse, Early Ramshorn cowpea plants were inoculated with AMV-K when plants were 13-20 cm high. Seeds were collected at maturity from healthy and infected plants to determine effects of virus infection on seed yield and the possibility of seed transmission.

RESULTS

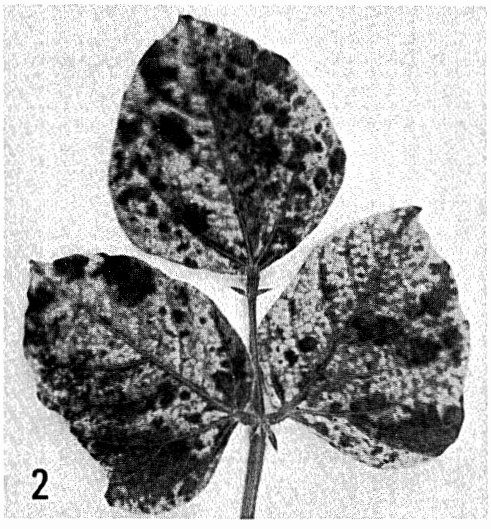
Symptoms of disease: Cowpea and mung bean plants naturally infected with AMV generally exhibited yellow mosaic (calico) foliage symptoms (Figs. 1, 2). At times, leaf yellowing was accompanied by leaflet deformation and dwarfing and by plant stunting. Occasionally, yellow mosaic symptoms were observed on the pods of AMV-infected cowpea plants (Fig. 3); however, seeds from these pods were not discolored. Alfalfa plants infected with yellow mosaic-inducing strains of AMV from cowpea exhibited leaf deformation and mosaic symptoms and frequently were stunted. Symptoms on AMV-infected alfalfa were generally less pronounced during the hot summer months.

Distribution and severity of disease: Different AMV strains infected naturally most of the food legumes cultivated in Iran for human consumption. These were broadbean, chickpea (Cicer arietinum), cowpea, lentil (Lens culinaris), and mung bean. Cowpeas infected with AMV were observed in field plantings in several regions of Iran, including the vicinities of Karaj, Shiraz, Mashad, Rezaiyeh, and Varamin. The prevalence of AMV-infected cowpeas generally was less than 10%, although a field with 50% infected plants was observed near Shiraz. Mung beans infected with AMV were observed at Karaj, Borojerd, and Varamin. Infected plants were scattered through plantings and disease prevalence was usually less than 1%.

At Karaj, prevalence of AMV in cowpea and mung bean variety trials in 1966 was 8.7% for cowpea (92 lines infected out of 1054) and 3.6% for mung bean (43 lines infected out of 1186). Infection with AMV rarely exceeded 5% in any susceptible cowpea or mung bean accession in Karaj field trials between 1966 and 1971.

Host range: The host ranges of the four AMV isolates from alfalfa, cowpea, and mung bean are listed in Table 1. The four isolates produced similar symptoms in 7 of 13 test plants. Symptoms induced by the Iranian AMV isolates on four diagnostic plant species (bean, cowpea, tobacco, and Chenopodium amaranticolor) were similar to those reported by others for different strains of the virus (1, 2, 3). The yellowing isolates from cowpea were distinguishable as a group from the mung bean and alfalfa isolates by their reaction on cowpea and bean. Only the cowpea isolates produced calico symptoms on inoculated cowpea plants. Calico symptoms also developed on mung bean plants inoculated with AMV-K, AMV-S, and AMV-M. Symptoms that developed on most of the test plants inoculated with the Karaj and Shiraz cowpea isolates were indistinguishable, except for the reactions on cucumber and lima bean. Although not included in Table 1, Iranian cowpea AMV yellowing isolates also produced yellow mosaic symptoms in pigeon pea (Cajanus cajan), soybean (Glycine max), and urd bean (Vigna mungo) in greenhouse inoculation tests.

Seed transmission: Transmission of a cowpea yellowing strain of AMV (AMV-K) was not detected in 60 seeds collected from two Mashad cowpea plants naturally infected at Karaj before flower initiation. No virus was detected in 200 seeds from diseased Early Ramshorn cowpea plants inoculated in the field at Karaj with AMV-K at prebloom or full bloom, or in 100 seeds from greenhouse-grown plants inoculated in the seedling stage.



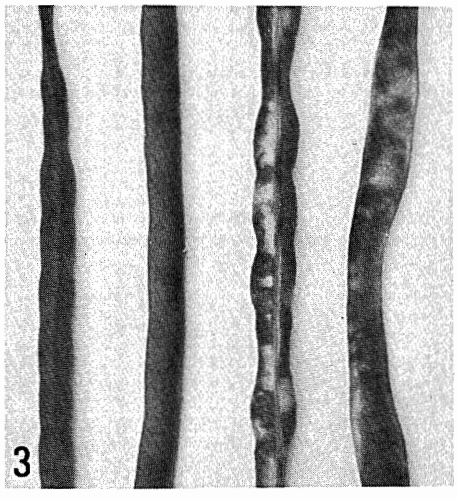


FIGURE 1. Cowpea plant (left) naturally infected with a strain of alfalfa mosaic virus at Shiraz, Iran, which produces yellow mosaic (calico) symptoms; healthy plant on right. FIGURE 2. Yellow mosaic symptoms on the foliage of a mung bean plant naturally infected at Karaj, Iran with alfalfa mosaic virus. FIGURE 3. Yellow mosaic symptoms on two pods (on right) which were harvested from a cowpea plant artificially inoculated with a yellow mosaic-inducing strain of alfalfa mosaic virus from Karaj, Iran. Two pods from healthy plants on left.

Effect of virus infection on yields and plant survival: Field inoculations of Early Ramshorn cowpea at prebloom or full bloom with AMV-K reduced seed yields by 47 and 17%, respectively; inoculation with AMV-A reduced yields by 55 and 14%, respectively (Fig. 4). In the same field trial, CAMV reduced seed yields of cowpea plants inoculated at prebloom and full bloom by 44 and 43%, respectively. Seed yields of Early Ramshorn cowpea inoculated with AMV-K at prebloom in the greenhouse were reduced by 76%, and those of plants inoculated with CAMV were reduced by 80%. Plant survival either in the greenhouse or the field was not reduced by AMV inoculation, although plants were markedly stunted by prebloom inoculation. Plants inoculated at prebloom typically developed pods with yellow mosaic symptoms.

Serology: Agar double-diffusion tests were used to detect AMV and CMV infection in foliage of naturally infected alfalfa, cowpeas, and mung beans collected in various areas of Iran. This test could distinguish AMV-infected cowpeas and mung beans from those naturally infected with CMV, the symptoms of which were often similar. Agar double-diffusion tests also were used to confirm AMV infection in artificially inoculated plants with questionable foliar symptoms.

Table 1. Host range and reactions of alfalfa, cowpea, and mung bean isolates of alfalfa mosaic virus (AMV) from Iran.

Host[a]	Host reaction to AMV isolates			
	Cowpea (Karaj)	Mung bean (Karaj)	Cowpea (Shiraz)	Alfalfa (Karaj)
Amaranthaceae				
Gomphrena globosa	LL, M[b]	LL, M	LL, M	LL, M
Chenopodiaceae				
Chenopodium amaranticolor	LL	LL	LL	LL
Cucurbitaceae				
Cucumis sativus 'National Pickling'	LL, M, St	M	M	M
Leguminosae				
Cicer arietinum 'Ghazvin'	W, St	W, St	W, St	W, St
Glycine max 'CNS-4'	LD, M, YM	-	-	NS
Lens culinaris 'Ghazvin'	LC, M	LC, M	LC, M	LC, M
Medicago sativa 'Africa'	LD, M[c]	LD, M	LD, M	LD, M
Phaseolus lunatus 'Jackson Wonder'	VN, LD, M	NS	M	NS
P. vulgaris 'Pinto 111'	LL, VN, LC, M	LL, M, SYS	LL, VN, LC, M	LL, M
Vicia faba 'Algerian'	LL, M	NS	LL, M	LL, M
Vigna radiata 'Oklahoma 12'	LL, VN, YM	LL, VN, YM	LL, VN, YM	NS
V. unguiculata 'Early Ramshorn'	LL, YM, St	LL, M	LL, YM, St	LL, M
Solanaceae				
Nicotiana tabacum 'Samsun NN'	M	M	M	M

[a]Hosts are listed alphabetically by family and genus.

[b]-, not inoculated; LC, leaf curling; LD, leaf deformation; LL, necrotic local lesion; M, mosaic; NS, not susceptible; St, stunting; SYS, systemic yellow spots; VN, vein necrosis; W, wilting; YM, yellow mosaic.

[c]Foliar symptoms on AMV-infected alfalfa frequently became less pronounced during the hot summer months.

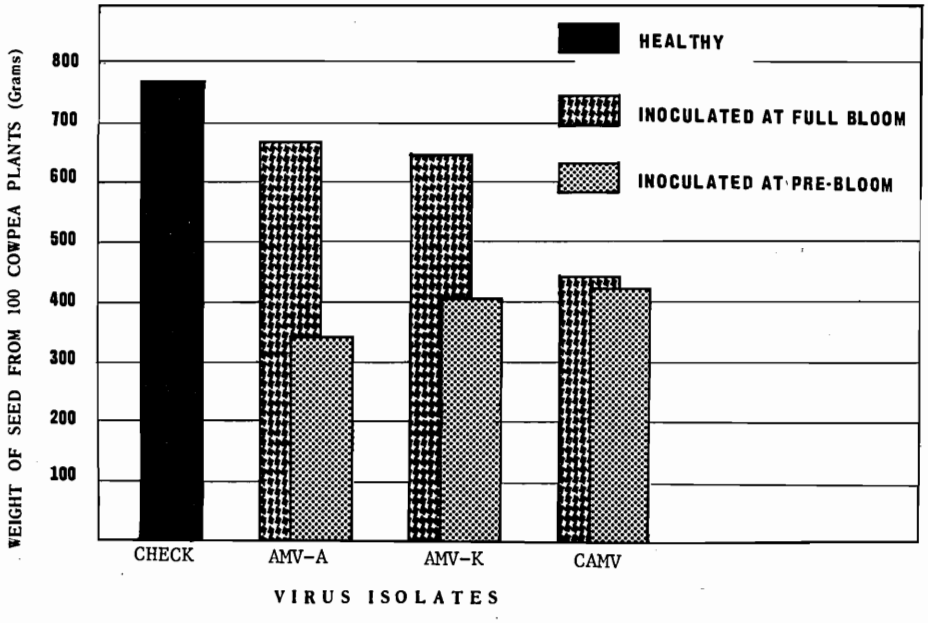


FIGURE 4. Effects of two Karaj isolates of alfalfa mosaic virus from alfalfa (AMV-A) and cowpea (AMV-K) and one of cowpea aphid-borne mosaic virus (CAMV) on seed yields of Early Ramshorn cowpea artificially inoculated in the field at Karaj, Iran, at two stages of plant growth.

Vector transmission: The AMV cowpea yellowing isolate (AMV-K) was transmitted by adult apterae of Acyrthosiphon pisum from virus-infected alfalfa, cowpea, and tobacco to healthy seedlings of Early Ramshorn cowpea in a stylet-borne (noncirculative) manner. Transmission ranged from 10-50%, depending on the virus source plant and the number of aphids used in test feedings. Transmission of AMV was highest when aphids fed for 1-5 min on virus-infected 'Samsun' tobacco plants before transfer to cowpea test plants in groups of 10 aphids per plant. Transmission by single pea aphids occurred, but rarely exceeded 10%. Disease symptoms resulting from aphid transmission of AMV-K to cowpea in greenhouse tests were identical to those that developed on naturally infected plants in the field.

Resistance: No resistance to AMV-K was detected among 56 cowpea lines inoculated with cowpea yellowing isolate AMV-K under greenhouse conditions. The foliage of most accessions infected with AMV-K exhibited yellow mosaic symptoms, as did the pods of many infected lines. Some systemically infected lines were severely stunted and the foliage was generally deformed and reduced in size. Plants in a few lines, although exhibiting symptoms, were AMV tolerant and not severely stunted. Cowpea lines tolerant to AMV-K were PI (USDA Plant Introduction accession number) 227829 (Guatemala) and PI 293474 (U.S.A.).

DISCUSSION

In the period from 1966-1971, I (unpublished data) isolated several strains of AMV, some of which induced yellow mosaic symptoms in cowpea, from Iranian alfalfa plantings. In the present study, cowpea and mung bean plants infected with yellowing strains of AMV invariably were found to be growing near alfalfa plantings. Cowpea fields were characteristically more severely affected by AMV than mung bean plantings. The disease of mung bean caused by AMV appears to be of limited agricultural importance in Iran.

The experimental host range of AMV is extensive (1, 2, 3). Cowpea and mung bean are 2 of over 305 plant species that are susceptible to strains of AMV by artificial inoculation (1, 3). Natural infection of cowpea and mung bean by AMV appears to be restricted and apparently has been reported only from Iran (5, 6). Under Iranian field conditions, yellow mosaic symptoms induced by strains of AMV were observed only on cowpea and mung bean.

Although yellowing symptoms were useful in locating cowpeas and mung beans naturally infected by AMV, confirmation of the identity of AMV required other tests. This testing was necessary because in certain regions of Iran, particularly in the southwest, both crops were naturally infected with yellow-inducing strains of cucumber mosaic virus, the symptoms of which could not be distinguished from those produced by cowpea yellowing strains of AMV. Agar double-diffusion serology tests were found to be effective in establishing the identity of plants naturally or artificially infected with either virus. Host-range studies were also useful in separation and identification of AMV and CMV from naturally or artificially infected plants.

Most of the cowpea lines tested for resistance to the Karaj cowpea yellowing isolate of AMV were highly susceptible to virus infection. Additional greenhouse and field screening trials will be required to locate suitable sources and levels of resistance in cowpea to this and other important cowpea viruses, like CAMV. Sources of resistance to Iranian cowpea viruses may be present in the cowpea germplasm collections maintained by the International Institute of Tropical Agriculture in Ibadan, Nigeria or the Southern Regional Plant Introduction Station, Experiment, Georgia, U.S.A.

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