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Biology of Bean Yellow Mosaic and Pea Leaf Roll Viruses affecting *Vicia faba* in Iran

By

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With 4 figures

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Introduction

Broadbeans (*Vicia faba* L.) are one of several pulse crops (food legumes) grown in Iran for human consumption. About 5,000 hectares of broadbeans are grown, primarily in the north around the Caspian Sea and in the south near the Persian Gulf. In Khuzestan Province (Southwestern Iran) broadbeans are the major pulse crop grown during the fall and winter. Annually, broadbean yields in this region are affected by various diseases, the most important of which are viruses (KAISER, MUELLER and DANESH 1967, KAISER, DANESH, OKHOVAT and MOSSAHEBI 1968). The viruses of major importance are bean yellow mosaic (BYMV) and pea leaf roll (PLRV) which are widely distributed in Iran and infect most pulses grown in the country (KAISER, DANESH, OKHOVAT and MOSSAHEBI 1968, KAISER and DANESH 1971, KAISER and DANESH 1971). Little is known concerning the spread, survival, vectors, alternate hosts, seed transmission, or effects on yield of viruses affecting *V. faba* in Iran. This study was initiated to investigate various aspects of the biology of these two viruses with regard to the diseases they cause in broadbean in Iran.

Materials and Methods

Isolates of BYMV and PLRV used in the inoculation studies were from diseased broadbeans from Dezful and Karaj, Iran, respectively. The isolate of BYMV differed from most others in Iran in host range and symptomatology and in being seed-borne in *V. faba* (unpublished data). The viruses were maintained on Algerian broadbean in the greenhouse where temperatures ranged from 10 to 30 °C.

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Aphid species used in the transmission studies were reared on healthy plants of Algerian broadbean which were indexed periodically to determine whether they were virus free. In virus transmission studies with BYMV, a stylet-borne virus (KAISER and DANESH 1971), aphids were starved for 1 to 3 hr before being given acquisition feeding periods of less then 1 min (duration of a single probe) on virus-infected broadbean leaves. They were then transferred to healthy broadbeans in leaf cages, 1 aphid/plant for a 24-hr feeding period. In studies with PLRV, a circulative virus (KAISER and DANESH 1971, QUANTZ 1954, TINSLEY 1959), aphids reared on virus-infected broadbeans were transferred to healthy test plants, 1 aphid/plant, for a 72-hr inoculation feeding period. All plants were sprayed with an insecticide at the termination of the inoculation feeding period. Aphid species used in these studies were collected from broadbeans naturally colonized in the field and were identified as *Aphis craccivora* Koch, *Aphis fabae* Scopoli, *Acyrthosiphon pisum* (Harris), *Acyrthosiphon sesbaniae* David, and *Myzus persicae* (Sulzer).

In replicated field trials at Karaj, Algerian broadbeans were inoculated in different stages of growth with BYMV and PLRV, singly and in combination. The plots were two rows wide and 5 m long with 4 replications/virus/inoculation date. Inoculum of BYMV was prepared by grinding virus-infected plant tissue with a mortar and pestle to which had been added 0.01 M phosphate buffer, pH 7.0 and 320-mesh Carborundum. Immediately after trituration, the homogenates were rubbed on young, fully expanded leaflets of broadbean plants with the thumb and forefinger. With PLRV, one young, fully expanded leaflet of each plant was exposed to 10 to 20 viruliferous aphids in a leaf cage for a 3-day inoculation feeding period. Doubly inoculated plants were exposed to viruliferous aphids (PLRV) within 30 min of being inoculated with BYMV. Plants were inoculated at 3 stages of plant growth: pre-bloom, 41 days after planting; full bloom, 61 days after planting when plants were flowering; and post bloom, 76 days after planting when pods were enlarging. Prior of each inoculation, plots were rouged of all unhealthy appearing plants. Fifteen to 20 days after inoculation, plants exhibiting virus symptoms were tagged. Plots were sprayed at 10 to 15 day intervals with liquid formulations of two broad spectrum insecticides. At harvest time (105 days after planting) data on the number of pods and seeds, and seed weight were recorded from 25 tagged plants in each plot.

Broadbean plantings in Southwestern Iran at the Khuzestan Water and Power Authority's Agricultural Experiment Station, Safiabad, were surveyed at periodic intervals for virus incidence and spread. Diseased annual and perennial plants growing in or adjacent to broadbean plantings were collected and indexed on various test plants, including bean (*Phaseolus vulgaris* L. 'Bountiful'), chickpea (*Cicer arietinum* L. 'Ghazvin'), Algerian broadbean, cowpea (*Vigna sinensis* [L.] Savi ex Hassk. 'Early Ramshorn'), cucumber (*Cucumis sativus* L. 'National Pickling'), *Nicotiana glutinosa* L., and at times on *Cassia occidentalis* L. and *C. obtusifolia* L. Non-viruliferous aphids were also fed on portions of diseased plants for 72 hr before being transferred to healthy broadbeans in groups of 5 to 20 aphids for a 3-day feeding. Other techniques, including serology and electron microscopy, were also utilized in identification of isolates of BYMV and PLRV (KAISER and DANESH 1971).

Seed from virus-infected broadbeans was treated with N-Trichloromethylthio-4-cyclohexene-1,2-dicarboximide (captan) and planted in pasteurized greenhouse soil to observe for seed transmission. Diseased plants were tagged at periodic intervals and back inoculations were made to healthy Algerian broadbean and Bountiful bean. Electron micrographs were made of leaf dip preparations of virus-infected seedlings which had been negatively stained with 2 % potassium phosphotungstic acid, pH 7.0.

Results

Effect of virus infection on yields

Seed yields from broadbean plants inoculated at Karaj with BYMV and PLRV, singly or in combination, at different stages of plant growth were

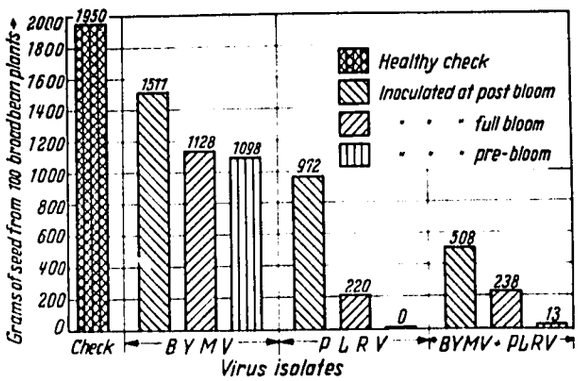


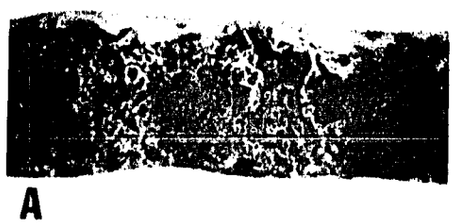
Fig. 1. Effects of infection by bean yellow mosaic (BYMV) and pea leaf roll (PLRV) viruses at three stages of plant growth on seed yield of broadbean (*Vicia faba*) in field inoculation trials at Karaj, Iran

reduced by 23 to 100 % (fig. 1). Largest yield reductions of 99 to 100 % occurred in plants inoculated at pre-bloom (fig. 1). All inoculations with PLRV reduced yields by 50 % or more. Inoculation with BYMV at pre-bloom and full bloom decreased seed yields by 44 and 42 %, respectively.

Fig. 2. Symptoms of pea leaf roll virus in broadbean (right) are stunting, yellowing, leaf rolling, and absence of flowers or pods; healthy plant, left

Plants inoculated at pre-bloom with PLRV or BYMV + PLRV developed distinctive symptoms of severe stunting, chlorosis, leaf rolling, and absence of flowers or pods (fig. 2). Mortality of diseased plants in these inoculation treatments exceeded 80 %. There was little mortality in plants inoculated with BYMV, singly, although stunting was particularly prevalent in the first inoculation date. In plants inoculated with both viruses, symptoms of BYMV were often masked by those of PLRV 3 to 4 weeks after inoculation.

Necrotic rings developed on pods of some plants infected at pre-bloom with BYMV (fig. 3 A). Only BYMV was reisolated from pods exhibiting these symptoms. Discolored seeds were found in some pods from the pre- and full



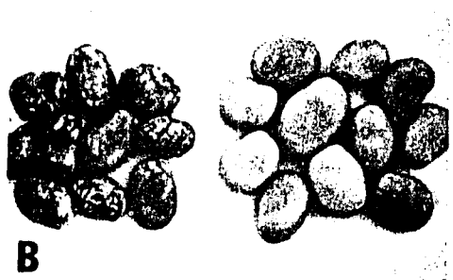


Fig. 3. Symptoms of bean yellow mosaic virus in pods and seeds of Algerian broadbean. A) Necrotic rings on broadbean pod from a diseased plant. B) Discoloration of seeds from virus-infected plants (left); seeds from virus-free plants, right

bloom inoculation treatments, although not necessarily from pods with ring spotting symptoms (fig 3 B). The discoloration was confined to the seed coat.

Seed transmission

Seed from the different inoculation treatments was planted in the greenhouse for observations on seed transmission of BYMV and PLRV. BYMV was transmitted in seed of plants inoculated singly at pre-, full, and post bloom and in combination with PLRV at full bloom (table 1). Highest transmission occurred at full bloom where 0.95 % of the seedlings were infected. Mosaic symptoms were discernible in the primary leaves and became more pronounced in the compound leaves which developed later (fig. 4). PLRV was not seed transmitted, although only a few seeds from plants inoculated at pre-bloom with BYMV + PLRV were available for testing.

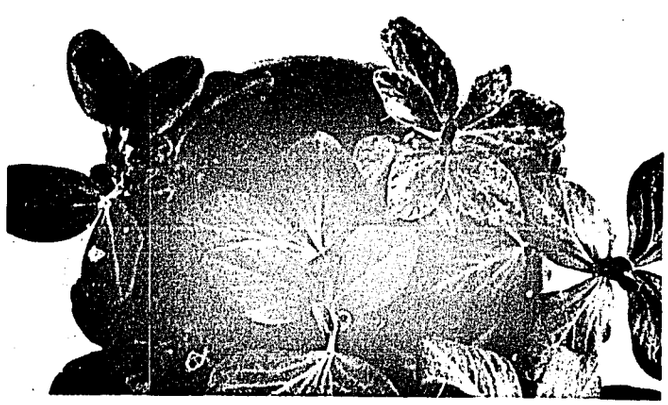


Fig. 4. Mosaic symptoms in an Algerian broadbean seedling (upper right) infected from seed with bean yellow mosaic virus

Table 1

Transmission of bean yellow mosaic (BYMV) and pea leaf roll (PLRV) viruses in seed of Algerian broadbean (*Vicia faba*) inoculated in the field at three stages of plant growth

Virus	Stage of Plant Growth When Inoculated [a]	No. Seeds Planted	No. Emerged	No. Virus Infected Seedlings	% Transmission
Check (non-inoculated)	-	1220	1180	0	0
BYMV	Pre - bloom	874	794	2	0.25
BYMV	Full bloom	1210	1160	11	0.95
BYMV	Post bloom	1090	1065	2	0.19
PLRV	Pre - bloom	0 [b]	0	0	0
PLRV	Full bloom	570	428	0	0
PLRV	Post bloom	740	672	0	0
BYMV + PLRV	Pre - bloom	40	34	0	0
BYMV + PLRV	Full -bloom	579	437	1 [c]	0.23
BYMV + PLRV	Post bloom	540	480	0	0

a) Pre-bloom, plants 8—12 cm tall; full bloom, plants flowering and early fruiting stages; post bloom, late fruiting stages.

b) No seed was harvested from virus-infected plants.

c) Seedling infected with BYMV.

Table 2

Seed transmission and subsequent spread of bean yellow mosaic virus in 20 lines of broadbean (*Vicia faba*) in replicated field trials in Southwestern Iran[a])

Line No.	Country of Origin	No. Plants	No. Seed-borne [b]	% Transmission	% Virus Infection (Weeks after planting)		
					15	19	22
1	Poland	903	1	0.1	20	78	100
2	England	903	0	0	15	49	100
3	West Germany	881	0	0	22	58	100
4	Italy	982	1	0.1	6	38	100
5	Spain	954	0	0	20	64	100
6	Japan	1086	1	0.1	4	28	100
7	Iran	910	3	0.3	17	43	99
8	Iran	963	0	0	9	49	100
9	Turkey	1067	3	0.3	8	39	99
10	Turkey	941	3	0.3	11	48	100
11	Iran	1085	4	0.4	21	60	100
12	Iran	875	0	0	11	30	100
13	Cyprus	826	2	0.2	6	36	100
14	Iran	950	0	0	10	44	100
15	Iran	1189	0	0	15	53	100
16	Iran	1088	4	0.4	26	73	100
17	Turkey	928	8	0.9	34	73	100
18	Iran	971	0	0	7	26	93
19	Iran	1039	4	0.4	25	74	100
20	Iran	1072	3	0.3	20	65	98

a) Plots (4 rows wide, 5 m long with 4 replictions/line) were seeded October 12, 1967 and harvested May 20, 1968.

b) Seedlings were assessed for BYMV symptoms when plants were in the 2—4 leaf stage.

Seed transmission and spread of BYMV in nature

Seed transmission and subsequent spread of BYMV was observed in 20 broadbean lines in a replicated yield trial at Safiabad (table 2). Seed of all lines included in this test was harvested from BYMV-infected plants which were grown at Safiabad the previous year. Seed transmission occurred in 60 % of the lines, ranging from 0.1 to 0.9 %. Spread of BYMV increased from 4 to 34 % at 15 weeks to 93 to 100 % after 22 weeks (table 2). By harvest time (31 weeks) all plants were infected with BYMV. The incidence of PLRV was less than 5 %.

Broadbean seed of 56 lines from 14 countries was planted in replicated yield trials at Safiabad in 1967 and 1968. In both years the incidence of BYMV at harvest time was over 90 %. A survey of a similar trial in 1969 (seed from 1968 Safiabad trial) revealed that BYMV was seed-borne in 80 % of the lines. Seed transmission of 0.1 to 0.5 % occurred in 32 of the 56 lines. The incidence of seed-borne infection exceeded 2 % in only one line. At maturity, over 95 % of the plants were virus infected. No seed transmission of PLRV was observed, and at harvest less than 4 % of the plants were infected with this virus.

Effect of natural infection by BYMV and PLRV on yield of broadbean and subsequent seed transmission

Largest yield reductions with BYMV and PLRV in a 5-hectare planting of Algerian broadbean at Safiabad occurred in plants infected in the seedling stage (table 3). Yields of plants infected with PLRV were reduced by 80.5 to 100 %, and those with BYMV by 0 to 36 % (table 3). Since no virus-free plants were found at the end of the trial, yield comparisons were made with the BYMV pod set series which had the highest yield. Most PLRV-infected broadbeans were probably infected with BYMV due to the high incidence of this virus and because the symptoms of BYMV were usually masked in plants infected with both viruses.

Table 3

Effect of natural infection by bean yellow mosaic (BYMV) and pea leaf roll (PLRV) viruses at three stages of plant development on yield of Algerian broadbean (*Vicia faba*) in South-western Iran, and subsequent observations on seed transmission of these viruses

Virus	Stage of Plant Development	Mean per plant			% Decrease in Seed Weight [a]	% Seed Transmission [b]
		No. Pods	No. Seeds	Seed Weight (g)		
BYMV	Pot set	12.9	32.6	35.3	-	0.17
BYMV	Flowering	10.5	28.7	27.9	20.8	0.43
BYMV	Seedling	8.8	21.9	22.6	36.0	0.25
PLRV	Pot set	7.7	16.8	7.1	80.5	0
PLRV	Flowering	0.4	0.5	0.6	98.3	0
PLRV	Seedling	0	0	0	100	0

a) Yield comparisons were made with BYMV at pod set as there were no virus-free plants at the time of harvest.

b) Each figure for % seed transmission of BYMV was based on 1150 to 2000 seeds.

Transmission of BYMV from seed of infected plants ranged from 0.17 % at pod set to 0.43 % at flowering (table 3). PLRV was not transmitted in seed of infected plants.

Aphid vectors of BYMV and PLRV

Aphid species collected from healthy and diseased broadbeans in Iran were *Aphis craccivora*, *A. fabae*, *Acyrthosiphon pisum*, *A. sesbaniae*, and *Myzus persicae*. All aphid species transmitted BYMV in a stylet-borne manner, acquiring and transmitting the virus in brief feeding periods of less than 1 min (table 4). Transmission of BYMV ranged from 70 % with *Aphis fabae* to 20 % with *Acyrthosiphon pisum* and *A. sesbaniae* (table 4).

PLRV was transmitted by aphid species in a circulative manner (table 4). However, in several transmission trials using single aphids, *M. persicae* failed to transmit the virus. With groups of 10 aphids, there was less than 3 % transmission of PLRV. Aphids needed longer feeding periods (1 to 3 hr) on PLRV-infected plants to acquire the virus. *Aphis craccivora* was the most efficient vector of PLRV, with 71.4 % of the aphids transmitting the virus.

Table 4

Transmission of bean yellow mosaic (BYMV) and pea leaf roll (PLRV) viruses by different aphid species to broadbean (*Vicia faba*)

Aphid species	Transmission [a]			
	BYMV		PLRV	
	No.	%	No.	%
Aphis craccivora Koch	13 / 20 [b]	65	65 / 91	71.4
Aphis fabae Scopoli	14 / 20	70	25 / 119	21.0
Acyrthosiphon pisum (Harris)	4 / 20	20	50 / 125	40.0
Acyrthosiphon sesbaniae David	4 / 20	20	78 / 123	63.4
Myzus persicae (Sulzer)	12 / 20	60	0 / 133	0

[a]) At the termination of the acquisition feeding period, aphids were transferred to healthy broadbeans, 1 aphid/plant, for 24 hr (BYMV) or 72 hr (PLRV). With BYMV both nymphs and adult aperae were used, whereas with PLRV, only adult apterae were used.

[b]) Numerator is the number of plants infected; denominator is the number of plants inoculated.

Alternate hosts of BYMV and PLRV

In the broadbean growing region of Southwestern Iran only annual and perennial legumes were found to be naturally infected with BYMV and PLRV. Hosts of PLRV were *Cicer arietinum*, *Lens esculenta* Moench, *Medicago hispida* Gaertn., *M. lupulina* L., *M. sativa* L., *Melilotus indica* (L.) All., *Phaseolus vulgaris*, *Pisum sativum* L. and *Vigna sinensis*. BYMV infected all plants, except *M. sativa* and *V. sinensis*. Many of these alternate hosts were also important reservoirs of aphid vectors of both viruses.

Plant resistance

To locate sources of resistance to BYMV and PLRV, 110 broadbean lines from 19 countries were screened in greenhouse and field trials over a 5-year

period. All lines were susceptible to both viruses. The greatest reduction in growth and yields and the highest plant mortality was caused by PLRV. Some broadbean types appeared to be tolerant to BYMV, especially when infection occurred later in plant growth.

Discussion

Although most investigators have not found BYMV or its related strain, pea mosaic virus (PMV) (BOS 1970) to be transmitted in broadbean seed (AZAD, NAGAICH and SEHGAL 1961, HEATHCOTE and GIBBS 1962, KVÍČALA 1965, NITZANY and COHEN 1964, NOUR and NOUR 1962, NOUR-ELDIN, EL-BANNA, EL-ATTAR and EID 1966 and TAPIO 1970), QUANTZ (1954) in Germany and NITZANY and COHEN (1962) in Israel found that PMV was occasionally seed-borne in broadbean. HAMPTON and HANSON (1968) in the United States reported that seed transmission which occurred in 5 of 380 broadbean seedlings of a commercial seed lot was associated with rod-shaped virus particles of 520 or 750 nm. In Southwestern Iran seed transmission of BYMV in local broadbean varieties cultivated by farmers was usually less than 0.5 %. In yield trials at Safiabad, BYMV was seed-borne in 45 of 56 broadbean types and transmission ranged from 0.1 to 2.4 %, although a majority of these lines contained less than 0.5 % infected seed. PLRV was not found to be seed-transmitted in broadbean or other pulse crops in Iran which agrees with BENNETT's (1969) statement that no circulative (persistent) aphid-transmitted viruses were known to be seed-borne. Seed transmission of PLRV in *V. faba* or other hosts has not been reported in the literature (HEATHCOTE and GIBBS 1962, QUANTZ 1954, QUANTZ and VÖLK 1954 and TINSLEY 1959).

The author has isolated several pathogenic strains of BYMV from broadbeans in various pulse growing areas of Iran which differed in host range, symptomatology and virulence (unpublished data). In Fars Province, Iran, IZADPANAH et al. (1969) isolated a strain of BYMV from virus-infected broadbeans which was more virulent and caused higher mortality and greater yield reductions than the strain of BYMV that I commonly found affecting broadbeans in Southwestern Iran. A difference in the strain (BENNETT 1969) of BYMV may account for the lack of seed transmission in their trials (K. IZADPANAH, personal communication).

Seed transmission is an important mechanism of survival and spread of BYMV in broadbeans in Southwestern Iran. Although alternate hosts contribute to the spread of BYMV into broadbean fields later in the growing season, the most important initial source of the virus in broadbean plantings appears to be infected seed. BYMV was not found to be seed-borne in other pulse crops cultivated in Iran. With PLRV which is not seed-transmitted, alternate hosts are essential in perpetuating the virus from one broadbean crop to another. In all areas of Iran where PLRV affects food legumes, alfalfa was found to be the major perennial overwintering host of the virus and several of its aphid vectors.

It is not known whether different strains of PLRV occur in Iran. Isolates in Southwestern Iran appear to be more virulent than those infecting *V. faba* in England (HEATHCOTE and GIBBS 1962, TINSLEY 1959), in effects on plant growth, yield, and mortality. Infection of broadbeans with BYMV and PLRV in the early stages of plant development drastically reduced plant vigor and productivity with PLRV causing the largest yield reductions and highest plant mortality. Similarly, in England where broadbeans are commonly infected by PLRV, PMV, and pea enation mosaic virus, yields are reduced most by early infections, especially with PLRV (HEATHCOTE and GIBBS 1962). Broadbeans infected naturally with PLRV in Iran appeared to be more susceptible to *Botrytis fabae* Sardiña (TINSLEY 1959) and root rot diseases.

In Iran broadbeans are colonized by several aphid species, most of which are vectors of several virus diseases affecting this crop (KAISER et al. 1968), including BYMV and PLRV. Broadbeans and other leguminous crops and weeds in Southwestern Iran are colonized most frequently and abundantly by *Aphis craccivora* which is an important vector of BYMV and PLRV. Chemical control of aphid vectors transmitting BYMV and PLRV from reservoir hosts to broadbean plantings is not feasible due to economic reasons. A more practical approach would be to cultivate varieties containing resistance to these and other diseases. Although all broadbean lines tested in Southwestern Iran were susceptible to BYMV and PLRV, a few local types consistently out-yielded introduced lines, even though most plants were virus-infected prior to harvest. Until higher yielding, disease or insect resistant lines are found, farmers in Southwestern Iran are encouraged to plant the local types which are preferred by the inhabitants of this region.

Summary

The biology of two viruses, bean yellow mosaic (BYMV) and pea leaf roll (PLRV), affecting broadbeans (*Vicia faba*) was studied under field conditions in Iran. Seed yields from broadbeans inoculated with both viruses in the seedling, flowering, and pod set stages of growth were reduced by 23 to 100%. Largest yield reductions and highest mortality occurred with PLRV when plants were in the early stages of development. Pods infected with BYMV occasionally developed necrotic ring spotting symptoms and seeds were often discolored. BYMV was transmitted in 0.19 to 0.95% of the seed from plants inoculated at pod set and flowering, respectively. In field trials with 20 broadbean types, BYMV was transmitted in less than 1% of the seeds, but was subsequently spread by aphids to 4 to 34% of the plants in 15 weeks to 93 to 100% after 22 weeks. Seed transmission of BYMV ranged from 0.1 to 2.4% in 45 of 56 broadbean lines from 14 countries included in replicated yield trials. There was no transmission of PLRV in seed of inoculated or naturally infected broadbean plants. In nature, broadbeans were colonized by several aphid species which were vectors of both viruses. BYMV was stylet-borne by *Aphis craccivora, Aphis fabae, Acyrthosiphon pisum, Acyrthosiphon sesbaniae,* and

Myzus persicae. PLRV was transmitted by all aphid species in a circulative manner. Various annual and perennial legumes were alternate and overwintering hosts of BYMV and PLRV and their aphid vectors. No resistance was found to BYMV or PLRV in 110 broadbean lines tested in greenhouse or field trials.

Zusammenfassung

Bohnengelbmosaikvirus (BYMV) und Erbsenblattrollvirus (PLRV) auf *Vicia faba* in Iran

Die Biologie der beiden Viren wurde unter Feldbedingungen in Iran untersucht. Die Samenausbeute von Ackerbohnen wurde durch Infektion mit den beiden Viren im Keimlings-, Blüte- und Fruchtansatzstadium um 23 bis 100 % vermindert. Die stärkste Verminderung der Samenausbeute und höchste Sterblichkeit trat mit PLRV an Pflanzen früher Entwicklungsstadien ein. Mit BYMV infizierte Hülsen zeigten gelegentlich nekrotische Ringflecke und oft verfärbte Samen. BYMV wurde durch 0,19 bzw. 0,95 % der Samen von im Hülsenansatz- oder Blütenstadium infizierten Pflanzen übertragen. In Feldversuchen mit 20 Ackerbohnensorten wurde BYMV durch weniger als 1% der Samen übertragen, aber in der Folge durch Blattläuse auf 4 bis 34 % der Pflanzen (in 15 Wochen) und 93 bis 100 % (nach 22 Wochen) verbreitet. Samenübertragung von BYMV erfolgte zu 0,1 bis 2,4 % bei 45 von 56 Ackerbohnensorten aus 14 Ländern. PLRV wurde in Samen von natürlich oder experimentell infizierten Ackerbohnen nicht übertragen. Im Felde fanden sich auf Ackerbohnen mehrere Blattlausarten, welche beide Viren übertrugen (*Aphis craccivora*, *Aphis fabae*, *Acyrthosiphon pisum*, *A. sesbaniae* und *Myzus persicae*; BYMV nicht persistent, PLRV persistent). Verschiedene einjährige und ausdauernde Leguminosen sind Neben- und Überwinterungswirte der beiden Viren und ihrer Vektoren. In 110 Ackerbohnensorten konnte keine Resistenz gegen die beiden Viren festgestellt werden.

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