ISOLATION AND IDENTIFICATION OF SOME BROADBEAN MOSAIC VIRUSES IN JORDAN

By

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INTRODUCTION

Faba bean (*Vicia faba* L.) is an important winter crop in Jordan planted mainly for fresh market consumption and/or as fodder crop. Moreover, Jordan exports fresh faba beans to neighboring countries (6). The nutritional value of faba bean is considerable since it is rich in protein (24,44,52).

The crop is planted to 8457 donums in Jordan, approaching 2.5% of the total area planted to vegetables and 41% of the total area planted to legumes (6). It is affected by many plant diseases of which mosaic diseases are seemingly the most limiting factors in production (28).

Faba bean plants in Jordan are increasingly showing symptoms apparently of viral etiology. Infected plants are stunted and show mosaic of variable intensity, leaf rolling and narrowing. The mosaic diseases can have a market effect on the yield. Plants that are naturally infected with mosaic diseases yielded 17-90% less than uninfected plants, depending on the stage of infection (28).

More than 30 viral diseases have been recorded infecting faba beans naturally in different parts of the world (14,28). Some of these viruses are seed-borne in faba beans, which if coupled with aphid transmission may play a critical role in virus preservation and long or short distant spread of the disease they induce.

In Jordan many viruses are seemingly involved in mosaic disease complex of faba bean. Bean yellow mosaic and broadbean wilt viruses had been isolated from faba bean plants showing

mosaic (3,4,38).

This study aims at extending knowledge about the identity of other possible virus(es) involved in the mosaic disease complex of faba bean in Jordan. Identification of such virus(es) requires studying the host range, general properties, aphid transmissibility, inclusion bodies, electron microscopy and serology. Incidence of the mosaic disease in the faba bean fields will be also assessed. Moreover, the study will furnish more information about some virus(es) reported earlier.

LITERATURE REVIEW

Several viruses are apparently involved in the mosaic disease complex of faba beans. These viruses are different in their distribution, symptoms and transmission. Bean yellow mosaic virus (BYMV), is one of the most prevalent mosaic-causing viruses in faba bean in the world (11,28). Other viruses in the mosaic group are pea enation mosaic (PEMV), alfalfa mosaic (AMV), broadbean mottle (BBMV), clover yellow vein (CYVV), cucumber mosaic (CMV), pea seed-borne mosaic (PSbMV) and broadbean wilt (BBWV) (28).

Bean common mosaic (BCMV) is rarely encountered on faba beans. It had been recorded on *V. faba* in Syria, USA (28) and in Lebanon (37), but it is found in all parts of the world where beans are grown (12,19), and is economically important in many areas (19). It is a potyvirus with a flexuous filamentous particles of 730-750 nm long (12,28,43) and 15 nm wide (12). The virus, as a member of the potyvirus group, has cylindrical cytoplasmic inclusions in epidermal strips associated with the pinwheel inclusions (12,39,54). In crude sap, the thermal inactivation point is 55-60 C, the dilution end point is 10^{-3}-10^{-4} and the longevity *in vitro* is 1-4 days (12). BCMV has very limited host range. Symptoms induced by the virus depend greatly on host cultivar, virus strain and environmental conditions (12,18,19). On faba beans some strains induce systemic symptoms. Most other strains, however, seemed unable to infect faba bean or caused symptomless infection (28). On beans the International Working Group on Legume Viruses (IWGLV)

suggested a standard set of bean cultivars for differentiating strains of BCMV (19). The Federation of British Plant Pathologists classified symptoms caused by BCMV on beans to two couples namely, resistance-susceptible and sensitive-tolerant. Susceptible cultivars are characterized by systemic infection whereas the resistant cultivars develop no systemic infection. A susceptible cultivar is sensitive if infection results in moderate to severe disease symptoms and considerable damage. It is tolerant if it carries the virus systemically, but shows slight or no visible symptoms. Cultivars are immune if they were not infected by the virus. Drijfhout *et al* (19) reported that variation in response of beans to infection with BCMV is due to cultivar and virus strain differences. Accordingly, he was able to assign twenty two BCMV isolates into seven groups with six subgroups using nine bean cultivar groups. The differential bean cultivar groups are listed in tables 1 and 2. The necrotic reactions of bean cultivars were considered as secondary criteria for classification and were used to distinguish subgroup (b and a) within a strain group. Virus strains in subgroup b induced systemic necrosis more readily than those in subgroup a. Florida strain (IV a) for instance, unlike the strain of (IV b), did not produce systemic necrosis at any temperature. The difference between NL3 (VI a) and NL5 (VI b) was in systemic necrosis produced on cultivar Amanda by NL5. The temperature plays an important role in symptoms severity. Cultivars, susceptible to some strains, may exhibit resistance at certain temperatures to other strains. Grogan and Walker in 1948 reported that at temperature above 30 C some

Table 1. * Systemic reaction of cultivars from host groups 1 to 5, inoculated with 22 isolates of bean common mosaics virus, arranged according to strain group of the virus.

		BCMV strain group and isolates																					
		I				II				III	IV					V			VI		VII		
Host group	Cultivar	Type	NL1	PR9M	IRAN	NL7	R220	S74	PV25	NL8	FLOR.	IDA.	WEST.	NL6	BAILIF	NY15	NL2	IMUNA	NL3	NL5	NL4	Mex.	Chile
1	Dub. Wit.	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	Sutter pink	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	RLGL-C	R	R	R	R	S	T	S	T	R	S	S	S	S	S	T	S	S	T	S	S	T	S
	Purgold	R	R	R	R	S	T	S	T	R	S	S	S	S	S	T	S	S	T	S	S	S	S
3	RLGL-B	R	R	R	R	R	R	R	R	R	S	S	S	S	S	R	R	R	S	S	S	S	S
	GN-123	R	R	R	R	R	R	R	R	R	S	S	S	S	S	R	R	R	T	T	S	S	S
4	Sanilac	R	R	R	R	R	R	R	R	S	R	R	R	R	R	S	S	S	S	S	R	R	R
	RM-34	R	R	R	R	R	R	R	R	S	R	R	R	R	R	S	S	S	S	S	R	R	R
5	Monroe	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S
	GN-31	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S

S: Sensitive. R: Resistant. T: Tolerant. RLGL-B: Redlands Greenleaf B. RLGL-C: Redlands Greenleaf C. GN-123: Great Nortern 123. GN-31: Great Northern 31. RM-34: Red Mexican 34. Dub. Wit.: Dubbele Witt. Flor. : Florida. West.: Western . Mex.: Mexican. Ida.: Idaho .

* From Drijfhout, E. et al (19).

Table 2. * Systemic reaction of cultivars from host groups 6 to 9, inoculated with 22 isolates of bean common mosaic, arrange according to strain groups of the virus.

		BCMV strain group and isolates																					
		I				II				III	IVa	IVb				Va	Vb	VIa	VIB		VII	
Host group	Cultivar	Type	NL1	PR9M	IRAN	NL7	R220	S74	PV25	NL8	FLOR.	IDA.	WEST.	NL6	BAILIF	NY15	NL2	IMUNA	NL3	NL5	NL4	Mex.	Chile
6	Jubila	I	I	I	I	I	I	I	I	I	I	N	N	N	N	I	N	N	N	N	I	I	I
7	Topcrop	I	I	I	I	I	I	I	I	I	I	V	V	V	V	I	V	V	N	N	I	I	I
	Imp. Tgn	I	I	I	I	I	I	I	I	I	I	V	V	V	V	I	V	V	N	N	I	I	I
8	Widusa	I	I	I	I	I	I	I	I	N	I	V	V	V	V	I	I	I	N	N	I	I	I
	Blk. Tur.	I	I	I	I	I	I	I	I	N	I	V	V	V	V	I	I	I	N	N	I	I	I
9	Amanda	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	N	I	I	I

I: Resistant at 20-30C temperatures. No systemic symptoms. N: Necrotic tip kill of most or all plants . V: Variably sensitive : some plants may show systemic necrosis at 20-30C temperatures. Ipm.Tgn: Improved Tendergreen. Blk.Tur: Black Turtle Soap. Flor. : Florida . Ida. : Idaho . West. : Western . Mex. : Mexican .

* From Drijfhout, E. *et al* (19).

resistant cultivars developed severe vascular necrosis in the leaves, stem, shoot tips and roots. This reaction was referred to as "black root". However at normal growing temperatures of 20-28 C, those cultivars were resistant to the Type and NY15 strains (19).

BCMV, is readily transmitted by mechanical inoculation and it spreads in nature by different species of aphids as *Aphis fabae* (Scopoli), *Myzus persicae* (Sülzer) and *Acyrthosiphon pisum* (Harris) in a stylet-borne manner (12). The aphid transmits the virus more readily from chlorotic leaf area than from dark green areas (12,54). BCMV is seed transmitted in beans. Infected seeds are considered as the most important source for initial crop infection (12). Morales and Castano (44) reported that transmission by seeds depends greatly on the stage of infection of mother plants and on the bean cultivar. They found that maximum seed transmission was observed for plants inoculated at the primary leaf stage. Bean cultivars: Dubbele Witte, Redlands Greenleaf B, Michelite 62, Sanilac and Red Mexican 35 carried the US1, US2, US5, NL3 and NL4 strains of BCMV at a rate ranging from 39.7 to 54.4%. The virus was transmitted in less than 1% in Imuna and Great Northern lines 31 and 123. On the contrary US2 strain of BCMV was not transmitted in 1000 tested seeds from Pinto 114. The necrosis-inducing BCMV NL3 strain proved highly transmissible in the seeds of Sanilac and Michelite 62 cultivars. Bos (12) found that the virus is located in the embryo and the cotyledons but rarely in seed coat.

Moderate infection with BCMV caused 50% reduction in number of pods per plant and 53% reduction in seed yield, while severe

infection caused 64% reduction in number of pods per plant and 68% reduction in seed yield (23).

The virus is not reported to be seed transmitted in faba bean.

BCMV, is serologically related to several viruses in the potyvirus group as bean yellow mosaic (BYMV) (12,53), soybean mosaic (SMV), cowpea aphid-borne mosaic (CAMV) (12) and blackeye cowpea mosaic (BlCMV) (37,47). Partial or complete cross-protection in plants has some times been found between BCMV and BYMV (12).

BYMV, another important member of potyvirus group, is a world wide distributed virus (11,28). It has filamentous particles of 750-800 nm long (10,11,28,36,40), and 15 nm wide (28) and is readily transmitted by sap inoculation (11,40). Although BYMV infects mainly leguminous plants, it can infect several non-legumes (11). Bos *et al* (15) divided BYMV isolates into three distinct groups on the basis of host range, symptoms and reaction of some cultivars of bean and pea. These groups were typical bean yellow mosaic virus isolates, pea yellow mosaic strain isolates and pea necrosis strain isolates. The three groups did not differ in their serological properties. Jones and Diachun (30) classified BYMV into three distinct subgroups on the basis of serological and biological differences. Members of subgroup I induce infection on *Nicotiana tabacum* CV. Burley 21, and cause yellow mosaic, necrosis and death in susceptible bell bean and Dwarf Gray Sugar pea cultivar. In addition they cause tip necrosis and death in susceptible bean cultivars as Bountiful. Members in the second subgroup cause mild-dark

green mosaic in pea and bell bean and moderate mosaic in susceptible cultivars as Bountiful (30). The Type and Jordanian isolates of BYMV belong to subgroup II (4,30). The members of subgroup II usually infect Red Mexican U136 cultivar which appeared to be resistant to BYMV isolates of the other subgroups. Members in the third subgroup generally do not infect tobacco, but infect susceptible peas and bell bean causing either mild light-green mosaic to severe yellow-green mosaic. They can not induce death in them (40). Symptoms of BYMV in *V. faba* depend greatly on the virus strain (28). For instance the symptoms induced by the Type strain is characterized by transient vein chlorosis followed by obvious green or yellow mosaic with no leaf distortion (11). The necrotic-lesion strain of BYMV produces solid brownish-red necrotic local lesions on faba bean, while the severe-yellow mosaic virus produces systemic mottle (28,50). The virus induced large amount of amorphous cytoplasmic granular inclusions (4,20,48) and intranucleolar crystalline inclusions that could be seen under the compound microscope. In thin sections, cylindrical inclusions are found specially in the pinwheel forms (11,40).

BYMV is inactivated when heated to 55-60 C for 10 minutes. It withstands 10^{-4} but not 10^{-5} dilutions and is infectious after storage for 48 hours *in vitro* at 20 C but not after 72 hours (4,11,40). The virus can be transmitted by more than 20 aphid species in non-persistent manner (11). *Aphis fabae*, *A. craccivora* (Koch) and *Myzus persicae* are the most efficient vectors (32).

BYMV is transmitted through the seeds of faba bean at a

sativum and *V. faba* (26). The incidence of the disease depends greatly on the temperature. At low temperature the incidence is apparently low, and under greenhouse conditions fewer inoculated plants developed symptoms when grown at 18 C but not at 29 C (41). Extracts from infected roots remain infectious for more than 96 h. The dilution end point is between 10^{-3} - 10^{-4} and only traces of infectivity remained after 10 min. heating at 55 C. The infectivity is lost by freezing (26).

PSbMV is aphid transmitted in stylet-borne manner by several aphids as *M. persicae, Acyrthosiphon pisum, Macrosiphum euphorbiae* (Thomas), *Aphis craccivora* but not by *Periphyllus lyropictus* (Kessler) (26). The Japanese isolate of PSbMV was not transmitted by *A. pisum* which is an active vector of the American isolates (42). The virus is readily transmitted by sap. Transmission by seeds is recorded in *Pisum sativum* at a ratio that reached 30% if infection occurred prior to flowering. No seed transmission was found when the plants were infected after flowering (26,35). In addition the virus is transmitted through a low percentage of seeds of *Vicia articulata, V. narbonensis* and *V. pannonica* (26). In Japan and the United States no wild species had been found that serves as reservoir host. The virus spreads in the field by aphids that feed on seedlings which originate from infected seed (42).

PSbMV is serologically related to BYMV. Isolates from U.S.A and Japan were serologically related. The relationships to other legume-infecting potyviruses are not determined (26).

Although BLRV is not in the mosaic group, it is among the most important viruses known to infect *V. faba*. The infected faba

MATERIALS AND METHODS

SAMPLE COLLECTION AND VIRUS ISOLATION. About 100 leaf samples of faba bean showing mosaic symptoms were collected from different fields along the Jordan Valley at weekly intervals starting from late autumn till the end of the growing season. Moreover, leaf samples from weeds belonging to different families were collected from faba bean fields and/or adjacent areas (Appendix 1).

Each sample was tested for virus(es) presence using the following assay plants: *Chenopodium quinoa*, *C. amaranticolor*, *Pisum sativum* CV. Alaska pea, Onward pea, *Phaseolus vulgaris* CV. Pinto 114, Bountiful, Monroe, *Vigna unguiculata* CV. California blackeye, *Nicotiana tabacum* "Havana 423", *Medicago sativa* and *Trifolium alexandrinum*. Inoculum was prepared by macerating part of the collected tissue in 0.01M neutral phosphate buffer containing 0.01M sodium diethyldithiocarbamate (Na DIECA) and 0.01M cystine hydrochloride, using sterilized mortar and pestle. The assay plants were dusted with 6000-mesh carborundum. Inoculation of the test plants was done by forefinger at the cotyledonary or at the 3rd-4th leaf stage. The plants were observed 4 weeks for symptoms development. All tested plants were back indexed on faba beans 30 days after inoculation.

HOST RANGE. Fourty three plant species from 7 families used in this study were grown in plastic pots filled with methyl bromide fumigated or pastuerized soil (75 C) under glasshouse conditions. At the proper stage of growth (cotyledonary or first true leaf stage), the plants were inoculated with the selected isolates. The symptoms were recorded 30 days after inoculation. Samples

from inoculated and tip leaves of the tested plants were back indexed on faba bean.

PROPERTIES IN CRUDE SAP. Tip leaves of infected faba bean were extracted in 0.01M neutral phosphate buffer containing 0.01M DIECA and cystine (1 gm tissue / 1 ml buffer). The extract was strained through two layers of cheesecloth. The dilution end point (DIP) was determined by subjecting 1ml sap to 10-fold serial dilutions. For the thermal inactivation point (TIP), 0.5 ml aliquots were heated separately in water-bath to 50,55,60,65,70 C for 10 minutes. All heated aliquots were stored in an ice bath till the end of the experiment. The longevity *in vitro* was determined by storing 4 ml of sap at room temperature and assayed daily for infectivity. The assay plants used in all aforementioned experiments was faba bean.

APHID TRANSMISSION. Aphids, collected from different hosts and locations, were reared on different host plants (Table 3). In all aphid transmission experiments, adult apterous nonviruliferous aphids were transferred with camel's hair brush to a petri-dish, starved for 1 h and given an acquisition feeding period of 10 minutes. Five aphids per plant were transferred to 20 healthy plants of faba bean seedlings. The aphids were killed with an insecticide* (at rate of 5 gm / 10 L H2O) at the end of 5 minutes

* Pirimor (Pirimicarb: 2-Dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate). ICI. Ltd.

inoculation feeding period. The inoculated plants were placed in the glasshouse for 20 days for symptoms development.

INCIDENCE OF MOSAIC DISEASES ON FABA BEANS. Incidence of mosaic diseases on faba beans was determined in three fields selected randomly along the Jordan Valley in the following areas: Masharee, Sawalha, and South-Shuneh. The selected fields were visited at weekly intervals between Nov.17,1987 and March 20,1988. For each field, number of plants showing mosaic symptoms were counted each time out of 200 faba bean plants selected at random in four rows. The percentage of plants with visual symptoms was calculated for the area on a given date of collection,

One hundred or fifty four leaf samples selected randomly from the surveyed fields, were tested serologically against BYMV or BCMV respectively.

RESPONSE OF SOME FABA BEAN AND BEAN CULTIVARS TO VIRUS ISOLATES. Fourty faba bean cultivars, (Table 4), supplied by ICARDA, and 27 bean cultivars (Table 5), supplied by Dr. M. Silbernagel, were tested for their susceptibility to infection by virus isolates 87-23,87-24 and 87-25. The cultivars were inoculated mechanically at cotyledonary stage for beans or at 3-4 leaf stage for faba bean by the virus isolates. For each isolate, 8 plants from each cultivar were inoculated. Plants were observed daily for symptoms during a period of 30 days. All tested cultivars were then back indexed on faba bean and bean.

Table 4. Faba bean cultivars *, supplied by International Center for Agricultural Research in the Dry Areas (ICARDA), challenged with 87-23, 87-24 and 87-25 isolates.

No.	Code number	Origin and pedigree
1.	BF 2/2	Sudan variety
2.	Giza 3 Improved	Egypt variety
3.	Giza 402	Egypt variety
4.	Hudeiba 72	Sudan variety
5.	Reina Blanca	Egypt (Spain)
6.	Sm-L	Sudan variety
7.	ILB 2785	Sudan landrace
8.	ILB 2786	Sudan landrace
9.	ILB 2788	Sudan landrace
10.	ILB 2789	Sudan landrace
11.	NEB 2727/75	Egypt selection
12.	2095/76	Egypt selection
13.	187/2324/79	Egypt selection
14.	187/1104/80	Egypt selection
15.	314/1188/81B	Egypt selection
16.	343/1131/82	Egypt selection
17.	345/1197/82	Egypt selection
18.	SEI-1(1)	Sudan selection
19.	SEI-1(2)	Sudan selection
20.	SEI-4(1)	Sudan selection
21.	SE7-8(2)	Sudan selection
22.	SE7-9(1)	Sudan selection
23.	SE12-1(1)	Sudan selection
24.	SE13-2(2)	Sudan selection
25.	SE14-7(1)	Sudan selection
26.	SE14-9(1)	Sudan selection
27.	SNA1-7	Sudan selection
28.	SNA5-2(1)	Sudan selection
29.	SNA12-2(1)	Sudan selection
30.	SP10-4	Sudan selection
31.	SP3-6(1)	Sudan selection
32.	SP8-3	Sudan selection
33.	SP23-1	Sudan selection
34.	SP25-2	Sudan selection
35.	SE1-6(3)	Sudan selection
36.	SE6-5	Sudan selection
37.	SN-8-7	Sudan selection
38.	SNAI-8	Sudan selection
39.	SNA9-2(1)	Sudan selection
40.	SN11-1(2)	Sudan selection

* Information is provided by ICARDA.

Table 5. Origin of bean cultivars that had been challenged with 87-23, 87-24 and 87-25 virus isolates.

No.	Bean cultivar	Origin
1.	Apollo	*
2.	Amanda	Netherlands
3.	Black Turtle 1	*
4.	Black Turtle Soap	Mexico
5.	Bountiful	*
6.	California Light Red Kidney	*
7.	Dubbele Witte	Netherlands
8.	Great Northern 31	USA
9.	Great Northern 123	USA
10.	Imuna	Germany
11.	Improved Tendergreen	USA
12.	Jubila	Germany
13.	Michelite 62	USA
14.	Monroe	USA
15.	Puregold	USA
16.	Pinto 114	USA
17.	Redlands Greenleaf B	Australia
18.	Redlands Greenleaf C	Australia
19.	Red Mexican 34	USA
20.	Red Mexican 35	USA
21.	Red Mexican 36	USA
22.	Sanilac	USA
23.	Sutter Pink	USA
24.	Stringless Green Refugee	USA
25.	Topcrop	USA
26.	Tormine	*
27.	Widusa	Netherlands

* No information.

ELECTRON MICROSCOPY. Formvar coated grids were floated for 5 minutes on infected tissue extract in 0.01 M neutral phosphate buffer. The grids were then washed with distilled water before they were stained with 5-6 drops of 2% potassium phosphotungstic acid (PTA, PH 6.5) or 1% uranylacetate. The grids were viewed with ZEISS EM 10B electron microscope.

INCLUSION BODIES. Inclusion bodies were demonstrated by floating the peeled underside of epidermal strips from underside of infected and healthy leaves of faba bean in 18 drops Azure-A mixed with 2 drops from 0.2 M disodium phosphate for 15 minutes. The strips were then washed with ethanol for 30 seconds, before they were floated in 2-methoxy acetate for 10 minutes (Christie and Edwardson 1977). Good preparation were mounted in a D.P.X media and examined with the Balplan compound microscope.

SEROLOGY. Antisera for BCMV, CYVV, and SMV, were provided by Dr. G. I. Mink of Washington State University. Antisera to BlCMV was provided by Dr. K. Makkouk of ICARDA. Antisera to PSbMV was provided by Dr. R. O. Hampton of Oregon State University and the antisera for BYMV was supplied by Dr. L. Bos of Instituut Voor Plantenziektenkundig Oinderzoek. Other types of BYMV and CYVV antisera were provided by Dr. F. Zettlere of the University of Florida.

The virus isolates were tested serologically against BCMV, BYMV, CYVV, PSbMV, and SMV antisera using agar double diffusion tests. Plates for these tests were prepared by dissolving 0.8%

Noble agar containing 1% sodium azide and 0.5% sodium lauryl sulfate (SDS) as described by Purcifull and Batchelor (46). Virus antigens were prepared from diseased faba bean by grinding the tissue with neutral phosphate buffer (1gm/1ml) diluted with an equal amount of 3% SDS.

The indirect enzyme-linked immunosorbent assay (ELISA) was also employed in serological tests for BCMV, BYMV, BlCMV and PSbMV. Antisera for BYMV, BlCMV and PSbMV used in ELISA test were the same antisera used in agar double diffusion test, whereas the BCMV antiserum was a monoclonal antibodies (197 A) supplied by Dr. G. I. Mink. Buffers adopted in this test were used by Koenig (33) (Appendix 2). Antisera and conjugate dilutions were compared in preliminary ELISA tests using sets of positive and negative controls. The dilutions that gave the best reaction were selected in further tests.

The infected faba bean leaves from isolates 87-21, 87-23, 87-24 and 87-25 were macerated at 1:50 dilution for BCMV or at 1:10 dilution for BYMV, BlCMV and PSbMV, in grinding buffer (CEP) (Appendix 2) containing 0.45% diethyldithiocarbamate trihydrate (DIECA). ELISA plate wells were separately charged with 0.3 ml sap extract. Healthy sap extract and PBS-Tween buffer were used as control. The plate was then incubated for 1 h at room temperature. After the sap extract was discarded, the plate was washed with PBS-Tween and the wells were then charged with 0.2 ml of antisera diluted in PEP buffer (Appendix 2) to 1:5000, 1:2000, 1:8000 or 1:10000 specific to BYMV, BlCMV, PSbMV or BCMV respectively. The plate was incubated 1 h at room temperature, after which the antisera was discarded and the plate was washed 3

times with PBS-Tween. 0.2 ml of Goat Anti-Mouse or Goat Anti-Rabbit conjugate, diluted to 1:3000 in PEP, buffer were added to wells that had been coated with BCMV monoclonal antibodies or with BYMV, PSbMV and BlCMV antisera, respectively. The plate was incubated 3 h at 37 C. The wells were washed with PBS-Tween and 0.3 ml of substrate was added to each well. The substrate was prepared by dissolving 0.1 gm of p-Nitrophenyl Phosphate Disodium in 100 ml of substrate buffer (Appendix 2).

Results of the reaction were taken 30 minutes after adding the substrate, visually or by using ELISA reader EIA-TEK model 308. The results were considered positive if the substrate color turn to yellow as compared to control wells or if the absorbance values at 405 nm were two times more than that of the values in control wells under similar conditions.

Serotyping of isolates that had been identified as BCMV were further tested against three BCMV monoclonal antibodies, namely 197 A, I-2 and I-59 provided by Dr. G. I. Mink.

RESULTS

VIRUS ISOLATION. Ninty-three virus isolates were recovered from diseased faba bean leaves showing mosaic symptoms collected through 1987. All attempts to isolate viruses from fourty weed samples collected through 1987-1988 failed (Appendix 1). Since most isolates reacted in similar pattern when assayed on selected host plant species (Table 6), four isolates, namely 87-21, 87-23, 87-24 and 87-25 were chosen to represent the remaining isolates. Isolate 87-21 was selected on the basis of its reaction on *C. amaranticolor* and isolate 87-23 was selected on the basis of the difference that was shown on *C. amaranticolor* and *V. unguiculata*. Isolate 87-24 was differentiated from the remaining isolates by its symptoms on *C. amaranticolor* and isolate 87-25 was selected or differentiated on the basis of its reaction on *C. amaranticolor*, *P. sativum* CV. Alaska and *P. vulgaris* CV. Stringless blue lake. The first three isolates (87-21,87-23 and 87-24) were passed through single lesion transfers in *C. amaranticolor*. The fourth isolate 87-25 was recovered from a faba bean plant which comes from apparently infected seed.

HOST RANGE AND SYMPTOMOLOGY. Fourty three plant species from seven families were inoculated separately with each of the four isolates. The isolates have host range that is restricted to few plants belonging to leguminoseae and chenopodiaceae, including the following plants: *Chenopodium amaranticolor* *C. quinoa*, *Lathyrus odoratus*, *Melilotus indica*, *Phaseolus vulgaris* CV. Stringless blue lake, *Pisum sativum* CV. Alaska and IFPI and *Vicia faba*.

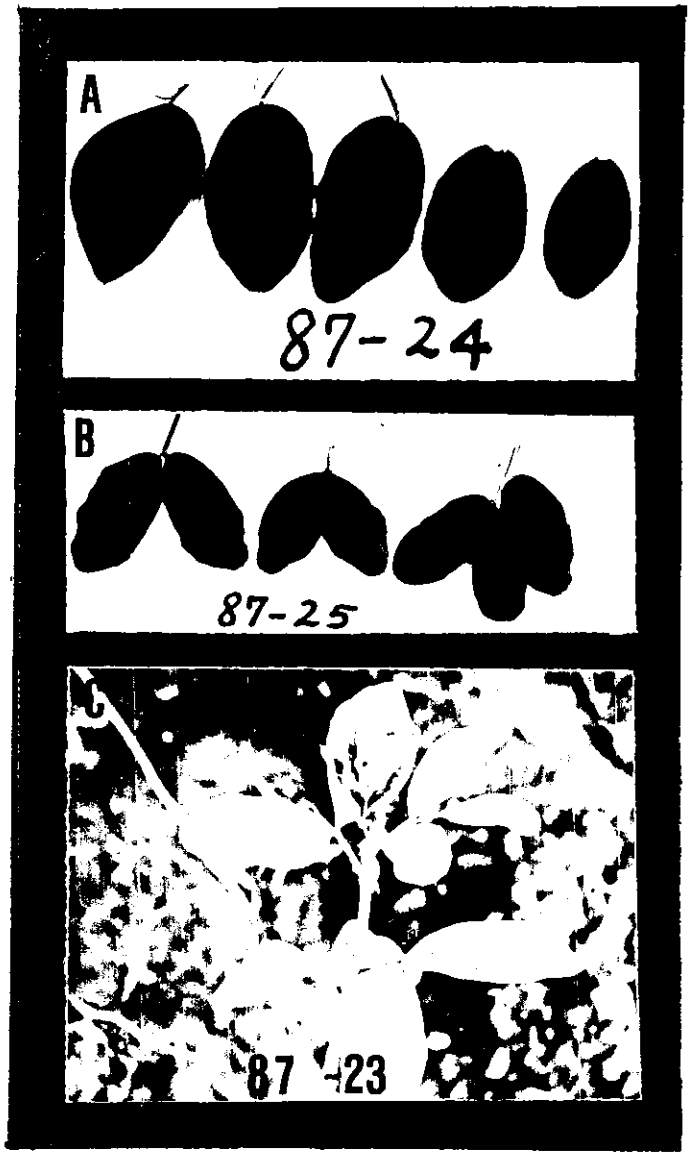

Plate 1. Symptoms of BCMV on faba bean are severe systemic mottling. A: isolate 87-24, B: isolate 87-25 and C: isolate 87-23.

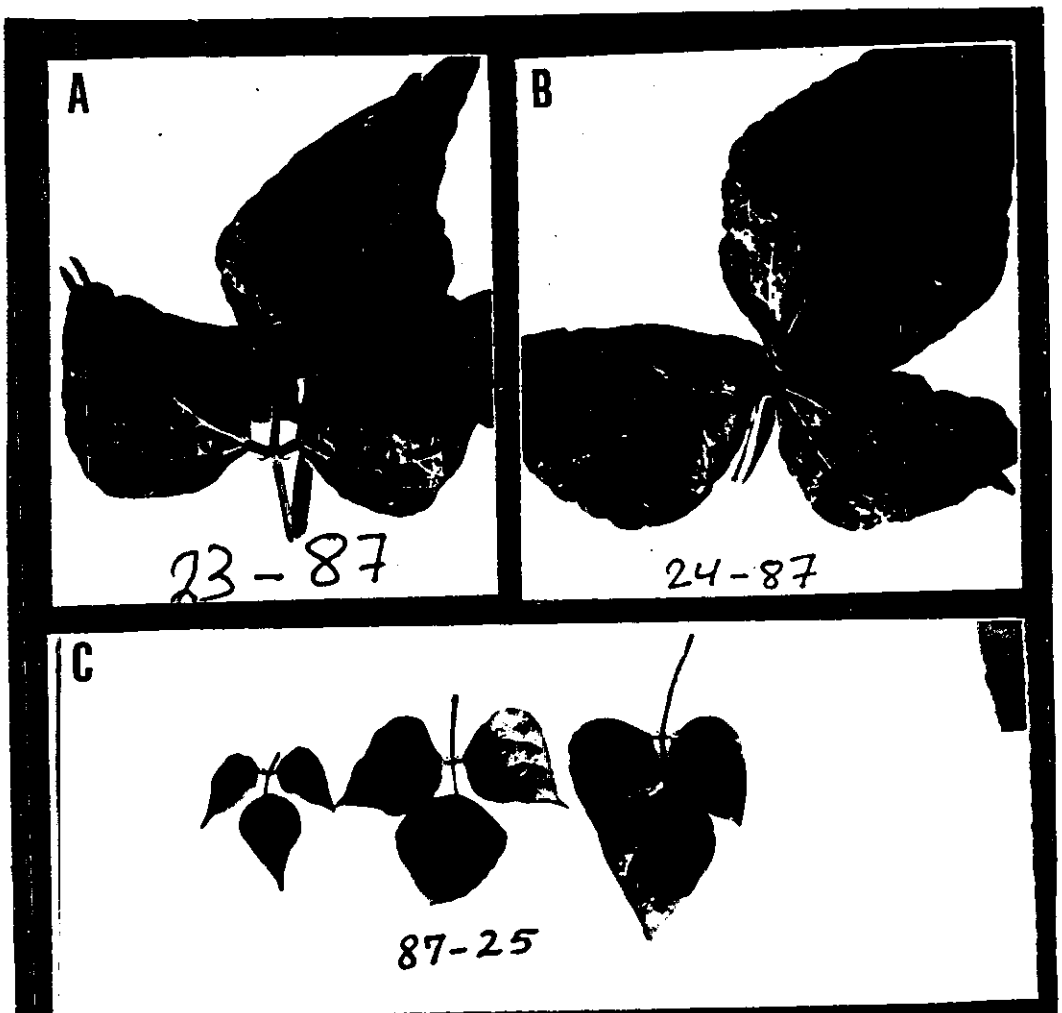

Plate 2. Symptoms of BCMV on leaves of *P. vulgaris* CV. Stringless blue lake. A: isolate 87-23, B: isolate 87-24 and C: isolate 87-25.

Isolate 87-24 caused pinpoint necrotic local lesions on *C. amaranticolor* two weeks after inoculation. The lesions became reddish in color one month after inoculation (Plate 3). Isolate 87-21 induced chlorotic local lesions.

Chenopodium quinoa. The four isolates showed chlorotic local lesions on *C. quinoa*. No systemic symptoms occurred.

Pisum sativum "Alaska" and "IFPI". Symptoms for the four isolates started 10 days after inoculation as vein clearing and mild mosaic that developed into severe mottling. In addition the size of the leaves of infected plants was reduced as compared to that of healthy plants. Generally the infected plants were stunted. Isolate 87-25 showed severe stunting and the older leaves started to die 25 days after inoculation. Necrosis moved upwards until the whole plant die.

Lathyrus odoratus. The four isolates induced similar symptoms on sweet pea. The symptoms started as mild mosaic two weeks after inoculation. Twenty days later, small blisters appeared on the tip leaves. The infected plants were generally stunted.

Lupinus luteus. Isolate 87-25 caused severe symptoms on lupin (Plate 4). The symptoms started as mild mottle 12 days after inoculation. Twenty days after inoculation the tip leaves showed malformation and narrowing. The malformed leaves became yellowish with few portions remained green. In addition the plants were severely stunted. Isolate 87-24 induced mild mosaic 12 days after inoculation. Isolate 87-21 caused no visible symptoms, while isolate 87-23 did not infect lupin.

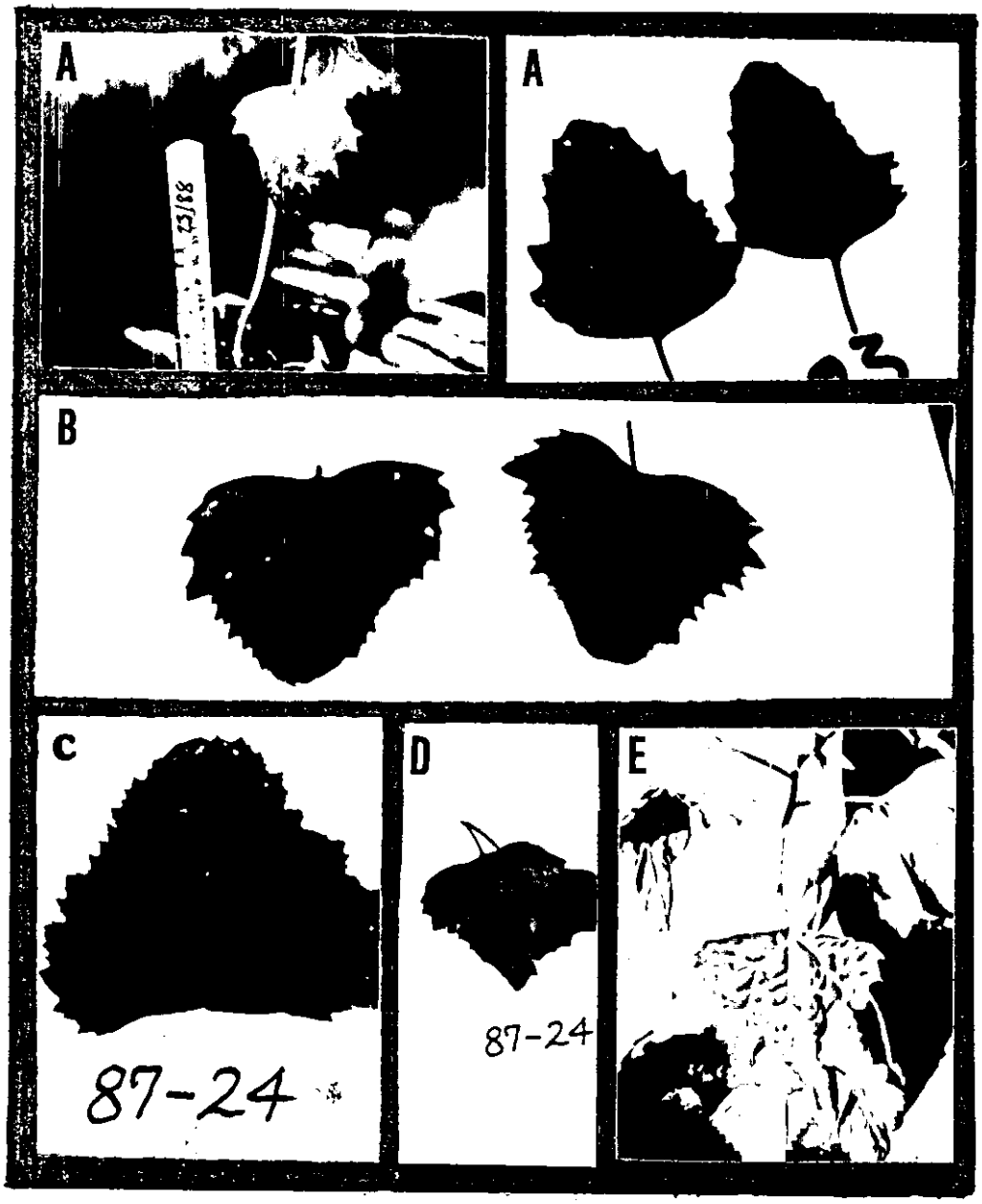

Plate 3. Symptoms of BCMV on *C. amaranticolor*. A: chlorotic local rings induced by isolate 87-23. B: the chlorotic rings spreading along the veins (left), healthy *C. amaranticolor* (right). C: pinpoint necrotic local lesions produced by isolate 87-24. D: the lesions became reddish in color. E: numerous chlorotic local lesions induced by isolate 87-25.

Plate 4. Symptoms of BCMV isolate 87-25 on lupin showing mottling, general yellowing and stunting (left). Healthy lupin (right).

PROPERTIES IN CRUDE SAP. Isolate 87-21 was found to withstand heating up to 55 C but not 60 C, had a dilution end point of 10^{-4} and retained infectivity in crude sap for 48 h but not for 72 h.

Isolate 87-23 had thermal inactivation point of 55-60 C, dilution end point of 10^{-3} and its longevity *in vitro* was 3 days.

The general properties of 87-24 and 87-25 were: the dilution end point 10^{-5}, 10^{-4} respectively. The thermal inactivation point ranged between 55-60 C for both isolates. The longevity *in vitro* was three days or five days for isolates 87-25 and 87-24 respectively.

ELECTRON MICROSCOPY. Flexuous rod particles were detected in crude sap from *V. faba* leaves infected with 87-23, 87-24 and 87-25 (Plate 5).

The length of particles were found to be 750-850 nm, 733 nm and 773 nm for isolates 87-23, 87-24 and 87-25 respectively.

INCLUSION BODIES. The three isolates of 87-23, 87-24 and 87-25 produced cytoplasmic amorphous inclusion bodies (Plate 6). The inclusions were never seen in healthy strips of faba bean.

TRANSMISSION BY APHIDS. Isolate 87-21 was readily transmitted by *M. persicae.*

The three isolates of 87-23, 87-24 and 87-25 were also readily transmitted by *Aphis fabae*, *A. craccivora* and *M. persicae. A. gossypii* (Glover) can readily transmit isolate 87-23 and 87-25, whereas it failed to transmit isolate 87-24 to faba bean but can do so to one plant out of 20 tested bean plants.

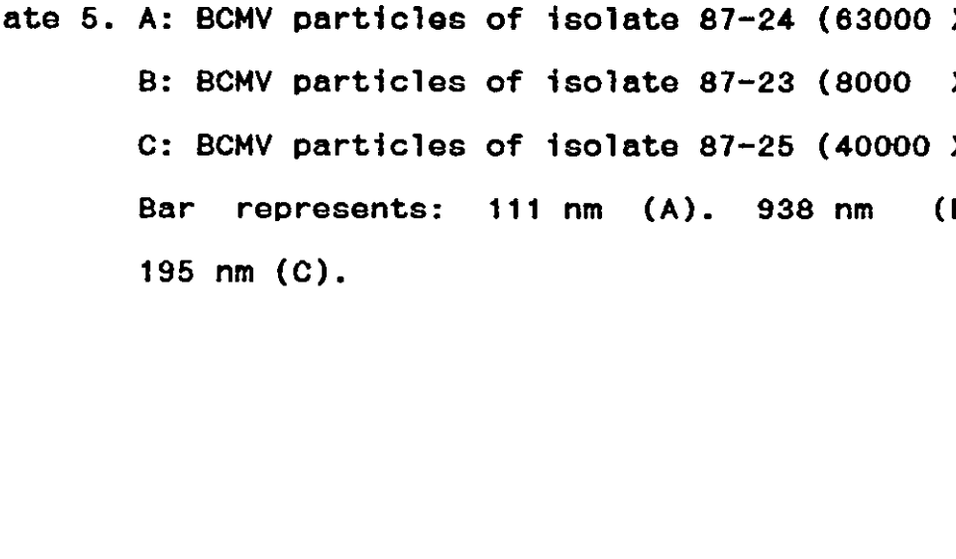

Plate 5. A: BCMV particles of isolate 87-24 (63000 X).
B: BCMV particles of isolate 87-23 (8000 X).
C: BCMV particles of isolate 87-25 (40000 X).
Bar represents: 111 nm (A). 938 nm (B).
195 nm (C).

Efficiency of transmission by aphids varied with aphid species and the virus isolate (Table 7). For instance isolate 87-23, the most efficient vector was *A. fabae*, and for isolates 87-24 and 87-25, *M. persicae* was the most efficient vector.

INCIDENCE OF MOSAIC DISEASE ON FABA BEANS. The incidence of mosaic disease affecting faba beans was studied in three fields. The disease built up to complete infection within two to three months depending on the date of planting. Incidence of mosaic disease in Masharee field, increased slower and over longer period of time compared to the two fields in South-Shuneh and Sawalha respectively. The progress of the disease with elapse of time was characterized by a sigmoid curve, suggestive of a disease at epidemic proportion (Fig. 1).

9% of the leaf samples collected from faba beans with mosaic symptoms, were found infected with BCMV and 97% of them were found infected with BYMV (Fig. 1)

SEROLOGY. In agar double diffusion test the four isolates 87-21, 87-23, 87-24 and 87-25, did not react with antisera specific to , BYMV, CYVV (Plate 7), PSbMV or BCMV. In ELISA test, however, the four isolates (87-21, 87-23, 87-24 and 87-25) did not react with any of PSbMV or BlCMV antisera. Isolate 87-21 reacted with BYMV antisera whereas isolates 87-23 and 87-24 reacted with BCMV and cross reacted with BYMV. Isolate 87-25 strongly reacted with BCMV with no cross-reaction with BYMV.

Table 7. Efficiency of different aphid species in transmitting the 87-23, 87-24 and 87-25 isolates .

Vector	87-23 # of inefected plants/ # of tested plants	87-23 % of infected plants	87-24 # of infected plants/ # of tested plants	87-24 % of infected plants	87-25 # of infected plants/ # of tested plants	87-25 % of infected plants
Aphis fabae	34/38	89.5 %	8/20	40.0 %	13/22	59.1 %
Myzus persicae	30/39	76.9 %	14/21	66.7 %	19/27	70.4 %
A. craccivora	17/34	50.0 %	8/18	44.4 %	12/21	57.1 %
A. gossypii	6/20	30.0 %	0/34	00.0 %	2/31	6.5 %
			1/20*	5.0 %		

Source plant : Broadbean .
Assay plant : Broadbean .
* Assay plant : Bean .

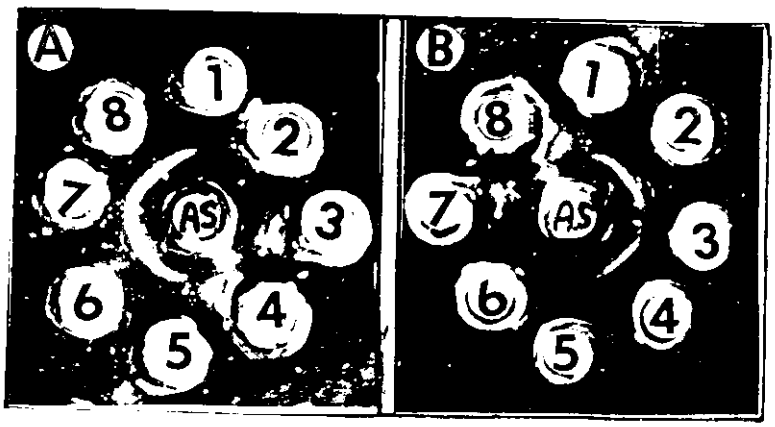

plate 7: Serological test in agar Ouchterlony agar-gel diffusion test. A: Central well contain antisera for BYMV. The peripheral wells contain sap from, 1: Healthy bean, 2: Healthy bean, 3: 87-23, 4: 87-24, 5: 87-25, 6: BYMV antigen, 7: 87-25, 8: BYMV antigen. B: Central well contain antisera for CYVV. The peripheral wells contain sap from, 1: 87-23, 2: CYVV antigen, 3: 87-23, 4: CYVV antigen, 5: 87-24, 6: 87-25, 7: Healthy bean, 8: Healthy bean.

The three isolates of BCMV failed to react with I-2 and I-59 monoclonal antisera which are specific for some BCMV isolates but reacted with a broadspectrum monoclonal antisera of BCMV (197 A) (Table 8).

RESPONSE OF SOME FABA BEAN AND BEAN CULTIVARS TO VIRUS ISOLATES.

Bean cultivars were divided to 4 groups with respect to their response to BCMV infection (Tables 9 & 10).

The first group included bean cultivars that are not infected with the challenging virus isolates (87-23, 87-24 and 87-25). These are called the immune cultivars. Of particular importance is Monroe cultivar which was found to be immune to the three virus isolates. Red Mexican 36 and Michelite 62 were found immune to 87-23 and 87-25 but not to 87-24. Pinto 114 was immune to 87-25 but not to 87-23 and 87-24. Dubbele Witte, Sanilac and Topcrop were found immune only to 87-23, while California Light Red Kidney, Jubila, Redlands Greenleaf B and Apollo were immune to 87-24. Imuna and Great Northern 123 were immune to 87-25.

The second group was the resistant cultivars. Those cultivars develop no systemic infection. Amanda, Bountiful and Red Mexican 35 showed resistance to the three virus isolates, while Redlands Greenleaf B, Redlands Greenleaf C and Improved Tendergreen were resistant to 87-23 and 87-25 but not to 87-24. Pinto 114, Imuna and Great Northern 123 were resistant to virus isolates 87-23 and 87-24 but not to 87-25. Puregold and California Light Red Kidney were resistant to isolate 87-23, Red Mexican 34 was resistant to isolate 87-24. Tormine,

Table 9. Response of bean cultivars to faba bean virus isolates 87-23, 87-24 and 87-25.

Bean cultivars	87-23	87-24	87-25
Apollo	mSM	NI	LL
Amanda	LL	LL	LL
Black Turtle 1	SSM+ST	SSM+ST	SSM+ST
Black Turtle Soap	SSM+ST	SSM+ST	SSM+ST
Bountiful	LL	LL	LL
California Light Red Kidney	LL	NI	LS+L
Dubbele Witte	NI	SM+ST	SM+ST
Great Northern 31	mSM	LL	LL
Great Northern 123	LL	LL	NI
Imuna	LL	LL	NI
Improved Tendergreen	LL	LS+L	LL
Jubila	SM	NI	LL
Michelite 62	NI	SM	NI
Monroe	NI	NI	NI
Puregold	LL	mSM	LS+L
Pinto 114	LL	LL	NI
Redlands Greenleaf B	LL	NI	LL
Redlands Greenleaf C	LL	SM	LL
Red Mexican 34	SM	LL	SM
Red Mexican 35	SNLL+VN	SNLL+VN	LL
Red Mexican 36	NI	SM	NI
Sanilac	NI	SSM	SM
Sutter Pink	SM	SSM	SSM
Stringless Green Refugee	SSM	SM	LL
Topcrop	NI	LL	LL
Tormine	mSM	LS+L	LL
Widusa	SM	SM	SM

mSM: mild systemic mottle, NI: not infected, LL: latent local infection, SSM: severe systemic mottle, ST: stunted, SM: systemic mottle, LS+L: latent systemic and local infection, SNLL: severe necrotic local lesions, VN: vein necrosis.

Table 10:Bean mosaic virus

Resistance level	
Immune	Du Mc Sa
Resistant	Am Gr le Ki Im Im 11
Sensitive	Bl So St Wi
Tolerant	Ap To

Immune: No in infection res harbour the v

reaction of deferential bean cultivars varied with the isolates as showen in table 9. Generally the isolates reaction with these bean cultivars does not fit any pattern for previously described groups. Moreover, Monroe cultivar was resistant when challenged with all BCMV isolates in the seven reported groups (19), but was immune to all BCMV isolates from faba bean. This may justify creating a new group that accommodate them.

Moreover, the efficiency of transmission by different aphid species varied with the virus isolate (17). Although the efficiency of transmission by *M. persicae* and *A. craccivora* were more or less similar to the three isolates of BCMV (87-23, 87-24, 87-25), *A. gossypii* failed to transmit isolate 87-24 to faba bean but was able to transmit it to one bean plant. However, *A. gossypii* transmitted isolates 87-23 and 87-25 at very low efficiency when faba beans were used as assay plant. *A. fabae* transmitted isolate 87-23 efficiently as compared with other two isolates (87-24 and 87-25).

The differences among isolates 87-23, 87-24 and 87-25 are substantiated by serological reaction of these isolates with different antisera. Although the three isolates belong to serogroup B, they differ in their behavior. Isolate 87-23 and 87-24 cross reacted with BYMV antisera whereas isolate 87-25 failed to do so.

The incidence study, indicated that the spread of mosaic disease is very high. These results might be attributed to the high population of winged aphids in autumn and winter of 1987, particularly in Novmber and February (9). Survey study of BYMV indicated the presence of the virus throughout the

Jordan Valley, suggesting that virus sources are similarly as widespread. The incidental recovery of BCMV from faba bean fields was low and that may be due to less availability of virus sources.

SUMMARY

A study was conducted to identify virus(es) involved in the mosaic disease complex in Jordan .

Ninty three virus isolates were recovered from diseased faba bean plants showing mosaic symptoms collected through 1987. Four isolates were chosen to represent the remaining isolates on the basis of their reaction on selected preliminary host plant species. The study extended information on their host range, physical properties, electron microscopy, inclusion bodies, serological behavior and the response of different bean and faba bean cultivars to infection by these isolates.

One isolate was identified as BYMV. The other three isolates were identified as three different strains of BCMV on the basis of their serological and biological behavior. The three strains differ from all 22 BCMV strains described earlier in the literature .

Incidence of the mosaic diseases affecting faba bean in Jordan Valley reached up 100% at the end of March. The incidence survey indicated that the BYMV is the most prevalent virus in the Jordan Valley. BCMV did not play an important role in the mosaic disease complex compared to BYMV.

عزل وتعريف بعض امراض التبرقش الفيروسية على محصول الفول في الاردن

ملخص

اجريت دراسة على محصول الفول في وادي الاردن وذلك للتعرف على امراض فيروسية جديدة غير تلك التي عرفت سابقا ، ولدراسة مدى انتشارها ونسبة الاصابة بها بالاضافة الى ان الدراسة وفرت معلومات اكثر عن بعض الامراض التي عرفت سابقا .

لقد جمعت ثلاث وتسعون عينة من اوراق الفول التي ظهرت عليها اعراض تبرقش وذلك في ربيع عام ١٩٨٧ وبناء على الاعراض التي ظهرت على بعض النباتات الكاشفة ، تم اختيار اربع عزلات درست بتوسع من حيث المدى العائلي الذي شمل ثلاثة واربعين عائلا والحشرات الناقلة التي شملت اربعة انواع مختلفة من المن والخواص الطبيعية ودراسة شكل الفيروس وطوله بواسطة المجهر الالكتروني ، ودراسة الاعراض الداخلية للفيروس في النبات والاختبارات المصلية . وكنتيجة لهذه الدراسة تم تعريف احدى هذه العزلات على انها مرض اصفرار وتبرقش الفاصوليا . والعزلات الثلاث الاخرى عرفت على انها سلالات مختلفة من مرض تبرقش الفاصوليا العام . وقد اثبتت الدراسة البيولوجية لهذه السلالات بانها تختلف عن الاثنتين والعشرين سلالة المعرفة عالميا لهذا الفيروس .

ومن ضمن هذه الدراسة ايضا تم جمع اربعين عينة من الاعشاب النامية في حقول الفول ، وفي حقول اخرى في فترة وجود محصول الفول ، وفي فترات غيابه وذلك لمحاولة عزل اي فيروس منها ولكن جميع المحاولات باءت بالفشل .

وكنتيجة لهذه الدراسة فان امراض التبرقش الفيروسية كانت تنتشر بنسبة ١٠٠ % عل طول وادي الاردن على محصول الفول في نهاية الموسم . ويعتبر مرض اصفرار وتبرقش الفاصوليا الفيروس السائد على محصول الفول، حيث انه كان ينتشر بنسبة ٩٧ % بينما مرض تبرقش الفاصوليا العام كان منتشرا بنسبة ٩% فقط . وقد تعزى هذه النسبة العالية لانتشار امراض التبرقش الفيروسية الى توافر اعداد كبيرة من المن المجنح والذي ينقل هذه الامراض بكفاءة .

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Appendix 1. Location, date of collection and number of weed samples collected from different faba bean fields and adjacent areas in Jordan.

SCIENTIFIC NAME *	# OF SAMPLES	FAMILY **	LOCATION	DATE OF COLLECTION
Amaranthus hybridus	1	Amaranthaceae	Baka'a	9.15.1987
Amaranthus hybridus	2	Amaranthaceae	S.Shuneh	9. 7.1987
Anagallis arvensis	1	Primulaceae	S.Shuneh	3. 1.1988
Berteroa incana	1	Cruciferae	Wadi-Shaib	9. 7.1987
Beta vulgaris	1	Chenopodiaceae	S.Shuneh	2.11.1987
Beta vulgaris	1	Chenopodiaceae	Dair-Alla	2.11.1987
Chenopodium album	1	Chenopodiaceae	S.Shuneh	9. 7.1987
Chenopodium murale	2	Chenopodiaceae	Dair-Alla	2.10.1988
Chenopodium quinoa	1	Chenopodiaceae	Baka'a	9.15.1987
Chrozophora tinctoria	2	Euphorbiaceae	Al-Karama	9.15.1987
Chrozophora tinctoria	1	Euphorbiaceae	S.Shuneh	9. 7.1987
Chrozophora tinctoria	1	Euphorbiaceae	Wadi-Shaib	9. 1.1987
Crepis aspera	1	Compositae	Dair-Alla	3.15.1988
Datura metel	2	Solanaceae	Al-Karama	9.15.1987
Datura metel	1	Solanaceae	Wadi-Shaib	9. 7.1987
Eremostachys laciniata	1	Labiatae	S.Shuneh	3. 1.1988
Erodium malacoides	1	Geraniaceae	Dair-Alla	2.11.1988
Erodium gruinum	1	Geraniaceae	S.Shuneh	3. 1.1988
Eruca sativa	1	Cruciferae	S.Shuneh	3.15.1988
Euphorbia geniculata	1	Euphorbiaceae	S.Shuneh	9. 7.1987
Malva parviflora	2	Malvaceae	Dair-Alla	2.12.1988
Malva parviflora	1	Malvaceae	N.Shuneh	3. 1.1988
Malva parviflora	1	Malvaceae	N.Shuneh	9. 1.1987
Malva parviflora	1	Malvaceae	U.J.farm	9. 7.1987
Mercurialis annua	1	Euphorbiaceae	S.Shuneh	3. 1.1988
Oxalis corniculata	1	Oxalidaceae	S.Shuneh	3. 1.1988
Portulaca oleraceae	1	Portulaceae	N.Shuneh	9.15.1987
Portulaca oleraceae	1	Portulaceae	Wadi-Shaib	9. 7.1987
Prosopis fracta	1	Mimosaceae	Wadi-Shaib	9. 7.1987
Prosopis fracta	1	Mimosaceae	N.Shuneh	9.15.1987
Sinapis arvensis	1	Cruciferae	S.Shuneh	3. 1.1988
Sisymbrium irio	1	Cruciferae	U.J.farm	3.15.1988
Solanum alatum	1	Solanaceae	N.Shuneh	9. 1.1987
Solanum nigrum	1	Solanaceae	S.Shuneh	9. 7.1987
Sonchus oleraceus	1	Compositae	S.Shuneh	9. 7.1987
Sonchus oleraceus	1	Compositae	U.J.farm	3.15.1988
Sonchus oleraceus	1	Compositae	S.Shuneh	3. 1.1988
Trifolium clusii	1	Leguminosae	U.J.farm	9. 7.1987
Urospermum picroides	1	Compositae	Wadi-Shaib	3.15.1988
Withania somnifera	1	Solanaceae	Wadi-Shaib	9. 1.1987

* The weeds were indentified with the help of Dr. B.Abu Irmeileh.

** -Abu-Irmeilh, B. (2)
-Anonymous (7).