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Broad bean mottle virus: identification, host range, serology, and occurrence on faba bean (Vicia faba) in West Asia and North Africa

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Abstract

One of the faba bean viruses found in West Asia and North Africa was identified as broad bean mottle virus (BBMV) by host reactions, particle morphology and size, serology, and granular, often vesiculated cytoplasmic inclusions. Detailed research on four isolates, one each from Morocco, Tunisia, Sudan and Syria, provided new information on the virus.

The isolates, though indistinguishable in ELISA or gel-diffusion tests, differed slightly in host range and symptoms. Twenty-one species (12 legumes and 9 non-legumes) out of 27 tested were systemically infected, and 14 of these by all four isolates. Infection in several species was symptomless, but major legumes such as chickpea, lentil and especially pea, suffered severely from infection. All 23 genotypes of faba bean, 2 of chickpea, 4 of lentil, 11 out of 21 of *Phaseolus* bean, and 16 out of 17 of pea were systemically sensitive to the virus. Twelve plant species were found to be new potential hosts and cucumber a new local-lesion test plant of the virus.

BBMV particles occurred in faba bean plants in very high concentrations, and seed transmission in this species (1.37%) was confirmed.

An isolate from Syria was purified and two antisera were produced, one of which was used in ELISA to detect BBMV in faba bean field samples. Two hundred and three out of the 789 samples with symptoms suggestive of virus infection collected in 1985, 1986 and 1987, were found infected with BBMV: 4 out of 70 (4/70) tested samples from Egypt, 0/44 from Lebanon, 1/15 from Morocco, 46/254 from Sudan, 72/269 from Syria and 80/137 from Tunisia. This is the first report on its occurrence in Egypt, Syria and Tunisia. The virus is a potential threat to crop improvement in the region.

Additional keywords: chickpea, ecology, electron microscopy, ELISA, inclusion bodies, lentil, pea, *Phaseolus* bean, seed transmission.

Introduction

Faba bean (*Vicia faba*) is one of the major crops dealt with by ICARDA. When surveying the crop in the region served by ICARDA (West Asia and North Africa) for actually and potentially important viruses (Makkouk et al., 1987), we soon came across virus isolates resembling broad bean mottle virus (BBMV).

The virus was first described in England from a severely diseased crop of broad bean

(now preferably called faba bean) in Nottinghamshire (Bawden et al., 1951) and was later reported from a crop in Cambridge (Tinsley, 1957). It has long remained of mere academic interest for its unusually high concentration in infected cells (Bawden et al., 1951; De Zoeten and Schlegel, 1967), its inclusion bodies and possible sites of infection (Rubio and Van Slogteren, 1956; De Zoeten and Schlegel, 1967; Lastra and Schlegel, 1975), and its being one of a few Bromoviruses (Gibbs, 1972; Lane, 1974, 1979).

Further incidental reports have meanwhile appeared on its transmission by three species of beetles (Walters and Surin, 1973) and its occurrence in faba bean in Portugal (Borges and Louro, 1974), Sudan (Murant et al., 1974), Morocco (Assou, 1978; Bourbah and Fezzaz, 1979; Fischer, 1979) and Algeria (Ouffroukh, 1985), however with little information on incidence and ensuing damage. Although BBMV was claimed not to be seed-transmissible (Bawden et al., 1951), Murant et al. (1974) later reported that it could be transmitted in faba bean seeds when occurring together with bean yellow mosaic virus (BYMV), and Assou (1978) found diseased plants in the field from the beginning of crop development and in seedlings grown from seed from an infected crop.

Possible seed transmission, incidence and effect on faba bean and some other crop species made the virus seem important to ICARDA's crop improvement program. We therefore further identified representative isolates from the region, looked for variation of the virus, studied some of its characteristics, and further surveyed the region for its distribution and incidence.

Materials and methods

Field collections and virus isolates. Samples of faba bean leaves with symptoms suggestive of virus infection were collected from farmers' fields and experimental plots of agricultural research stations in Egypt, Lebanon, Morocco, Sudan, Syria and Tunisia during 1985-1987. A portion from each sample was extracted in 0.2 M phosphate buffer, pH 6.0, and used for testing for the presence of BBMV by ELISA. The other portion was desiccated over calcium chloride ($CaCl_2$) to be mailed for electron microscopy and for later recovery for further testing. Four isolates tentatively identified as BBMV, namely Mo (= MV90-85 from Morocco), Tu (= TV75-85 from Tunisia), Su (= SuV94-86 from Sudan) and Sy (= SV48-86 from Syria), were used for further characterization of the virus. Most of the work was with Mo and Tu, both in Beirut and Wageningen.

Host-range studies. In Beirut 28 plant genotypes belonging to 20 species were inoculated with all four isolates. Four weeks after inoculation, non-inoculated leaf samples were harvested from all species and tested by ELISA for the presence of BBMV. The tissue was extracted in 0.2 M phosphate buffer, pH 6.0, at a dilution of 1 : 10 (w/v). In Wageningen 10 of the above species and 7 others as well as a number of cultivars of faba bean, pea and *Phaseolus*-bean were tested with isolates Mo and Tu. Several of those not reacting with symptoms were back inoculated onto *Chenopodium amaranticolor* and *V. faba* 'Compacta' or tested with ELISA.

Seed-transmission tests. To study possible seed transmission in faba bean, plants of cv. Syrian Local were inoculated with a mixture of BBMV and BYMV in the field, 76, 107 and 140 days after sowing. Harvested seeds were germinated in sterile sand in the glasshouse. Embryo axes were obtained by cutting off the cotyledons, with the seed

coat attached to them, and tested in groups of 10 by ELISA.

Microscopy. For electron microscopy small pieces of leaf, either fresh or dried, were chopped in a few drops of 2% sodium phosphotungstate (PTA), pH 6.5. The resulting liquid was transferred to the carbon-coated formvar-covered grids, incubated for one minute, whereafter excess liquid was removed.

Light microscopy was with isolate Mo. Epidermal strips from faba bean plants were stained for ca 15 minutes in a mixture of 1% phloxine and 1% methylene blue, both in equal volumes of distilled water, ethylene glycol monomethyl ether and ethanol according to Christie (1969; Bos, 1969).

Virus purification. The isolate Sy was propagated in *Vicia faba* 'Syrian Local'. Systemically infected leaves were harvested 2-3 weeks after inoculation. The virus was purified by a procedure derived from Campbell (1971) and summarized as follows. Clarification was by adjusting the pH of the tissue homogenate with citric acid to 5.0 and subsequent low-speed centrifugation. Virus was then concentrated by adding 8% polyethylene glycol. Further purification was by sucrose density-gradient centrifugation. Purified preparations thus obtained were used for antiserum production.

Serology and antiserum production. A BBMV antiserum (titre 256) against the British type strain and another (titre 64) against a Sudanese isolate, provided by Dr A.F. Murant, Invergowrie, Scotland, were used to confirm the identity of our isolates. Two antisera were prepared in Beirut against the Sy isolate; one with 0.2% glutaraldehyde-treated purified virus and the other with untreated purified virus. Two rabbits were given four weekly intramuscular injections of 2 mg virus each. The virus preparation for the first injection was emulsified with an equal volume of Freund's complete adjuvant and for the three subsequent injections with Freund's incomplete adjuvant. A booster injection was given four weeks after the fourth injection. The rabbits were bled eight times at weekly intervals starting one week after the fourth injection.

BBMV antiserum produced against untreated Sy isolate was used in the gel-diffusion test. Plates for this test were prepared by dissolving 1% Noble agar in 0.1 M phosphate buffered saline, pH 6.0, containing 0.05% sodium azide.

The ELISA procedure followed was that of Clark and Adams (1977), but gammaglobulins were isolated by the caprylic-acid method (Steinbuch and Audran, 1969). Faba bean field samples were considered BBMV-infected when the A_{405} values exceeded the healthy controls by three standard deviations. Inoculated host species, however, were considered infected when the A_{405} values exceeded those of the healthy controls by five standard deviations. The ELISA threshold value was increased for the latter test to reduce the chance of including false positives in the host-range study.

Results

Host-range tests and symptomatology. The results of host-range tests conducted in Beirut and Wageningen are summarized and compared with those of other workers in Table 1.

One or more of the isolates studied systemically infected 21 (12 legume and 9 non-legume) species out of the 27 tested. Fourteen species became systemically infected by

Table 1. Host-range tests with four isolates of broad bean mottle virus form Morocco (Mo), Tunisia (Tu), Sudan (Su) and Syria (Sy) in Beirut, Lebanon (Be), and Wageningen, the Netherlands (Wag), as judged by observation for symptoms (Be and Wag) and testing of non-inoculated leaves with ELISA (Be) or by back inoculation (Wag), and data from the literature.

Species and cultivars or lines tested[1]	BBMV isolates investigated						Data from the literature		
	Mo		Tu		Su	Sy	type strain Bawden et al. (1951)[3]	Walters and Surin (1973)[4, 5]	Ouffroukh (1985)[6]
	Be	Wag	Be	Wag					
Legume species									
* *Cicer arietinum*									
ICARDA ILC 263		– S[2]							
ICARDA ILC 482		L S							
Glycine max	– * s		– * s		– * s	– * s	+	L – (several cvs)	
								– – (other cvs)	
Lathyrus odoratus	S		S		S	S	+	L S	– S
**Lens culinaris*									
Preco 3 ILL 4605		L S							
Syrian Local Small (ILL 4401)		– S							
Syrian Local Large (ILL 4400)		– S							
78 S 26013 (ILL 5587)		L S							
Lupinus albus								L S	– S
Medicago sativa	– * –		– * –		– * –	– * –	–	– – (5 cvs)	– –
**Melilotus albus*		L S		L S					
Melilotus officinalis								S	– S
Phaseolus vulgaris									
Bataaf		L –		– –					
Bountiful	– * s		– * s		– * s	– * s		– * –	– * –
Monroe		L S		L S					
Canadian Wonder	– * S		– * S		– * S	– * S	S		
Great Northern		L S		– –					
Red Mexican	– * s		– * s		– * s	– * s			
Sutter Pink	– * s		– * s		– * s	– * s			
Topcrop		L –		L –					
other cvs		L S (7 cvs)					L S	L – (7 cvs)	– –
		– S (1 cv)						– – (6 cvs)	
		– – (1 cv)						l – (1 cv)	
		L – (4 cvs)							

Pisum sativum																
ICARDA 1LP 205	S				S				S	S						
ICARDA 1LP 2903	S				S				S	S						
Early Perfection		L	S			L	S									
Jewel		L	S			L	S									
Koroza		L	S			L	S									
Perfection		L	S			L	S									
Rondo		L	S			L	S									
other cvs		L	S (9 cvs)										–	S (3 cvs)	–	S
		– *	S (1 cv)										–	– (1 cv)		
Trifolium incarnatum	s	l	S		S	l	S		S	S		S	–	S (2 cvs)	–	S
Trifolium pratense		l	–			l	–					S	–	S (3 cvs)	–	–
Trifolium repens		l	–			l	–						–	S (1 cv)		
Trifolium subterraneum 1FTL 704			S				S		s	S		S			–	–
Vicia faba																
Compacta			S				S									
Syrian Local	S				S				S	S						
other cvs		L	S (1 cv)								– *	S (2 cvs)	–	S (1 cv)	–	S
		– *	S (11 cvs)													
Vicia sativa	s				s				s	s		–			–	S
**Vigna unguiculata*																
California Blackeye No 5	s				s				s	s					–	–
other cvs													–	– (4 cvs)		
Non-legume species																
Amaranthaceae																
**Gomphrena globosa*	s	– *	S		s	– *	–		s	s			l	–	–	–
Chenopodiaceae																
Chenopodium album													L	–	L	–
Chenopodium amaranticolor		L	–			L	–						L	–	L	–
Chenopodium capitatum	–				–				–	–						
Chenopodium quinoa	S	L	–		S	L	–		S	S			L	–	L	–
Spinacia oleracea													–	–	–	–
**Tetragonia expansa*	s	–	–		s	–	–		s	s						
Cruciferae																
**Capsella bursa-pastoris*	–				s				–	–						
Cucurbitaceae																
Cucumis sativus		L	–			L	–						–	–	–	–

Table 1. (Continued).

Species and cultivars or lines tested[1]	BBMV isolates investigated								Data from the literature			
	Mo			Tu			Su	Sy	type strain Bawden et al. (1951)[3]	Walters and Surin (1973)[4, 5]		Ouffroukh (1985)[6]	
	Be	Wag		Be	Wag								
Non-legume species (continued)													
Solanaceae													
**Datura stramonium*	–			–			s	–	–			–	–
Lycopersicon esculentum									–			–	–
**Nicotiana benthamiana*	s	L	S	s	L	S	s	s					
**Nicotiana clevelandii*	–	L	S	–	L	s	s	–					
**Nicotiana glutinosa*	s			–			–	–	–	–	–	–	–
Nicotiana occidentalis		–	–		–	–							
Nicotiana rustica										–	–	–	–
**Nicotiana tabacum*													
Havana 423	s			–			–	–					
Havana 425	s			–			s	s					
Samsun	–	–	–	s	–	–	s	–				–	–
Turkish	s			s			s	–					
Xanthi	–	l	–	–	l	–	s	–				–	–
other cvs	–									–	– (2 cvs)	–	–
Solanum melongena										–	–		

[1] Species marked with an asterisk are new systemic hosts of the virus.

[2] L = local symptoms; S = systemic symptoms; l = latent local infection; s = latent systemic infection; – = no infection as demonstrated by back inoculation onto indicator plants or by serological assay; * = no symptoms observed but not assayed for infection.

[3] Authors concerned only reported whether symptoms and infection occurred, but for most plant species they did not discriminate between local and systemic symptoms and infection.

[4] Authors concerned only reported that test species were observed for symptoms and were assayed for infection on indicator plants, without indication whether such testing was of inoculated or non-inoculated leaves or both.

[5] Other legume species tested by Walters and Surin (1973) with negative results are: *Desmodium paniculatum, Galactia elliottii, Lespedeza sericea, Mucuna deeringianum, Phaseolus atropurpureus, P. lathyroides, P. limensis, Pueraria thunbergiana, Sesbania exaltata* and *Strophostyles helvola*.

[6] Author only reports local and/or systemic symptoms. Sixteen other legume species were listed to react with systemic symptoms and 14 species not listed here did not react.

all four isolates. Nine of the legume species and 4 of the non-legume species reacted with systemic symptoms, at least in one of their cultivars tested and at least with one of the four virus isolates studied. Twelve plant species (in Table 1 indicated with an asterisk) were found to be new systemic hosts of the virus. They include the legumes *Cicer arietinum*, *Lens culinaris*, *Melilotus albus* and *Vigna unguiculata*. Eleven legume species other than faba bean have earlier been reported as potential natural hosts including *Glycine max*, *Lathyrus odoratus*, *Lupinus albus*, *Melilotus officinalis*, *Phaseolus vulgaris*, *Pisum sativum*, *Trifolium incarnatum*, *T. pratense*, *T. subterraneum* and *Trigonella foenum-graecum* (Bawden et al., 1951; Walters and Surin, 1973; Fischer, 1979). Ouffroukh (1985) has recently listed 16 additional legume species showing systemic symptoms after inoculation with an Algerian isolate of the virus, and 14 other plant species not mentioned in Table 1 not reacting with symptoms. This would bring the total number of species systemically susceptible to the virus to 40, but Ouffroukh's data are uncertain since he did not test his species for infection.

Fourteen cultivars and nine promising ICARDA breeding lines of *Vica faba* were tested for their reaction to Mo and Tu. In addition to 'Compacta' and 'Syrian Local', regularly used as test species in Wageningen and Aleppo, respectively, these included the cultivars 'Beryl', 'Eureka', 'Felix', 'Futura R2', 'Giza 3', 'Medes', 'Metissa', 'Minica', 'Optica', 'Proprix', 'Reina Blanca' and 'Statissa'. 'Giza 3' and 'Compacta' were the only cultivars together with some of the breeding lines that sometimes reacted with diffuse chlorotic or necrotic local lesions, appearing in ca 4 days, and often only with lesions remaining green when leaves turned yellow (Fig. 1). All genotypes reacted in ca 7 days with severe systemic vein chlorosis to chlorotic vein banding developing into overall leaf chlorosis in a couple of leaves following the inoculated leaves, sometimes with some remaining interveinal islands of green tissue and in some genotypes with

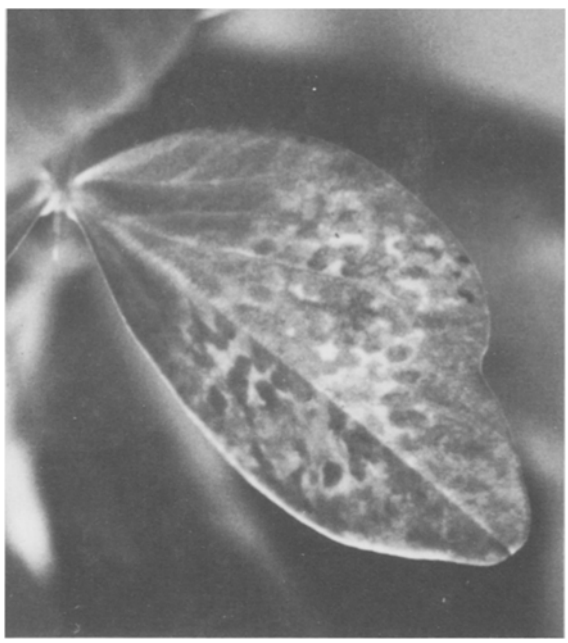

Fig. 1. Local lesions in faba bean breeding line FLIP 84-239 FB nine days after inoculation with isolate Tu.

Fig. 2. Systemic symptoms in faba bean; left, 'Compacta'; right, breeding line FLIP 84-239 FB; 22 and 24 days, respectively, after inoculation with isolate Tu. Plant at left shown from third leaf from bottom onward; note intermediate phase of temporary recovery.

necrosis along leaf edges. Plants thereafter more or less recovered temporarily, with a persistent mottling, marbling or diffuse mosaic recurring in leaves that developed later (Fig. 2, left). In some breeding lines the mosaic and mottling was severe (Fig. 2, right) and accompanied by leaf malformation, reduction in leaf size, plant stunting and bushy growth. Symptoms were more severe with Tu than with Mo. Some genotypes showed irregular vein chlorosis or vein mosaic rather than mottling, and others showed additional necrotic streaks on stems.

The two breeding lines of *Cicer arietinum* (chickpea) tested reacted with systemic symptoms to Mo (one with vein chlorosis followed by mosaic and the other with necrotic stem streaking with tip necrosis). With all four isolates systemic infection of *Glycine max* (soybean) was symptomless. *Lathyrus odoratus* reacted with systemic mottle to all four isolates. All four genotypes of *Lens culinaris* (lentil) inoculated with Mo reacted with systemic interveinal chlorosis, mottling and/or mosaic (Fig. 3, right). Some local necrosis was produced in 'Preco 3' and '26013'.

Twenty-one cultivars of *Phaseolus vulgaris* (common bean) were tested with isolate Mo, four of these with all four isolates (Table 1). Fourteen cultivars reacted with local lesions, which were small and chlorotic, and appeared as soon as 8 days after inoculation ('Bataaf') or later ('Top Crop'), or were green rings appearing only when the inoculated leaves turned yellow ('Monroe'). Several of these cultivars reacted with striking systemic yellow stippling or blotching ('Monroe' and 'Dubbele Witte', Fig. 4, left).

Fig. 3. Systemic symptoms in *Melilotus albus*, more severe with isolate Tu (left, upper row) than with isolate Mo (left, lower row), and in *Lens culinaris* (right, isolate Mo), 39 and 25 days after inoculation, respectively.

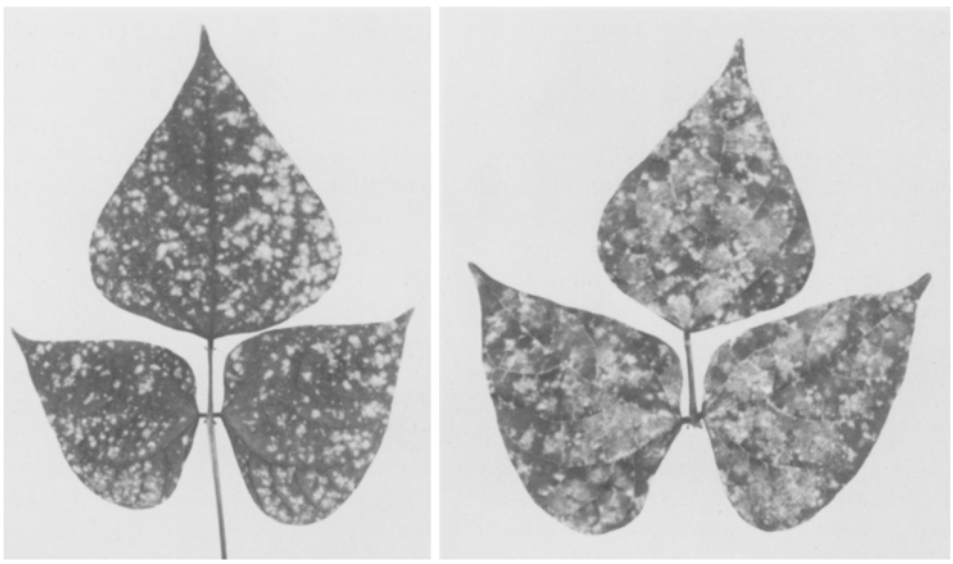

Fig. 4. Systemic yellow stippling or blotching in *Phaseolus vulgaris* 'Dubbele Witte' (left) and 'Great Northern 123' (right), 43 days after inoculation with isolate Mo.

In 'Great Northern 123' this blotching developed into a diffuse mosaic (Fig. 4, right) (and this cultivar was immune to isolate Tu). No symptoms could be observed in four cultivars, namely 'Bountiful', 'Imuna', 'Red Mexican' and 'Sutter Pink' (all but 'Imuna' tested with all four isolates), but virus could be detected in non-inoculated leaves when all but 'Imuna' were tested with ELISA. 'Bataaf' did not produce symptoms when inoculated with two isolates, nor could virus be recovered or detected with ELISA.

All 15 cultivars and two ICARDA lines of *Pisum sativum* (pea) tested were susceptible to Mo, and the ICARDA lines to the other three isolates as well. Some were also tested with Tu. Most genotypes reacted with desiccating, rapidly enlarging local lesions soon leading to withering of inoculated leaves, later followed by necrosis (often first one-sidedly extending downward), tip necrosis and premature plant death. In 'Koroza' and 'Rondo' both isolates often caused partially systemic necrosis, and virus could no more be detected with ELISA in recovered plants. 'Vitalis' reacted with systemic green vein banding or interveinal mosaic when tested with Mo.

Melilotus albus (white sweet clover) reacted to Mo and Tu with systemic chlorotic spotting and striking irregular vein yellowing or vein mosaic, which was severest and accompanied by leaf distortion with Tu (Fig. 3, left).

Trifolium incarnatum and *T. subterraneum*, tested with all four isolates, reacted with systemic mottling to most isolates, and virus could be detected with ELISA with all isolates.

Chenopodium amaranticolor, tested only with Mo and Tu, rapidly reacted with many chlorotic or dry pin-point local lesion sometimes appearing as early as two days after inoculation (Fig. 5, left). *C. quinoa* reacted to these isolates also with pin-point local lesions (Fig. 5, middle). The genotype used in Beirut reacted with diffuse systemic chlorotic lesions, and the virus could be detected with ELISA in non-inoculated leaves.

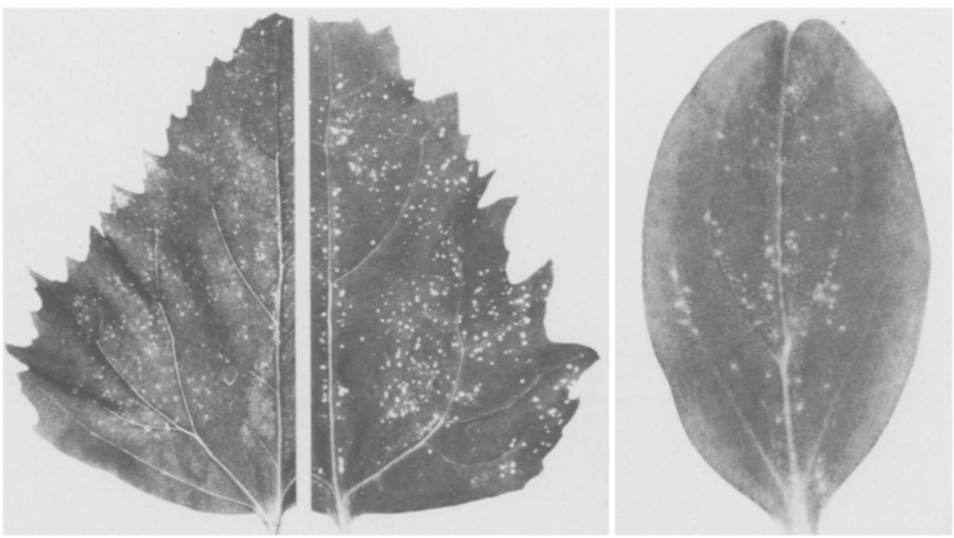

Fig. 5. Small chlorotic to pin-point local lesions of isolate Mo in *Chenopodium amaranticolor* (left) and *C. quinoa* (middle) 14 days after inoculation, and of isolate TV18-75 in *Cucumis sativus* 'Gele Tros' 12 days after inoculation (right).

Cucumis sativus (cucumber) 'Gele Tros' was found as a new local-lesion host of the virus reacting to isolates Mo and Tu on inoculated cotyledons at the earliest in 5 days with small distinct chlorotic lesions (Fig. 5, right), turning dry when the cotyledons later became yellow.

Seed-transmission tests. When embryo axes of 930 germinated seeds from plants inoculated with BBMV and BYMV were tested by ELISA, 12 out of 93 groups of 10 seeds each were found infected with BBMV and 11 with BYMV. Supposing that each positive group contained one infected seed, the percentage of seed (embryo) infection was 1.29 (12/930). Using the formula $p = [1 - (\frac{Y}{N})^{1/n}] \times 100$ of Maury et al. (1985), where p = percentage of infection, Y = number of groups free of virus, N = number of groups tested, n = number of seeds per group, the calculated incidence of BBMV infection amounted to 1.37%.

Microscopy. Isometric particles ca 25 nm in diameter were readily seen with the electron microscope in crude sap preparations from faba bean field samples, either fresh or dried, or from faba bean plants inoculated and maintained in the glasshouse. They mostly occurred in very high concentrations and a high proportion of them were penetrated by stain (Fig. 6).

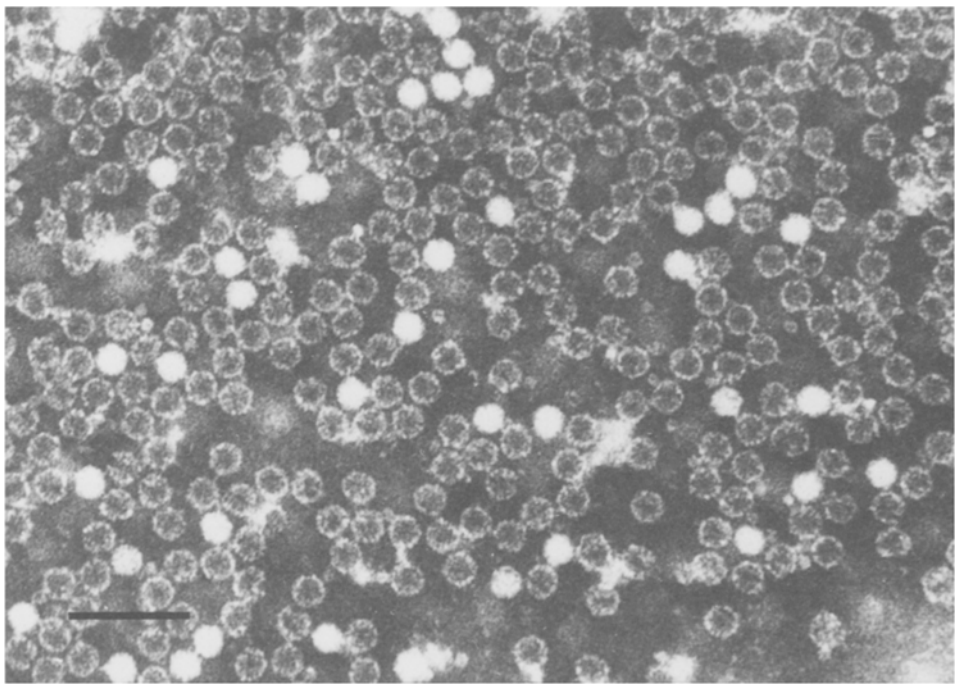

Fig. 6. Electron micrograph of high concentration of virus particles (isolate TV20-86) in crude-sap preparation of faba bean leaves; note penetration of many particles by PTA. Magnification bar represents 100 nm.

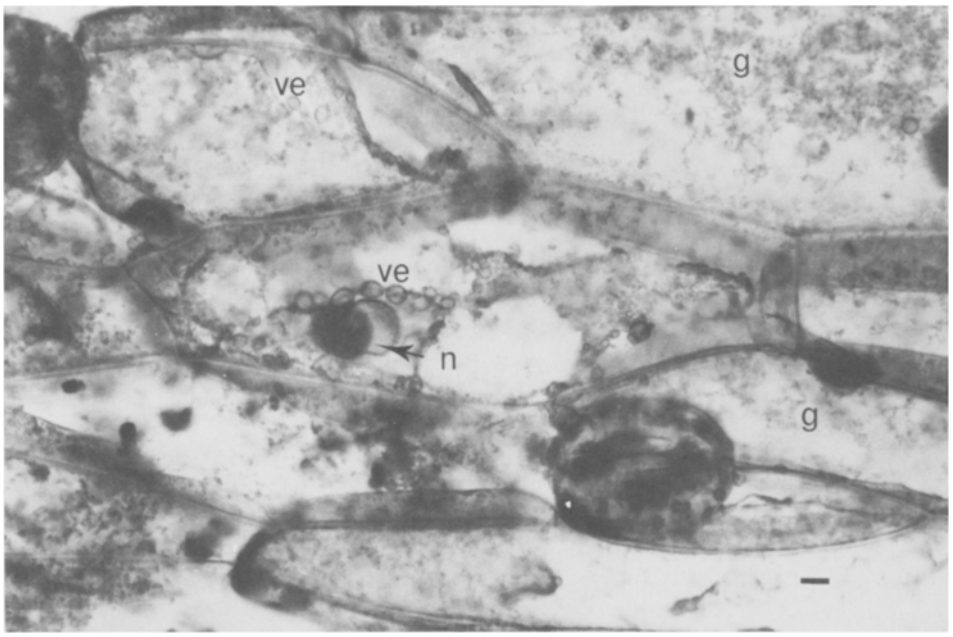

Fig. 7. Accumulations of granular (g) and vesiculated (ve) material in epidermal cells of petioles of faba bean with isolate Tu after staining with phloxine and methylene blue (n = nucleus). Magnification bar represents 10 μm.

Cytological aberrations could readily be observed with a light microscope in epidermal strips of infected plants of faba bean after staining with phloxine and methylene blue. In non-inoculated leaves they appeared as soon as three days after inoculation of lower leaves, and at 22 days after inoculation they were more prevalent in lower and top leaves than in middle leaves of plants inoculated on the two lowest leaves. Nucleoli were enlarged and the cytoplasm contained granular and often vesicular material which was first widely distributed (Fig. 7) but later often condensed into granular, partly 'vacuolated' or vesiculated inclusion bodies, one or more per cell. They were of varying, sometimes globular, shape and often exceeded the nucleus in size. They persisted throughout the period of observation (30 days), but incidence was highest from 7-20 days after inoculation, with a peak at 10 days.

Persistence of infectivity. The persistence of infectivity in expressed sap of plants of faba bean 'Compacta', systemically infected with Mo was determined in the conventional way and infectivity was tested by inoculation onto *C. amaranticolor* for local symptoms, and onto *V. faba* 'Compacta' for systemic symptoms. Most infectivity was lost after dilution $\times 10^4$ and all after $\times 10^5$, most after heating at 80 °C but all between 90-95 °C, and most after 37 days of storage at room temperature but all after 42 days.

Isolates Mo and Tu were still highly infectious after desiccation of virus-containing faba bean leaves over $CaCl_2$ at 4 °C and two years of storage over anhydrous $CaSO_4$.

Virus purification and antiserum production. With Sy, virus yields of up to 100 mg of purified virus per kg of infected leaves of faba bean ('Syrian Local') were obtained after two cycles of sucrose density-gradient and high-speed centrifugation. The antisera produced against the two different virus preparations of Sy, though of low titre (16 and 16-64, respectively, in gel-diffusion tests) were of good quality for ELISA, as indicated by the high values (A_{405} 1.0-2.0) obtained with BBMV-infected tissue and low values (< 0.1) with healthy tissue.

The amount of gammaglobulin isolated from the antiserum produced against glutaraldehyde-treated Sy was ca twice that from the antiserum against the untreated preparation. This held for all eight bleedings of the two antisera and on an average amount to 6.67 and 3.38 mg/ml, respectively. However, when the titers of the BBMV-specific gammaglobulins of the different bleedings were assayed by ELISA and gel-diffusion no differences between the two antisera were observed.

Serology. Serological identity of the isolates Mo and Tu with BBMV was soon proved in gel-diffusion tests in Wageningen with these isolates and the antisera to the type isolate and a Sudanese isolate (Murant et al., 1974) obtained from A.F. Murant. Both antisera reacted with both isolates up to and including antiserum dilution 16.

The four BBMV isolates were later found to be serologically identical when tested in gel diffusion and ELISA with our own antiserum to isolate Sy. In the agar-gel-diffusion test with the antiserum against untreated Sy virus precipitin lines of different isolates fused with each other without spur formation. Likewise, in ELISA the curves obtained with the four isolates ran parallel when different sap dilutions of faba bean leaves with each of the isolates were tested against one gammaglobulin concentration (1 μg/ml), or when the same sap dilution (1 : 20) from infected leaves was tested against different gammaglobulin concentrations. Moreover, when infected leaves were harvested four weeks after inoculation, the highest virus concentration was consistently found for isolate Mo and the lowest for Tu (Fig. 8).

All five extraction buffers (0.1 M potassium phosphate, pH 6.0; 0.1 M PO_4 + 0.005 M $MgCl_2$, pH 6.0; 0.1 M PO_4, pH 7.0; 0.1 M PO_4 + 0.1 M EDTA, pH 7.0; 0.2 M PO_4, pH 6.0) used for the detection of BBMV in faba bean tissue by ELISA were as efficient as the standard extraction buffer recommended by Clark and Adams (1977).

Virus survey. Of 789 faba bean samples with virus-like symptoms collected from Egypt, Lebanon, Morocco, Sudan, Syria and Tunisia, 203 were found to be infected when tested by ELISA with the Sy antiserum. BBMV was detected in 5.7, 0.0, 6.6, 18.1, 26.7 and 58.4% of the samples tested from each of the above countries, respectively. The figures were 4/70 (4 infected out of 70 samples tested) for Egypt, 0/44 for Lebanon, 1/15 for Morocco, 46/254 for Sudan, 72/269 for Syria and 80/137 for Tunisia. Since the number of samples tested from Lebanon and Morocco was small, further sampling and testing is required for more reliable reporting of the relative occurrence of BBMV in these countries. Complex infection with BYMV was detected in 37.4% (76/203) of the samples infected with BBMV. Results reported here show that BBMV naturally infects faba bean in Egypt, Morocco, Sudan, Syria and Tunisia.

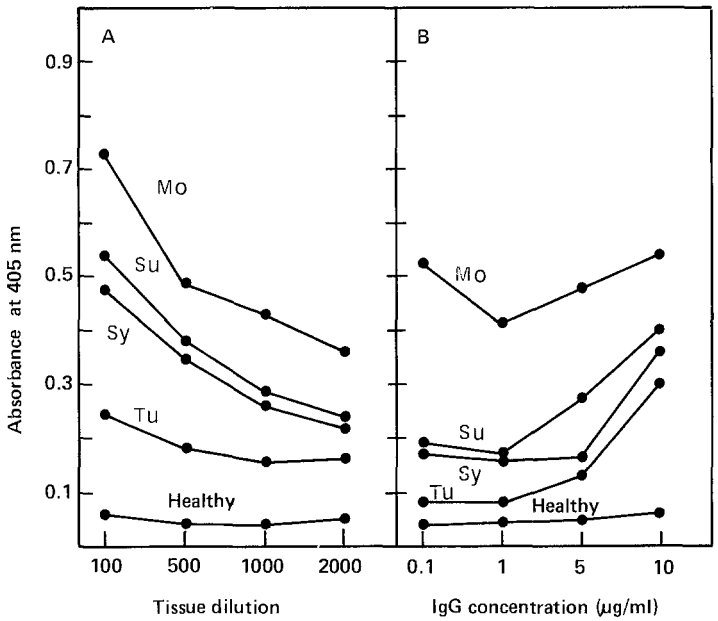

Fig. 8. ELISA values obtained with antiserum against Sy and sap from faba bean plants four weeks after inoculation with four different isolates of broad bean mottle virus from Morocco (Mo), Sudan (Su), Syria (Sy) and Tunisia (Tu). Curves in (A) represent values for different sap dilutions, and in (B) values for the same sap dilution (1 : 20, W/V) tested against different gamma-globulin concentrations.

Discussion

The host range and symptoms of the four isolates studied, their particle morphology and size, the reaction of Mo and Tu with an antiserum to the type strain of BBMV described by Bawden et al. (1951) and reviewed by Gibbs (1972), and the serological reactions of the four isolates when using an antiserum to one of them (Sy), led us to identify all isolates as BBMV.

In *Phaseolus* bean the symptoms resembled those of the beetle-transmitted bean yellow stipple virus, partially described in the USA (Zaumeyer and Thomas, 1950), also reported from Costa Rica (Gamez, 1972), but later claimed to be a serologically related strain of cowpea chlorotic mottle virus (Fulton et al., 1975), another bromovirus. That virus, however, did not infect species outside the Leguminosae, including 'Turkish' tobacco, and species highly susceptible to BBMV, such as *Melilotus albus*, *Trifolium incarnatum*, *T. pratense*, *T. repens*, and *Vicia faba*, were resistant.

The isolates studied, though serologically indistinguishable, slightly differed in their host reactions, suggesting the existence of different strains. This may have a bearing upon control and resistance breeding although all genotypes of faba bean tested were uniformly infected by two isolates tested. Isolate Tu was more virulent on *V. faba*, especially ICARDA breeding lines, and on *Melilotus albus* (Fig. 3, left) than Mo which at Wageningen was the only of the two isolates infectious to and causing systemic symptoms on *Gomphrena globosa* and *Phaseoulus vulgaris* 'Great Northern'. 'Bataaf' reacted

to Mo with local lesions and was immune to Tu. Some of our host reactions differed from those reported earlier (Table 1: Bawden et al., 1951; Walters and Surin, 1973; Ouffroukh, 1985). Such discrepancies also held for results obtained in Beirut and Wageningen. They might be due to the use of different test plant genotypes as of *C. quinoa* and to considerably different growing conditions. Twelve plant species were found to be new suscepts to systemic infection. The virus is now known to be able to systemically infect at least 23 plant species (or 40, when data by Ouffroukh (1985) are included). Among the 15 susceptible legume species (31, when data by Ouffroukh (1985) are taken into account) are important crops.

All 23 genotypes, including ICARDA breeding lines, of faba bean tested were vulnerable to infection, with symptoms often being severe (see also Bawden et al., 1951), especially when plants get infected during early stages of growth e.g. from the seed (Bourbah and Fezzaz, 1979). The virus is potentially important to other major legume crops, susch as chickpea, lentil and pea. Systemic necrosis, as in pea, may easily be ascribed to other causes. Symptomless systemic infection in many other legumes, such as cowpea, several cultivars of *Phaseolus* bean, and in soybean and vetch, suggest a much wider, yet unnoticed, natural host range.

The virus occurs in extremely high concentrations in infected plants as shown by most electron micrographs of crude sap preparations (Fig. 6), yields resulting from purification, and earlier observations (Bawden et al., 1951; Yamazaki et al., 1961). Granular and often vesiculated cytoplasmic inclusion bodies indeed differ from those of bean yellow mosaic virus (see also Rubio and Van Slogteren, 1956). They have earlier been found to largely consist of virus particles in cytoplasm at the point of saturation (De Zoeten and Schlegel, 1967), and may be useful for diagnosis.

High persistence of infectivity in expressed sap further demonstrated the stability of the virus. The ageing in vitro in more than 37 days was considerably longer than the 21 days or less reported by Bawden et al. (1951). The virus preserved well over $CaCl_2/CaSO_4$ at 4 °C.

The purification procedure yielded fairly pure virus preparations. The virus is moderately immunogenic (Gibbs, 1972) and titers of our antisera did not exceed 64 in gel-diffusion tests. The antisera produced a precipitin line with extracts from BBMV-infected faba bean and no reaction with healthy extracts. The antisera against the glutaraldehyde-treated and non-treated preparation of isolate Sy produced high BBMV-specific and low 'healthy' readings in ELISA.

Although antigen stabilization with formaldehyde has been reported for some viruses (Von Wechmar and Van Regenmortel, 1968; Francki and Habili, 1972), our glutaraldehyde treatment did not reliably improve immunogenicity of BBMV.

The severity of symptoms in faba bean 2-4 weeks after inoculation more or less paralleled virus concentation as judged by the A_{405} values obtained in ELISA with leaf extracts made four weeks after inoculation. The Moroccan isolate (Mo) caused severest symptoms 2-4 weeks after inoculation when the A_{405} value was highest. The Syrian and Sudanese isolates were milder and produced lower A_{405} values, whereas the Tunisian isolate (Tu) produced symptoms similar to the Syrian and Sudanese isolates but consistently produced the lowest A_{405} values. ELISA might thus be useful for the evaluation of and distinction between aggressiveness and virulence of BBMV isolates in faba bean.

BBMV was detected in leaf samples of faba bean from five of the six countries

surveyed. Incidence of the virus was relatively low in all countries surveyed except in Tunisia, where it was detected in nearly 60% of the samples collected. Within the region the virus has been reported earlier in Sudan, Morocco, and Algeria. Assou (1978) and Bourbah and Fezzaz (1979), surveying crops in the Meknès region, Morocco, found it in 8 out of 16 and all 10 crops examined respectively, with final incidences up to 9 and to 5%, respectively. By symptom observation Ouffroukh (1985) found the virus, serologically identical to an unidentified virus earlier reported from Algeria by Zagh and Férault (1980), to be widespread in that country though not of high incidence (1-3%). Although the latter authors did not obtain a reaction with antiserum to BBMV, the symptoms they describe in faba bean indeed are highly characteristic of BBMV. The present publication is the first report on the occurrence of BBMV in Tunisia, Egypt and Syria. It may well occur throughout the faba bean growing area of West Asia and North Africa. It thus is of more than mere academic interest, also because all faba bean genotypes tested so far are vulnerable to infection.

Surveying of other potentially vulnerable legume crops, such as chickpea, lentil and pea, is likely to reveal its importance in them as well. Other species, especially perennial clovers, as already suggested by Bawden et al. (1951), and even non-legumes might play a role in the perennation of the virus.

Perennation and long-distance spread may also be in seed, as already suspected by Bawden et al. (1951), Assou (1978) and Bourbah and Fezzaz (1979) but not confirmed by Bawden et al. (1951) when testing 130 seeds of faba bean and 30 seeds of *Phaseolus* bean. Detection of BBMV infection in 1.3% of the embryonal axes of faba bean seeds, as reported in this study, corroborates the tentative report by Murant et al. (1979) of such seed transmission when the virus occurred together with bean yellow mosaic virus. We found such complex infections to be prevalent in the region (37% of the samples with BBMV).

Occurrence in the field and spread from apparent sources of infection suggested the involvement of vectors (Bawden et al., 1951; Bourbah and Fezzaz, 1979). In the USA, where the virus is not reported to occur naturally, it could be experimentally transmitted by the striped cucumber beetle (*Acalymma trivittata*), the spotted cucumber beetle (*Diabrotica undecimpunctata*) and the grape colaspis (*Colaspis flavida*). Transmission by the second vector was only during the first 24 h following acquisition feeding (Walters and Surin, 1973). In Portugal Borges and Louro (1974) reported a low rate of transmission by the leaf weevil *Sitona lineatus*. The actual role of vectors requires further study.

Wide geographical distribution, potentially wide host range, symptomless infection in many species, seed transmissibility, and pathogenicity to a number of major legume crops make BBMV a potential threat to crop improvement in the ICARDA region.

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Samenvatting

Tuinbonevlekkenvirus: identificatie, waardplantenreeks, serologie en voorkomen in faba-boon (Vicia faba) in West-Azië en Noord-Afrika

Eén van de in West-Azië en Noord-Afrika in faba-boon aangetroffen virussen werd geïdentificeerd als het tuinbonevlekkenvirus ('broad bean mottle virus') op grond van waardplantreacties, deeltjesvorm en -grootte, serologische eigenschappen en granulaire, vaak 'gevacuoliseerde' celinsluitsels. Verder onderzoek aan vier isolaten uit respectievelijk Marokko, Tunesië, Soedan en Syrië verschafte nieuwe informatie over het virus.

De in ELISA of gel-diffusietoetsen serologisch niet te onderscheiden isolaten verschilden enigszins in waardplantenreeks en symptomen. Van 27 getoetste plantesoorten werden 21 systemisch geïnfecteerd (12 vlinderbloemigen en 9 niet-vlinderbloemigen) waarvan 14 door alle vier isolaten. In vele ervan was de infectie symptoomloos, maar belangrijke als gewas geteelde vlinderbloemigen, zoals erwt, linzen en kekererwt, leden ernstig onder aantasting. Alle 23 getoetste faba-boongenotypen, beide van kekererwt, alle vier van linzen, 11 van de 21 getoetste van *Phaseolus*-boon en 16 van de 17 van erwt bleken systemisch gevoelig voor het virus. Twaalf plantesoorten bleken nieuwe potentiële waardplanten en komkommer een nieuwe lokale-lesietoetsplant voor het virus te zijn.

In faba-boneplanten kwam het virus in hoge concentratie voor en overdracht met zaad (1.37%) in deze soort kon worden bevestigd.

Een Syrisch isolaat werd gezuiverd en twee antisera werden bereid, waarvan één werd gebruikt voor de detectie van het virus in te velde verzamelde monsters. Van 789 in 1985 tot en met 1987 verzamelde bladmonsters, met symptomen die deden denken aan virusinfectie, bleken 203 het virus te bevatten en wel 4 van de 70 (4/70) uit Egypte, 0/44 uit Libanon, 1/15 uit Marokko, 46/254 uit Soedan, 72/269 uit Syrië en 80/137 uit Tunesië. Het virus was nog niet eerder aangetoond in Egypte, Syrië en Tunesië.

De grote verbreiding, grote kunstmatige waardplantenreeks, overdracht met zaad, en pathogeniteit voor een aantal belangrijke vlinderbloemige gewassen maken het virus tot een potentiële bedreiging van de programma's tot verbetering van de teelt van de bedoelde gewassen in het betrokken gebied.

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