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Broad bean mottle virus in Morocco; variability, interaction with food legume species, and seed transmission in faba bean, pea, and chickpea

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Abstract

Biological indexing of faba-bean samples collected during an earlier virus survey in Morocco revealed variation in symptom severity among isolates of broad bean mottle virus (BBMV). When seven selected isolates from Morocco and three from Algeria, Sudan, and Tunisia were further compared, they could be divided into mild, severe, and intermediate isolates, according to their pathogenicity on a number of food-legume genotypes tested. The Moroccan isolate SN1 and the Sudanese SuV256 were very mild, and deviant also in their effect on *Gomphrena globosa*, whereas the Tunisian TV75-85 and the Moroccan VN5 were virulent. Representative isolates were indistinguishable, however, in coat-protein molecular weights, and they reacted similarly to the antisera to a Moroccan and a Syrian isolate in electro-blot immunoassay.

Promising ICARDA breeding lines and accessions – ten each of pea and lentil, nine of chickpea, and twelve of faba bean – were all found vulnerable (susceptible and sensitive) to all isolates. Within each food-legume species, vulnerability varied from high to moderate, and no immunity was detected. Virus concentrations in faba-bean lines suggest that isolates differ in virulence rather than in aggressiveness, and that the differences in vulnerability among the lines are due to differences in sensitivity rather than in susceptibility.

When pooled seed samples were germinated and seedlings were tested for BBMV in DAS-ELISA, the virus was found seed-transmitted in faba bean, chickpea, and pea at transmission rates of ca 1.2, 0.9, and 0.1%, respectively. This is the first report on seed transmission of BBMV in faba bean, when occurring on its own, and the first record of such seed transmission in chickpea and pea.

Additional keywords: sensitivity, susceptibility, pathogenicity, vulnerability.

Introduction

Broad bean mottle virus (BBMV) was first described from faba bean (*Vicia faba* L.) in England by Bawden et al. (1951). Much later, it was reported from the same crop in Portugal (Borges and Louro, 1974), and then occasionally from Sudan and North Africa (Murant et al., 1974; Fischer, 1979; Ouffroukh, 1985; Makkouk et al., 1988a), and from China (Ford et al., 1981) and West Asia (Makkouk et al., 1988a). The virus is now known to be able to infect a wide range of legume species and to be widespread in faba bean in West Asia and North Africa, the outreach region of the International Center for

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Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria (Makkouk et al., 1988b).

During systematic surveys of faba bean for viruses in Morocco, BBMV was found to be widely distributed there and to occur in high incidences in farmers' fields (Fortass and Bos, 1991). The biological indexing of the survey samples revealed BBMV isolates which differed in symptom severity. Information on such pathogenic variation is essential for breeding programmes. The variability of the virus in Morocco, the interaction of Moroccan isolates with agronomically promising ICARDA breeding lines of major food legume species, and seed transmission in faba bean, pea, and chickpea were therefore studied in detail.

Materials and methods

Virus isolates. Seven Moroccan isolates of BBMV, collected from different faba-bean growing areas and found to differ in symptom severity on some host plants, were used in this study. They were given the codes BN1, F2, FN1, ON4, SN1, UN2, and VN5, referring to the areas of collection during the survey (Fortass and Bos, 1991). The isolates were screened by ELISA, electron microscopy, and biological indexing for absence of other viruses infecting faba bean. Three other isolates from Algeria (AlgB1), Sudan (SuV256), and Tunisia (TV75-85) were included for comparison. TV75-85 and SuV256 were from the IPO-DLO collection, and were described earlier by Makkouk et al. (1988b) and Bos et al. (1992). AlgB1 was provided by A. Ouffroukh (INPV, El Harrach, Algiers, Algeria). All virus isolates were obtained from faba-bean field samples, and were stored dessicated over calcium chloride. They were revived and maintained in the glasshouse on *Vicia faba* 'Compacta'.

Host-range studies. The virus isolates were extracted in 0.03 M potassium phosphate buffer, pH 7.7, and inoculated onto four plants of selected test species. Carborundum, 400 mesh, was used as abrasive. The plants were kept in a glasshouse and observed for symptom development for at least four weeks. The plants, or parts of them showing no visible symptoms, were tested for latent infection in double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) as described by Clark and Adams (1977). The antiserum used was the one to the Moroccan isolate MV90-85 (Makkouk et al., 1988b), since it was found to react with all the isolates under investigation when tested in preliminary assays.

Virus purification. Two selected Moroccan isolates (SN1 and VN5) and the isolates AlgB1, SuV256, TV75-85, and MV90-85 were propagated in *Nicotiana clevelandii*, and purified by two cycles of differential centrifugation according to Hollings and Horváth (1981).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE of purified virus preparations was carried out according to Laemmli and Favre (1973). The separating gel contained 12% acrylamide, and about 2 µg of virus preparation was loaded per slot.

Electro-blot immunoassay (EBIA). EBIAs of the purified virus preparations were carried out as described earlier (Fortass et al., 1991), using the antiserum to the Moroccan isolate MV90-85 and an antiserum to the Syrian isolate SV48-86 provided by K. M. Makkouk (ICARDA, Aleppo, Syria).

Interaction with food-legume breeding lines. A number of agronomically promising breeding lines and accessions of faba bean, chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), and pea (*Pisum sativum*) were received from the Genetic Resources Unit of ICARDA. Ten lines each of pea and lentil, nine of chickpea, and twelve of faba bean were used to investigate their behaviour to the BBMV isolates, and to evaluate possible differences between isolates in pathogenicity and between host genotypes in vulnerability (susceptibility and sensitivity). Five plants per entry were mechanically inoculated with each virus isolate as described before.

Virus concentration in faba-bean lines. In order to detect possible differences in aggressiveness among virus isolates and in susceptibility among faba-bean lines, the concentrations of all isolates (except SuV256) in inoculated faba-bean breeding lines were evaluated weekly, until five weeks after inoculation, using DAS-ELISA. At a sampling date, tip leaves were selected and ground. The dilutions of the crude extract were the same for all lines, isolates, and sampling times.

Seed-transmission tests. All faba-bean, chickpea and pea plants used for studying the interaction between food-legume breeding lines and BBMV isolates were kept in the glasshouse until seed maturity. All seeds were harvested and sown in steam-sterilized soil. The developing seedlings were collected and tested for BBMV in groups of three, five, or ten in DAS-ELISA. The rates of seed transmission were calculated using the formula of Maury et al. (1985):

$p = [1 - (Y/N)^{1/n}] \times 100$, where p is the percentage of infection, Y the number of seedling groups free of virus, N the number of groups tested, and n the number of seedlings per group.

Results

Host range and symptomatology

The test-plant reactions to the virus isolates are summarized in Table 1. All test plants inoculated became infected except *Nicotiana tabacum* 'Samsun'. Infection remained restricted to the inoculated leaves in *Chenopodium amaranticolor*, *Chenopodium quinoa*, both cucumber cultivars, and *Phaseolus vulgaris* 'Bataaf'. These species and cultivars were found good local-lesion hosts, reacting as early as 2–3 days after inoculation. No differences between isolates were recorded on the basis of the reactions of non-legume species, except that SN1 and SuV256 appeared deviant in their effect on *Gomphrena globosa*.

On the basis of the reactions of the food-legume lines and cultivars tested, the BBMV isolates could be divided into three categories, viz. mild (poorly pathogenic) isolates (FN1, SN1, and SuV256), severe (highly pathogenic) isolates (ON4, TV75-85, and VN5), and intermediate isolates (AlgB1, BN1, F2, and UN2). A selected set of genotypes, in-

Table 1. Reactions[a] of selected host plants to different BBMV isolates.

Host plants	BBMV isolates									
	AlgB1	BN1	F2	FN1	ON4	SN1	SuV256	TV75-85	UN2	VN5
Non-legume species										
Chenopodium amaranticolor	L,–	L,–	L,–	L,–	L,–	L,–	L,–	L,–	L,–	L,–
Chenopodium quinoa	L,–	L,–	L,–	L,–	L,–	L,–	L,–	L,–	L,–	L,–
Cucumis sativus										
'Chinese Slangen'	L,–	L,–	L,–	L,–	L,–	L,–	L,–	–,–	L,–	L,–
'Gele Tros'	L,–	L,–	L,–	L,–	L,–	L,–	*,*	L,–	L,–	L,–
Gomphrena globosa	L,S	L,S	L,S	L,S	L,S	l,–	–,s	L,S	L,S	L,S
Nicotiana clevelandii	L,S	L,S	L,S	L,S	L,S	L,S	L,S	L,S	L,S	L,S
Nicotiana tabacum										
'Sumsun'	–,–	–,–	–,–	–,–	–,–	–,–	–,–	–,–	–,–	–,–
Legume species										
Cicer arietinum										
FLIP 85-15	Ln,S	Ln,S	Ln,S	–,S	Ln,S	–,S	–,S	Ln,S	Ln,S	Ln,S
Lens culinaris										
ILC 5876	–,S	–,S	–,S	–,S	–,Sn	–,S	–,S	–,Sn	–,S	Ln,Sn
ILC 6437	Ln,Sn	–,S	Ln,S	Ln,S	Ln,Sn	–,S	–,S	Ln,Sn	Ln,Sn	Ln,Sn
Paseolus vulgaris										
Bataaf	L,–	L,–	L,–	L,–	L,–	L,–	L,–	L,–	L,–	L,–
Pisum sativum										
Acc. 21	Ln,Sn	Ln,S	–,S	–,S	Ln,Sn	Ln,S	–,S	Ln,Sn	Ln,Sn	Ln,Sn
Acc. 167	Ln,–	Ln,–	Ln,–	Ln,S	Ln,Sn	Ln,–	Ln,–	Ln,–	Ln,Sn	Ln,Sn
Trifolium incarnatum	L,S	L,S	L,S	L,S	L,S	L,S	l,S	L,S	L,S	L,S
Vicia faba										
FLIP 84-230	L,S	–,S	L,S	–,S	Ln,S	–,S	–,S	Ln,S	L,S	Ln,Sn
FLIP 86-122	L,S	L,S	–,S	–,S	Ln,S	–,S	–,S	Ln,S	–,S	Ln,S

[a] L, local symptoms; S, systemic symptoms; l, latent local infection; s, latent systemic infection; n, necrotic; –, no infection; *, not tested.

cluding *Lens culinaris* ILC 6437 and ILC 5876, *Pisum sativum* Acc. 21 and Acc. 167, and *Vicia faba* FLIP 84-230 and FLIP 86-122, differentiated between the mild and severe isolates.

The severe isolates could be distinguished by the systemic necrosis induced on both pea-breeding accessions 21 and 167, and by the stem necrosis on faba-bean FLIP 84-2037. In addition, isolate ON4 was the only one causing a systemic necrosis followed by wilting on pea 'Castro' (not listed in Table 1), which reacted typically with necrosis and withering of the inoculated leaves and stem necrosis (Fig. 1) to the remaining isolates. The mild isolates, on the other hand, did not induce any systemic necrosis on any of the food legume lines and cultivars tested. They did not infect the pea accessions systemically, and both lentil lines did not react locally. Moreover, the Sudanese isolate SuV256 appeared still milder, as it failed to induce a local reaction on the chickpea lines tested. The remaining isolates behaved differently from the severe and mild ones, depending on the line or cultivar inoculated. All the isolates behaved similarly on *Phaseolus vulgaris* 'Bataaf', and also on *Trifolium incarnatum*, except for SuV256, which did not induce a local reaction on the latter.

Fig. 1. Withering of inoculated leaves and stem necrosis induced by BBMV-FN1 on *Pisum sativum* 'Castro', 17 days after mechanical inoculation.

Electrophoretic analysis

All purified isolates revealed, on SDS-polyacrylamide gels, a single polypeptide with a molecular weight of ca 22 kD. They behaved similarly and were indistinguishable in coat-protein molecular weights.

EBIA

The reactivity of the purified isolates to the antiserum to the Moroccan isolate MV90-85 in EBIA is shown in Fig. 2. All isolates reacted similarly and with the same intensity. The reactions were also found similar when an antiserum to the Syrian isolate SV48-86 was used (data not shown).

Food-legume genotype reactions

Faba bean. The reactions of the different ICARDA breeding lines of faba bean to the virus isolates under investigation are summarized in Table 2. All lines were found vulnerable to all the isolates. They all reacted with the mottling characteristic of BBMV infection (Fig. 3), but differences could be observed on the basis of presence or absence of local symptoms and the severity of stunting. On faba bean, the isolates VN5, ON4, TV75-85, and AlgB1 were more pathogenic than the remaining isolates. They induced a local reaction (necrotic in some combinations) and varying degrees of stunting on most lines. The isolate VN5 appeared to be the most pathogenic one as judged by the necrotic local reaction and stunting it induced. The Sudanese isolate SuV256 and the Moroccan SN1 induced neither a local reaction (except on the most sensitive lines) nor a stunting on any of the lines tested. The ICARDA breeding lines FLIP86-114, 86-117, and 86-119 appeared to be the most vulnerable, and the lines FLIP 87-26, 86-146, and 86-122 the least.

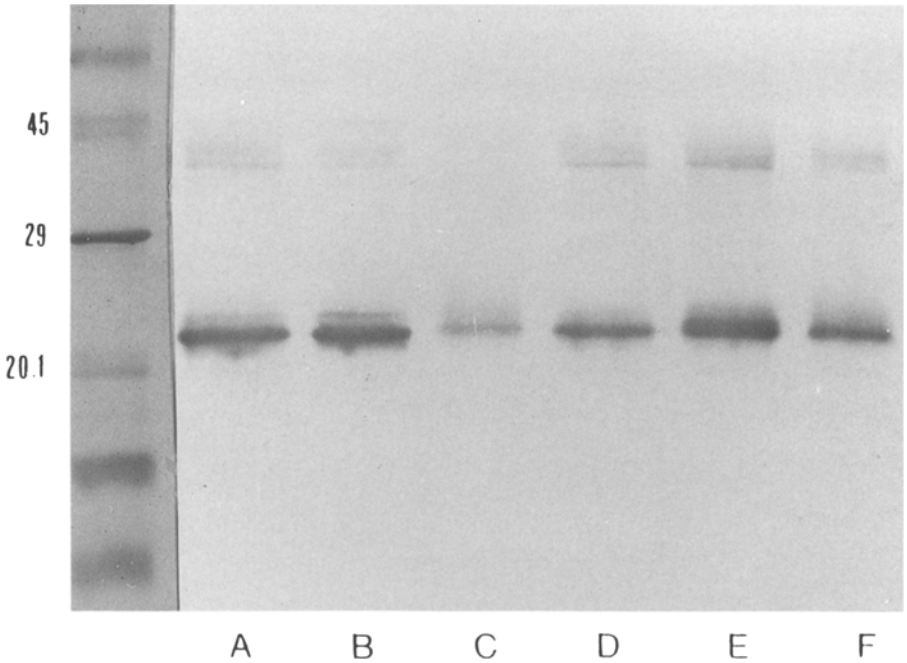

Fig. 2. Reactivity in EBIA of BBMV isolates to the antiserum to the Moroccan isolate MV90-85. A: SN1, B: VN5, C: AlgB1, D: SuV256, E: TV75-85, and F: MV90-85.

Fig. 3. Systemic mottling on *Vicia faba* 'Compacta' induced by BBMV-FN1, three weeks after mechanical inoculation. Healthy control on the left.

Table 2. Reactions[a] of the faba-bean breeding lines, arranged from top to bottom according to decrease in vulnerability, to the BBMV isolates arranged from left to right according to increase in severity.

	SN1	F2	SuV256	FN1	BN1	UN2	AlgB1	ON4	TV75-85	VN5
FLIP 86-117	C/Mo	C/Mo	N/Mo	N/Mo	N/Mo	C/Mo	C/Mo ST	N/Mo SN	C/Mo ST	N/Mo sST
FLIP 86-119	C/Mo	C/Mo	N/Mo	N/Mo	N/Mo	C/Mo	C/Mo ST	N/Mo	C/Mo ST	N/Mo sST
FLIP 86-114	–/Mo	C/Mo	N/Mo	N/Mo	–/Mo Nc	C/Mo	N/Mo	N/Mo	N/Mo sST	N/Mo sST
FLIP 86-107	–/Mo	C/Mo	–/Mo	N/Mo	–/Mo	C/Mo	N/Mo ST	N/Mo SN,sST	C/Mo sST	N/Mo SN,sST
FLIP 86-115	–/Mo	C/Mo	–/Mo	–/Mo	N/Mo	C/Mo	N/Mo ST	N/Mo	C/Mo sST	N/Mo SN,sST
FLIP 86-116	–/Mo	–/Mo	–/Mo	N/Mo	–/Mo	C/Mo	N/Mo ST	N/Mo SN	C/Mo sST	N/Mo sST
FLIP 84-230	–/Mo	C/Mo	–/Mo	–/Mo	–/Mo	C/Mo	C/Mo	N/Mo	N/Mo ST	N/Mo VN, ST
FLIP 84-237	–/Mo	–/Mo	–/Mo	–/Mo	N/Mo	C/Mo	C/Mo ST	N/Mo	C/Mo ST	N/Mo sST
FLIP 85-172	–/Mo	–/Mo	–/Mo	–/Mo	–/Mo	N/Mo	C/Mo ST	N/Mo	–/Mo ST	N/Mo SN, ST
FLIP 87-26	–/Mo	–/Mo	–/Mo	–/Mo	C/Mo	N/Mo	C/Mo	N/Mo	C/Mo ST	N/Mo ST
FLIP 86-146	–/Mo	C/Mo	–/Mo	–/Mo	C/Mo	C/Mo	C/Mo	–/Mo	N/Mo ST	N/Mo ST
FLIP 86-122	–/Mo	–/Mo	–/Mo	–/Mo	C/Mo	–/Mo	C/Mo	N/Mo	N/Mo ST	N/Mo ST

[a] Local reaction/systemic reaction: C, local chlorosis; N, local necrosis; –, no reaction; Mo, mottle; SN, stem necrosis; ST, stunting; sST, severe stunting; Nc, necrosis; VN, vein necrosis.

Table 3. Reactions[a] of the pea accessions, arranged from top to bottom according to decrease in vulnerability, to the BBMV isolates arranged from left to right according to increase in severity.

	FN1	SuV256	F2	BN1	SN1	AlgB1	TV75-85	ON4	VN5	UN2
Acc. 22	W/Mo ST	–/Mo	W/YMo sST	W/Mo ST	W/Mo SN,ST	W/Mo SN	W/YMo ST	W/Mo SN,ST	W/YMo ST	W/Mo SN,ST
Acc. 125	W/SN	W/Mo SN	W/SN	W/SN	W/Nc W	W/SN Nc,W	W/SN Nc,W	W/Mo SN	W/Nc W	W/SN Nc,W
Acc. 62	W/SN	W/SN	W/SN	W/SN	W/SN	W/SN W	W/SN W	W/SN W	W/SN W	W/SN W
Acc. 21	–/YMo	–/Mo	–/YMo	W/YMo	W/YMo	W/Nc W	W/Nc W	W/YMo Nc	W/Mo Nc	W/Nc W
Acc. 8	–/YMo	W/Mo	–/YMo ST	–/YMo	W/YMo	W/Nc W	W/Nc W	W/YMo	W/Mo	W/Nc W
Acc. 101	W/SN	W/SN	W/SN	W/SN	W/SN	W/SN	W/SN	W/SN	W/SN	W/SN
Acc. 154	W/SN	W/SN	W/SN	W/SN	W/SN	W/SN	W/SN	W/SN	W/SN	W/SN
Acc. 30	W/SN	W/SN	W/SN	W/SN	W/SN	W/SN	W/SN	W/SN	W/SN	W/SN
Acc. 169	W/SN	W/SN	W/–	W/–	W/SN	W/SN	W/–	W/SN	W/SN	W/SN
Acc. 167	W/–	W/–	W/–	W/–	W/–	W/–	W/–	W/SN	W/SN	W/SN

[a] Local reaction/systemic reaction: W, wilting; –, no reaction; Mo, mosaic; YMo, yellow mosaic; ST, stunting; sST, severe stunting; SN, stem necrosis; Nc, necrosis.

Pea. All tested accessions of pea were found vulnerable (Table 3). The isolates VN5, TV75-85, and UN2 were highly pathogenic on this host species. In addition to the typical reaction consisting of wilting of the inoculated leaves and stem necrosis, some highly vulnerable accessions reacted with stunting, systemic necrosis, or wilting (Fig. 4). The pea lines tested can be grouped into highly vulnerable (ICARDA accessions 21, 22, 62, and 125) and less vulnerable (accessions 101, 154, 167, 169, and 30). No seeds were produced by the plants of accession 22 inoculated with the different BBMV isolates. The accessions 8, 21, and 22 did not react with symptoms typical of BBMV, but with an unusual yellow mosaic (Fig. 4, middle).

Chickpea. The reactions of the chickpea lines tested are summarized in Table 4. These data show that the isolates VN5, TV75-85, and to a lesser extent UN2, are the most pathogenic on chickpea, and that the isolates SuV256, FN1, and SN1 are mild (Fig. 5). All lines tested were found vulnerable to all isolates. They can be grouped into highly vulnerable (ILC 482, FLIP 82-150, and FLIP 83-47), less vulnerable (FLIP 87-69, and 85-15) and intermediate lines, with reactions varying according to isolate.

Lentil. All lentil lines tested were found vulnerable, except ILC 5845, 5999, 6442, and 6773, which did not react to the mild isolate SuV256 (Table 5). Isolates VN5, TV75-85, ON4, and UN2 were the most pathogenic. They often induced a local necrotic reaction and systemic yellow mosaic and necrosis (depending on the line). Isolates SuV256, SN1, BN1, and FN1 did not induce any local reaction on most lines tested, while the systemic reaction to them was limited to a mosaic or mild stunting. The breeding lines ILC 5845 and 6437 were found highly vulnerable, whereas ILC 6442, 6216, 6763, and 6773 were less vulnerable. The remaining lines behaved differently, depending on the isolate.

Table 4. Reactions[a] of the chickpea breeding lines, arranged from top to bottom according to decrease in vulnerability, to the BBMV isolates arranged from left to right according to increase in severity.

	SuV256	SN1	FN1	BN1	F2	AlgB1	ON4	UN2	VN5	TV75-85
FLIP 82-150	–/mMo	N/VY	N/Mo	N/Mo	N/Mo	N/Mo	N/Mo	N/Mo	N/Mo	N/sMo
	ST	ST	ST	ST	ST	ST	ST,FL	ST,FL	ST,FL	sST,FL
FLIP 83-47	–/mMo	N/VY	N/Mo	N/Mo	N/Mo	N/Mo	N/Mo	N/Mo	N/Mo	N/sMo
		ST	ST	ST,FL	ST	ST	ST	ST,FL	ST,FL	sST,FL
ILC 482	–/mMo	N/VY	N/Mo	N/Mo	N/Mo	N/Mo	N/Mo	N/Mo	N/Nc	N/sMo
		ST	ST,FL	ST	ST,FL	ST	ST	ST	W	sST,FL
FLIP 84-15	–/mMo	N/VY	N/mMo	N/Mo	N/Mo	N/Mo	N/Mo	N/Mo	N/Mo	N/sMo
		ST	ST	ST	ST	ST	ST	ST	ST,FL	sST
FLIP 84-92	–/mMo	–/VY	N/Mo	N/Mo	N/Mo	–/Mo	N/Mo	N/Mo	N/Mo	N/sMo
			ST	ST	ST	ST	ST	ST	ST	sST
ILC 263	–/Mo	–/VY	–/Mo	N/Mo	N/Mo	N/Mo	–/Mo	N/Mo	N/Mo	N/sMo
		ST	ST	ST	ST	ST	ST	ST	ST	sST
FLIP 81-293	–/mMo	–/Mo	–/Mo	N/Mo	N/Mo	–/Mo	–/Mo	N/Mo	N/Mo	N/sMo
		ST	ST	ST	ST	ST	ST	ST	ST	sST
FLIP 87-69	–/mMo	–/VY	–/VY	N/Mo	–/Mo	N/Mo	–/Mo	N/Mo	N/Mo	N/Mo
		ST	ST	ST	ST	ST	ST	ST	ST	ST
FLIP 85-15	–/mMo	–/VY	–/VY	N/Mo	N/Mo	N/Mo	N/Mo	N/Mo	N/Mo	N/Mo
						ST	ST	ST	ST	ST

[a] Local reaction/systemic reaction: N, local necrosis; –, no reaction; Mo, mosaic; mMo, mild mosaic; sMo, severe mosaic; VY, vein yellowing; ST, stunting; sST, severe stunting; FL, filiform leaves; W, wilting; Nc, necrosis.

Fig. 4. Reaction of the pea Acc. 8 to BBMV-UN2 (middle) and BBMV-SN1 (right), 17 days after mechanical inoculation. Uninoculated control on the left.

Fig. 5. Reaction of the chickpea line FLIP 84-92 to two isolates of BBMV, TV75-85 (middle) and SN1 (right), four weeks after inoculation. Uninoculated control on the left.

Table 5. Reactions[a] of the lentil breeding lines, arranged from top to bottom according to decrease in vulnerability, to the BBMV isolates arranged from left to right according to increase in severity.

	SuV256	BN1	SN1	FN1	F2	AlgB1	ON4	TV75-85	UN2	VN5
ILC 6437	–/Mo	–/Mo	–/Mo	N/Mo	N/Mo	N/Mo Nc	N/Mo Nc	N/Mo Nc	N/Nc	N/Mo Nc
ILC 5845	–/–	–/Mo	N/Mo	N/Mo	N/Mo	–/Mo	N/Nc	N/Mo Nc	N/Nc	N/Nc
ILC 5876	–/ST	–/Mo	–/Mo	–/Mo	–/Mo	–/Mo	–/Mo Nc	–/Mo Nc	–/Mo	N/Mo Nc
ILC 5722	–/ST	–/Mo	–/Mo ST	–/Mo	–/Mo ST	N/Mo	N/Mo Nc	–/Mo Nc	N/Mo Nc	N/Mo Nc
ILC 5999	–/–	–/Mo	–/Mo	–/Mo	–/Mo	–/Nc	–/Mo	–/Mo	N/Mo Nc	W/Mo Nc
ILC 6246	–/Mo	N/–	–/Mo	–/Mo	N/Mo	N/–	–/Mo	–/Mo Nc	–/Mo	N/Mo Nc
ILC 6442	–/–	–/Mo	–/Mo	–/Mo	–/Mo	–/Mo	N/Mo	–/Mo Nc	–/Mo	–/Mo Nc
ILC 6763	–/ST	–/Mo	–/Mo	–/Mo	–/Mo	–/Mo	N/Mo	–/Mo	N/Mo	–/Mo Nc
ILC 6216	–/Mo	–/Mo	–/Mo	–/Mo	–/Mo	–/Mo	–/Mo	–/Mo	–/Mo	N/Mo Nc
ILC 6773	–/–	–/VY	–/Mo	–/Mo	–/Mo	–/Mo	–/Mo	–/Mo	–/Mo	–/Mo

[a] Local reaction/systemic reaction: N, local necrosis, –, no reaction; W, wilting; –, no reaction; Mo, mosaic; ST, stunting; VY, vein yellowing; Nc, necrosis.

Virus concentration in faba-bean lines

The evolution of the isolate concentrations in faba-bean lines shows that they reach the maximum around the second week after inoculation (Fig. 6). No clear differences could be seen between isolates or between breeding lines. The virus concentration appears independent of the vulnerability of the line and the pathogenicity of the isolate.

Seed transmission

The results of seed-transmission tests are shown in Table 6. From the 330, 315, and 1300 seedlings grown from pooled seeds produced by inoculated faba bean, chickpea, and pea plants, respectively, groups were found infected after ELISA testing. The transmission rates were calculated and found to be 1.21, 0.95, and 0.07% in faba bean, chickpea, and pea, respectively.

Table 6. Seed-transmission rates of BBMV in faba bean, chickpea, and pea.

Food-legume species	Number of seedlings tested	Number of seedlings per group	Number of groups positive in ELISA	Rate of transmission in %
Faba bean	330	3	4	1.21
Chickpea	315	3	3	0.95
Pea	1300	10	1	0.07

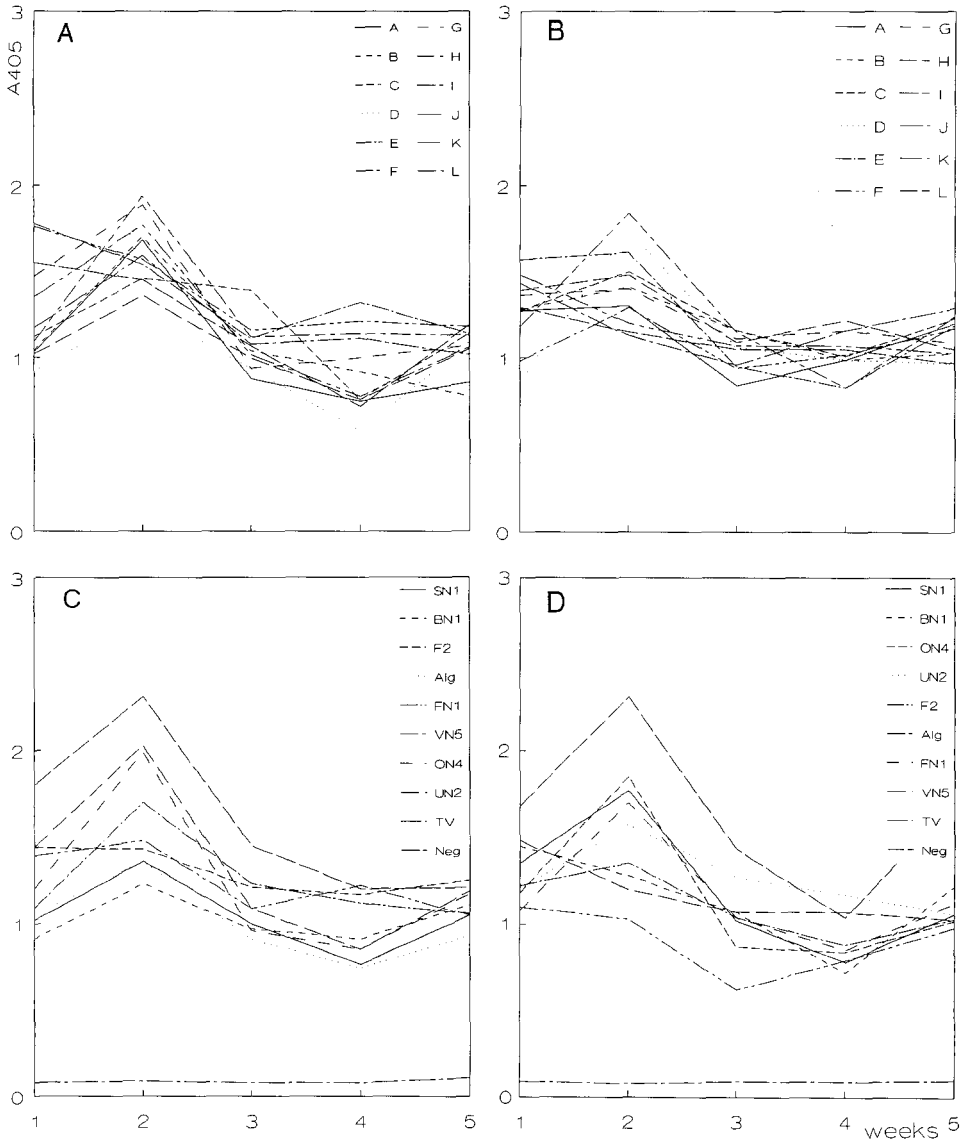

Fig. 6. Virus concentration measured by optical density (405 nm) at different weeks following mechanical inoculation. A: Concentration of the mild isolate SN1 in different faba-bean lines (A to L). B: Concentration of the severe isolate VN5 in the same faba-bean lines. A to L are the FLIP lines 84-230, 84-237, 85-172, 86-107, 86-114, 86-115, 86-116, 86-117, 86-119, 86-122, 86-146, and 87-26, respectively. C: Concentration of different isolates in the highly vulnerable faba-bean line FLIP 86-117. D: Concentration of different isolates in the less vulnerable line FLIP 86-146. Neg is the uninoculated control.

Discussion

The ten BBMV isolates investigated can be divided into three groups based on their pathogenicity on selected test species (Table 1). These results are largely corroborated by the

reactions of the groups of genotypes of four food-legume species when tested separately (Tables 2–5), indicating overall differences in virulence between the BBMV isolates. The severe isolates (VN5, ON4, including the Tunisian TV75-85, and to a lesser extent UN2) are characterized by the systemic necrosis induced on pea Acc. 21 and on the lentil lines ILC 5876 and ILC 6437, and by the local necrosis induced on the faba-bean line FLIP 86-122. On the other hand, the mild isolates (FN1, SN1, including the Sudanese SuV256, and to a lesser extent F2) did not cause local necrosis on chickpea line FLIP 85-15 and on lentil line ILC 6437, and did not produce local symptoms on faba-bean line FLIP 86-122. The remaining isolates assumed an intermediate position, but varied largely in the reaction they induced, depending on the food-legume species and lines tested. BN1, for instance, appeared less pathogenic on lentil than on chickpea. This indicates that clear differences in host genetic vulnerability further add to the variation in symptoms produced. The severe isolates appeared more pathogenic on some food-legume species than on others: VN5 is more pathogenic on lentil and faba bean, whereas TV75-85 is so on chickpea, and UN2 on peas. Although all isolates were obtained initially from faba-bean fields, four other food legumes are potential natural hosts, and some isolates are even more pathogenic on food legumes other than faba bean. Within the cluster of mild isolates, SuV256 was the most deviant in its extremely low pathogenicity. It did not induce a local reaction on any of the nine chickpea lines tested, but produced a systemic mild mosaic (Table 4). It is the only isolate which did not induce any symptom on four lentil lines (including ILC 5845, highly vulnerable to all other isolates). It also differs from the remaining mild isolates by the mild reactions it induced on *Gomphrena globosa* and *Trifolium incarnatum*, and by absence of systemic symptoms on the former and of local symptoms on the latter (Table 1).

The investigated virus isolates behaved identically in EBIA when using antisera to a Moroccan and a Syrian isolate, and their coat proteins exhibited identical molecular weights. They appeared to differ in pathogenicity only. Virus concentrations determined in infected faba-bean lines show that mild isolates occur also in high concentration, and that less vulnerable lines contain virus concentrations comparable with those in highly vulnerable ones. This suggests that the differences in pathogenicity between isolates are due to differences in virulence rather than aggressiveness, and that differences in vulnerability between genotypes are due to differences in sensitivity rather than susceptibility. The terminology used to describe the relationships between virus isolate and host genotype are according to Bos (1983). The vulnerability of a host is its inability to defend itself and overcome the effects of a virus. It depends on its susceptibility (readiness to accept the virus and assist its multiplication) and its sensitivity (severity of the reaction to the attack). The pathogenicity of a virus is its ability to cause disease and is determined by its aggressiveness (readiness to infect and multiply in the host) and by its virulence (ability to incite disease symptoms).

Consequently, the isolates VN5, TV75-85, ON4, and UN2 should be regarded as virulent isolates of BBMV, and SuV256, SN1, FN1, and F2 as mild isolates of this virus. More differentiating parameters and the reproducibility of the severity of symptoms in relation to the temperature are needed in order to consider the extremes as virulent and mild strains. When considering the Moroccan isolates, VN5 and SN1 are considered the extremes. For screening of food-legume germplasm for resistance to BBMV in Morocco, VN5 should be used for faba bean, chickpea, and lentil, and the isolate UN2 for pea.

Further surveying of food legumes, including biological testing of samples, in Algeria, Tunisia, and Sudan is likely to reveal the existence of mild and severe isolates in these countries also. This indicates, as reported earlier (Fortass and Bos, 1991), the importance of supplementing ELISA testing with biological indexing, as it allows the detection of variation in pathogenicity between virus isolates.

The food-legume breeding lines tested were all found susceptible to all the isolates used, except that ILC-lentil lines 5845, 5999, 6442, and 6773 did not react to the mild strain SuV256. Within each food legume, highly and less vulnerable, or more specifically, highly and less sensitive lines can be distinguished. A cluster of intermediate genotypes sensitive to the virulent isolates and less sensitive to the mild ones, with varying degrees of interactions, is found within each food legume species. This necessitates the use of virulent isolates when screening germplasm for resistance to the virus. Moreover, local germplasm should be tested and compared with the genetic material to be introduced from international programmes in order to avoid the risks of introduction of genetic vulnerability, as was, for example, the case with Asian genotypes of rice introduced into Africa and found vulnerable to rice yellow mottle virus previously endemic in the continent (John et al., 1986). The food-legume breeding lines and accessions we have tested were all found vulnerable to highly vulnerable, and no immunity could be detected. This implies that within international programmes, before introduction into new regions, germplasm should be screened for resistance to the viruses occurring there.

In this study, BBMV was found to be seed-transmitted in faba bean, pea, and chickpea. The seed transmission in lentil could not be tested since no seed was produced by the infected plants. This is the first conclusive report on seed transmission of BBMV in faba bean, and the first record of such transmission in pea and chickpea. The seed transmission of this virus in faba bean was already suspected (Bawden et al., 1951), and has been later reported for mixed infections of the virus with bean yellow mosaic virus (Murant et al., 1974; Makkouk et al., 1988b). The rates of transmission now found differed according to species, and the highest rate was recorded in faba bean (1.2%). The number of seedlings tested was relatively low, and more testing is needed to better quantify the rates of seed transmission of the virus, especially in faba bean and chickpea. The numbers of seeds available were too low to study the effect of genotype and virus isolate on seed transmission. The use of different genotypes of food legumes, and of different isolates of the virus has undoubtedly enhanced the chance of detecting seed transmission of BBMV in our pooled seed samples.

High vulnerability of legumes other than faba bean and high incidence of this virus already found in faba bean suggest widespread occurrence of BBMV also in other legumes in Morocco and other countries in West Asia and North Africa. Symptoms in these species, including necrosis as described here and before (Makkouk et al., 1988b), are likely to be overlooked as being caused by BBMV or by virus at all. Seed transmission in faba bean and other legume crops may well explain the widespread occurrence of the virus throughout West Asia and North Africa (Makkouk et al., 1988b), and its prevalence in Morocco (Fortass and Bos, 1991). On-going studies have shown already that *Sitona lineatus* is a vector of BBMV in Morocco, and the natural occurrence of the virus in other food-legume species and wild hosts is under investigation.

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