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Survey of faba bean (Vicia faba L.) for viruses in Morocco

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Abstract

A total of 52 faba-bean (*Vicia faba* L.) fields, located in the main growing areas in Morocco were surveyed for viruses. From 240 samples with symptoms suggestive of virus infection, the following viruses were detected using electron microscopy, serology, and biological indexing: Alfalfa mosaic virus (AMV), bean yellow mosaic virus (BYMV), broad bean mottle virus (BBMV), broad bean stain virus (BBSV), broad bean true mosaic virus (BBTMV), pea early-browning virus (PEBV), pea enation mosaic virus (PEMV), pea seed-borne mosaic virus (PSbMV), and a complex of luteoviruses including bean leafroll virus (BLRV). This is the first report of the occurrence of BBTMV, PEMV, PSbMV, and the luteoviruses (including BLRV) on faba bean in Morocco.

The luteoviruses and BBMV were found to be the most prevalent. They were detected in 56 and 50%, respectively, of the surveyed fields; while AMV, BBSV, and PEBV were found in single fields only. The remaining viruses were less prevalent, and were detected in a range of 4 to 15% of the fields surveyed. The incidences per field of the prevalent viruses varied and ranged from 1 to 33% for BBMV and up to 20% in the case of luteoviruses. BBMV was found confined to the central and northern parts of the country, BBTMV and PEMV mainly occurred in the central area, while the luteoviruses and BYMV were spread over the faba-bean growing regions of the country.

Additional keywords: Alfalfa mosaic virus, bean leafroll virus, bean yellow mosaic virus, broad bean mottle virus, broad bean stain virus, broad bean true mosaic virus, pea early-browning virus, pea enation mosaic virus, pea seed-borne mosaic virus, luteoviruses, virus incidence, geographical distribution.

Introduction

Faba bean (*Vicia faba* L.) is the major food legume grown in Morocco. It occupies around 200 000 ha yearly, and is extensively grown in the central part (Meknès area), the plain of Chaouia (Settat area), and the northwest of the country (Anonymous, 1990). Faba bean is a major component in the cropping system on small farms and in non-irrigated areas, where the recurrent annual crop rotation is cereal-faba bean.

The yields are generally very low, and viruses represent one of the most important

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constraints (Schluter et al., 1976). On a world basis, 44 viruses have been reported to infect the crop naturally (Bos et al., 1988), but only six viruses have been reported from faba bean in Morocco without information on their incidence and geographical distribution (El Maataoui and Fischer, 1976). Later, following testing of incidentally collected samples, it was claimed that broad bean stain virus (BBSV) and bean yellow mosaic virus (BYMV) are economically the most important viruses infecting faba bean in the country (Fischer, 1979). Recently, a limited survey revealed the occurrence of three viruses only, viz. BYMV, broad bean mottle virus (BBMV), and BBSV (Makkouk et al., 1988a). However, the number of samples tested so far is extremely low, and no survey covering the different faba-bean growing areas in the country has been conducted.

This paper describes a systematic survey of the major faba-bean growing regions of Morocco for viruses naturally occurring in faba-bean crops and reports on their geographical distribution, incidence and relative importance.

Materials and methods

Survey planning

In March 1988, a preliminary survey was conducted in order to locate the main faba-bean growing areas, to examine the development of the crop with time in different regions, and to make a first evaluation of the occurrence of virus infections. The resulting information, and the national crop statistics (Anonymous, 1990), helped to develop the itinerary of the survey to representatively cover the main faba-bean regions in the country (Fig. 1). The southern area has thereafter been surveyed during the third week of March 1990, and the central and northern areas, one week later. At that time, crops were at the stage of flowering up to early podding.

Choice of fields

The fields to be sampled were chosen systematically by making a stop after each 25 km along the itinerary, and then taking the nearest field. The total number of fields surveyed was 52. Field sizes varied from 0.25 to 2 ha.

Field observations and sample collection

The survey was limited to the viruses causing apparent infections. In each field sampled, the different syndromes suggestive of virus infection in the whole field were described. A sample representing each syndrome was thereafter collected, sealed in a plastic bag, labelled, and put in an ice box for transport. Then, the incidence of plants with each syndrome was assessed by counting the number of plants showing that syndrome in 25% of the total number of rows. These rows were regularly distributed over the field beginning with the first one, and were examined systematically (Barnett, 1986). In the laboratory, all the collected samples (240 in total) were dried over calcium chloride for later virus identification in Wageningen.

Virus identification

The viruses were identified by electron microscopy, biological indexing to a limited number of test plants, and serology.

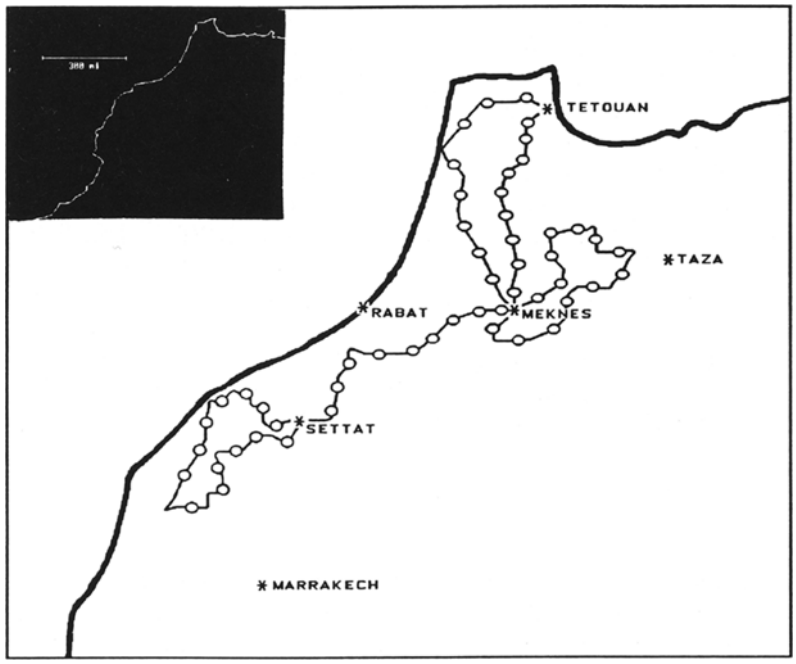

Fig. 1. Itinerary of the survey of faba-bean viruses in Morocco (- o -: surveyed field).

Electron microscopy. The samples were chopped in 0.2% sodium sulfite, stained in 2% uranyl acetate and viewed in a Philips CM 12 transmission electron microscope.

Biological indexing. The samples were extracted in 0.03 M potassium phosphate buffer, pH 7.7, and inoculated to four plants each of: *Chenopodium amaranticolor*, *C. quinoa*, *Phaseolus vulgaris* 'Bataaf', *Pisum sativum* 'Castro', and *Vicia faba* 'Compacta'. The plants were kept in an insect-free glasshouse for symptom development for at least four weeks.

Serology. The samples were extracted in phosphate-buffered saline containing 0.1% Tween 20 and 2% polyvinylpyrrolidone, and tested with all antisera listed below in DAS-ELISA as described by Clark and Adams (1977). For pea enation mosaic virus (PEMV), the biotin-avidin ELISA was adopted according to Zrein et al. (1986).

The antisera to BYMV and clover yellow vein virus (CYVV) were our own (Fortass et al., 1991). Antisera to BBMV, broad bean wilt virus (BBWV), pea early-browning virus (PEBV), alfalfa mosaic virus (AMV), pea seed-borne mosaic virus (PSbMV), and cucumber mosaic virus (CMV) were provided by D.Z. Maat (IPO-DLO, Wageningen, the Netherlands), to PEMV by G. Adam (BBA, Braunschweig, Germany), to BBSV by K.M. Makkouk (ICARDA, Aleppo, Syria), to broad bean true mosaic virus (BBTMV) by H. Rohloff (BBA), and to bean leafroll virus (BLRV) by L. Katul (BBA).

The samples showing symptoms suggestive of luteovirus infection were tested in DAS-ELISA with antisera to potato leafroll virus (PLRV) and BLRV, and in triple-antibody sandwich ELISA (TAS-ELISA) with a panel of monoclonal antibodies to PLRV (Van den Heuvel et al., 1990) provided by J.F.J.M. van den Heuvel (IPO-DLO).

Results

Viruses identified

Of the 52 fields surveyed, 42 (81%) were found to harbour at least one virus. The viruses identified from the collected samples are: AMV, BBMV, BBSV, BBTMV, BYMV, PEBV, PEMV, PSbMV, and a number of luteoviruses including BLRV. The viruses BBTMV, PEMV, PSbMV, and the luteoviruses have not been previously reported from faba bean in Morocco.

BBMV was readily identified by its reactions on test plants. Both *Chenopodium* species reacted with pin-point local lesions two to three days after inoculation, and pea 'Castro' reacted also rapidly with necrotic local lesions, which enlarged and led to withering of inoculated leaves, and with stem necrosis. Thus, sample infection by BBMV was recognized as early as three days after inoculation, and identity of the isolates was confirmed serologically. Also in the electron microscope, this virus was readily detected by its high concentration and the dark center of the particles. The variability of the symptoms in 'Compacta' demonstrated the existence of different isolates of BBMV, indistinguishable by ELISA.

Characteristic test-plant reactions also enabled the identification of BYMV, and three isolates, varying in their symptoms on 'Bataaf' and 'Castro', were distinguished.

The AMV isolate detected was virulent on bean 'Bataaf', which reacted with vein necrosis and wilting of the inoculated leaves as early as two days after inoculation. Both *Chenopodium* spp. reacted with systemic chlorotic lesions, severe stunting and leaf deformation. The PEBV isolate was found of low pathogenicity compared to Dutch isolates. The remaining viruses were not easily identified by biological indexing.

The reactivity in ELISA of the samples with field symptoms suggestive of luteovirus infection was complex. Some samples reacted with BLRV antiserum only, others with PLRV polyclonal antiserum and the PLRV monoclonal antibodies (MAb) WAU-A12 and WAU-A13, whereas twelve samples reacted with WAU-A12 only. This MAb was known to react strongly with beet western yellows virus and not with the Dutch isolate of BLRV (Van den Heuvel et al., 1990). These serological patterns suggest that, in addition to BLRV and its possible deviant strains, other luteovirus(es) occur naturally on faba bean in Morocco. In this report, they are treated as a group.

Field symptoms

The originally observed field symptoms produced by the viruses isolated were extremely variable. This may have been due to the genetical variability of the faba-bean landraces grown, and to differences in time of infection.

The symptoms produced by BBMV varied from green mottling or distinct green veinbanding mosaic in most of the leaves (most likely due to early infection) to vein chlorosis or interveinal mosaic on the upper leaves of the plant (in case of late infection). BYMV induced symptoms varying from severe green blotching to green or yellow mosaic in the upper leaves. The plants infected with PEMV showed either vein clearing or characteristic translucent leaf spotting. Symptoms induced by the luteoviruses consisted of interveinal yellowing, upward rolling, and brittleness of the leaves. The early infected plants were generally stunted, necrotic, and produced few or no pods at all (Fig. 2).

For the remaining viruses, the symptoms consisted of mosaic and reduction in

Fig. 2. Field symptoms induced by early infection of a luteovirus on faba bean. Healthy plant on the right.

growth and leaf size in case of BBSV infection, leaf narrowing and growth reduction in case of PSbMV, and clear mosaic of the upper leaves in case of BBTMV.

Virus incidence

The number of samples from which the viruses have been identified and the percentages of samples infected with them are shown in Table 1. BBMV and the luteoviruses appear to be the most prevalent viruses in faba bean in Morocco. Next in incidence rank BYMV, PEMV, and BBTMV. The remaining viruses were found incidentally only.

Because of the great variation in symptoms, especially of BBMV, the number of samples from a given field in which a virus was detected does not reflect the actual incidence of that virus. Table 2 records the incidence of the fields infested with the respective viruses. It shows that the viruses found can be grouped into three categories based on their incidence. The first category includes the luteoviruses and BBMV which were detected in 56 and 50%, respectively, of the surveyed fields. They are thus the most prevalent viruses in faba-bean crops in Morocco. The second category comprises the viruses PEMV, BBTMV, BYMV, and PSbMV which were less frequent (detected in a range decreasing from 15 to 4% of the fields). The third category of viruses includes AMV, BBSV, and PEBV, each of these was detected in one field only.

Table 1. Viruses identified from the faba-bean samples collected during the survey arranged according to incidence.

Viruses[1]	Number of infected samples	% of total number of samples
BBMV	73	30.4
Luteoviruses	28	11.6
BYMV	9	3.7
PEMV	8	3.3
BBTMV	6	2.5
PSbMV	2	0.8
AMV	1	0.4
BBSV	1	0.4
PEBV	1	0.4

[1] AMV : alfalfa mosaic virus
BBMV : broad bean mottle virus
BBSV : broad bean stain virus
BBTMV: broad bean true mosaic virus
BYMV : bean yellow mosaic virus
PEBV : pea early-browning virus
PEMV : pea enation mosaic virus
PSbMV: pea seed-borne mosaic virus

Table 2. Prevalence of the viruses identified from the survey.

Virus(es)[1]	Number of fields where found	% of total number of fields
Luteoviruses	28	55.7
BBMV	26	50.0
PEMV	8	15.4
BBTMV	5	9.6
BYMV	5	9.6
PSbMV	2	3.8
AMV	1	1.9
BBSV	1	1.9
PEBV	1	1.9

[1] For explanation of acronyms, see Table 1.

Mixed infections

Most viruses were found to occur in single infections. The mixed infections and their relative incidences are listed in Table 3. The mixed infections by BBMV and BBTMV and by PEMV and BBMV were the most prevalent. They were found each in 8% of the surveyed fields.

Number of viruses detected per field

The number of viruses detected per field varied from one to four (Table 4). Among the surveyed fields, 48% harboured two viruses, generally BBMV and a luteovirus, and 36% of the fields harboured only one virus. There was only one field in which four viruses were detected. Thus, the number of fields harbouring more than one virus is high.

Table 3. Mixed infections detected in faba-bean samples collected during the virus survey.

Virus mixture[1]	Number of positive samples	Number of fields where found	% of total number of fields
AMV - BYMV	1	1	1.9
BBMV - BBTMV	4	4	7.7
BBMV - PEMV	4	4	7.7

[1] For explanation of acronyms, see Table 1.

Table 4. Number of viruses detected per field, and incidences of multiple infestation.

Number of viruses[1]	Number of fields	% of infested fields
1	15	35.7
2	20	47.6
3	6	14.3
4	1	2.4

[1] For explanation of acronyms, see Table 1.

Geographical distribution

The geographical locations at which the individual viruses were detected in faba bean in Morocco are shown in Fig. 3. BBMV (Fig.3A) appears to be more or less confined to the central and northern parts of the country, although also found in the area of Settat. The luteoviruses (Fig. 3B) were spread nearly all along the route of the survey. BYMV (Fig. 3E) occurred incidentally only and in different regions. BBTMV (Fig. 3C) and PEMV (Fig. 3D) were found in the central part, and the remaining viruses were detected in one or two fields in the southern part of the surveyed area.

Field incidences of the prevalent viruses

The number of virus-infected plants per field is generally low, except for the prevalent viruses (BBMV and the luteoviruses). The variability of their incidences is represented in Fig. 4. The incidence of BBMV per field is generally less than 10%, with a maximum of 33% recorded in one field. The incidence of the luteoviruses recorded in the majority of the fields ranged between 1 and 10%, the highest incidence recorded is 20%.

Discussion

Of the viruses identified from the samples collected during the survey, PEMV, PSbMV, BBTMV, and the luteovirus complex (including BLRV) have not been previously reported from Morocco. The first three have already been reported from most of the countries in West Asia and North Africa (Makkouk et al., 1988a), and this paper now extends information on their natural occurrence to faba bean in Morocco. Our results show that the luteoviruses and BBMV are the most prevalent viruses in faba bean crops in Morocco.

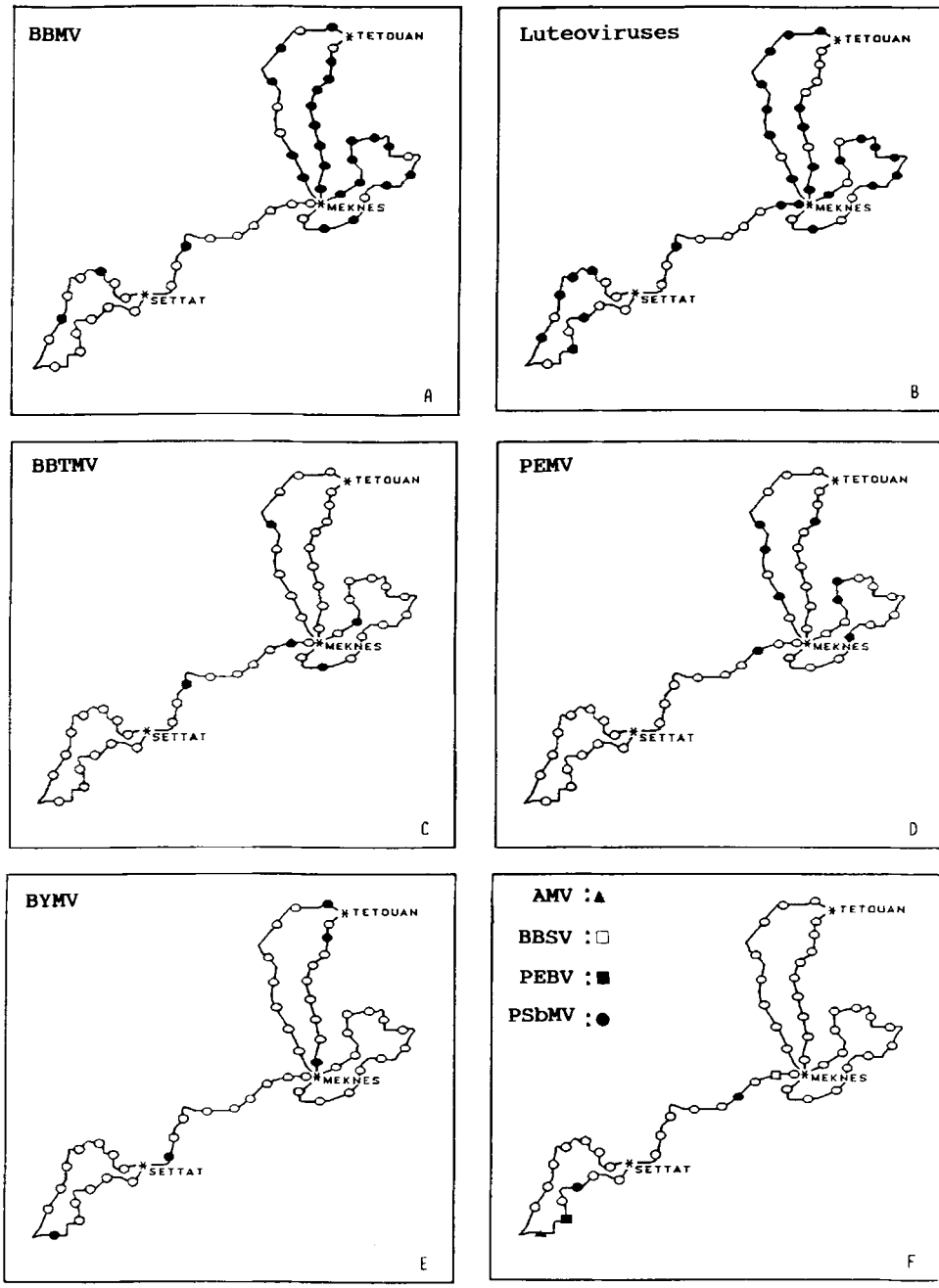

Fig. 3. Geographical distribution of the faba-bean viruses in Morocco.

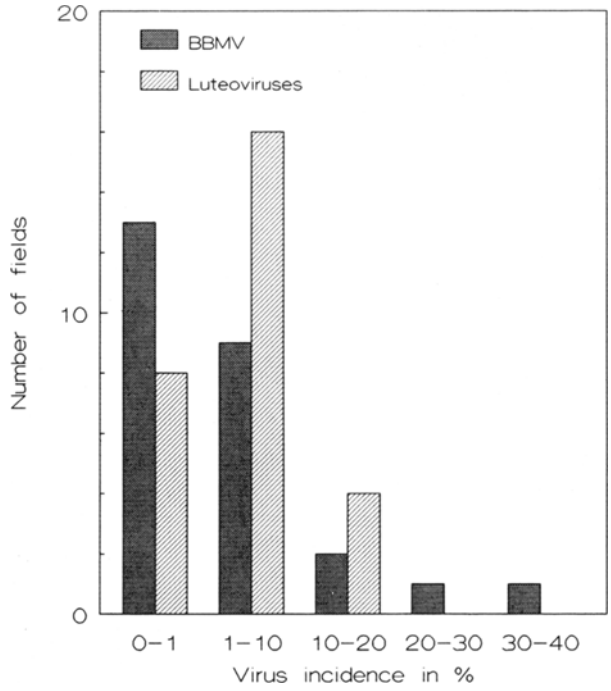

Fig. 4. Incidence of BBMV and the luteoviruses in surveyed faba-bean fields.

Although earlier reports considered BBMV to be of limited distribution and of no economical importance (Fischer, 1979), it now appears to be prevalent in the country. On a world basis, BBMV seemed to be of restricted geographical distribution. It has been reported for the first time from England (Bawden et al., 1951) and later incidentally from Portugal (Borges and Louro, 1974), Sudan (Murant et al., 1974), Morocco (El Maataoui and Fischer, 1976), China (Ford et al., 1981), and Algeria (Ouffroukh, 1985). It was recently reported for the first time from Tunisia, Egypt, Lebanon, and Syria (Makkouk et al., 1988b). Although so far considered of mere academic interest, it is now known to be widespread throughout West Asia and North Africa, and to have high incidence in Morocco. The actual transmission of BBMV in the field is not yet known. Seed transmission is suspected (Bawden et al., 1951; N'Ait Mbarek, 1978), and has been reported but at low rate when the virus occurs in mixed infection with BYMV (Murant et al., 1974; Makkouk et al., 1988b). Information on the role of vectors is also limited. Walters and Surin (1973) reported experimental inefficient transmission by the beetles *Acalymma trivittata*, *Diabrotica undecimpunctata* and *Colaspis flavida*, while Borges and Louro (1974) reported a similarly poor transmission by the weevil *Sitona lineatus*.

The prevalence of BBMV in Morocco and its high incidence recorded in some fields may be due to a high rate of seed transmission and/or the existence of (an) efficient vector(s). Its reported wide host range, mainly among legume species (Makkouk et al., 1988b), suggests the existence of natural sources of infection contributing to its potentiel importance. Further investigations are needed to determine the ways of transmis-

sion of this virus in Morocco, survey other legumes for natural infection, study the variability of the virus, and evaluate the genetic vulnerability of the promising breeding lines of major food legume species to the variants of the virus.

Our serological data revealed the occurrence of a complex of luteoviruses including BLRV, or deviant strains of the latter, in faba bean in Morocco. The recorded incidences of this group of viruses did not exceed 20%, but fields in the area of Meknès visited late in the season showed much higher incidences. Thus a field survey around May would give an assessment of the incidence (number of fields infested and number of plants infected per field) of the luteoviruses different from the one in March reported here. In addition, the transmission by aphids in a persistent manner, and the dramatic damage to faba bean make the luteoviruses of prime importance. Further studies are required for their complete identification, including specificity of aphid transmission and host range (Johnstone et al., 1984), and serology.

BBSV which was considered earlier to be widespread in Morocco (Fischer and Lockhart, 1976) has been found in our survey in one single field only, and is now considered of no economical importance. This decrease in the incidence of the virus may be due to a reduction in the population density of its vector, and could therefore represent a situation of self-elimination as reported for Scotland by Jones (1978).

BYMV, also previously reported as an economically important faba-bean virus in Morocco (Fischer, 1979), appears to be less prevalent. However, it occurs in different areas of the country, is seed and aphid transmitted, and therefore remains of potential importance.

The newly reported viruses PEMV and BBTMV occur in widely separate regions, mainly in the central-northern part of the country, while PSbMV was encountered in two fields only. Further surveys would evaluate the dynamics of their occurrence, and surveying of peas for viruses would most probably reveal infection by PEMV and PSbMV of this crop.

In our survey we did not come across BBWV, although the virus has been reported from Morocco in faba bean (El Maataoui and Fischer, 1976) and in peppers (Lockhart and Fischer, 1976). CMV, which has been reported from faba bean in most of the countries in West Asia and North Africa, is still not encountered in faba bean in Morocco, neither does CYVV. More and repeated surveying is undoubtedly needed to monitor the dynamic occurrence of these viruses. Surveys at different times of the year may give an assessment of their build-up during the season. More detailed studies on the prevalent viruses, i.e. BBMV and the luteoviruses, including yield loss assessement are essential for faba-bean improvement in Morocco.

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