Neth. J. Pl. Path. 98 (1992) 237-252

Identification and properties of a deviant isolate of the broad bean yellow band serotype of pea early-browning virus from faba bean (Vicia faba) in Algeria

M.A-M. MAHIR[1], M. FORTASS[2] and L. BOS

DLO Research Institute for Plant Protection (IPO-DLO), P.O. Box 9060, 6700 GW, Wageningen, the Netherlands

[1] Permanent address: Agricultural Research Corporation, P.O. Box 126, Wad Medani, Sudan

[2] Permanent address: Department of Plant Pathology, École Nationale d'Agriculture (ENA), B.P. S/40, Meknès, Morocco

Accepted 22 May 1992

Abstract

A virus, isolated from faba bean (*Vicia faba*) obtained from Algeria, was readily recognized as a tobravirus by its particle sizes and morphology. Pea (*Pisum sativum*) and French bean (*Phaseolus vulgaris*) characteristically reacted to the isolate like pea early-browning virus (PEBV), but faba bean, *Antirrhinum majus*, *Nicotiana rustica*, and *N. tabacum* reacted with line-pattern symptoms which were unusually brilliant on the *Nicotiana* species. In electron-microscope decoration tests, the isolate did not react with an antiserum to the Dutch type strain of PEBV, but with one to the broad bean yellow band (BBYB) serotype from Italy. It resembles this serotype in reaction on faba bean, but seems to differ appreciably on *N. rustica*, *N. tabacum*, and *Petunia hybrida*. It is described as a deviant isolate of the BBYB serotype of PEBV.

All thirteen faba-bean genotypes tested were found to be susceptible to the Algerian isolate and two Dutch type strain isolates of the virus, and to react with erratic line-pattern symptoms to the Algerian isolate only. All ten genotypes of chickpea (*Cicer arietinum*) tested reacted hypersensitively, and four out of ten genotypes of lentil (*Lens culinaris*) were susceptible to the virus but reacted differentially to the three isolates. Seed transmission of PEBV, including the new isolate, in faba bean is confirmed (9% for the Algerian isolate, and over 45% for one of the Dutch type strain isolates), and seed transmission of the virus in a non-legume (*N. rustica*, 4%) is herewith first reported. This is the first report on the occurrence of the BBYB serotype of PEBV outside Italy, and of PEBV outside Morocco in North Africa.

Additional keywords: chickpea, hypersensitivity, lentil, *Nicotiana rustica*, *N. tabacum*, *Phaseolus vulgaris*, *Pisum sativum*, seed transmission, soil-borne virus, strain differentiation.

Introduction

Pea early-browning virus (PEBV) is a tobravirus (Harrison, 1973) originally described in Western Europe (Bos and Van der Want, 1962; Gibbs and Harrison, 1964) as a soil- and seed-borne virus able to cause severe damage in pea (*Pisum sativum*) but often symptomlessly infecting other plant species, including alfalfa (*Medicago sativa*) and faba bean (*Vicia faba*). British isolates were serologically only distantly related to the

Dutch type strain (Gibbs and Harrison, 1964). The virus was later reported from pea in other European countries (for references see Harrison, 1973), and from other legume crops such as faba bean in Poland (Fiedorow, 1980, 1983), yellow lupin (*Lupinus luteus*) also in Poland (Pospieszny and Frencel, 1985), and French bean (*Phaseolus vulgaris*) in Sweden (Gerhardson and Ryden, 1979). It was also found to be seed-transmitted in faba bean (Fiedorow, 1980, 1983; Cockbain et al., 1983). Occurrence of the virus in pea and faba bean at widely separated locations in Morocco was reported by Lockhart and Fischer (1976). Russo et al. (1984) isolated a virus from faba bean in Southern Italy which they considered a distinct tobravirus named broad bean yellow band virus (BBYBV), serologically unrelated to PEBV. Soon thereafter, Robinson and Harrison (1985) provided evidence that it is a third serotype of PEBV, then designated PEBV-BBYB, rather than a distinct tobravirus.

Within the framework of the cooperation between IPO-DLO and the International Centre for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria, and further to a survey for viruses of faba bean in West Asia and North Africa (Makkouk et al., 1988), we recently isolated a tobravirus from a faba-bean leaf sample obtained from Algeria, so far not included in the survey. The virus caused symptoms in faba bean reminiscent of BBYBV, but typical of PEBV in pea and other hosts. This paper reports on the identification of the isolate as a deviant isolate of the BBYB serotype of PEBV and provides new information on the virus, its variability and its potential importance in legume crop species which are of major importance to crop-improvement projects in the region.

Materials and methods

Virus isolates and maintenance. The Algerian isolate under investigation (AlgR10) was derived from a sample of faba-bean leaves desiccated over calcium chloride ($CaCl_2$) and kindly provided by A. Ouffroukh, Institut National de Protection des Végétaux (INPV), El Harrach, Algiers, Algeria. E116 is the Dutch type strain of PEBV (Bos and Van der Want, 1962), and E413 a slightly different isolate from pea (Bos and Huijberts, unpublished results, 1984), both stored over $CaCl_2$. The three virus isolates were separately propagated and maintained in *Phaseolus vulgaris* 'Bataaf'. Sap transmission was usually from inoculated primary leaves, either fresh or stored at −20 °C, and ground in 0.01 M potassium phosphate buffer, pH 7.6. Carborundum was used as an abrasive.

Host-range experiments. At least two, but usually eight, plants of leguminous and non-leguminous plant species were sap-inoculated. Plants were kept in an insect-proof glasshouse (ca. 20 °C) for symptom development. Those showing questionable or no symptoms were tested for infection by back-inoculation onto *Chenopodium amaranticolor.*

Electron microscopy. Leaf samples from infected plants were chopped in 2% (w/v) sodium phosphotungstate (PTA, pH 6.7) for negative staining, and viewed in a Philips CM12 transmission electron microscope. Particles were measured from prints at magnifications ×6500 and 8500 in classes of 0.25 mm, and normal lengths estimated were converted into nm.

Serologically specific electron microscopy. Antisera to E116 and BBYBV were kindly supplied by D.Z. Maat (IPO-DLO, Wageningen) and G.P. Martelli (Bari, Italy), respectively. The tests were performed as described by Walkey and Webb (1984) except for the incubation of the grids at room temperature for 5 instead of 20 min in a moist chamber.

Food legume genotypical reaction. For detecting possible strain differences, the AlgR10 and the two Dutch isolates were compared on a range of food legume genotypes. Emphasis was on legume species that are of major importance in the ICARDA crop-improvement programmes, i.e. pea, faba bean, chickpea, and lentil, and on promising genotypes of these to predict the behaviour of future legume crop genotypes in the region. Of these crops, 13, 12, 10 and 10 gene-bank entries and/or genotypes, respectively, were obtained from ICARDA's Genetic Resources Unit. At least four plants per entry were inoculated, and symptoms were recorded as described before. Back inoculations were made from symptomless and questionably reacting plants.

Seed-transmission tests. Dry seeds were collected from plants of faba bean two months after inoculation (pooled from all genotypes listed on p. 247 for the three isolates separately), and of *Phaseolus*-bean 'Bataaf', *Nicotiana rustica*, and *Petunia hybrida* about three months after inoculation with AlgR10 only. Seeds of *N. rustica* were treated with gibberellic acid to facilitate germination. The developing seedlings were inspected for symptoms, and the presence of virus was checked by inoculation onto *C. amaranticolor.*

Results

Host reactions

Isolate AlgR10 was easily sap-transmitted from inoculated 'Bataaf' leaves to 25 plant species belonging to 6 families. Their reactions together with the data on BBYBV from the literature (Russo et al., 1984) are summarized in Table 1. AlgR10 resembled the Dutch isolates of PEBV on several hosts, but differed substantially in its reaction on at least five plant species. Symptoms in some important host species which reacted characteristically or differentially are described below.

Antirrhinum majus. Local and systemic concentric rings and line patterns were induced by AlgR1O (Fig. 1), whereas local and systemic infection by both Dutch isolates was symptomless in spite of high virus concentration in uninoculated leaves.

Chenopodium amaranticolor reacted similarly to the three viruses. On inoculated young leaves, numerous small chlorotic lesions, many with a pin-point dry centre, were produced in three days. The dry centres gradually enlarged into necrotic lesions, often with a brownish or reddish outline, further surrounded by a chlorotic halo. Lesions were largest with E413. On inoculated bottom leaves, local lesions were sometimes chlorotic or necrotic rings surrounding normal green tissue and/or consisted of etched, later whitish dry or necrotic tissue. Necrosis of petioles and stem tissue near inoculated leaves was occasionally produced by the Dutch isolates only, but none of the isolates was detected in uninoculated leaves.

Table 1. Summary of host reactions.

Host species	Virus isolates			
	Dutch isolates		AlgR10	BBYBV[a]
	E116	E413		
Amaranthaceae				
Celosia argentea	L^nS^n	L^nS^n	L^nS^n	
Gomphrena globosa	L^nS^n	L^nS^n	L^n –	L^n –
Chenopodiaceae				
Chenopodium amaranticolor	L^n –	L^n –	L^n –	L^n –
Chenopodium murale			L^n –	
Chenopodium quinoa	L^n –	L^n –	L^n –	L^n –
Cucurbitaceae				
Cucumis sativus				L –
'Chinese Slangen'	L/L^n –	L/L^n –	L/L^n –	
'Gele Tros'			L –	
Leguminosae				
Lathyrus odoratus	l s	l s	l s	
Medicago sativa	L^n –	L^n –	L^n –	
Phaseolus vulgaris				
'Bataaf'	L^nS/S^n	L^nS/Sn	L^nS/S^n	
'Bountiful'				L^nS
'Black Turtle'				L^nS
'Pinto III'				L^nS
'La Victoire'				L^nS
Pisum sativum				
'Castro'	L^nS^n	L^nS^n	L^n –/S^n	
'Dark Skin Perfection'	(L^n)ls	(L^n)ls	(L^n)ls	L^ns
'Perfected Wales'				L^ns
'Rondo'	L^ns/S^n	L^ns/S^n	L^nS^n	
Trifolium incarnatum	L^n –	L^n –	L^n –	
Trifolium repens	– –	l –	– –	
Vicia faba				L^nS
'Compacta'	l s	l s	L^nS	
Scrophulariaceae				
Antirrhinum majus	l s	l s	L S	
Solanaceae				
Datura stramonium	l –	l –	l –	L^n –
Nicotiana benthamiana	l (S)	l (S)	L S	L^nS
Nicotiana clevelandii	L^nS/l s	L^nS/l s	L(^{n})S	S
Nicotiana glutinosa	l S	l S	L^nS	
Nicotiana occidentalis P1	L^nS^n	L^nS^n	L^nS^n	
Nicotiana rustica	l s	l s	L S	– * –
Nicotiana tabacum				
'White Burley'	L(S^n)	L –	L S(^{n})	– * –
Petunia hybrida	l s	L^n(S^n)	(L^nS^n)	– * –

Explanation of symbols: L = local symptoms; S = systemic symptoms; l = latent local infection; s = latent systemic infection; – = no infection as verified by back inoculation; – * = infection not tested for; ^{n} = necrotic. Symbols in brackets refer to inconspicuous or erratic symptoms depending on growing conditions.

[a] Data by Russo et al. (1984).

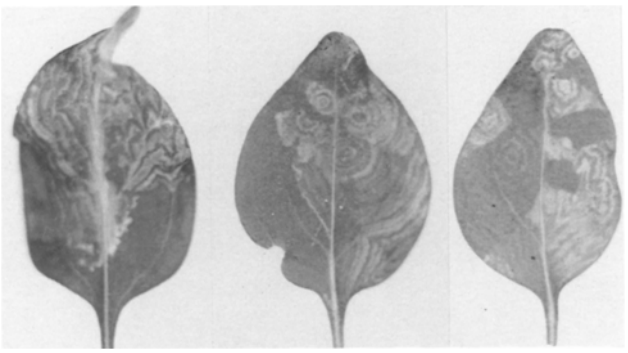

Fig. 1. Systemic concentric ringspotting in *Antirrhinum majus* after inoculation with an Algerian isolate of pea early-browning virus (AlgR10).

Chenopodium quinoa. All isolates produced etched local lesions which soon enlarged, coalesced and turned necrotic, causing withering and premature shedding of inoculated leaves. With the Dutch isolates, there were few chlorotic and/or necrotic local lesions in addition to the etched lesions. Restricted systemic stem necrosis around the petioles of inoculated leaves was observed with AlgR1O and E413 only. None of the isolates could be detected in uninoculated leaves.

Nicotiana benthamiana. AlgR10 induced many diffuse chlorotic spots and rings with a green centre, sometimes as early as one day after inoculation, followed by chlorotic ringspotting and marbling associated with leaf rugosity. With the Dutch isolates, systemic infection usually did not result in clear symptoms, but it was sometimes associated with irregular mottling and leaf malformation.

Nicotiana glutinosa. Only AlgR1O produced a varying number of small chlorotic to etched or necrotic local lesions, slightly enlarging and gradually turning necrotic. All three isolates induced diffuse systemic mottling.

Nicotiana rustica. Although all three isolates locally and systemically infected the inoculated plants, symptoms were produced by AlgR1O only, and systemic symptoms were very striking. Few chlorotic local lesions, 3–4 mm in diameter, were soon followed by an irregular whitish systemic vein chlorosis developing into a brilliant pattern of parallel lines following the larger veins or occurring in concentric rings. At several places, interveinal tissue remained normal or assumed a dark green colour, thus developing into a mosaic pattern. Leaves often showed a whitish marbling in some of them or in certain parts of the leaves (Fig. 2). Symptoms persisted for life and plants were considerably reduced in size.

Nicotiana tabacum 'White Burley'. Local and systemic symptoms caused by AlgR10 were similar to those in *N. rustica*, but the pattern lines were finer, and sometimes islands of white tissue were formed (Fig. 3). With E116 and AlgR10, the local and systemic infections were associated infrequently, often late during plant development,

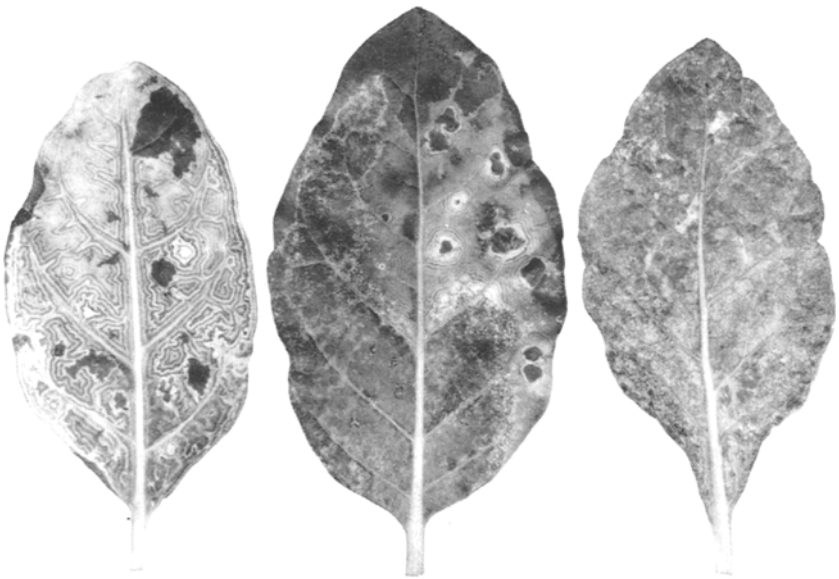

Fig. 2. Systemic yellow to whitish line patterns and marbling in *Nicotiana rustica* after inoculation with an Algerian isolate of pea early-browning virus (AlgR10).

Fig. 3. Fine systemic line patterns and white discolourations in *Nicotiana tabacum* 'White Burley' after inoculation with an Algerian isolate of pea early-browning virus (AlgR10).

Petunia hybrida. AlgR1O induced local and systemic interveinal necrotic lesions, necrotic streaks on veins and stems, and severe stunting in a limited number of the inoculated plants, presumably because of genetic heterogeneity. Although both Dutch isolates infected plants systemically and reached high concentrations in them, only E413 produced symptoms consisting of leaf and severe necrotic stem streaking.

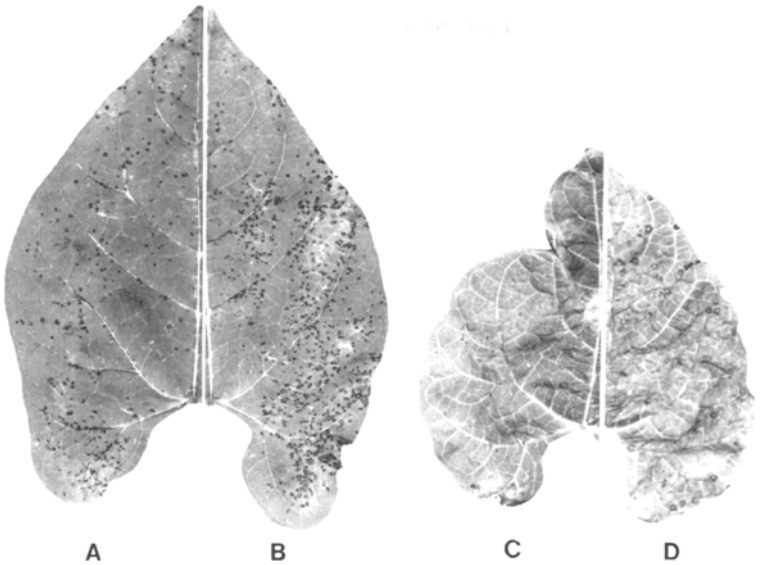

Fig. 4. Local lesions of an Algerian isolate of pea early-browning virus (AlgR10) (A,C) and a Dutch isolate (E413) (B,D) in *Phaseolus vulgaris* 'Bataaf', 10 days after inoculation. (A, B) Primary leaves, relatively old when inoculated; (C, D) primary leaves, relatively young when inoculated.

Phaseolus vulgaris 'Bataaf'. Inoculated primary leaves reacted to all isolates after two days with localized reddish vein necrosis, and after one more day with many necrotic rings surrounding normal green tissue. This remained green with AlgR10 and E116, and quickly turned necrotic with E413. The local necrotic rings were smaller, the older the primary leaves were at the time of inoculation (Fig. 4). The irregular veinal necrosis often led to leaf crumpling, if the leaves were young when inoculated. Systemic symptoms consisted of diffuse chlorotic lesions with a necrotic centre, or of irregularly shaped chlorotic rings or flecks and some associated leaf malformation (Fig. 5). In older plants, the local reaction to E413 was usually more rapid and more necrotic than that to the other isolates, and subsequent systemic symptoms of E413 were more erratic and often absent. No virus could be recovered from symptomless leaves.

Pisum sativum. In 'Rondo', AlgR10 induced few necrotic local lesions followed by systemic necrosis and often by premature plant death. With the Dutch isolates, similar necrotic local lesions were produced, and some localized stem necrosis followed; systemic infection was largely symptomless but still associated with high virus content. In 'Castro', however, the effect of AlgR10 was least severe. Dry or necrotic local lesions developed and remained smaller or enlarged more slowly as compared to those of the other isolates (Fig. 6, upper part), and no virus could be demonstrated in symptomless leaves. Systemic symptoms rarely occurred. With the Dutch isolates, in this cultivar

Fig. 5. Systemic symptoms in trifoliolate leaves of *Phaseolus vulgaris* 'Bataaf', one month after inoculation with an Algerian isolate of pea early-browning virus (AlgR10) (left), and Dutch isolates E413 (middle) and E116 (right).

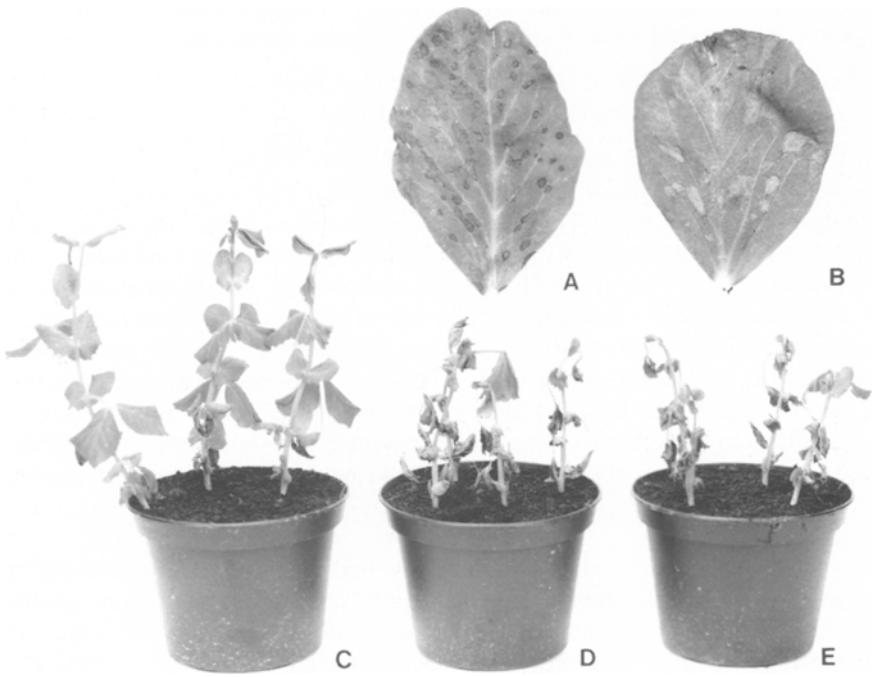

Fig. 6. Local and systemic symptoms of an Algerian isolate of pea early-browning virus (AlgR10) (A, C), and Dutch isolates E116 (B, D) and E413 (E) in *Pisum sativum* 'Castro', 5 days (A, B) and 11 days (C, D, E) after inoculation.

Fig. 7. Systemic line-pattern symptoms of an Algerian isolate of pea early-browning virus (AlgR10) in *Vicia faba* 'Compacta'.

many etched local lesions developed and enlarged rapidly. They were soon followed by severe systemic vein, stem and apical necrosis, and by plant death (Fig. 6, lower part).

Vicia faba 'Compacta'. No obvious symptoms were produced by the two Dutch isolates despite a high virus concentration throughout inoculated plants, as apparent from several back inoculations. In contrast, AlgR10 produced occasional local necrotic spots (1–2 mm in diameter) and local chlorotic and/or necrotic rings, and erratically occurring but recurrent systemic chlorotic concentric ring spots and oak-leaf type line patterns (Fig. 7). No systemic infection resulted when many necrotic local lesions were formed, as after inoculation with a high dose of AlgR10.

Electron microscopy

Electron-microscopic observations of crude sap from inoculated 'Bataaf' leaves revealed straight rod-shaped tubular particles with predominant lengths of 83 and 211 nm, respectively, for AlgR10 when 219 particles were measured. Obvious end-to-end aggregation also occurred (Fig. 8). Normal lengths determined for E116 were 85 and 206 nm, and for E413 83 and 208 nm. Distribution curves showed narrow peaks. AlgR10 showed a small secondary peak of short particles ca. 58 nm long.

Serologically specific electron microscopy

Decoration tests showed that the antiserum to the Dutch E116 did not coat AlgR10 particles in crude sap preparations, whereas BBYBV antiserum clearly decorated the particles of AlgR10. The Dutch isolate E413 was also densely decorated with the antiserum to E116, indicating that the two Dutch isolates belong to the same serotype. No decoration was recorded for E116 and E413 with the antiserum to BBYBV (Fig. 8).

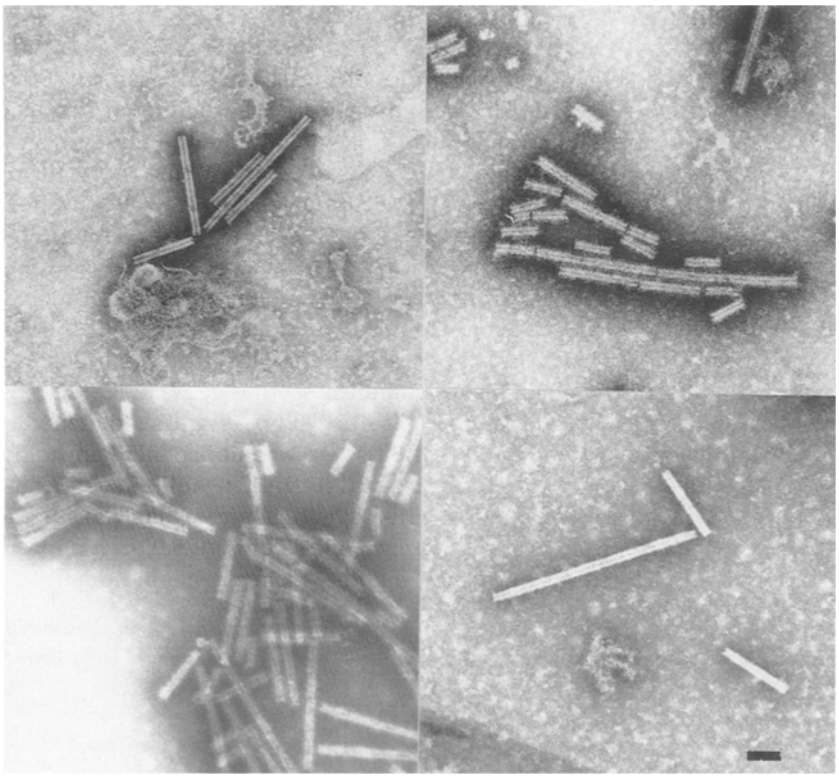

Fig. 8. Electron-microscope decoration tests with an Algerian isolate of pea early-browning virus (AlgR10) (above) and a Dutch isolate E116 (below) and antisera to E116 (left) and BBYBV (right). Magnification bar represents 100 nm.

Food legume genotypical reaction

Pea. The data summarized in Table 2, together with those for the pea cultivars included in Table 1, clearly show that the three virus isolates are infectious to all pea genotypes tested, including ICARDA lines, and pathogenic to most of them. All isolates incited similar reactions on most genotypes and were indiscriminately extremely virulent on three ICARDA lines, i.e. accession No. 62, 125 and 154, and all were lethal on accession No. 62. Systemic infection remained symptomless in 'Dark Skin Perfection' and 'Early Perfection' as well as in three ICARDA accessions (No. 22, 30, and 169), and in 'Jewel' infection remained local.

There were no clear differences between the two Dutch isolates except in accession No. 8 in which E116 remained local, but AlgR10 behaved differently in 'Castro', 'Rondo', and ICARDA accession No. 30 and 167. AlgR10 was less pathogenic than the Dutch isolates in 'Castro', because of the rapid and severe local reaction indicating hypersensitivity (Fig. 6). In 'Rondo', AlgR10 was more pathogenic than the other isolates, i.e. it induced clear local and systemic necrosis and caused relatively severe

Table 2. Summary of reactions of pea genotypes.

Genotypes[a]	Virus isolates		
	E116	E416	AlgR10
8	L^n –	L^nS^n	L^nS^n
21	L^nS^n	L^nS^n	L^ns
22	l s	l s	l s
30	l s	l s	L S
62	L^nS^n	L^nS^n	L^nS^n
101	L^nS^n	L^nS^n	(L^n)lS^n
125	L^nS^n	L^nS^n	L^nS^n
154	L^nS^n	(L^n)lS^n	L^nS^n
167	L^nS^n	L^nS^n	L^nS^n
169	(L^n)ls	(L^n)ls	L^ns
'Jewel'	L^n –	L^n –	L^n –
'Dark Skin Perfection'	(L^n)ls	(L^n)ls	(L^n)ls
'Early Perfection'	(L^n)ls	(L^n)ls	L^ns

For explanation of symbols, see Table 1.
[a] ICARDA accession numbers and internationally available cultivars.

stem necrosis and occasional plant death. In both cultivars, the Dutch isolates behaved in the opposite way. They were extremely pathogenic in 'Castro' and weakly pathogenic in 'Rondo'. The Algerian isolate was more pathogenic than the Dutch isolates in the ICARDA accessions No. 30 and 167. AlgR10 caused some localized stem necrosis and stunting in No. 30, but severe local and systemic necrotic stem streaking, severe stunting and occasional death in No. 167. In these two pea lines, the Dutch isolates were less pathogenic.

Faba bean. The three virus isolates were found to be equally infectious to all twelve ICARDA faba-bean genotypes tested. Back inoculations from inoculated as well as from uninoculated leaves demonstrated high virus contents. However, erratic but recurrent systemic symptoms, as described for 'Compacta' (Fig. 7), and an inconspicuous reduction in plant growth, were only observed with AlgR10, and similarly in all genotypes. The ICARDA Food Legume Improvement Program (FLIP) lines tested were FB No. 84-230 and -237, 85-172, 86-107, -114, -115, -116, -117, -119, -122, -164, and 87-26.

Chickpea (Cicer arietinum). All three virus isolates were found to be locally, but not systemically pathogenic to all ten ICARDA chickpea genotypes tested, and the virus could not be isolated from uninoculated leaves. No difference was observed between the isolates. The local symptoms varied from confined small necrotic (E413) to occasionally enlarging and coalescing lesions (AlgR10 and E116) (Fig. 9). No reduction in plant vigour was observed as compared to uninoculated controls. The ICARDA genotypes tested were ILC No. 263 and 482, and FLIP C No. 81-293, 82-150, 83-46, -47, 84-15, -92, 85-15, and 87-69.

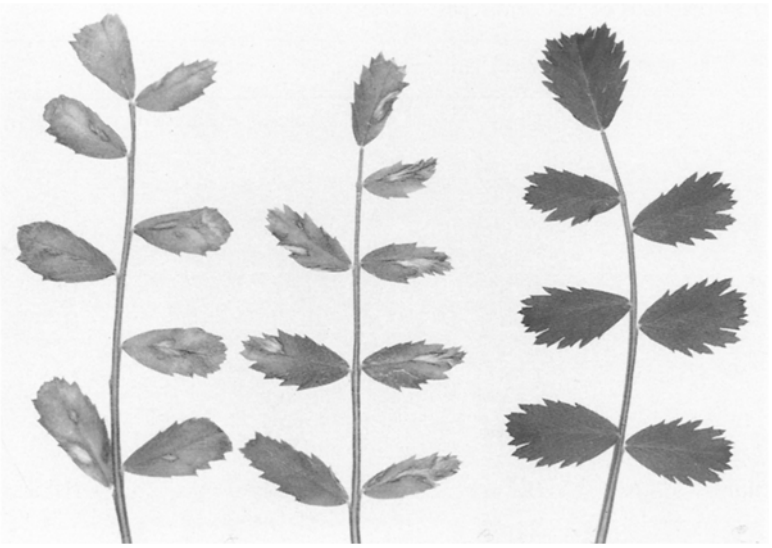

Fig. 9. Hypersensitivity reaction of chickpea (*Cicer arietinum*, ICARDA line FLIP 85-15C) to an Algerian isolate of pea early-browning virus (AlgR10) (middle) and a Dutch isolate (E116) (left), 4 days after inoculation. Uninoculated control on the right.

Lentil (Lens culinaris). A great deal of similarity was observed between the three isolates on the ten ICARDA genotypes tested (Table 3). Back inoculations have shown that six lines were immune to all three isolates. Only two lines were highly susceptible (ILC5845 and 5876). However, on these lines, AlgR10 and E116 were pathogenic and induced symptoms varying from necrotic stem streaking, withering, stunting and occasional plant death, to rosetting of sprout tips of ILC5876. AlgR10 and E413 were recovered in low and moderate concentration from uninoculated tissue of normal-looking plants of ILC6442 and 5999, respectively.

Seed-transmission tests

Out of 44, 33, and 43 seedlings of faba bean from plants infected with AlgR10, E116, and E413, respectively, only 1 seedling with AlgR10 showed line-pattern symptoms characteristic of the isolate. However, at testing, 4, 15, and 0 seedlings were found to contain the virus in high concentrations, which amounts to 9.1, 45.5, and 0% seed transmission, respectively.

Out of 141, 48, and 56 seedlings grown from plants of *P. vulgaris* 'Bataaf' infected early with AlgR10, E116, and E413, respectively, none showed symptoms, and all developed into vigorous plants. Biological assay of all seedlings in groups of 8 did not reveal infection.

Out of the 3283 seedlings grown from seeds of plants of *N. rustica* infected with AlgR10, 130 seedlings showed cotyledonary chlorosis followed by considerable persistent growth reduction and overall leaf discolouration or marbling due to white pin-points or line patterns. Back inoculation proved that the virus was only present

Table 3. Summary of reactions of ICARDA lentil genotypes.

ILC Genotypes	Virus isolates		
	E116	E413	AlgR10
5722	– –[1]	– –	– –
5845	l S^{st} (+ +)	l s (+ + +)	l S^{st} (+ + +)
5876	l S^{st} (+ + +)	l s (+ + +)	l S^{st} (+ + +)
5999	– –	l s (+ +)	– –
6216	– –	– –	– –
6246	– –	– –	– –
6437	– –	– –	– –
6442	– –	– –	l s (+)
6763	– –	– –	– –
6773	– –	– –	– –

For explanation of symbols see Table 1; st = stunting; (+ + +) high virus concentration; (+ +) moderate virus concentration; (+) low virus concentration as demonstrated by back inoculation.

in these diseased seedlings, in very high concentrations, and that symptomless seedlings did not contain the virus. The seed-transmission rate in *N. rustica* was determined to be ca. 4% (3.96%).

A growing-on test with 200, 130, and 250 seeds from plants of *P. hybrida* infected with AlgR10, E116, and E413, respectively, including bioassay of the seedlings in groups of 10, revealed no seed transmission.

Discussion and conclusions

The isolate AlgR10 clearly behaved like PEBV, especially in pea and *P. vulgaris*, in symptomlessly infecting several other plant species, and in the type and two sizes of particles observed in the electron microscope. Sizes of the long component of the three isolates are similar to those reported earlier (Bos and Van der Want, 1962: about 105 and 210 nm; Harrison, 1966: 103 and 212 nm). The short component of all three isolates now studied, including the Dutch type strain E116, is shorter (ca. 80 nm), but this size agrees with one of the preparations of Bos and Van der Want (1962). Van Hoof (1969) had found that short particles could vary largely with isolate (from 54 to 99 nm). AlgR10 differs considerably from the Dutch type strain (E116) of PEBV, as well as from the later isolated E413, especially in the reaction of its natural host *V. faba*, and of *A. majus* and *Nicotiana* species, particularly *N. rustica* and *N. tabacum* 'White Burley', and basically in serology. The unusual and brilliant line-pattern symptoms in *N. rustica* and *N. tabacum* are highly characteristic. The Dutch isolate E413 is quite similar to the type strain isolate E116 in the reactions evoked in most genotypes of pea and some other legumes. However, there are slight differences in other species, such as *P. hybrida*, and *P. vulgaris* 'Bataaf', and a considerable difference in two lines of lentil, as well as in the rate of seed transmission in faba bean.

The symptoms obtained with AlgR10 in *V. faba* are reminiscent of, although less

severe than, those described for the BBYBV in Italy (Russo et al., 1984), later reidentified as the BBYB serotype of PEBV (Robinson and Harrison, 1985). The latter authors already pointed out that BBYBV invades pea and *P. vulgaris* systemically, like the Dutch and British strains of the virus, but that it differed in the severe mottling it induced in *N. benthamiana* and *N. clevelandii.* The close relationship of AlgR10 to this serotype was confirmed by the dense coating of AlgR10 particles with BBYBV antiserum in electron-microscope decoration tests. Host-plant reactions reported by Russo et al. (1984) for BBYBV suggest considerable biological differences between the Italian PEBV-BBYB serotype and our AlgR10. For example, *N. rustica*, *N. tabacum*, and *P. hybrida* did not react in their tests, and they could not detect the virus in inoculated plants by electron microscopy. However, Italian host-range tests were limited, and *A. majus*, characteristically reacting to our AlgR10, was not included in the investigations by Russo et al. (1984). They considered BBYBV to differ from PEBV, based on absence of clear systemic necrosis in two pea cultivars tested. One of their pea cultivars ('Dark Skin Perfection') did not react with necrosis to our three isolates either, and systemic necrotic reaction was often erratic in pea. Biological and serological evidence have led us to conclude that the Algerian PEBV isolate is a deviant isolate of the BBYB serotype of the virus. Unfortunately, the original BBYBV isolate was not available at our request for direct comparison.

This paper is the first report on pathogenicity of PEBV to lentil, although several genotypes tested seem to be immune to the virus. The ten chickpea genotypes tested, although rapidly reacting with local lesions, did not contract systemic infection. If hypersensitivity is also effective in roots subject to natural infection via nematodes, these genotypes may escape infection in the field.

Infectivity of PEBV to faba bean, its extremely high concentration in infected plants, and seed transmissibility in this host (see also Fiedorow, 1980, 1983; Cockbain et al., 1983), make this crop species of great potential importance to the ecology of the virus, i.e. in maintaining or increasing inoculum potential or infection pressure in a given area, and in the spread from one location to another. The virus is also of potential importance to faba bean and faba-bean improvement programmes in the region because of the high degree of susceptibility of a number of promising ICARDA breeding lines to all three isolates. This holds especially for the BBYB-strain since it may also damage the crop. Even when symptomless, as usual in faba bean and in some lentil genotypes, the virus may aggravate the symptoms of other viruses when occurring in complex, taking into account the high concentrations of the virus in infected plants. Mixed infection of faba bean with bean leafroll luteovirus, prevalent in Arab countries (Makkouk et al., 1988) including Morocco (Fortass and Bos, 1991), has earlier been found often to induce leaf and stem necrosis and sometimes early plant death, not caused by the viruses individually (Cockbain et al., 1983).

None of the faba-bean genotypes tested here showed any indication of resistance to infection. In pea, some genotypes have shown some degree of resistance or tolerance ('Dark Skin Perfection', 'Early Perfection', pea accession No. 22), and even systemic immunity (perhaps because of hypersensitivity) to all isolates ('Jewel') and usually to AlgR10 ('Castro'). However, resistance to one isolate appeared not necessarily to hold for other isolates or strains (as in pea 'Rondo', and 'Castro'and accession No. 30) and in lentil. AlgR10 was also sometimes found to be excluded from systemic infection in faba bean through hypersensitivity.

This paper is the first report on seed transmission of PEBV in a non-legume, that is *N. rustica*. Such seed transmission may also hold for other non-legumes not yet tested for seed transmission. Seed transmission in cultivated and wild species is known to play an important role in the ecology of nematode-transmitted soil-borne viruses in general (Murant and Lister, 1967).

This is also the first report on the occurrence of PEBV in Algeria and in North Africa outside Morocco, and on the occurrence of the BBYB serotype of the virus outside Italy. No information is available as to the origin and possible distribution of the virus in Algeria. Earlier information about the occurrence of PEBV in pea and faba bean at widely different locations in Morocco (Lockhart and Fischer, 1976), and its previously undetected occurrence in the first faba-bean sample we tested from Algeria, suggest that in West Asia and North Africa it may be more widespread than supposed so far. Usually symptomless infection in faba bean, and symptoms in pea usually not suggestive of virus infection, indicate that occurrence is likely to be overlooked even by plant pathologists. The virus escaped detection during the ICARDA survey for viruses in faba bean in the region (Makkouk et al., 1988) since no antiserum to it was included in the testing of samples, and serological testing for tobraviruses remains difficult because of serological variation.

Seed-transmitted and soil-borne viruses may endanger crop improvement programmes. Once introduced at places where nematode vectors are present, the viruses may remain there forever, either directly threatening existing crops, or doing so at any time after the introduction of new sensitive crop genotypes. The viruses therefore have to be taken into consideration in crop improvement programmes, including seed production schemes. However, risks of establishment greatly depend on the occurrence and incidence of potential nematode vector species.

Acknowledgments

We are greatly indebted to Dr G.P. Martelli and Dr M. Russo, Bari, Italy, for sending the BBYBV antiserum and to Dr K.M. Makkouk, ICARDA, Aleppo, Syria, for providing seeds of the promising ICARDA breeders' genotypes of chickpea, faba bean, lentil, and pea. Financial support by the Directorate General for International Cooperation (DGIS) of the Netherlands Ministry of Foreign Affairs via the IPO/ICARDA Linkage Project for Plant Virology is gratefully acknowledged.

References

Bos, L. and Van der Want, J.P.H., 1962. Early browning of pea, a disease caused by a soil- and seed-borne virus. Tijdschrift over Planteziekten 68: 368–390.

Cockbain, A.J., Woods, R.D. and Calilung, V.C.J., 1983. Necrosis in field beans (*Vicia faba*) induced by interactions between bean leaf roll, pea early-browning and pea enation mosaic viruses. Annals of Applied Biology 102: 495–499.

Fiedorow, Z.G., 1980). Some virus diseases of horse bean. Tagungs-Bericht Akademie der Landwirtschaftswissenschaften der Deutschen Demokratischen Republik, Berlin. 184: 361–366.

Fiedorow, Z.G., 1983. Pea early-browning virus on horse bean (*Vicia faba* L. ssp. *minor*). Zeszyty Problemowe Postepow Nauk Rolniczych 291: 97–109.

Fortass, M. and Bos, L., 1991. Survey of faba bean (*Vicia faba* L.) for viruses in Morocco. Netherlands Journal of Plant Pathology 97: 369–380.

Gerhardson, B. and Ryden, K., 1979. An isolate of pea early browning virus from field-grown *Phaseolus vulgaris*. Phytopathologische Zeitschrift 95: 93–96.
Gibbs, A.J. and Harrison, B.D., 1964. A form of pea early-browning virus found in Britain. Annals of Applied Biology 54: 1–11.
Harrison, B.D., 1966. Further studies on a British form of pea early-browning virus. Annals of Applied Biology 57: 121–129.
Harrison, B.D., 1973. Pea early-browning virus. C.M.I./A.A.B. Descriptions of Plant Viruses. No. 120: 4 pp.
Lockhart, B.E.L. and Fischer, H.U., 1976. Some properties of an isolate of pea early browning virus occurring in Morocco. Phytopathology. 66: 1391–1394.
Makkouk, K.M., Bos, L., Azzam, O.I., Koumari, S. and Rizkallah, A., 1988. Survey of viruses affecting faba bean in six Arab countries. Arab Journal of Plant Protection 6: 61–53.
Murant, A.F. and Lister, R.M., 1967. Seed transmission in the ecology of nematode-borne viruses. Annals of Applied Biology 59: 63–76.
Pospieszny, H. and Frencel, I., 1985. Viruses in natural infections of yellow lupin (*Lupinus luteus* L.) in Poland. VI. Pea early-browning virus. Acta Phytopathologica Academiae Scientiarum Hungaricae 20: 91–95.
Robinson, D.J. and Harrison, B.D., 1985. Evidence that broad bean yellow band virus is a new serotype of pea early-browning virus. Journal of General Virology 66: 2003–2009.
Russo, M., Gallitelli, D., Vovlas, C. and Savino, V., 1984. Properties of broad bean yellow band virus, a possible new tobravirus. Annals of Applied Biology. 105: 223–230.
Van Hoof, H.A., 1969. Enige eigenschappen van Nederlandse isolaten van het vroege-verbruiningsvirus van de erwt. Mededelingen Rijksfakulteit Landbouwwetenschappen Gent 34: 888–894.
Walkey, D.G.A. and Webb, M.J.W., 1984. The use of a simple electron microscope serology procedure to observe relationships of seven potyviruses. Phytopathologische Zeitschrift 110: 319–327.