Neth. J. Pl. Path. 80 (1974) 113–123

A strain of cucumber mosaic virus, seed-transmitted in beans

L. BOS and D. Z. MAAT

Institute of Phytopathological Research, Wageningen,

Accepted 4 February 1974

Abstract

From bean plants (*Phaseolus vulgaris*) grown near Valencia, Spain, a virus was isolated that is easily transmitted by sap and by leaf contact to beans and 23 of 37 other plant species tested. In most species symptoms were mild or absent. Symptoms in bean could be easily confused with those of bean common mosaic virus, but were usually mild and diseased plants often recovered. All bean cultivars tested were susceptible. One of twelve varieties investigated showed 7% seed transmission. Seed remained infective after 27 months of storage. Two antisera (titre 64) were prepared against purified, formalin-treated virus. Serologically the virus was found to be closely related to normal cucumber mosaic virus and hardly or not to the chrysanthemum aspermy virus. This shows that it differs from peanut stunt virus which is known to cause a severe disease in beans in the USA.

Partial masking of symptoms, high infectivity, wide host range and seed transmission make the virus potentially important to bean cultivation.

Introduction

In 1970 our attention was drawn to a severe disease in beans (*Phaseolus vulgaris*) that was spreading rapidly in fields near Valencia, Spain. From material sent to us for diagnosis we easily isolated a virus (coded B32) from both leaves and pods. The virus was readily sap-transmitted. It turned out to be seed-borne in bean and to resemble cucumber mosaic virus (CMV, cryptogram R/1:1/18:S/S:S/Ap). We have studied it in some detail, because of the potential threat imposed by the virus, although the naturally occurring disease syndrome could not be reproduced in the greenhouse.

Materials and methods

Most work was done with the isolate B32 from beans. Other virus isolates used for serology and purification are listed in the sections concerned.

Test plant work was performed in an insect-proof greenhouse in the conventional way (Bos et al., 1960). For purification, virus was propagated in *Nicotiana rustica*. Virus was partially purified according to a slight modification of the method described by Scott (1963).

The resulting preparations were further purified by sucrose density-gradient centrifugation in a Ti-14 zonal rotor. The virus-containing fraction was concentrated by ultracentrifugation, dialysed overnight against 7% formalin and subsequently dialysed against buffer. The final suspension was stored with 50% glycerin at –20 °C.

Antisera were prepared by injecting rabbits 2 or 3 times intravenously at 2 or 3 day

intervals followed by one intramuscular injection using Freund's incomplete adjuvant 2-3 weeks later. After another 2-3 weeks titres were estimated and new injections given if necessary.

For serology, agar gel-diffusion tests were employed and antisera were diluted with saline. Formalin-fixed purified virus or crude sap of *Nicotiana rustica* or *N. glutinosa* was used.

Results

Host range and symptoms

Results of host range tests were determined by visual observation and back inoculation onto *Chenopodium amaranticolor* or *C. quinoa* (Table 1). Most plant species that gave positive reactions when back inoculated to *Chenopodium* contained high virus concentrations as judged by the number of local lesions produced.

Phaseolus vulgaris 'Bataaf' showed epinasty of the inoculated primary leaves and a slight chlorosis of the main veins of tip leaves about one week after inoculation. One week later a striking interveinal chlorosis and dark green veinbanding appeared that resembled bean common mosaic (Fig. 1). Later on plants usually recovered. Most of the bean cultivars tested reacted similarly. 'Gazelle' and 'Tendercrop' were observed

Table 1. Results of host range tests.

Plant species	Reaction	Plant species	Reaction
Brassica chinensis	– –	*Nicotiana tabacum* 'White Burley'	lS
Brassica oleracea capitata	– –	*Nicotiana tabacum* 'Xanthi'	lS
Browallia sp.	– –	*Ocimum basilicum*	lS
Capsicum annuum	–S	*Petunia hybrida*	l–
Chenopodium album	LS/–	*Phaseolus aureus*	L–
Chenopodium amaranticolor	L–	*Phaseolus vulgaris* 'Bataaf'	LS
Chenopodium quinoa	L–	*Physalis floridana*	ls
Crotalaria spectabilis	–	*Pisum sativum* 'Koroza'	– –
Cucumis sativus 'Gele tros'	lS	*Pisum sativum* 'Rondo'	– –
Dianthus chinensis	– –	*Rudbeckia* sp.	– –
Glycine soja 'Hawkey'	– –	*Solanum melongena*	ls
Glycine soja 'Illem'	– –	*Spinacia oleracea*	lS
Glycine soja 'Tokio'	– –	*Trifolium incarnatum*	– –
Lathyrus odoratus	ls	*Trifolium pratense*	– –
Lycopersicon esculentum		*Trifolium repens*	l/– –
'Ailsa Craig'	ls	*Tropaeolum majus*	– –
Medicago sativa	–	*Valerianella locusta*	– –
Nicotiana clevelandii	ls	*Vicia faba* 'Compacta'	ls
Nicotiana glutinosa	lS	*Vicia faba* 'Driemaal wit'	l–
Nicotiana hybrida	lS	*Vinca rosea*	– –
Nicotiana megalosiphon	l–	*Zinnia elegans*	l–
Nicotiana rustica	lS		

L = local symptoms
S = systemic symptoms
l = symptomless local infection
s = symptomless systemic infection
– = no infection, as demonstrated by back inoculation

Tabel 1. Resultaten van het waardplantonderzoek.

Fig. 1. Systemic symptoms in bean *(Phaseolus vulgaris)* 'Metis', 14 days after inoculation with B32.

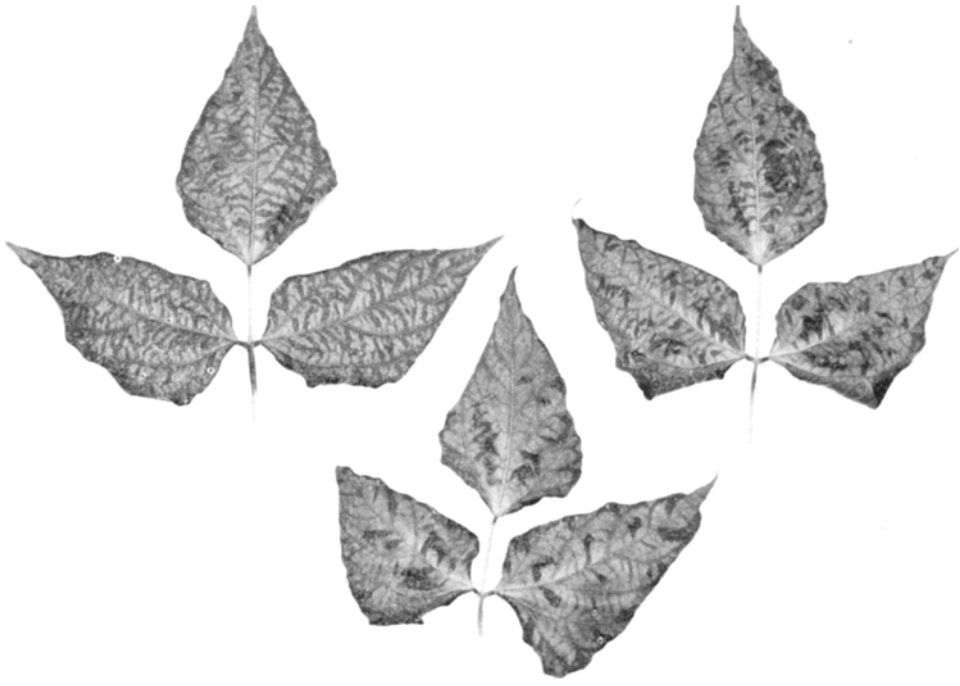

Fig. 1. Systemische symptomen in boon (Phaseolus vulgaris) 'Metis', 14 dagen na inoculatie met B32.

in the greenhouse in more detail. Although infected plants recovered almost completely, and the numbers of pods per plant were not clearly reduced, fewer seeds were formed per pod.

Cucumis sativus 'Gele Tros' produced a faint and irregular vein chlorosis to vein mosaic in the first foliage leaves but plants soon recovered.

Nicotiana species usually showed some systemic vein chlorosis later followed by a pronounced mottling or irregular flecking with some leaf malformation. Often plants completely recovered.

Chenopodium amaranticolor, especially the older plants, reacted in 4 days with numerous chlorotic pinpoint lesions that sometimes had dry centres and other times developed into diffuse chlorotic rings about 2 mm in diameter. *C. quinoa* also produced many small yellow or chlorotic local lesions in 4 to 5 days. These sometimes enlarged and coalesced and lead to desiccation of the inoculated leaves. In *C. album* numerous chlorotic local lesions in young leaves often extended along the veins. The virus could sometimes be recovered from non-inoculated tip leaves.

Bean varietal tests and influence of conditions

In one experiment with 15 bean cultivars all but two showed symptoms similar to those described above for 'Bataaf'. Most tip leaves temporarily showed rugosity but later recovered from disease. The virus could always be easily demonstrated by back inoculation (Table 2).

Table 2. Bean varietal reaction and seed transmission.

Cultivar name	Reaction	Numbers of seeds infected out of seeds tested
Amanda	lS[1]	0/19[3]
Bataaf	LS	
Cordon	LS	0/20
Double White Princess	oS[2]	1/7
Dubresco	oS	0/20
Flits	–S	0/16
Great Northern	oS	
Imuna	oS	0/15
Jubila	lS	0/20
Metis	lS	6/29
Michelite	lS	0/12
Prelude	–s	0/20
Processor	ls	
Sanilac	lS	0/20
Topcrop	oS	0/11

[1] For explanation of symbols see Table 1.
[2] o = no back inoculation from inoculated leaves.
[3] Number of seeds yielding infected seedlings out of number of seedlings from infected plants; absence of infection was checked by back inoculation of average samples onto *C. amaranticolor* and *C. quinoa*; in case of symptoms all seedlings of the cultivar concerned were back inoculated individually.

Tabel 2. Reactie van bonerassen en zaadoverdracht.

In another test 18 bean cultivars were tested. Among these were 11 cultivars that had not been tested before (viz. Donares, Gazelle, Largo, Remo, Sanocrop, Slenderwhite, Slimbel, Slimgreen, Sprite, Tempo and Tendercrop). Plants were grown in a greenhouse (18–22°C) or in growth chambers at 23 or 16°C during the day and 17 or 12°C during the night, respectively. At high and low temperatures symptoms did not differ appreciably but recovery was slower at low temperatures, presumably because of slower growth.

In a small-scale experiment with five of the last-mentioned cultivars in a growth chamber with a 16 h day of 30°C and an 8 h night of 22.5°C, the infected plants although recovered from symptoms were generally smaller, more branched, flowered irregularly and produced fewer pods and seed.

Virus transmission

Mechanical transmission. In a preliminary experiment 4 bean plants were inoculated by 1) thoroughly touching leaves with fingers previously rubbed on diseased bean plants, 2) thoroughly touching and wiping leaves with diseased leaves, 3) rubbing leaves with a roll of infected leaves without using abrasives, and 4) sap inoculation without abrasive. All inoculated plants produced clear symptoms.

In a more detailed test, groups of eight bean plants each were inoculated in a number of ways (nine treatments in total) with finger and cloth previously rubbed on diseased plants. All groups of test plants were back-inoculated onto *Chenopodium amaranticolor* and *C. quinoa* and this confirmed infection of symptom-bearing bean plants

only. Fingers were infective immediately and 5 min after rubbing on diseased plants (treatment 1 and 2). Infectivity was removed by washing the fingers with plain water (tr. 3), and with water and soap (tr. 4). After such a cleaning no infectivity could be demonstrated even when test leaves were dusted with carborundum (tr. 5 and 6). With cloth the virus could be transferred immediately (tr. 7) and 5 min after rubbing the source plants (tr. 8), but no more 15 min after such rubbing (tr. 9). Non-treated control plants (tr. 10) remained symptomless.

These tests indicate that the virus may also be transferred on cloth (and perhaps clothes) and on hands when handling plants. The virus may remain infectious on hands and clothes for at least 5 minutes. However, hands can be easily decontaminated by washing, even without soap.

Seed transmission. From some thoroughly diseased plants of the bean cultivars listed in Table 2, seeds were harvested and sown in the greenhouse. The seedlings were tested for infection through the seed by visual observation and back inoculation as indicated in Table 2 (note 3).

In a more extensive test, seeds were harvested from diseased plants of 'Double White Princess' and 'Metis', inoculated soon after germination. Three samples of 10 seeds from each cultivar were soaked in water overnight and then ground with water and the pulp inoculated onto 'Bataaf' bean and *C. quinoa*. Clear symptoms were obtained in bean and many local lesions on *C. quinoa* with one out of three groups of seeds from 'Metis'. When seeds were sown in three groups of 30 and three groups of 10, with 'Metis' eight plants out of 120 showed symptoms, and all 'DWP' plants remained healthy. Back inoculation using average samples from the above six groups of plants per cultivar proved the absence of virus in symptomless groups.

Thus, seed transmission was proved in 'Metis' (in the above-mentioned and some other tests in total 16 out of 230 seedlings or 7% were infected), and was questionable in 'DWP' (1 out of some over 200).

After storage at room temperature for 27 months the same lot of 'Metis' seeds still produced infected plants.

Persistence of infectivity in crude sap

The persistence of infectivity was determined a number of times in sap expressed from systemically infected bean plants ('Bataaf') by inoculation onto *C. amaranticolor* and *C. quinoa*, two plants each. Results of local lesion countings indicated that infectivity rapidly declined at dilutions around 10^4 (completely gone between 10^5–10^6), during heat treatment at 55–60°C (gone at 60°C) and after ageing between 6 and 24 h (gone after two days).

Cross protection

In a cross-protection experiment with *Nicotiana glutinosa*, four plants, first inoculated with the B32 isolate, were super-inoculated on tip leaves 15 days later with Price's yellow strain of cucumber mosaic virus. All plants remained free of the severe yellow symptoms, as did the four plants inoculated with B32 only whereas the plants inoculated only with the yellow strain produced striking yellow symptoms in 10-14 days, followed by severe growth reduction.

Electron microscopy

In chop preparations using 2% PTA for negative staining virus particles were rarely seen. When present, they clearly demonstrated a tendency to disintegrate (Fig. 2). Purified preparations dialysed against 7% formalin showed many particles (Fig. 3) about 30 nm in diameter.

Serology

Two antisera prepared against formalin-fixed purified B32 had titres of 16–64 when tested against purified antigen, and of 2–4 when tested against crude sap of infected *N. rustica* plants. Sometimes one antiserum also reacted with sap of healthy *N. rustica* (titre 2).

The isolate B32 was compared with a number of viruses related to cucumber mosaic virus. The results of these tests as well as the virus isolates and antisera are given in Table 3. They indicate a close relationship between B32 and CMV. The antisera designated 'Lisse' were kindly provided by Ir D. H. M. van Slogteren, Lisse, the Netherlands. The antisera against peanut stunt virus were obtained from Dr G. I. Mink (PSV-W) and Dr T. L. Steepy (PSV-E), USA.

Fig. 2. (Left). Chop preparation in PTA of *Chenopodium quinoa* with B32, 8 days after inoculation. Bar represents 100 nm.
Fig. 3. (Right). Purified preparation of B32 after formalin fixation and staining with PTA.

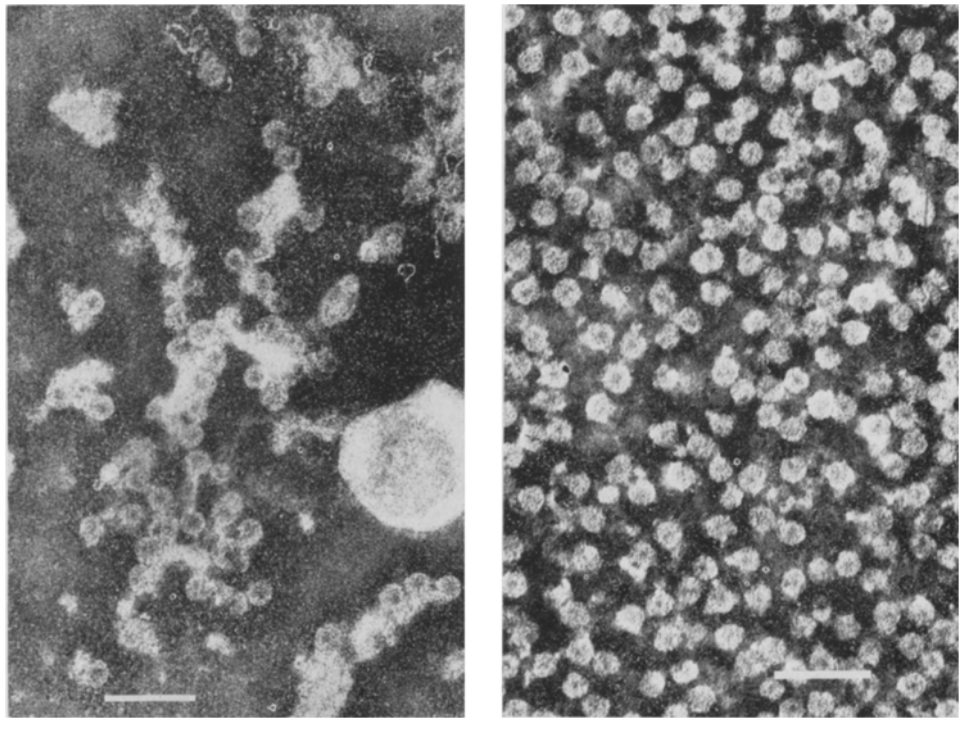

Fig. 2. (Links). Hakselpreparaat in PTA van Chenopodium quinoa met B32, 8 dagen na inoculatie. Staafgeeft 100 nm weer.
Fig. 3. (Rechts). Gezuiverd preparaat van B32 na formaline-fixatie en kleuring met PTA.

Table 3. Results of serological experiments performed to identify B32 virus. The figures represent the titres of the antisera.

Antigens		Antisera										
		PSV-W	PSV-E	**B32	**B32	**CMV-Y	*CMV-red currant	CMV-Lisse	CV-Noordam-Lisse	**CV-Noordam	*CV	Normal serum
B32	purified	–	(8)	64	64	128	64	–	–	–	–	–
B32	purified	–	4	16	16	64	16	–	–	–	–	–
B32	crude sap	–	4	4	2	8	4	64	–	–	–	–
B32	crude sap	–	8	4	4	8	8	128		–	–	
CMV-Y	purified	–	–	32	16	64	–	–	–	–	–	–
CMV-Y	crude sap	–	8	2	2	8	8	64	–	–	–	–
CMV-Y	crude sap	–	4	4	4	8	8	128	–	–	–	–
CMV-Lisse	crude sap	–	4	–	–	–	16	128	2	–	(64)	–
CMV-Lisse	crude sap	–	8	–	2	–	32	256	–	–	–	–
CV-Noordam	purified	–	–	–	–	–	4	8	256	1024	4096	–
CV-Noordam	crude sap	–	4	–	–	–	–	16	64	1024	4096	–
N. rustica	crude sap	–	4	–	2	–	–	–	–	–	–	–
N. glutinosa	crude sap	–	4	–	–	–	–	–	–	–	–	–

* antisera prepared at Wageningen; ** as * but against formalin-fixed antigen;
– no reaction; ( ) reactions questionable; PSV: peanut stunt virus;
CMV-Y: cucumber mosaic virus, Y-strain (Scott, 1963);
CV-Noordam: CMV-related chrysanthemum virus (Noordam, 1952); CV: chrysanthemum virus, origin not well known.

Tabel 3. Resultaten van de serologieproeven ter identificatie van B32. De getallen geven de titers van de antisera weer.

Discussion

Biological tests described here, especially the cross-protection experiment, have already proved beyond doubt that the B32-virus is closely related to cucumber mosaic virus (CMV).

Although little-known, some data on natural infection of legume crops by CMV have been reported e.g. azuki bean (*Phaseolus angularis*; Tsuchizaki et al., 1970), broad bean (*Vicia faba*; Kovachevsky, 1965), chick pea (*Cicer arietinum*; Kovachevsky, 1965; Kaiser and Danesh, 1971), cowpea (*Vigna unguiculata*; a.o. Anderson, 1955; Tsuchizaki, et al., 1970), crown vetch (*Coronilla varia*; Ostazeski and Scott, 1967), lucerne (*Medicago sativa*, Doolittle and Zaumeyer, 1953; Babović, 1968), pea (*Pisum sativum*; Whipple and Walker, 1941; Hagedorn, 1950; Quantz, 1957), sweet pea (*Lathyrus* spp.; Kovachevsky, 1965) white lupine (*Lupinus albus*; Kovachevsky, 1965) and yellow lupine (*L. luteus*; Köhler, 1935). Whipple and Walker (1941) also found the virus to cause a mosaic in beans symptomatologically suggesting infection by bean

common or bean yellow mosaic virus. All 16 bean cultivars tested by them were found susceptible, and masking often occurred. Recently Tsuchizaki (1973) mentioned the isolation of CMV from beans in Japan. Several legume isolates seem to go systemic in bean and some other legumes, which is not typical of normal CMV (Gibbs and Harrison, 1970). Tomaru and Udagawa (1967) have isolated such a legume strain from tobacco.

In recent years peanut stunt virus (PSV), reacting with some CMV and (tomato) aspermy type virus antisera (Mink, 1969), caused considerable concern in the USA because it causes a destructive epidemic disease in beans in North Carolina. All commercial bean varieties were found to be susceptible (Hebert, 1967; Echandi and Hebert, 1971; Zaumeyer and Goth, 1967). The latter authors concluded the PSV to be a potential threat to beans. Mink et al. (1969) considered a new virus disease of beans, detected and briefly described by Silbernagel et al. (1966) in Washington, to be a western strain of the PSV. The PSV has also been detected in beans in Japan (Tsuchizaki, 1973).

Seed transmission of CMV-B32, the virus we discovered in bean leaves and pods from Spain, was demonstrated in at least one cultivar. As only a few seeds were tested it is possible that a low percentage of seed transmission could occur in other varieties. Since the virus is seed-borne, it could have been easily introduced from other countries. It was therefore compared with the PSV. Our serological tests, however, have proved the virus to be closely related to normal CMV. In this respect it clearly differs from PSV (Mink, 1969; Mink, et al., in manuscript). This is further supported by the fact that in our experiments the PSV-W antiserum did not react. The PSV-E antiserum available reacted similarly with virus preparations and healthy plant sap, thus not permitting any conclusion as to relationships. The CV antisera, prepared at Wageningen and Lisse, clearly differentiated between B32 and CMV strains on the one hand and the chrysanthemum virus isolates on the other.

The serological tests indicate that B32 is a strain of normal CMV. However, symptomatologically it differs considerably from normal CMV strains, in that it causes systemic disease in beans and is very mild in cucumber and *Nicotiana* species, hardly causing symptoms in the latter. Whipple and Walker (1941) and Tsuchizaki (1973) are the only authors recording the natural occurrence of CMV in bean plants. The first authors stated that the beans concerned were resistant to bean common mosaic virus. The two strains they studied remained infectious in vitro for seven days, whereas our virus persisted only for one day. No seed transmission could be proved with the American virus in 440 bean seedlings.

Finally, careful study of a preliminary communication by Marrou et al. (1969) from southern France suggests that their 'new bean disease caused by an unidentified virus' (which was lost in the meantime; personal communication by Dr J. Marrou) was due to CMV, perhaps identical to B32. It infected 80% of the plants of 'Coco nain rose d'Eyragues' in a varietal bean field at Bellegarde and caused symptoms indistinguishable from those by B32. It was later also found in a varietal test at Montfavet causing bean common mosaic-like symptoms in varieties resistant to that virus. In the first-mentioned cultivar the authors found 22% seed transmission. It was also transmitted by *Myzus persicae* in a non-persistent way. After purification it turned out to be a spherical virus and it was concluded to differ from all viruses reported so far from beans.

Recently a similar virus may have been isolated from field beans in Puerto Rico. It caused dark green veinbanding and mild mosaic symptoms on beans and other legumes and gave a positive reaction with CMV antisera, among others one prepared by the junior author of the present paper (Dr J. Bird, personal communication, 1973).

Seed transmission of CMV in a leguminous crop seems to be new, although earlier reported for certain non-leguminous weeds (Häni et al., 1970; Tomlinson and Carter, 1970).

In no bean variety has a really destructive disease been reproduced with our CMV strain in the greenhouse nor at some temperatures in growth chambers. This suggests that the identified B32 virus is not the actual cause of the reported bean calamity near Valencia. However, the CMV strain isolated is worth attention, since it may constitute a potential danger. This is because of its contagiousness, its wide host range and especially because of its seed transmission in beans. Weak symptoms and masking or confusion of symptoms with those of bean common mosaic virus may easily help infection to escape notice, thus permitting spread of the virus. Often viruses latent or semi-latent in certain hosts may cause severe symptoms in special cultivars or in other host species, either alone or in complex with other viruses.

Acknowledgments

We thank Miss M. P. Schor for skilful assistance with the greenhouse experiments.

Samenvatting

Een stam van het komkommermozaïekvirus die met bonezaad overgaat

Uit boneplanten, geteeld in de buurt van Valencia, Spanje, werd een virus geïsoleerd dat met sap gemakkelijk overgaat op bonen en 23 andere van de 37 getoetste plantesoorten (Tabel 1). In de meeste soorten waren de symptomen zwak of zelfs afwezig.

De verschijnselen in boon konden gemakkelijk worden verward met die van het bonerolmozaïekvirus (Fig. 1), maar ze waren meestal zwak, terwijl de geïnfecteerde planten zich doorgaans min of meer herstelden. Alle 26 getoetste bonerassen (o.a. Tabel 2) bleken vatbaar te zijn.

Bij boon ging het virus door aanraking met de vingers of aan een doekje over, ook wanneer na aanraking van de zieke plant 5 minuten werd gewacht alvorens een gezonde aan te raken. Na 15 minuten wachten kon evenwel geen virusoverdracht meer worden aangetoond. Door wassen met alleen water, of met water en zeep, bleken de handen gemakkelijk van virus te reinigen. In één van de 12 hierop onderzochte bonerassen bleek het virus met zaad over te gaan (7%). Het zaad was nog geïnfecteerd toen het opnieuw werd getoetst na bewaring gedurende 27 maanden.

De verdunningsgrens van het virus lag bij 100.000, de inactiveringstemperatuur bij ongeveer 60 °C en de houdbaarheid in vitro bij 24 uur.

In *Nicotiana glutinosa* beschermde het virus tegen latere infectie met de gele stam van het komkommermozaïekvirus.

In hakselpreparaten van geïnfecteerde planten waren de virusdeeltjes slechts met moeite aantoonbaar (Fig. 2). Gezuiverde, met formaline behandelde preparaten bleken echter veel, ongeveer 30 nm grote deeltjes te bevatten (Fig. 3).

Tegen gedeeltelijk gezuiverde, met formaline gefixeerde preparaten werden twee antisera met een titer van 64 gemaakt. Serologisch bleek het virus nauw verwant te zijn aan het normale komkommermozaïekvirus en nauwelijks of niet aan het chrysante-aspermievirus (Tabel 3). Het verschilt daarom van het 'peanut stunt virus', waarvan bekend is dat het in de USA een ernstige ziekte in boon kan veroorzaken.

Gedeeltelijke symptoommaskering, hoog infectievermogen, uitgebreide waardplantenreeks en overgang met zaad doen het virus voor de boneteelt van potentiële betekenis zijn.

References

Anderson, C. W., 1955. Vigna and Crotalaria viruses in Florida I. Preliminary report on a strain of cucumber mosaic virus obtained from cowpea plants. Pl. Dis. Reptr 39: 346–348.

Babović, M., 1968. Virus diseases of alfalfa in Yugoslavia. Zašt. Bilja (Beograd) 102: 335–410.

Bos, L., Hagedorn, D. J. & Quantz, L., 1960. Suggested procedures for international identification of legume viruses. Tijdschr. PlZiekt. 66: 328–343.

Doolittle, S. P. & Zaumeyer, W. J., 1953. A pepper ringspot caused by strains of cucumber mosaic virus from pepper and alfalfa. Phytopathology 43: 333–337.

Echandi, E. & Hebert, T. T., 1971. Stunt of beans incited by peanut stunt virus. Phytopathology 61: 328–330.

Gibbs, A. J. & Harrison, B. D., 1970. Cucumber mosaic virus. C.M.I./A.A.B. Descriptions of plant viruses. No 1: 4 pp.

Häni, A., Pelet, F. & Kern, H., 1970. Zur Bedeutung von *Stellaria media* (L.) Vill. in der Epidemiologie des Gurkenmosaikvirus. Phytopath. Z. 68: 81–83.

Hagedorn, D. J., 1950. A cucumber virus strain with a wide leguminous host range. Phytopathology 40: 11.

Hebert, T. T., 1967. Epidemiology of the peanut stunt virus in North Carolina. Phytopathology 57: 461.

Kaiser, W. J. & Danesh, D., 1971. Etiology of virus-induced wilt of *Cicer arietinum*. Phytopathology 61: 453–457.

Köhler, E., 1935. Untersuchungen über die Lupinenbräune (Viruskrankheit). NachrBl. dt. PflSchutzdienst, Stuttg. 15: 90–91.

Kovachevsky, I. C., 1965. Die Gurkenmosaikvirose in Bulgarien. Bulg. Acad. Sci. Sofia: 80 pp.

Marrou, J., Navatel, J. C. & Férault, C., 1969. Mise en évidence d'une nouvelle maladie à virus du haricot dans le Sud-Est de la France. Ann. Phytopath. 1: 409–415.

Mink, G. I., 1969. Serological relationship among cucumber mosaic virus, tomato aspermy type viruses and peanut stunt virus. Phytopathology 59: 1889–1893.

Mink, G. I., Iizuka, N. & Kiriyama, K. Some cucumber mosaic virus antisera contain antibodies specific for peanut stunt virus and chrysanthemum mild mottle virus. In manuscript.

Mink, G. I., Silbernagel, M. J. & Saksena, K. N., 1969. Host range, purification and properties of the western strain of peanut stunt virus. Phytopathology 59: 1625–1631.

Noordam, D., 1952. Virusziekten bij chrysanten in Nederland. Tijdschr. PlZiekt. 58: 121–190.

Ostazeski, S. A. & Scott, H. A., 1967. Natural occurrence of cucumber mosaic virus in crown vetch (*Coronilla varia*). Phytopathology 57: 648.

Quantz, L., 1957. Über das Auftreten des Gurkenmosaikvirus auf Erbsen. Angew. Bot. 31: 166–173.

Scott, H., 1963. Purification of cucumber mosaic virus. Virology 20: 103–106.

Silbernagel, M. J., Mink, G. I. & Saksena, K. N., 1966: A new virus disease of beans. Phytopathology 56: 901.

Tomaru, K. & Udagawa, A., 1967. Strains of cucumber mosaic virus isolated from tobacco plants. IV. A strain causing systemic infection on legume plants. Bull. Hatano Tob. Exp. Stn 58: 69–77.

Tomlinson, J. A. & Carter, A. L., 1970. Studies on the seed transmission of cucumber mosaic virus in chickweed (*Stellaria media*) in relation to the ecology of the virus. Ann. appl. Biol. 66: 381–386.

Tsuchizaki, T., 1973. Peanut stunt virus isolated from beans in Japan. Ann. phytopath. Soc. Japan 1939: 67–72.

Tsuchizaki, T., Yora, K. & Asuyama, H., 1970. The viruses causing mosaic of cowpeas and azuki beans, and their transmissibility through seeds. Ann. phytopath. Soc. Japan 36: 112–120.
Whipple, O. C. & Walker, J. C., 1941. Strains of cucumber mosaic virus pathogenic on bean and pea. J. agric. Res. 62: 27–60.
Zaumeyer, W. J. & Goth, R. W., 1967. Beans and other leguminous hosts of peanut stunt virus. Phytopathology 57: 837.

Address

Instituut voor Plantenziektenkundig Onderzoek, Binnenhaven 12, Wageningen, the Netherlands.

Book review

D. Noordam: Identification of plant viruses; methods and experiments. Pudoc, Wageningen 1973; 207 pages of text, 101 black and white figures, 33 colour figures (4 plates), author and subject indexes. Plastic covered paper back. Price Dfl. 35.

The text, written by a staff member of the Department of Virology of the Agricultural University, regularly involved in teaching a course in virus identification, is meant mainly for students. It is based on a full-time course of 14 days or preferably a half-day course of 4 weeks and describes the various greenhouse and laboratory techniques involved in studying aspects of viruses.

Nearly all fields of virus research but field work are dealt with, such as the problem of contamination; mechanical inoculation; study of symptoms; virus multiplication in the host; local lesion virus assay; determination of dilution end-point; thermal inactivation and ageing in vitro; interaction with other viruses; purification; spectro-photometric virus assay; serology; electron microscopy; dry weight determination; transmission by aphids, leafhoppers, nematodes, fungi, through seed and by grafting and dodder; separation from mixed infections; separation of strains and mutants; and finally storage of viruses. Relatively much weight is laid on physical studies of viruses such as purification and electron microscopy and to serology (nearly half of the book) but analytical ultracentrifugation has not been included. Although the references at the end of each chapter are a selection from the literature only, they are carefully chosen and up to date. Hence the book contains a wealth of information also for researchers and research assistants more or less regularly using the various techniques.

The text has been carefully written, is easily legible and contains many valuable illustrations, some in colour. It has been well printed.

There are few apparent mistakes and errors. On p. 43, when discussing determination of virus concentration on local lesion hosts, it could have been more clearly stated that when relative concentrations are determined, the results much depend on all factors influencing test plant susceptibility and sensitivity. The same holds for determination of dilution end-point, thermal inactivation and ageing in vitro, dealt with in short separate chapters. On p. 88 it would have been more appropriate to speak of virus suspensions than of virus solutions. In the chapter on electron microscopy only the dip method is used as a technique to investigate crude sap for the presence of virus particles although several others are more frequently used nowadays. For making particle measurements, a periodical check of the microscope magnification with TMV as suggested on p. 147 is insufficient.

Unfortunately, the title of the book is slightly misleading. For most virologists, virus identification mainly means virus recognition or diagnosis of virus diseases. The term diagnosis, however, is not listed in the subject index, and techniques for routine indexing of crops or plant material for virus infection are not even mentioned. On the other hand, virus identification also means demonstration that an apparently new virus has an identity of its own, and thus the term also means virus characterization or description. The author should have explained this in the introduction of his book and outlined the scientific and practical implications of, and mutual relationships between the various chapters of the book.

Despite such shortcomings, the book will prove valuable to many students as well as to many researchers, who can profitably use it as not exhaustive laboratory guide, to 'methods in plant virology'.

L. Bos