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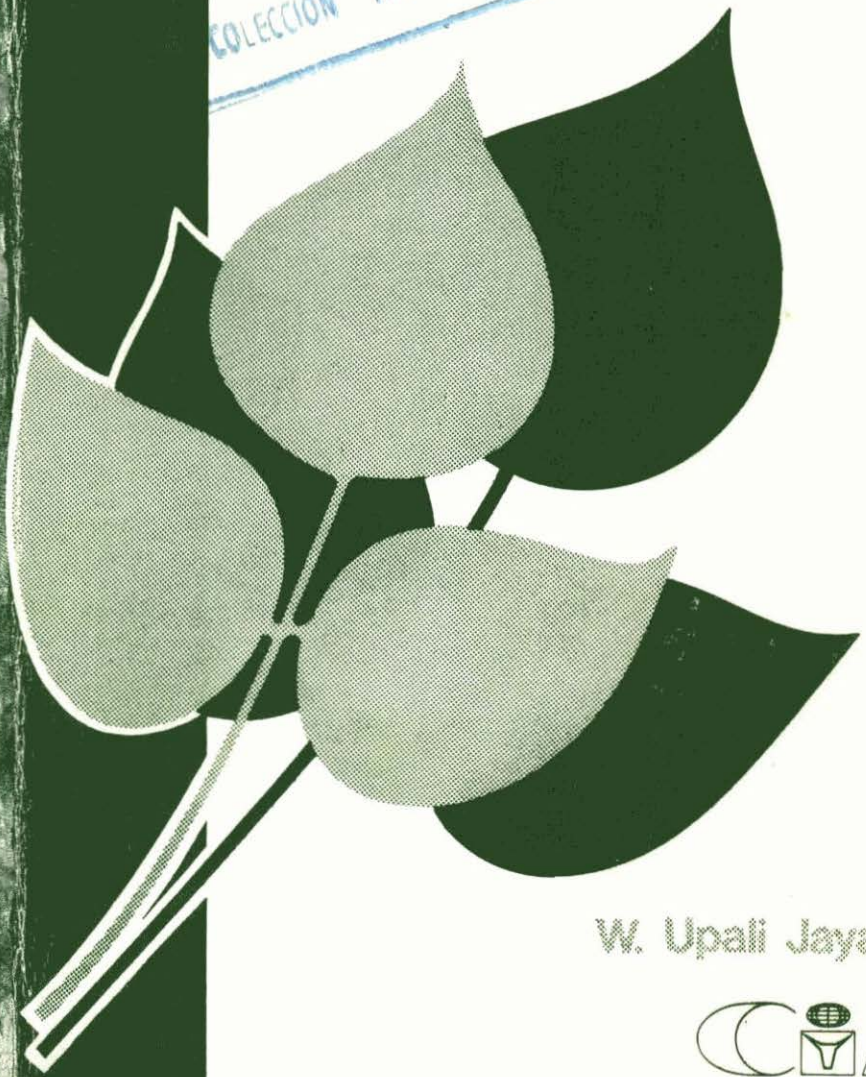
CHLOROTIC MOTTLE OF BEAN

(*Phaseolus vulgaris* L.)



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Chlorotic mottle of bean (*Phaseolus vulgaris* L.)

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Chlorotic mottle of bean (*Phaseolus vulgaris* L.)

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"Much learning, perfect handicraft, a highly trained discipline, and pleasant speech - this is the highest blessing"

- Lord Buddha -

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CONTENTS

CHAPTER 1

INTRODUCTION

Traditionally legumes have been an important protein source for human and animal nutrition in many parts of the world, especially in the developing countries. According to FAO reports for the years 1975-1977 the total world production was 12.4 million tons, of which 4.7 million tons (38%) were produced in Latin America. Though the production figures were for legumes in general, in Latin America the figures mainly represent the production of *Phaseolus vulgaris* (dry beans), an important staple in the diet of the Latin American people.

During the years 1973-1975, dry bean production in Latin America averaged 3973 million tons/year, an increase of about 15% over the production figures for 1962-1965 (Sanders and Alvarez, 1978). The average yield in Latin America is low at 600 kg/ha, which is less than a half of that in the U.S.A. (1400 kg/ha) or 3-5 tons/ha under experimental conditions (Cartree and Hanks, 1974). During the last decade the production growth rate in Latin America was less (0.27%) than the population growth rate (2.8%), resulting in bean imports and a decline in consumption, aggravating the nutritional and balance of payment problems of many Latin American countries (Sanders and Alvarez, 1978).

Total bean production in Latin America has been static for the last decade, despite the expanded production area. This decline in yield has been attributed to the variable climatic conditions, poor soil fertility, pests and diseases (CIAT, Ann. Repts. 1973-1979; Ruiz de Londoño and Pinstrup-Andersen, 1978; Graham, 1978; Van Schoonhoven, 1974; Zaumeyer and Thomas, 1957).

Soil problems, particularly those due to phosphorus and nitrogen deficiencies and unsuitable soil pH have contributed to the yield decline in Central America. In 110 soil analyses from this region

20% of the soils had a pH less than 6.0 (Muller et al., 1968), 66% were highly deficient in phosphorus (Fassbender et al., 1968) and 75% deficient in nitrogen (Diáz-Romeu et al., 1970). Central American bean fertilizer trials showed a pronounced response to nitrogen and phosphorus but not to potassium. Aluminium and manganese toxicity (Buol et al., 1975; Döbereiner, 1966) and molybdenum deficiency (Franco, 1977) complicated recommendation for fertilizers. Dry beans grown on such soils lacked vigour and reasonable productivity.

Phaseolus vulgaris is attacked by more than 250 diseases (Wellman, 1968) and more than 100 insect species (Ruppel and Idrobo, 1962; Mancía and Cortéz, 1975). The recent decline in production in Brazil was due to the severe epidemics of bean golden mosaic virus (BGMV), anthracnose and common bacterial blight (Sanders and Alvarez, 1978). Bean common mosaic virus (BCMV) appeared to be the most widely distributed virus in Latin American bean fields (Gutierrez et al., 1975), causing a yield loss of 35-98% (Burga and Scheffer, 1974; Gálvez and Cárdenas, 1974; Zaumeyer and Thomas, 1957). Bean yellow mosaic virus (BYMV) caused serious yield losses in Chile (Bruna de Tosso and Urbina de Vidal, 1976), in Argentina (Von der Phalen, 1962), in Brazil (Kitajima and Costa, 1974) and in Uruguay (Gálvez, 1980). Pests of bean caused by beetle species such as *Epilachna varivestris*, *Cerotoma trifurcata*, *Diabrotica balteata*, caused yield losses of 70% (Paz et al., 1975). In combination with the viruses that they transmit, such as southern bean mosaic virus (SBMV), bean yellow stipple virus (BYSV) (Gámez, 1976), bean curly dwarf mosaic virus (BCDMV) and bean mild mosaic virus (BMMV) (Meiners et al., 1977; Waterworth et al., 1977), yield losses substantially greater than those caused by insects alone, can occur. The severe outbreak of BGMV in Brazil in 1978 was also due to an increased population of the vector insect, *Bemisia tabaci*, a whitefly species (Costa, 1975).

Diseases of bean given high priority by the Bean Program of the Centro Internacional de Agricultura Tropical (CIAT), Palmira, Colombia, include those caused by bacteria (common bacterial blight, angular leaf spot), by fungi (anthracnose, rust) and by viruses (BCMV, BGMV; CIAT, 1979). Other fungal diseases such as white leaf spot, halo blight, white mold and web blight, which usually had a low incidence, have recently appeared in severe outbreaks in parts of Cen-

tral and South America (Schwartz, personal communication, 1980). Similar situations have also been reported for viruses in beans. Severe outbreaks of SBMV generally considered to be endemic in experimental fields of CIAT during 1979-1980 occurred in experimental field plots of *P. acutifolius* and twice caused the experiments to be abandoned. Similarly, the normal incidence in the field of a disease called bean chlorotic mottle is low, but in 1978 it caused 100% infection in CIAT susceptible breeding lines (Van Schoonhoven and Gálvez, personal communication, 1979).

The etiology of bean chlorotic mottle has not been established so far, but it is believed that this disease of bean is caused by the same whitefly-transmitted virus(es) which is (are) supposed to cause variegation in malvaceous plants, such as *Sida* spec., *Abutilon* spec., *Malva* spec., *Pavonia* spec., etc. (Gálvez and Cárdenas, 1980).

The aim of the present study was to identify and characterize the inciting agent of bean chlorotic mottle. To achieve this aim the following procedure was followed. First of all a study of the symptoms of the disease was made (Chapter 3), as this syndrome described in literature is rather confusing. Gálvez and Cárdenas (1980) mention that bean chlorotic mottle virus can cause a severe dwarfing, accompanied by proliferation of buds and bunchy or rosette type plant development. These authors further state, "If infection occurs in young plants, a witches' broom is produced and leaves often exhibit chlorotic mottling. Chlorotic spots or mottled areas may be produced on leaves of tolerant cultivars or older susceptible plants. These spots may be accompanied by a rugosing of leaves." As dwarfing and witches' broom can be brought about by many pathogens and even pests, as in the case of *Empoasca kraemeri*, the present author did not consider these symptoms to be characteristic of bean chlorotic mottle; thus they were of no diagnostic value. Hence only plants exhibiting characteristic chlorotic mottle (as defined in Chapter 3) were used throughout this study. With these plants transmission studies (both mechanical and by means of insects) were performed. In this way three sap-transmissible viruses were isolated, separated and tentatively identified (Chapter 4). These three viruses were further characterized and identified (Chapters 5, 6 and 7). Finally the three viruses were introduced into healthy bean plants (singly or in combination with each other) in order to reproduce the characteristic bean chlorotic mottle as observed in the fields (Chapter 8).

CHAPTER 2

REVIEW OF LITERATURE

The name "chlorotic mottle of bean" appeared in the literature for the first time in the English edition of the Annual Report of CIAT of 1975. In the Spanish edition of the same report the term "moteado clorótico del fríjol" was used. The causal agent of bean chlorotic mottle was supposed to be a virus with the whitefly *Bemisia tabaci* as its vector and *Rhynchosia minima*, a common tropical weed, as its natural host. In soybeans it was said to cause symptoms similar to those of bean golden mosaic.

Gálvez et al. (1975) stated that up to that moment "moteado clorótico" had been registered as the only virus disease of beans in Colombia. According to the latter authors the symptoms of "moteado clorótico" consist of small yellow spots in the leaves, severe rugosity and curling of the leaves, whereas in susceptible varieties there is pronounced reduction in the size of the leaflets, proliferation and witches' broom growth.

The name "moteado clorótico" was used earlier by Gámez (1969a, 1972) for a virus disease of bean in Costa Rica which later proved to be identical to bean yellow stipple (Gámez, 1976), a disease described by Zaumeyer and Thomas in 1950. Therefore, Gámez (1976) changed the name "moteado clorótico" into "moteado amarillo" (yellow mottle). Unfortunately, the same author had already given this name to a whitefly-transmitted disease of bean in El Salvador (Gámez, 1969b, 1970, 1971), which, according to him had many characteristics in common with the golden mosaic of bean reported from Brazil by Costa (1965).

Until now, whitefly-borne disease agents have been considered as viruses, though conclusive evidence for their virus nature has only been obtained in a few cases, such as, for instance in that of bean

golden mosaic. Therefore, it would be more correct to use the terms "disease agents", "causal agents" or incitants rather than "viruses". However, for the sake of convenience the last term will be used in this review.

In 1977 Gálvez gave a further description of the "moteado clorótico" of bean, which he also referred to as "enanismo moteado" (mottled dwarf). The latter name was chosen because often affected plants showed growth reduction and rosetting. Gálvez concluded on the basis of symptoms that the causal agent might be either *Abutilon* mosaic virus, which is known to affect many malvaceous plants, especially *Sida* spp., or *Rhynchosia* mosaic virus whose hosts are mainly legumes.

A number of whitefly-transmitted "rugaceous" diseases in Latin America have been distinguished on the basis of symptoms and on results from transmission experiments with whiteflies, mostly *B. tabaci*. Costa (1955, 1965) reported on the occurrence of three whitefly-transmitted diseases of beans in Brazil, viz. golden mosaic, mottled dwarf (caused by *Abutilon* mosaic virus) and crumpling (a disease which was first recorded in glasshouse experiments with a population of *B. tabaci* carrying *Euphorbia* mosaic virus). It is interesting to note that Costa could transmit *Abutilon* mosaic virus to bean, whereas Silberschmidt and Tommasi (1955) were unable to get disease symptoms on bean in whitefly transmission experiments with *Sida* affected with "infectious chlorosis of Malvaceae" (a group of diseases to which *Abutilon* mosaic is supposed to belong). In a later publication Costa (1975) described a fourth whitefly-transmitted bean disease, viz. soybean dwarf mosaic, caused by the same virus as *Euphorbia* mosaic. In the latter publication the name bean mottled dwarf had disappeared and bean dwarf mosaic was introduced, most likely as a synonym of the former, although the author did not mention that.

In El Salvador Gámez (1969b) obtained dwarfed beans after whiteflies from mosaic-diseased *Sida* plants had fed on them.

Bird and Sánchez (1971), Bird and López-Rosa (1973) and Bird et al. (1970, 1975) listed the following whitefly-transmitted diseases in Puerto Rico:
Euphorbia prunifolia mosaic, *Jacquemontia tamnifolia* mosaic, *Jatropha gossypifolia* mosaic, *Merremia quinquefolia* mosaic, *Phaseolus lunatus* mosaic, *Rhynchosia minima* mosaic and *Sida carpinifolia* mosaic. Out of these seven diseases only *S. carpinifolia* mosaic could not be

transmitted to bean by means of *B. tabaci* (Bird and López-Rosa, 1973; Bird et al., 1975).

Besides "moteado clorótico" thought to be caused by *Abutilon* mosaic virus or *Sida* mosaic virus, Gálvez (1977) described four more whitefly-transmitted virus diseases which affected beans under field conditions. These were: bean golden mosaic, *Euphorbia* mosaic, *Jatropha* mosaic and *Rhynchosia* mosaic.

Whether the above-mentioned mosaic diseases really had been transmitted by whiteflies to test plants, in other words, whether the symptoms observed in the test plants were in fact caused by the virus in the original, mosaic-showing source plant on which the whiteflies had fed, remains to be seen. Only in a few instances have back-transmission experiments been carried out. Costa (1955) was able to infect *Glycine max*, *P. vulgaris* and *Solanum tuberosum* with viruliferous *B. tabaci* reared on mosaic-diseased plants of *Sida micrantha* and *S. rhombifolia*. From these infected plants he could achieve retransmission to healthy *S. rhombifolia*, the indicator plant. Similar results were obtained by Silberschmidt and Tommasi (1956) who used *S. rhombifolia* with "infectious chlorosis of Malvaceae" as the source plant for the whiteflies and *Nicandra physalodes* as the test plant, and by Flores and Silberschmidt (1958) and Flores et al. (1960) who experimented with an even larger number of different test plants, including *Datura stramonium*, *Lycopersicon esculentum* and *Nicotiana tabacum*. Regarding the aforementioned positive results from retransmission tests obtained by Costa (1955) with beans it must be remarked that the same author seemed much less sure about these results in a later publication (Costa, 1965) in which he stated: "Attempts to recover the *Abutilon* mosaic virus from mottled dwarf bean plants back to seedlings of *S. micrantha* or *Malva parviflora* by means of the vector usually gave poor results." Therefore, in most of the cases, it is very doubtful whether the virus transmitted to the test plant was the same as the one causing the disease in the original source plant. Flores and Silberschmidt (1958) recognised the danger of arriving at conclusions about identity of viruses based on results from whitefly transmission experiments only, especially when the test plants used are known to be very susceptible to sap transmissible viruses, e.g., *D. stramonium*, *L. esculentum* and *P. vulgaris*. The latter authors, therefore, carefully checked for the presence of sap-transmissible viruses in the test plants which showed symptoms after viruliferous whiteflies had fed on them.

Schwartz et al. (1978) included under the name bean chlorotic mottle several other whitefly-transmitted diseases described by different workers and characterized by symptoms on bean which they considered to be similar to those of the former disease. These were: bean crumpling, possibly related to *Euphorbia* mosaic and causing necrosis in beans (Costa, 1975); bean dwarf mosaic, incited by *Abutilon* mosaic virus causing dwarfing and rosetting as well as yellow spots in the older leaves and greenish, yellow mottle in the younger ones (Costa, 1975); *Abutilon* mosaic (Costa, 1955); and *Rhynchosia* mosaic, a disease found to affect mainly legumes, although it also caused leaf curl and enations in tobacco (Bird et al., 1970). In the additional list of Spanish synonyms Schwartz et al. (1978) mentioned "enanismo del fríjol," which might refer to publications of Gámez (1969b, 1970) where the latter author described a disease of bean characterized by light green mottling besides stunting and proliferation. It is noteworthy that the sharp mosaic in bean depicted by Schwartz et al. (1978, Fig. 12, page 15) as one of the characteristic symptoms of bean chlorotic mottle has not been mentioned for any of the above bean diseases.

In a recent review Gálvez and Cárdenas (1980) accommodated bean chlorotic mottle virus into the same group as *Abutilon* mosaic virus, yellow dwarf mosaic virus (same as bean dwarf mosaic virus?) and the virus of "infectious chlorosis of Malvaceae." Although these authors referred to Costa (1965, 1975) while mentioning the incidence of bean chlorotic mottle in Brazil, it should be borne in mind that the latter author described diseases called mottled dwarf (Costa, 1965) and bean dwarf mosaic (Costa, 1975) which need not be necessarily identical to bean chlorotic mottle (the leaf symptoms of bean dwarf mosaic shown by Costa (1975) were much different from those of bean chlorotic mottle).

As most of the whitefly-transmitted viruses have been characterized on the basis of symptomatology and host plant range only, conclusions regarding a similarity between two virus diseases described in two different places or countries, are unwarranted, as has also been pointed out by Agudelo (1978).

From the literature on bean chlorotic mottle reviewed here, it is clear that a great confusion exists, both on the symptomatology of this disease and on its incitant.

CHAPTER 3

SYMPTOMATOLOGY OF BEAN PLANTS INFECTED WITH BEAN CHLOROTIC MOTTLE

As it was not clear from the literature which symptoms in beans were characteristic of bean chlorotic mottle, first of all a common symptom to this disease in affected plants had to be established. Such a symptom was a definite mosaic with discoloured areas bordered at veins giving these areas a sharply angular appearance. The discoloration was most often restricted to the region adjacent to the midrib and main veins (Fig. 1). The colour of the affected tissues ranged from pale-green to yellow. When the affected area was pale-green, it was not very different from the colour of the healthy leaf so that the symptoms were extremely difficult to detect under field conditions. In certain cultivars the chlorotic areas were more pronounced and contrasted markedly with the colour of the normal green parts of the leaf.

Fig. 1. Sharp mosaic characteristic of bean chlorotic mottle on P. vulgaris (CIAT breeding line BAT-01278).

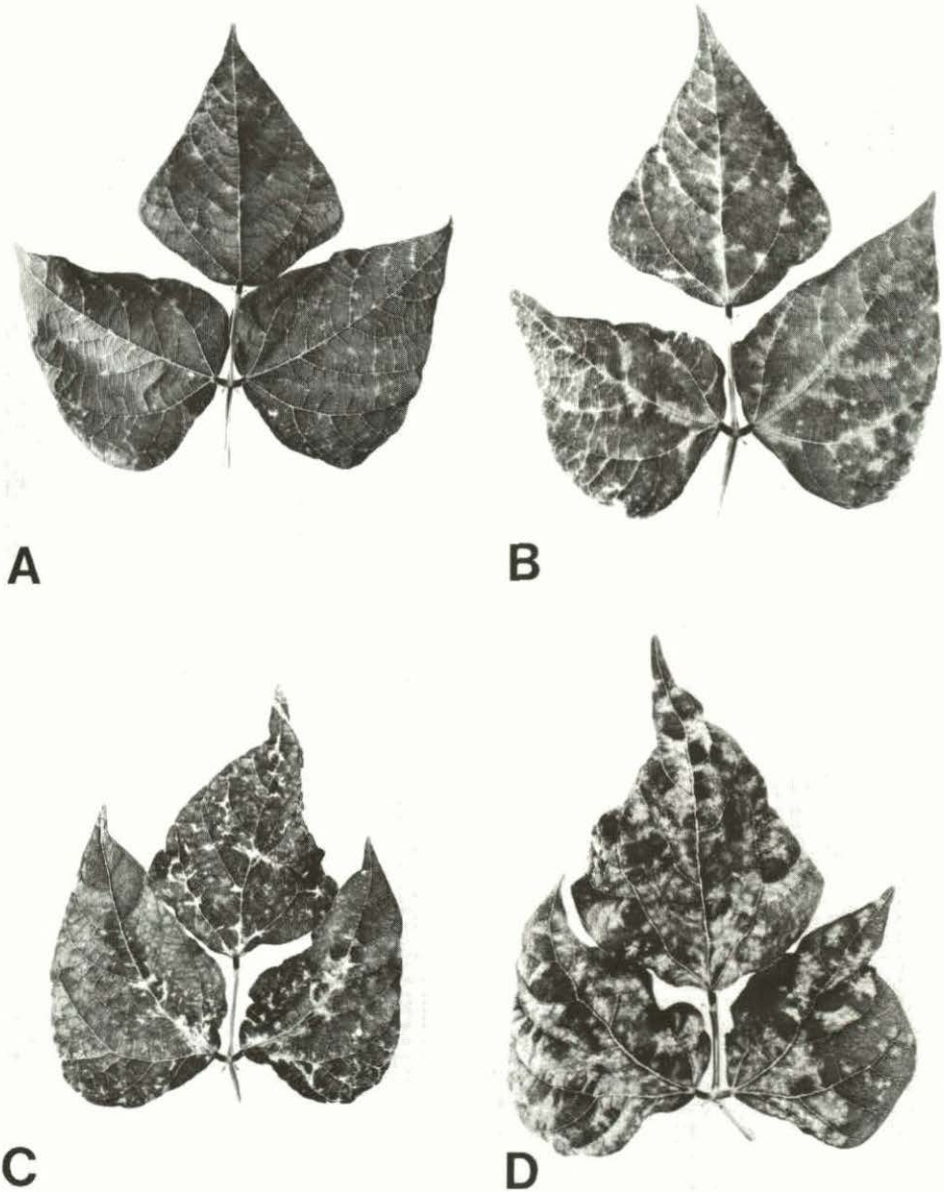


Fig. 2. Development of symptoms in leaves of a plant of P. vulgaris 'Guatemala 24' affected with bean chlorotic mottle. Vein mosaic (A) was follwed by vein banding (B). Sometimes, the chlorotic areas slightly dried up and formed translucent areas whereas the leaflets showed deformation (C). Eventually, the interveinal tissues became chlorotic resulting in a sharp mosaic (D).

These symptoms were considered to be characteristic for bean chlorotic mottle, as they invariably were referred to in the literature as part of the syndrome of this disease. These symptoms were used to recognize bean plants affected with bean chlorotic mottle in the field. The earliest signs of the disease on a bean plant which already had a few leaves with characteristic chlorotic mottle was a vein mosaic consisting of small chlorotic areas scattered on the leaf lamina, always associated with the midrib or the main veins (Fig. 2A). As the disease progressed the chlorosis spread into the tissues adjoining the veins causing vein banding (Fig. 2B). At times the chlorotic tissues became dried up and formed translucent areas, especially along the base of the leaf between veins (Fig. 2C). As the symptom severity increased the interveinal tissues became chlorotic, resulting in characteristic chlorotic mottle symptoms (Fig. 1) which in a later stage developed into a more general chlorosis with areas of unaffected tissues scattered in the lamina (Fig. 2D).

The affected leaves sometimes showed growth reduction and malformation such as drastic narrowing of the lamina. The growth reduction was associated with the chlorotic areas, at times leading to leaf margin serration, rugosity, blistering and epinasty of the lamina. The rugosity and blistering could be mild or extremely severe as in Figs. 3 and 4, respectively.

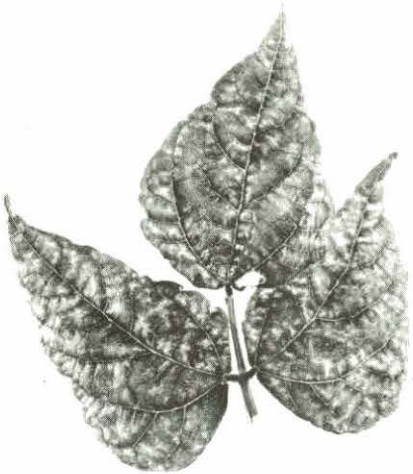


Fig. 3. Rugosity and slight chlorosis in a leaf of P. vulgaris 'Cajamarca' affected with bean chlorotic mottle.

Fig. 4. Severe blistering and curling of the leaflets of a leaf of P. vulgaris (CIAT breeding line BAT-01278) affected with bean chlorotic mottle.

Bean chlorotic mottle-affected plants of the same cultivar could be either normal in height (Fig. 5) or exhibit dwarfing and witches' broom-like growth (Fig. 7; Table 1). The plants which were normal in height sometimes showed the characteristic chlorotic mottle symptom in all the trifoliolate leaves. Depending on the cultivar the discoloration was bright-yellow (Fig. 5) or a mild greenish-yellow. The discoloured leaves did not always show rugosity or epinasty. In some cases the characteristic chlorotic mottle symptom was present only on the older leaves while the younger developing leaves were either practically symptomless or showed extreme rugosity and blistering (Fig. 6).

Fig. 5. P. vulgaris (CIAT breeding line BAT-01281) affected with bean chlorotic mottle. Sharp mosaic, ranging in colour from bright-yellow to pale-green in practically all the leaves.

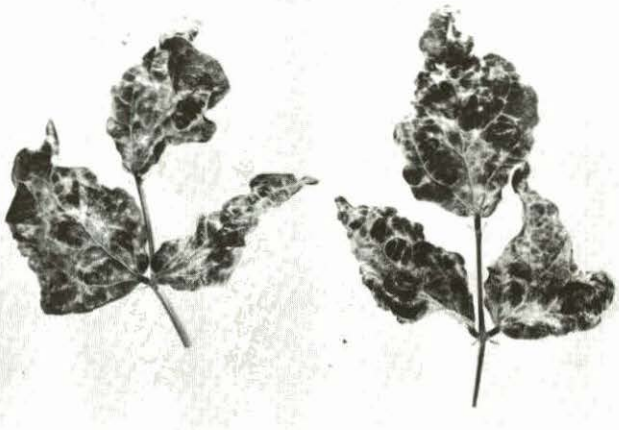


Fig. 6. Severe deformation and blistering of the leaflets of P. vulgaris 'Ancash 66' affected with bean chlorotic mottle.

In plants exhibiting witches' broom-like growth characteristic chlorotic mottle symptoms were present in at least one of the older leaves, usually the first to fourth trifoliolate leaves, which were normal in size as in Fig. 8 or showed size reduction with crowding and epinasty of the leaflets (Figs. 7 and 8). Plants with witches' broom-like growth had shortened internodes (Fig. 8) and some negative geotropism (Fig. 7). In the last mentioned case the erect branches carried very small malformed leaves, exhibiting mosaic, rugosity and severe epinasty. Such plants rarely produced flowers.

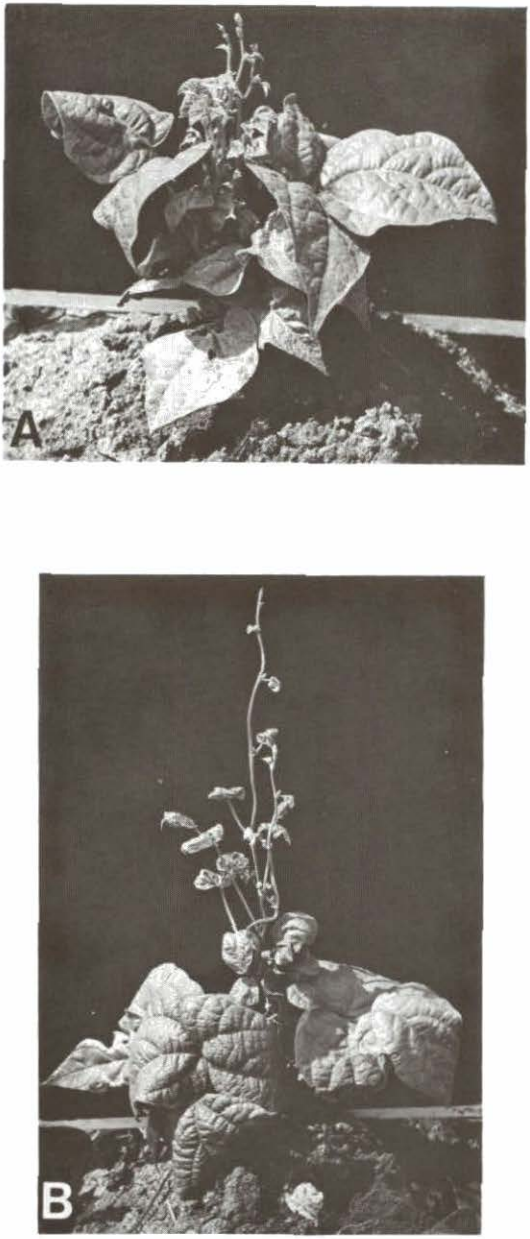


Fig. 7. Witches' broom-like symptoms in P. vulgaris 'El Cisne' affected with bean chlorotic mottle. (A) Shortening of the internodes and epinasty of the leaflets. (B) Epinasty of the leaflets, cladomania and negative geotropism.

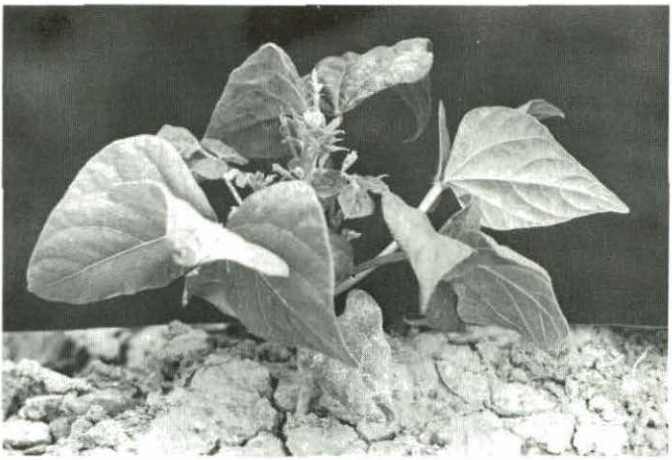


Fig. 8. P. vulgaris (CIAT breeding line BAT-23) affected with bean chlorotic mottle. Normal-looking leaves and witches' broom-like symptoms in the younger parts of the plants.

As all the above mentioned leaf and plant malformations did not always form part of the bean chlorotic mottle syndrome, they were not considered to be characteristic symptoms of the disease. Henceforth in this study the term chlorotic mottle will invariably refer to the characteristic mottle symptom consisting of the sharp regular mosaic shown by at least one leaf of affected bean plants.

Table 1. Experimental lines of P. vulgaris in the CIAT experimental field in October 1980 affected with bean chlorotic mottle.
1 = All plants showing only sharp mosaic. 2 = All plants showing sharp mosaic with dwarfing and witches' broom-like growth. 1.2 = Some plants showing the symptoms of 1, other plants showing those of 2.

Ahumados De Chirripo 2	(G-3391)	1.2	Guatemala 46	(G-5926)	2
Amarillo-Enredo	(G-2231)	1	Guatemala 47	(G-5928)	1.2
Ancash 66	(G-4727)	1	Guatemala 81	(G-5934)	1
Ant.-79 = Revoltura	(G-7233)	2	Guatemala 254	(G-6436)	2
Ant.-70B = Revoltura	(G-7318)	2	Guatemala 256	(G-5971)	2
Apetito	(G-1780)	2	Guatemala 335	(G-5991)	2
Apurimac 55	(G-7303)	2	Guatemala 372	(G-6006)	1
Atica (Gentry ≠ 20743)	(G-7128)	1	Guatemala 488	(G-6040)	2
Boy-117A	(G-8171)	1	Guatemala 565	(G-6069)	2
Cajamarca 126	(G-8216)	1	Guatemala 593	(G-6079)	1
Cal.-5 = Revoltura	(G-8172)	2	Guatemala 596	(G-6081)	1.2
Col. ≠ 103	(G-2602)	1	Higuerillo	(G-0811)	2
Col. ≠ 322	(G-2641)	1	Huevo De Pinche	(G-7437)	1
Colorado	(G-2302)	1	Mexico 347	(G-5347)	1
Colorado	(G-2371)	1	Mix. Imantac 22	(G-12251)	1.2
Comp. Neg. De Chima	(G-4252)	1	PI 310647	(G-1943)	1
Cun.-Chiguano	(G-7435)	2	PI 310811	(G-2053)	1.2
Cun.-41 = Criollo	(G-8183)	1	PI 312005	(G-2338)	1
El Cisne	(G-12378)	1.1	Puebla 240 C	(G-3367)	1
Enredo	(G-2337)	1	Pueblo 444	(G-3410)	2
F. Coral	(G-1794)	1	Santo Thomas 5n	(G-5272)	1
F. De Enredo	(G-0984)	1	S 412 AR	(G-3912)	1
Garoancillo	(G-2257)	1	SX-62-39	(G-6342)	2
Guatemala 24	(G-6435)	1	Turrialba 612-25	(G-3910)	1
Guatemala 41	(G-5972)	2			

CHAPTER 4

ISOLATION, SEPARATION AND TENTATIVE IDENTIFICATION OF VIRUSES PRESENT IN BEAN PLANTS WITH CHLOROTIC MOTTLE

4.1 *Introduction*

Attempts were made to isolate viruses from *P. vulgaris* plants which showed chlorotic mottle symptoms in the experimental fields at CIAT. From those plants two were selected for sap-transmission experiments, one with additional witches' broom-like growth and one with chlorotic mottle only. As mentioned in Chapter 3 the symptoms vary greatly in bean plants with chlorotic mottle. Therefore, an inoculation experiment was performed using top leaves and stems from plants with chlorotic mottle in their older leaves and various other symptoms in their younger leaves and stems, to find out whether these differences in symptoms were due to the presence of different viruses in these plants.

In the following the successive inoculation steps taken to isolate, separate and tentatively identify the viruses present in bean plants with chlorotic mottle are described.

4.2 *Materials and methods*

Young leaf material was ground in a cold sterilized mortar with cold 0.02 M sodium phosphate buffer pH 7.0 (buffer), containing 0.02 M Na_2SO_3. The sap obtained was directly inoculated onto the leaves of test plants previously dusted with carborundum powder (600 mesh) and then rinsed with tap water.

Inoculations were also performed with partially purified preparations from a single bean plant, collected from the field, showing chlorotic mottle only. This plant was processed as follows. The plant (c. 200 g) was homogenized in a Waring blender for 2 min. in 300 ml

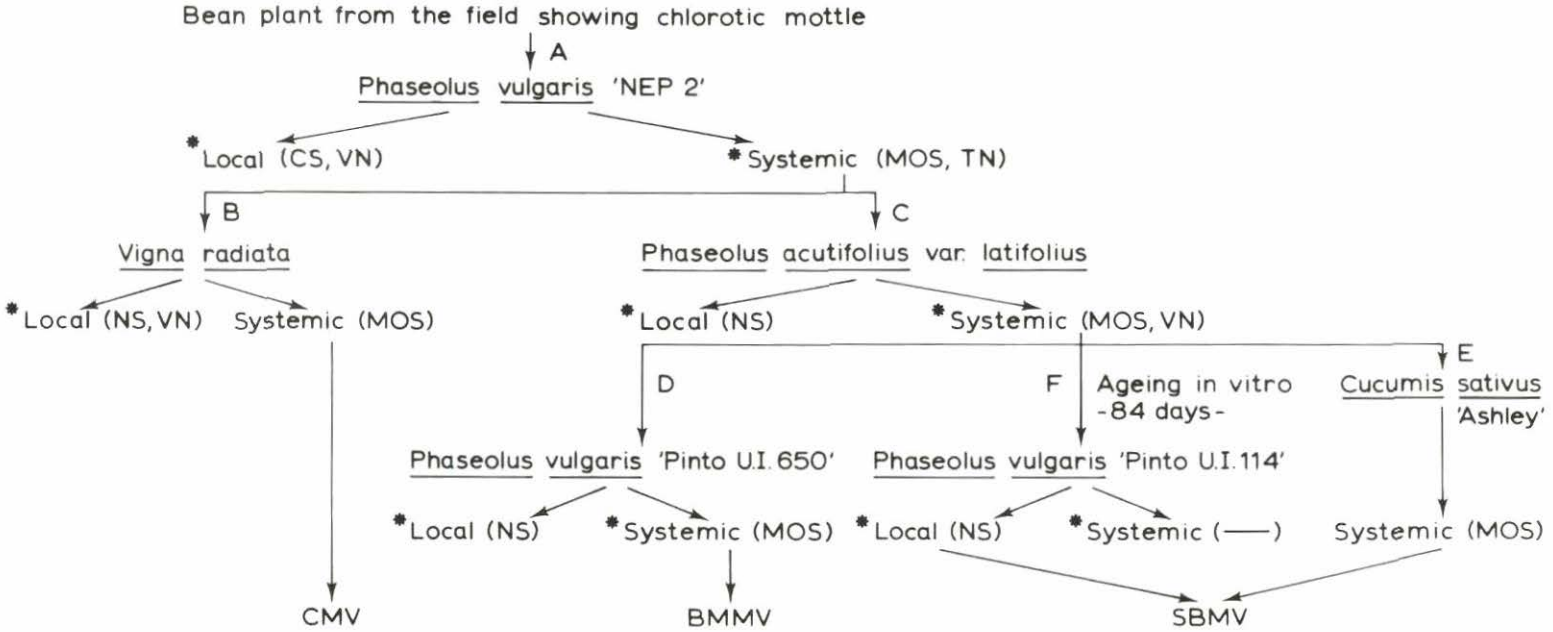
of cold buffer, containing 0.1% thioglycollic acid (TGA) and 1% 2-mercaptoethanol (2-ME). After adding 20% (v/v) of cold 1:1 chloroform-butanol mixture to the slurry, the latter was blended for another 2 min. The sap was then squeezed through a double-layered gauze and centrifuged at 7000 *g* in a Sorvall SS-34 rotor for 10 min. The supernatant was centrifuged for another 4 h at 41,000 *g* and the resulting pellets were suspended in 3 ml of buffer.

Besides the above mechanical transmission experiments, some experiments were performed with whiteflies. The exact identity of the genus of the whitefly could not be established, but most probably it was *Bemisia*. Whiteflies were collected from soybean fields in which their population is usually higher than in bean fields. The nearest soybean field available was 15 km from CIAT. To start the colony, adult whiteflies were collected from the field and then placed on *Ipomoea batatas*, a good host for rearing whiteflies in a glass cage.

As it was not possible to transplant chlorotic mottle-diseased bean plants into pots, the whiteflies were allowed to feed on the diseased plant in the field. Ten, 25 and 50 adult whiteflies were collected from the *I. batatas* plants and caged with a few chlorotic mottle-showing leaves for 48 h, followed by a similar period for inoculation feeding in the glasshouse on three healthy 7-day-old plants of *P. vulgaris* 'Porrillo 1', respectively. After 48 h the cages were removed and the plants sprayed with an insecticide to kill the whiteflies. The plants were observed for a period of one month after killing the whiteflies.

Serological tests were performed with the infected test plants by agar double-diffusion tests (Ouchterlony, 1962), using antisera to bean mild mosaic virus (BMMV) (received from Dr. H.E. Waterworth, U.S.D.A., Glenn Dale, Maryland, U.S.A.), southern bean mosaic virus (SBMV), and cucumber mosaic virus Y strain (CMV-Y) (both from the American type culture collection.

Fig. 9. Outline for the separation of three viruses in bean plants with chlorotic mottle by sap transmission



BMMV = Bean mild mosaic virus; CMV = Cucumber mosaic virus; SBMV = Southern bean mosaic virus
CS = Chlorotic spots, Mos = Mosaic
NS = Necrotic spots, TN = Top necrosis
VN = Veinal necrosis, —— = No symptoms
* = Symptoms on the test plants mentioned
Letters A-F represent the inoculation steps referred to in the text

4.3 *Results*

4.3.1 Mechanical inoculation with crude sap from a bean plant showing chlorotic mottle only

Sap from a bean plant showing chlorotic mottle only was inoculated onto the primary leaves of 7-day-old *P. vulgaris* 'Nep 2' plants (Fig. 9, A). This cultivar was chosen because according to a list available in het Entomology Section of CIAT Bean Program, it was very susceptible to chlorotic mottle. Seven days after inoculation the plants developed necrotic local lesions, systemic mosaic and top necrosis. The nature of the systemic mosaic observed was different from that described in Chapter 3 for chlorotic mottle. Sap from systemically infected leaves of the above *P. vulgaris* 'Nep 2' plants was inoculated onto a number of other leguminous plants, viz. *P. acutifolius* var. *latifolius* (Fig. 9, C), *P. vulgaris* 'Nep 2', *Vigna radiata* (Fig. 9, B) and *V. unguiculata* ssp. *sesquipedalis*. All test plants used proved to be susceptible and showed symptoms (Table 2).

Table 2. Reactions of test plants to inoculation with sap from P. vulgaris 'Nep 2' showing mosaic in the top leaves after inoculation with sap from chlorotic mottle-diseased bean plants.

Test plant	Symptoms	
	local	systemic
P. acutifolius var. latifolius	NS	MOS, VN
P. vulgaris 'Nep 2'	CS	MOS
V. radiata	CS, VN	MOS
V. unguiculata ssp. sesquipedalis	CS, VN	MOS

CS = chlorotic spots
MOS = mosaic
NS = necrotic spots
VN = veinal necrosis

Back-inoculations from the systemically infected leaves of *V. radiata*, *P. acutifolius* var. *latifolius* and *P. vulgaris* 'Nep 2' were then performed onto the same plant species as in Table 2.

The results in Table 3 show that inoculations from *V. radiata* caused both local and systemic symptoms in both *V. radiata* and *V. unguiculata* ssp. *sesquipedalis*, whereas no symptoms occurred after inoculations with sap from *P. acutifolius* var. *latifolius* in these *Vigna* species. On the other hand inoculation from *P. acutifolius*

var. *latifolius* caused a mosaic in *P. acutifolius* var. *latifolius*, whereas the inoculum from *V. radiata* induced only local lesions in this host. These results suggested the presence of at least two viruses in the bean plant with chlorotic mottle: one virus (I) caused local lesions in *P. acutifolius* var.*latifolius* and local and systemic infection in both *V. radiata* and *V. unguiculata* ssp. *sesquipedalis* and another virus (II) caused a systemic mosaic in *P. acutifolius* var. *latifolius* and no symptoms in *V. radiata* and *V. unguiculata* ssp. *sesquipedalis*. Dilution of sap from the systemically infected leaves of *P. vulgaris* 'Nep 2' inoculated onto *P. acutifolius* var. *latifolius*, *P. vulgaris* 'Nep 2'. *V. radiata* and *V. unguiculata* ssp. *sesquipedalis* also indicated the presence of at least the above two viruses. The local lesions of *P. acutifolius* var. *latifolius*, the local and systemic infection on *V. radiata* and that on *V. unguiculata* ssp. *sesquipedalis* appeared only up to a dilution of 10^{-3} (virus I). The systemic infection of *P. acutifolius* var. *latifolius* even occurred at a dilution of 10^{-7} (virus II).

Virus I isolated from systemically infected leaves of *V. radiata* (Fig. 9, B) caused a green mosaic in *Cucumis sativus* 'Ashley' and 'Marketer', systemic mosaic in *Nicotiana glutinosa*, *N. tabacum* 'Havana 425', a yellow mosaic in *N. rustica* and local lesions in *Chenopodium amaranticolor* and *C. quinoa*. The symptoms were reminiscent of those caused by cucumber mosaic virus (CMV) (Gibbs and Harrison, 1970). Therefore, virus I was tentatively identified as CMV.

Virus II isolated from systemically infected leaves of *P. acutifolius* var. *latifolius* (Fig. 9, C) caused necrotic local lesions and a systemic mosaic when inoculated onto the primary leaves of *P. vulgaris* 'Pinto U.I. 650' (Fig. 9, D). Sap from the systemically infected leaves of *P. vulgaris* 'Pinto U.I. 650' when back-inoculated onto the same bean cultivar failed to produce any local lesions but produced a systemic mosaic only. From this it was clear that virus II most likely consisted of two viruses: one producing local lesions in 'Pinto U.I. 650', the other failing to do so. Preliminary serological tests performed with sap from these systemically infected leaves gave a positive reaction with the antiserum to BMMV, but not with that to SBMV. Therefore, one of these viruses was tentatively identified as BMMV (Fig. 9). On the other hand, sap inoculated from the systemically infected leaves of *P. vulgaris* 'Nep 2' inoculated two weeks before with an inoculum from *P. vulgaris* 'Nep 2' (Table 3) reacted with an antiserum to BMMV and with that to SBMV. Inoculation

from *P. vulgaris* 'Nep 2' onto *P. vulgaris* 'Pinto U.I. 650' and *P. lunatus* 'Henderson Bush' caused necrotic local lesions characteristic of SBMV (Shepherd, 1971). Such locally infected leaves of *P. lunatus* 'Henderson Bush' when macerated and inoculated onto *P. vulgaris* 'Pinto U.I. 650' caused necrotic local lesions, which is consistent with the known reaction of SBMV (Sehgal, 1973). Therefore, this virus was tentatively identified as SBMV. However, judging from the systemic reaction on *P. vulgaris* 'Pinto U.I. 650', in addition to SBMV, which gives only local lesions on this cultivar (Sehgal, 1973; Zaumeyer and Harter, 1943 b), BMMV was also still present. In other words the original virus II isolate consisted of both BMMV and SBMV.

Table 3. Reactions of test plants to inoculation with sap from systemically infected leaves of P. acutifolius var. latifolius, P. vulgaris 'Nep 2', and V. radiata as mentioned in Table 2.

Test plant	Sap from systemically infected leaves of					
	P. acutifolius var. latifolius		P. vulgaris 'Nep 2'		V. radiata	
	Symptoms		Symptoms		Symptoms	
	L	S	L	S	L	S
P. acutifolius var. latifolius	-	MOS,ST	NS	MOS	NS	-
P. vulgaris 'Nep 2'	-	MOS	CS,VN	MOS	CS,VN	MOS
V. radiata	-	-	CS,VN	MOS	CS,VN	MOS
V. unguiculata ssp. sesquipedalis	-	-	CS	MOS	CS	MOS

CS = chlorotic spots
L = local
MOS = mosaic
NS = necrotic spots
S = systemic
ST = stunting
VN = veinal necrosis
- = no symptoms

According to Waterworth et al. (1977) *P. lunatus* is immune to BMMV. An attempt was made to use this characteristic to separate SBMV from BMMV. To this end *P. vulgaris* 'Pinto U.I. 650' was inoculated with sap from local lesions on *P. lunatus* 'Henderson Bush', which resulted in a systemic mosaic in addition to local necrotic spots. Sap from these systemically infected leaves reacted positively with BMMV antiserum. Infection with BMMV was first thought to be

due to surface contamination of the primary leaves of *P. lunatus* after inoculation with a mixture of BMMV and SBMV rather than to the presence of this virus inside the leaves. Therefore, in four consecutive local lesions transfers sap was inoculated onto *P. lunatus* 'Henderson Bush' and checked for the presence of BMMV by inoculating *P. vulgaris* 'Pinto U.I. 650'. Also in this case a systemic mosaic appeared in addition to local lesions. Obviously, this procedure was not suitable to separate SBMV from BMMV.

It proved possible to separate SBMV from BMMV by inoculating cucumber (*C. sativus* 'Ashley') with a mixture of the two viruses (Fig. 9, E). This plant is immune to BMMV and reacts with a systemic vein yellowing and mosaic to SBMV. However, this information was only obtained in the later part of this study at CIAT. The SBMV isolate used in most of the experiments was separated from bean plants infected with both BMMV and SBMV, in the Department of Virology, Wageningen in the following way. Sap from beans (*P. vulgaris* 'Bataaf') infected with BMMV and SBMV after inoculation with sap from systemically-infected leaves of *P. acutifolius* var. *latifolius* (Fig. 9, C) was exposed to ageing *in vitro* for 72 days and then mechanically inoculated onto *P. vulgaris* 'Pinto U.I. 114' (Fig. 9, F). This inoculation resulted in 10, 5, 15, 9, 16 and 9 necrotic local lesions on the six primary leaves of three plants respectively and only one plant developed a mild mosaic. A similar inoculation after 84 days resulted in 1, 2, 5, 4, 8 and 7 local lesions on the six primary leaves respectively, but no plant developed a systemic mosaic characteristic for BMMV. Sap from leaves with local lesions from the latter experiment was then inoculated onto *P. vulgaris* 'Bataaf' for propagation of SBMV.

4.3.2 Mechanical inoculation with crude sap from a bean plant showing chlorotic mottle and witches' broom-like growth

Sap from a bean plant showing chlorotic mottle and witches' broom-like growth was inoculated onto 7-day-old plants of *P. acutifolius* var. *latifolius*, *P. vulgaris* 'Honduras 46', *P. vulgaris* 'Pinto U.I. 650' and *V. unguiculata* 'California Blackeye'. The cultivars Pinto U.I. 650 and Honduras 46 were included as test plants because the former is a good indicator plant for SBMV and the latter was reported to be equally susceptible to chlorotic mottle as *P. vulgaris* 'Nep 2'.

Table 4. Reactions of test plants to inoculation with sap from bean plants showing both chlorotic mottle and witches' broom-like symptoms.

Test plant	Symptoms	
	local	systemic
P. acutifolius var. latifolius	NS	MOS, TN, WB
P. vulgaris 'Honduras 46'	-	MOS, TN
P. vulgaris 'Pinto U.I. 650'	NS	MOS
V. unguiculata 'California Blackeye'	CS?	MOS

CS = chlorotic spots
MOS = mosaic
NS = necrotic spots
TN = top necrosis
WB = witches' broom-like symptoms
- = no symptoms
? = doubtful symptoms

Sap from systemically infected leaves from the four test hosts in Table 4 were then inoculated onto 11 plant species and cultivars (Table 5). The latter test plants were chosen mainly because *Datura stramonium*, *V. unguiculata* 'California Blackeye' and *C. amaranticolor* are reported to be local lesion hosts for CMV (Gibbs and Harrison, 1970) and others (except *P. vulgaris* 'Porrillo Sintético') are indicator hosts for SBMV (Sehgal, 1973). The results of this inoculation experiment are given in Table 5.

For unknown reasons no symptoms developed in *V. unguiculata* 'California Blackeye' when inoculated with sap from systemically infected plants of the same cultivar. Sap from systemically infected leaves of *P. vulgaris* 'Pinto U.I. 650' inoculated with sap from *P. acutifolius* var. *latifolius* reacted positively with antiserum to BMMV indicating the presence of this virus. Sap from systemically infected leaves of *V. unguiculata* 'California Blackeye' inoculated 10 days before with sap from *P. vulgaris* 'Pinto U.I. 650' reacted positively with antiserum to CMV-Y indicating the presence of CMV. Sap from systemically infected leaves of *P. vulgaris* 'Porrillo Sintético' inoculated with sap from *P. vulgaris* 'Honduras 46' gave positive reactions with the antiserum to BMMV and to SBMV, indicating the presence of both viruses.

Table 5. Reactions of test plants to inoculation with sap from systemically infected leaves of P. acutifolius var. latifolius, P. vulgaris 'Honduras 46', P. vulgaris 'Pinto U.I. 650' and Vigna unguiculata 'California Blackeye'

Test plant	Sap from systemically infected leaves of							
	P. acutifolius var. latifolius		P. vulgaris 'Honduras 46'		P. vulgaris 'Pinto U.I. 650'		V. unguiculata 'California Blackeye'	
	Symptoms		Symptoms		Symptoms		Symptoms	
	L	S	L	S	L	S	L	S
C. amaranticolor	NS?	-	NS?	-	NS	-	x	x
D. stramonium	-	-	-	-	-	-	-	-
P. lunatus 'Henderson Bush'	NS	-	NS	-	-	-	x	x
P. lunatus 'Jackson Wonder'	NS	-	NS	-	-	-	x	x
P. vulgaris 'Pinto U.I. 111'	-	MOS	x	x	-	-	-	-
P. vulgaris 'Pinto U.I. 650'	NS	MOS	NS	MOS	-	MOS	-	-
P. vulgaris 'Pinto Colorado'	NS	MOS	NS	MOS	-	MOS	NS	MOS
P. vulgaris 'Pinto Olathe'	-	MOS	NS	MOS	NS?	MOS	NB	MOS
P. vulgaris 'Pinto Ovary'	NS	MOS	x	x	-	-	-	MOS
P. vulgaris 'Porrillo Sintético'	NS	MOS	NS	MOS	NS	MOS	x	x
V. unguiculata 'California Blackeye'	CS?	-	-	-	CS?	MOS	-	-

CS = chlorotic spots, MOS = mosaic, NB = necrotic blotching, NS = necroting spots, VN = veinal necrosis, x not tested, ? = doubtful symptoms, - = no symptoms.

4.3.3 Mechanical inoculation with partially purified preparations obtained from a bean plant in the field showing chlorotic mottle only

The partially purified preparations were inoculated onto the primary leaves of 7-day-old *P. vulgaris* 'Nep 2' plants. Two weeks after these plants developed a systemic mosaic serological studies were conducted. The systemically infected leaves gave a positive reaction with antiserum to BMMV and SBMV, but not to CMV-Y. Sap from these leaves was inoculated onto 14 different test plants. Table 6 shows that sap from the systemically infected leaves of *P. vulgaris* 'Pinto Olathe', back-inoculated onto the same bean cultivar, did not produce any local lesions, but produced a systemic mosaic only. Sap from these mosaic showing leaves gave a positive reaction with antiserum to BMMV indicating the presence of the latter virus. Sap from systemically infected leaves of *P. vulgaris* 'Porrillo Sintético' reacted positively with antiserum to BMMV and to SBMV. Back-inoculations from these systemically infected leaves onto *P. lunatus* 'Henderson Bush' and *P. vulgaris* 'Pinto Olathe' gave necrotic local lesions. From the results of serological tests and from the symptoms on *P. lunatus* 'Henderson Bush' and *P. vulgaris* 'Pinto Olathe' it was clear that SBMV was present in *P. vulgaris* 'Porrillo Sintético'. The absence of local infection in *P. acutifolius* var. *latifolius* and *C. amaranticolor* and that of a systemic infection in *V. radiata* and *V. unguiculata* ssp. *sesquipedalis* indicated the absence of CMV. These results were confirmed by the negative serological reaction, when sap from systemically infected leaves of *P. vulgaris* 'Porrillo Sintético' was tested against an antiserum to CMV-Y.

4.3.4 Mechanical inoculation with crude sap preparation from young leaves and stems of bean plants showing chlorotic mottle in their older leaves

An inoculation experiment was conducted using the younger leaves and stems from four different bean plants showing practically no symptoms, mild mottling, sharp mosaic (chlorotic mottle) and witches' broom-like symptoms respectively. All four plants had at least one older trifoliolate leaf with chlorotic mottle. The young leaf material was separately ground and inoculated onto the test plants mentioned in Table 7. *Phaseolus vulgaris* 'Noordhollandse Bruine' was chosen because this cultivar is very susceptible to BMMV, CMV and

SBMV. *Nicotiana glutinosa* and *N. tabacum* were used because they are very susceptible to CMV.

From Table 7 it can be seen that irrespective of the symptoms in the younger leaves, BMMV, SBMV and CMV were invariably found to be present in the chlorotic mottle-affected bean plants. Sap from systemically infected *P. vulgaris* 'Pinto U.I. 650' reacted positively with antiserum to BMMV. The systemic mosaic developed in *N. glutinosa*, *V. radiata* and *V. unguiculata* 'California Blackeye' indicated the presence of CMV, which could be confirmed by serology. Sap from systemically infected leaves of *P. acutifolius* var. *latifolius* reacted positively with the antisera to both BMMV and SBMV. The necrotic local lesions in *P. lunatus* 'Henderson Bush' and in *P. vulgaris* 'Pinto U.I 650' were indicative of SBMV.

Table 6. Reactions of test plants to inoculations with partially purified preparations from a bean plant with chlorotic mottle.

Test plant	Symptoms	
	local	systemic
Chenopodium amaranticolor	-	-
Datura stramonium	-	-
Glycine max	CS	MOS
P. acutifolius var. latifolius	VN, NS	MOS, VN, TN
P. lunatus 'Dixie Butterpie'	NS	-
P. lunatus 'Henderson Bush'	NS	-
P. lunatus 'Jackson Wonder'	NS	-
P. vulgaris 'Pinto Colorado'	NS	MOS
P. vulgaris 'Pinto Olathe'	NS	MOS
P. vulgaris 'Porrillo Sintético'	-	MOS
P. vulgaris 'Puebla'	-	MOS
Sesamum indicum	-	-
V. radiata	-	-
V. unguiculata ssp. sesquipedalis	-	-

CS = chlorotic spots
MOS = mosaic
NS = necrotic spots
TN = top necrosis
VN = veinal necrosis
- = no symptoms

Table 7. *Reactions* of test plants to inoculation with sap from younger leaves and stems of four bean plants showing chlorotic mottle in their older leaves. The symptoms *in* these young plant parts ranged from witches' broom-like, to sharp mosaic (chlorotic mottle), mild mottling and practically no symptoms. the non-inoculated leaves of the plants were tested serologically with antisera to BMMV (1), CMV (2), and SBMV (3), using agar double-diffusion tests.

Test plant	Practically symptomless				Sharp mosaic (Chlorotic mottle)				Witches' broom-like symptoms				Mild mottling			
	Symptoms L/S	Serology 1	2	3	Symptoms L/S	Serology 1	2	3	Symptoms L/S	Serology 1	2	3	Symptoms L/S	Serology 1	2	3
Nicotiana glutinosa	-[a]/VN,MOS,TN	-[b]	+[c]	-	-/MOS,TN	-	+	-	-/MOS,TN	-	+	-	-/MOS,TN	-	+	-
Nicotiana tabacum 'Havana 425'	-/-	-	-	-	-/-	-	-	-	-/-	-	-	-	-/-	-	-	-
P. acutifolius var. latifolius	-/MOS,TN	+	-	+	-/MOS,TN	+	-	+	-/MOS	+	-	+	-/MOS,TN	+	-	+
P. lunatus 'Henderson Bush'	NS/VB,EP,MOS	-	+	-	-/VB,EP	-	+	-	NS/ST,EP	-	+	-	NS/MOS	-	+	-
P. vulgaris 'Noordhollandse Bruine'	-/VC,MOS,R	-	+	+	-/MOS,R,EP	-	+	-	-MOS,R,EP	+	-	+	-/MOS,EP	-	+	-
P. vulgaris 'Pinto U.I. 650'	NS,VN, NLP/MOS	-	+	-	-/MOS	-	+	-	NLP/MOS	+	-	-	NS/MOS	+	-	-
V. radiata	NS/MOS	-	+	-	NS/MOS	-	+	-	-/-	-	-	-	NS/MOS	-	+	-
V. unguiculata 'California Blackeye'	-/MOS	-	+	-	-/MOS	-	+	-	-/MOS	-	+	-	-/-	-	-	-

a = no symptoms, b = negative serological reaction, c = positive serological reaction.
EP = epinasty, L = local, MOS = mosaic, NLP = necrotic line pattern, NS = necrotic spots, R = rugosity, S = systemic, ST = stunting, TN = top necrosis, VB = vein banding, VC = vein clearing, VN = veinal necrosis.

4.3.5 Transmission experiments with whiteflies

None of the healthy bean plants to which whiteflies from chlorotic mottle-diseased bean plants were transferred, showed symptoms 30 days after the onset of the inoculation access period.

4.4 *Discussion and conclusions*

Mechanical inoculation experiments conducted with the crude sap and partially purified preparations from bean plants showing chlorotic mottle in the field, showed more than one virus to be associated with the disease, viz. BMMV, CMV and SBMV. Using differential hosts the three viruses could be separated as shown in Fig. 9. From the partially purified preparations only BMMV and SBMV could be isolated. The failure to detect CMV in partially purified preparations could be understood, when the properties of CMV are taken into account. As CMV is a difficult virus to purify (Scott, 1963) the phosphate buffer used in the purification procedure followed might easily have desintegrated CMV particles, thus leading to the loss of this virus. For a possible explanation of the negative serological reaction with antiserum to CMV-Y see Chapter 6. Transmission experiments with whiteflies did not reveal the presence of any whitefly-transmitted virus in bean plants with chlorotic mottle.

CHAPTER 5

CHARACTERIZATION AND FURTHER IDENTIFICATION OF BEAN MILD MOSAIC VIRUS (BMMV) ISOLATED FROM BEAN PLANTS SHOWING CHLOROTIC MOTTLE

5.1 *Introduction*

Bean mild mosaic virus (BMMV) was first reported on beans by Waterworth et al. (1977) in El Salvador. They isolated the virus from a bean plant showing severe dwarfing, leaf curling and rugosity that was also infected with bean curly dwarf mosaic virus (BCDMV). The former virus could be readily transmitted mechanically and by chrysomelid beetles and had a host range restricted to leguminous plants. Up to now the publication by Waterworth et al. (1977) is the only literature on BMMV.

In the present study BMMV isolated at CIAT (designated BMMV-CIAT) was further characterized and identified.

5.2 *Materials and methods*

5.2.1 Maintenance and propagation of the virus

The virus was maintained and propagated in *P. vulgaris* 'Pinto 114' as a precaution against possible contamination with southern bean mosaic virus (SBMV) which gives only local lesions in this cultivar. For comparison BMMV from El Salvador (BMMV-W) received from Dr. H.E. Waterworth (U.S.D.A., Glenn Dale, Maryland, U.S.A.) maintained in *P. vulgaris* 'Topcrop' was used.

5.2.2 Mechanical inoculation and host range

The virus inoculum was prepared by grinding systemically infected *P. vulgaris* 'Pinto U.I. 114' leaves with cold 0.02 M sodium phosphate buffer pH 7.0, containing 0.02 M Na_2SO_3 (1 g leaves in 4 ml buffer) in a cold, sterilized mortar. The sap was then directly applied,

using the fingers, onto leaves previously dusted with carborundum powder (600 mesh) and then rinsed with tap water. Control plants were inoculated as above using buffer instead of sap. The plants which did not show any symptoms were back-inoculated onto *P. vulgaris* 'Honduras 46' or *P. acutifolius* var. *latifolius*. These plants developed a systemic mosaic when infected with BMMV.

All host range experiments were conducted under glasshouse conditions (mean day temperature 35-40°C; mean night range 18-22°C). On a bright sunny day at 2.30 p.m. the light intensity inside the glasshouse measured using a Lambda LI-170 Quantum-photometer was 1900 lux. All the plants used were grown from seeds directly planted in 10 cm plastic pots or 8 cm disposable styrofoam cups. In the case of small-seeded plants such as *Nicotiana* spec., the seeds were sown in a nursery bed placed in the glasshouse and the seedlings transplanted into pots when they were about 5 cm in height. While testing the CIAT advanced breeding lines of *P. vulgaris* the seeds were planted in 50 x 27 x 6 cm^3 plastic trays (7 breeding lines/tray). From every species or cultivar a minimum of seven plants were tested, except in the case of the CIAT advanced breeding lines (CIAT EP 1980) of *P. vulgaris* from which only five plants were tested. The plants were inoculated mechanically as described in Chapter 4. The plants were observed daily until flowering and the symptoms recorded once a week. The plants that did not show any symptoms were back-inoculated onto *P. acutifolius* var. *latifolius*. Periodically the inoculated plants were checked for the presence of BMMV-CIAT by serology (Chapter 4).

5.2.3 Transmission by insects and mites

Chrysomelid beetles, *Diabrotica balteata* Le Conte and *Cerotoma facialis* (Erickson) were kindly provided by the bean entomologists at CIAT. Transmission experiments were carried out in netted cylindrical cages (8.5 cm diameter x 11.5 cm height) as recommended by CIAT entomologists (Fig. 10). The beetles were given 48 h acquisition access periods on the trifoliolate leaves of 3-week-old *P. vulgaris* 'Pinto U.I. 114' plants infected with BMMV-CIAT and then transferred to *P. vulgaris* 'Honduras 46' plants for a 48 h inoculation access period. The plants were then sprayed with insecticide and left in the glasshouse.

Aphids, *Aphis gossypii* Glover reared on *Datura stramonium* were used. After starving 150 aphids for 30 min they were allowed to feed for 48 h on 2-week-old *P. vulgaris* 'Pinto U.I. 114' plants infected with BMMV-CIAT. After this acquisition period the aphids were transferred (25/plant) onto a piece of Whatman no. 1 filter paper (2.5 x 2.0 cm^2) to avoid mechanical contact. This piece of paper with 25 aphids was placed on a healthy leaf of *P. vulgaris* 'Honduras 46', using a camel hair brush. Once the aphids had moved from the filter paper to the leaf, the former was removed. One day later the plants were sprayed with an insecticide to kill the aphids.

Fig. 10. P. vulgaris 'Pinto U.I. 114' with a netted cylindrical cage used in experiments on beetle transmission.

Leafhoppers, *Empoasca kraemeri* Ross and Moore, one of the most important insect pests in beans in Colombia, were supplied by the bean entomologists at CIAT. Fifty adult leafhoppers were caged together with a *P. vulgaris* 'Pinto U.I. 114' plant infected with BMMV-CIAT in a netted cage (31 x 31 x 50 cm^3) for 48 h. After this period the cage was covered with a black cloth leaving a very small

area free. The positively phototropic leafhoppers migrating into this area were collected in groups of 10 using an aspirator. After 10 min of starving, the leafhoppers were transferred to healthy *P. vulgaris* 'Diacol Calima' for 48 h. Thereafter, the plants were sprayed with an insecticide and left in the glasshouse for observations.

Mites were collected from a non-inoculated naturally infested bean plant found in the glasshouse. They were identified as *Polyphagotarsonemus latus* Banks by Dr. C. Cardona of the CIAT entomology section. Mites were transferred to *P. vulgaris* 'Pinto U.I. 114' infected with BMMV-CIAT by replacing an infested leaf with mites over the petiole. After 48 hrs. the leaves of *P. vulgaris* 'Pinto U.I. 114' were checked under the stereomicroscope for infestation and then placed over a filter paper (in order to avoid direct contact between diseased and healthy leaf), which was carefully placed on a trifoliolate leaf of healthy *P. vulgaris* 'Diacol Calima'. Twenty-four hours later the filter paper was removed along with the dried diseased leaf and the plant was checked under the stereomicroscope for mites. The plants which were not infested were discarded and the others were sprayed against mites after another 24 h. The control plants were treated similarly but the mites were fed on healthy *P. vulgaris* 'Pinto U.I. 114'.

5.2.4 Transmission through seed

To determine whether BMMV-CIAT is transmitted through seed, different cultivars of 7-day-old *P. vulgaris* were inoculated mechanically on the primary leaves with BMMV-CIAT and kept in the glasshouse until the seeds were harvested. Occasionally, the plants were sprayed with biocides to control mites, aphids and powdery mildew. The mature seeds were harvested and planted in disposable styrofoam cups (1 seed/pot) with sterilized soil. The plants were observed for a period of five weeks. Evaluation was made usually on the basis of symptomatology, by serology or by back-inoculation to *P. acutifolius* var. *latifolius*.

5.2.5 Transmission by dodder

As *Cuscuta* spec. did not grow well on *P. vulgaris* it was first established on *Glycine max* 'ICA Pance' infected with BMMV-CIAT. After 8-12 days the newly formed shoots of *Cuscuta* spec. were trained onto *P. vulgaris* 'Diacol Calima' and *P. vulgaris* 'Nep 2'. Test plants were well separated from the infected *G. max* plant to prevent possible transmission through physical contact. Every week the test plants were checked serologically or by back-inoculation for infection.

5.2.6 Stability in sap

The BMMV-CIAT inoculum used in these experiments was obtained by grinding systemically infected leaves of *P. vulgaris* 'Pinto U.I. 114' in distilled water (1 g in 1 ml). The sap was then expressed through a double-layered gauze. *Phaseolus acutifolius* var. *latifolius* was used as assay plant.

5.2.6.1 Dilution end-point

A series of dilutions ($0\text{-}10^{-12}$) was made from the virus-containing sap using distilled water. The dilutions were then checked for infectivity.

5.2.6.2 Thermal inactivation point

The virus-containing sap was diluted to 10^{-2} using distilled water and from the resulting suspension 1 ml portions in thin-walled "Pyrex" test tubes were heated to different temperatures (30-100°C, at 2°C intervals) for 10 min. The samples were then rapidly cooled and their infectivity determined.

5.2.6.3 Longevity in vitro

The virus-containing sap was diluted to 10^{-2} using distilled water and from the resulting suspension 1 ml portions were kept at room temperature (22°C) in closed sterilized vials. Every week three *P. acutifolius* var. *latifolius* plants were mechanically inoculated with a 1 ml portion.

5.2.7 Purification

The virus was purified from systemically infected leaves of *P. vulgaris* 'Nep 2', 20 days after inoculation. In earlier experiments the purification method described by Waterworth et al. (1977) was used. However, the material associated with the green colour of the suspended pellet formed after high speed centrifugation greatly impeded the scanning of the bands visible after sucrose gradient centrifugation. Therefore a different procedure was followed to purify the virus. Fifty grams of leaves were homogenized in a Waring blender with 250 ml of 0.02 M sodium phosphate buffer pH 7.0 (buffer), containing 1% 2-mercaptoethanol (2-ME) and 0.15% thioglycollic acid (TGA) for 2 min. After adding 0.5% volume of 1:1 chloroform:butanol mixture to the slurry the latter was blended for another 2 min. After standing for 2 min the sap was squeezed through a double-layered gauze. The filtrate was clarified by centrifuging at 7000 *g* for 10 min in the Sorvall SS-34 rotor. The supernatant was filtered through glass-wool and then centrifuged at 41,000 *g* for 2½ h. The resulting pellets were suspended in 25 ml of buffer and clarified by low speed centrifugation at 7000 *g* for 10 min in the Sorvall SS-34 rotor. The supernatant was then layered over 1 ml of 40% (w/v) sucrose foot in the same buffer and centrifuged at 41,000 *g* for 3 h. The pellets were suspended in 3 ml of buffer and clarified by low speed centrifugation at 7000 *g* for 15 min. Occasionally the virus was also purified by precipitating with polyethylene glycol 6000 (PEG). The filtrate obtained by passing the supernatant through glass-wool was made up with PEG and sodium chloride to a concentration of 8% and 0.2 M, respectively, and stirred at room temperature for 4 to 6 h. The precipitate was suspended in buffer (40% v/v of the original sap volume) and clarified by centrifugation at 7000 *g* for 10 min. The supernatant was then layered over 1 ml of 40% sucrose foot in buffer, and centrifuged at 41,000 *g* for 3 h. The pellet was suspended in 3 ml of buffer and clarified by centrifuging at 7000 *g* for 15 min. Earlier purification experiments in which the method of Waterworth et al. (1977) was used, revealed the presence of large quantities of virus in the stems and roots. Therefore, in later experiments these plant parts were also included, and the purification method was modified as follows (Fig. 11). Three infected plants, approximately 300 g in weight, were homogenized in 250 ml of buffer, containing 1% 2-ME and 0.15% TGA in a Waring blender for

2 min. The extract was filtered through a double-layered gauze and the filtrate clarified by low speed centrifugation at 7000 *g* for 10 min. The supernatant thus obtained was heated to 60°C for 10 min in a waterbath, cooled rapidly and clarified again by low speed centrifugation at 7000 *g* for 10 min. The supernatant was then centrifuged at 41,000 *g* for 2½ h, after which the pellets were suspended in 25 ml buffer and clarified by low speed centrifugation at 7000 *g* for 10 min. The supernatant was then layered over 1 ml of a 40% sucrose foot in buffer, and centrifuged at 41,000 *g* for 3 h. The pellets were suspended in 3 ml of buffer, and clarified at 7000 *g* for 15 min. The partially purified virus suspensions obtained were further subjected to density gradient centrifugation.

The virus concentrations in purified preparations were calculated using the extinction coefficient at 260 nm ($E^{0,1\%}_{1\ cm,\ 260\ nm}$) to be 4.9 for BMMV-CIAT (calculated from the 260/280 ratio, according to Paul, 1959; Sehgal et al., 1970; and Gibbs and Harrison, 1976).

Rate-zonal centrifugation of partially purified BMMV-CIAT was performed in linear log sucrose gradients according to the method described by Brakke and Van Pelt (1970). Gradient columns were prepared by hand layering 3.40, 8.75, 8.00, 6.50 and 4.0 ml of solutions containing 292, 250, 200, 135 and 0 mg of sucrose/ml 0.02 M sodium phosphate buffer pH 6.25. After allowing the columns to stand at 4°C for 15 h, 1.5 ml were removed from the top of the column and 1 ml of partially purified BMMV-CIAT (5 mg/ml) was floated on it and centrifuged at 55,000 *g* for 2½ h. Centrifuged gradients were analysed in the ISCO density gradient fractionator model 640 equipped with an ISCO model 610 external recorder. Rate-zonal centrifugation of BMMV-CIAT and BMMV-W singly and in mixture was also performed in linear log sucrose gradients. Gradient columns were prepared by hand layering 1.20, 3.15, 2.70, 2.25, 1.50 and 1.20 ml of sucrose solutions containing 310, 275, 232, 182, 118 and 0 mg sucrose/ml 0.02 M sodium phosphate buffer pH 6.25 (Brakke and Van Pelt, 1970). After allowing the gradients to stand for 15 h at 4°C, 0.5 ml was removed from the top of three columns and replaced with 0.5 ml BMMV-CIAT (3 mg/ml), 0.5 ml of a mixture of BMMV-CIAT (3 mg/ml) and BMMV-W (3 mg/ml) in equal proportions and 0.5 ml BMMV-W (3 mg/ml), respectively. The gradients were centrifuged at 146,800 *g* for 1 h in a Beckman SW 41.1 rotor. The centrifuged gradients were analysed as above using an ISCO gradient fractionator.

Fig. 11. Method finally adopted to purify BMMV-CIAT from infected P. vulgaris 'Nep 2'.

5.2.8 Properties of particles

5.2.8.1 UV-absorption spectrum

The UV-absorption spectrum of purified BMMV-CIAT in 0.02 M sodium phosphate buffer pH 7.0, was measured in a Beckman DB spectrophotometer. The controls consisted of buffer only. Correction for light scattering was performed according to the optical method described by Englander and Epstein (1955) using the absorption between 320 and 360 nm.

5.2.8.2 Buoyant density

For determination of buoyant density, equilibrium centrifugation of purified BMMV-CIAT was carried out in caesium sulphate solution prepared by dissolving 5 g of caesium sulphate in 11.7 ml of buffer. The refractive index of the solution was measured by using a Zeiss refractometer. The density of this solution was 1.3631 at 25°C when calculated with the formula of Hearst and Vinograd (1961)

$$n_D^{25} = 0.0730\ \rho^{25} + 1.2646$$

where ρ^{25} is the density and n_D^{25} the refractive index of the solution at 25°C. A mixture was made of 0.2 ml of purified BMMV-CIAT (5 mg/ml) in buffer with 5 ml of caesium sulphate solution and centrifuged at 149,000 *g* for 17½ h. After centrifugation the bottom of the tube was punctured with a sharp needle and portions of three drops were collected. From each portion one drop was used to find the refractive index of the solution and the remainder was diluted to 1 ml with buffer, whereafter the absorbance was measured at 260 nm in a Beckman DB spectrophotometer using only buffer as blanks.

5.2.8.3 Sedimentation coefficient

The sedimentation pattern and the sedimentation coefficient of purified BMMV-CIAT suspensions were determined with an analytical ultracentrifuge Spinco model E. A series of different concentrations of BMMV-CIAT was prepared in 0.02 M sodium phosphate buffer pH 7.0, and was spun at 32,000 rpm with the rotor temperature kept at 20°C. Photographs were taken at 4 min intervals with Schlieren optics and under a diaphragm angle of 50°. With the graphical method of Markham (1960) the S value at each concentration was determined. The sedimentation coefficient at infinite dilution was calculated by extrapolation to zero virus concentration.

5.2.9 Particle morphology

For electron microscopic observations specimens of BMMV-CIAT were prepared according to Bos (1975). A small piece of *N. tabacum* 'White Burley' infected with tobacco mosaic virus (TMV) was chopped with five to six drops of 2% uranyl acetate pH 4.5, using a razor blade on a microscopic glass slide. A drop of this juice was mixed with three drops of a purified sample of BMMV-CIAT. With a glass capillary some of this mixture was transferred to a carbon-reinforced formvar coated copper grid (400 mesh). After a minute the excess liquid was removed by touching the grid with a piece of filter paper. The grid was then examined with a Siemens Elmiskop 101 electron microscope.

5.2.10 Particle composition

5.2.10.1 Nucleic acid

5.2.10.1.1 Extraction

The procedure applied for extracting the nucleic acid from BMMV-CIAT was based on the method described by Rushizky and Knight (1959) for tomato bushy stunt virus. To 10 ml of purified BMMV-CIAT (3 mg/ml) in buffer, was added 1.6 ml of 10% sodium dodecyl sulphate (SDS) and 0.1 ml of 2.5% bentonite in the same buffer, followed by sufficient glacial acetic acid to lower the pH to 4.1 at room temperature. The solution was then gently shaken until the opalescence disappeared. The pH of the solution was then adjusted to 6.1 with 1 M NaOH and an equal volume of water-saturated phenol added and the mixture shaken in a stoppered tube for five min. The mixture was then subjected to a low speed centrifugation at 1000 *g* for 5 min in the Sorvall SS-345 rotor. The three layers formed (top aqueous layer, middle interphase and the lower phenol layer) were separated with a syringe. The interphase was suspended in 3 ml of distilled water and 0.1 ml of 10% SDS and was extracted twice with water-saturated phenol as above. The aqueous layers formed after the three low speed centrifugations were pooled and extracted twice with 0.1 ml 10% SDS and an equal volume of water-saturated phenol. The aqueous layer was freed from phenol by extraction with an equal volume of cold ether, three times. The separation of phases was facilitated by centrifuging for 5 min

at 1000 *g* in the Sorvall SS-34 rotor. To the final aqueous phase 2 drops of 3.3 M sodium acetate pH 5.5, and 2.5 volumes of cold absolute ethanol were added and the mixture left at -20°C overnight. The precipitate formed was centrifuged at 17,000 *g* for 20 min in the Sorvall SS-34 rotor and the pellet obtained suspended in 5 ml of distilled water. The nucleic acid was again precipitated as above using cold absolute ethanol. After standing for 5 h at -20°C the nucleic acid was collected by centrifuging, freed from ethanol under vacuum for 15 min and then dissolved in distilled water.

5.2.10.1.2 UV-absorption spectrum

The UV-absorption spectrum of the purified BMMV-CIAT nucleic acid was measured in a Beckman DB spectrophotometer. The absorption between 320 and 360 nm did not give a satisfactory straight line. Therefore, correction for light scattering could not be made. The concentration of the nucleic acid preparation was calculated taking the extinction coefficient ($E^{0.1\%}_{1\ cm,\ 260\ nm}$) to be 25 (Gibbs and Harrison, 1976).

5.2.10.1.3 Type of nucleic acid

For the Mejbaum orcinol method (Putman, 1957) 0.5 mg/ml BMMV-CIAT nucleic acid, 1 mg/ml of TMV-RNA (prepared according to the method described by Knight (1975)), 1 mg/ml of DNA (fish sperm, Matheson Colman and Bell, Comp. N.J.) and distilled water (as control) were used. To 2 ml of the sample, an equal volume of 0.1% $FeCl_3$ was added, mixed and placed in a boiling water bath for 40 min and then cooled.

The diphenylamine reaction was performed according to the method described by Burton (1956). The reagent was prepared by dissolving 1.5 g of diphenylamine in 100 ml of glacial acetic acid and adding 1.5 ml of concentrated sulphuric acid. The solution was stored in the dark and just before use 20 ml of it mixed with 0.1 ml aqueous acetaldehyde (16 mg/ml). Solutions of BMMV-CIAT nucleic acid, TMV-RNA and DNA at concentrations of 0.2 mg/ml in 50 mM NaOH were employed. The controls contained 50 mM NaOH. To the samples an equal volume of 0.5 N $HClO_4$ was added and heated to 70°C for 15 min. After cooling to 30°C 1 ml of the test sample was mixed with 2 ml of diphenylamine reagent containing acetaldehyde and kept in the dark at 30°C for 18 h.

To study the effect of nucleases, 0.5 µg/ml of BMMV-CIAT nucleic acid in 0.02 M sodium phosphate buffer pH 7.0, was incubated with

pancreatic RNase-A (Sigma, 5x cryst., 1 µg/ml) for 90 min at 4°C and another volume of the BMMV-CIAT nucleic acid incubated at 100°C for 3 min with DNase-1 (Sigma, RNase-free, 5 µg/ml) in the presence of 2 mM Mg^{2+}. The samples were then bio-assayed on *P. acutifolius* var. *latifolius*.

5.2.10.1.4 Base composition

Purified BMMV-CIAT (16.3 mg) was used to determine the base composition according to the method described by Knight (1963). The nucleic acid was hydrolysed in 1 N HCl by heating the virus in a closed ampulla at 100°C for 1 h. The hydrolysate was centrifuged at 3000 *g* for 10 min and the supernatant chromatographed in Whatman no. 1 filter paper in a solvent consisting of t-butanol, 6 N HCl and water (70:13:17), at room temperature for 20 h. After chromatography, the paper was air-dried and the spots located and marked with a pencil under UV light. The spots and also non-spotted pieces of the same size and Rf-value to be used as controls were cut out and placed in closed test tubes together with 5 ml of 0.1 N HCl and eluted by standing at room temperature overnight. The absorption of each solution was read in a Beckman DB spectrophotometer at a wavelength of near maximum absorption for the nucleotide in question. The concentrations of bases were calculated according to the extinction coefficients geven by Sober (1970).

5.2.10.1.5 Phosphorus content and nucleic acid content

The phosphorus content of the nucleic acid was analysed according to the micro-method of Morrison (1964). For this experiment the virus was purified as in section 5.2.7 but instead of buffer, distilled water was used. All glassware used in the experiment was kept in bichromate-sulphuric acid overnight, rinsed in deionized water and dried before use. To make a standard curve, samples with 1, 2, 4 and 8 µg were taken from a solution containing 200 µg P/ml (0.89406 g of $NaH_2PO_4 \cdot 2H_2O$ in deionized water). Purified BMMV-CIAT (0.0786 mg) was dried in "Pyrex" test tubes calibrated at 5 ml level at 100°C. After cooling, the test tubes to which 0.3 ml of sulphuric acid (98%) was added, were heated over a gas flame until charring was completed. After adding with a pasteur pipette one drop of 30% (w/v) hydrogen peroxide the solution was gently shaken and boiled for 1 min. After cooling the tubes, 3.4 ml of deionized water was added. Thereafter, 0.1 ml of Na_2SO_3 (16.5% w/v) was added and the mixture

gently shaken. One ml of 2% (w/v) ammonium paramolybdate ($(NH_4)_6 \cdot Mo_7O_{24} \cdot 4H_2O$) was added directly to the solution followed by 0.1 ml of freshly prepared 10% (w/v) ascorbic acid. After shaking, the tubes were heated at 100°C for 10 min in a waterbath, cooled and the volume adjusted to 5.0 ml with deionized water. The tubes were gently shaken and the optical density measured at 822 nm against blanks containing only the reagents in a Bausch and Lomb Spectronic 20 spectrophotometer. Each determination was performed in triplicate.

The nucleic acid content of the virus was estimated using the 260/280 ratio (Gibbs and Harrison, 1976), buoyant density (Sehgal et al., 1970), the base composition and phosphorus content of the nucleic acid.

5.2.10.1.6 Molecular weight

The molecular weight of the BMMV-CIAT nucleic acid was determined by gel electrophoresis according to the method of Summer (1970) using Loening and Ingle (1967) buffer system in a Biorad model 150A gel electrophoresis cell. Gels of 2.6% acrylamide and 0.5% agarose were prepared as follows. To 9 ml of melted 1% agarose 0.20 ml of 10% ammonium persulphate was added and the mixture cooled to 48°C in a waterbath. In a separate beaker, 3 ml of a stock solution, containing 15% acrylamide and 0.75% N,N-methylene-bisacrylamide (BIS) were mixed with 3.8 ml of deionized water and 2.0 ml of nine times concentrated Loening's electrophoresis buffer. Just before casting the gel 0.02 ml of N,N,N,N,-tetramethylethylenediamine (TEMED) was added to the acrylamide solution, stirred gently, mixed with the agarose-ammonium persulphate solution, stirred and cast into glass tubes of internal diameter 5 mm and length 12.5 cm. A flat gel top was obtained by hand-layering deionized water over the agarose-acrylamide solution. The gel was allowed to polymerize for 30 min and the tubes were inserted into the electrophoresis apparatus. Reservoirs were filled with electrophoresis buffer containing 0.2% SDS. Prior to the application of the sample a current of 5 mA/tube was applied for 60 min in order to remove polymerization catalysts and other impurities. The nucleic acid for electrophoresis was prepared by dissociating intact BMMV-CIAT according to the method described by Lane and Kaesberg (1971) for broad bean mottle virus. Just prior to electrophoresis, 0.05 ml of purified BMMV-CIAT (at a concentration of 7.5 mg/ml in buffer), 0.1 ml of a solution of 1% SDS, 1 M urea,

5% sucrose and 0.05 M 2-ME in electrophoresis buffer were mixed and the mixture heated to 50°C in a waterbath for 10 min followed by cooling. Fifty microlitres of this solution were loaded over the gel just before electrophoresis. Cowpea chlorotic mottle virus (CCMV) nucleic acid species prepared according to the method described by Verduin (1978) were used as markers. Twenty microlitres of CCMV at a concentration of 10 mg/ml were mixed with 0.2 ml of a solution containing 10% glycerol, 2% SDS in deionized water and the mixture heated to 60°C for 10 min and then cooled. Fifty microlitres of this solution were layered over a gel just before electrophoresis, which was carried out for 4 h at 4 volts/cm of gel. After electrophoresis, the gels were removed, fixed for 15 min in 1 M acetic acid and stained for 1 h in a solution of 0.2% methylene blue in 0.4 M sodium acetate and 0.4 M acetic acid, pH 4.7. Thereafter they were destained in running tap water in the dark (Peacock and Dingman, 1967). The migration of the stained nucleic acid bands was measured from the top of the gel to the centre of each band. The linear relationship between the electrophoretic mobility and the logarithm of the molecular weight of the nucleic acid (Bishop et al., 1967; Peacock and Dingman, 1968; Gould et al., 1969) was used to calculate the molecular weight of the BMMV-CIAT nucleic acid. A standard line between the electrophoretic mobility and the logarithm of the molecular weight was drawn taking the molecular weights of CCMV-RNA_1 as 1.20×10^6, CCMV-RNA_2 as 1.07×10^6, CCMV-RNA_3 as 0.81×10^6 and CCMV-RNA_4 as 0.25×10^6 (Bancroft and Flack, 1972).

5.2.10.2 Coat protein

5.2.10.2.1 Molecular weight

The molecular weight of the BMMV-CIAT coat protein was determined by using polyacrylamide gel electrophoresis according to the method of Weber and Osborn (1969) in a Biorad model 150A gel electrophoresis cell. The gel buffer contained 7.8 g of sodium dihydrogen phosphate ($NaH_2PO_4 \cdot H_2O$), 38.6 g of disodium hydrogen phosphate ($Na_2HPO_4 \cdot 7H_2O$) and 2 g of SDS per litre. As markers 0.5 mg/ml solutions of bovine serum albumin (BSA*, mol. wt. 68,000), carbonic anhydrase (CA*, mol. wt. 32,000), purified coat protein preparations of cowpea chlorotic mottle virus (CCMV, mol. wt. 19,400) (Bancroft, 1971), cowpea mosaic

*Mol. wt. according to manufacturer, Polyscience Inc., U.S.A.

virus (CPMV, mol. wts. 37,000 and 22,000) (Van Kammen and De Jager, 1978), southern bean mosaic virus (SBMV bean strain, mol. wt. 29,000) (Tremaine, 1966) and bean common mosaic virus (BCMV-NL strain, mol. wt. 32,000) (Morales, 1979) were used. The marker proteins, BMMV-CIAT and BMMV-W were boiled in a dissociating buffer (0.02 M sodium phosphate buffer pH 7.0, 2% SDS and 1% 2-ME) for 2 min (Maizel, 1971). Before electrophoresis 50 µl of the treated protein suspensions were mixed with 3µl of 0.05% bromophenol blue in water, 1 drop of glycerol and 5 µl of 2-ME and layered over a 10% polyacrylamide gel. Electrophoresis was performed at a constant current of 8 mA per gel for 3½ h using 1:1 (v/v) diluted gel buffer. After electrophoresis the gels were removed from the tubes and stained overnight in 0.2% Coomassie brilliant blue (R 250) in 50% methanol and 7% glacial acetic acid by volume in water (Maizel, 1966). The gels were destained with a solution containing 75 ml glacial acetic acid, 50 ml of methanol and 875 ml of water. The distances the proteins migrated were measured from the top of the gel. The distances the marker proteins migrated were plotted against the logarithm of their known molecular weights and from the standard line thus obtained the molecular weight of BMMV-CIAT coat protein was determined using its migration.

5.2.11 Serology

An antiserum to BMMV-CIAT was prepared by administering four intramuscular injections to a white New Zealand rabbit. Before the first injection, 15 ml of blood were taken from the ear vein to obtain normal serum. The first injection consisted of 1 ml of purified BMMV-CIAT (3 mg/ml) emulsified with an equal volume of Freund's complete adjuvant and the remainder of 2 mg/ml each of purified BMMV-CIAT emulsified with an equal volume of Freund's incomplete adjuvant. One week after the last injection the rabbit was bled and the blood collected in sterilized 30 ml "Corex" test tubes which were then heated to 37°C for 1 h in a waterbath. The clotted blood was removed from the walls of the tube with a spatula and the serum was separated by centrifugation at 480 *g* for 10 min. The serum was collected and centrifuged again at 3000 *g* for 10 min (Purcifull and Batchelor, 1977). After adding 0.01% sodium azide as a preservative the clear serum was stored at -20°C in sterilized 1 ml glass vials until use.

Serological tests were carried out with the agar double-diffusion test (Ouchterlony, 1962) in 0.75% (w/v) Noble agar prepared in 0.01 M

Tris-nydrochloride buffer containing 0.85% (w/v) sodium chloride and 0.02% sodium azide. Gels were prepared in 9 cm diameter plastic petri dishes or on glass slides using a Gelman immunodiffusion kit. Wells were cut using either auto-gel T/M well cutters (Grafar Corp.; well to well distance 6.0 mm) or Gelman well cutters (well to well distance 5.0 mm). Dilutions of plant sap or purified virus were made in 0.85% (w/v) sodium chloride solution and the antiserum with normal serum (Purcifull and Batchelor, 1977). The tests were performed in a closed humid chamber at 22°C.

The virus was tested against the following antisera of which the titres, in so far as mentioned by the donors, are given within parentheses: *Arabis* mosaic virus (1024), broad bean wilt virus (1024), carnation mottle virus (1024), carnation ringspot virus (1024), CPMV-Nigerian isolate (1024), CPMV-Trinidad isolate (1024), raspberry ringspot virus (512), red clover mottle virus (1024), tobacco streak virus-soybean isolate (1024), tomato black ring virus (1024), turnip yellow mosaic virus (1024), (all of them kindly supplied by Ing. D.Z. Maat, IPO, Wageningen), BMMV-W (a gift from Dr. H.E. Waterworth, U.S.D.A., Glenn Dale, Maryland, U.S.A.), SBMV-bean strain and severe SBMV (a gift from Dr. K.A. Kimble, Department of Plant Pathology, University of California, Davis, California, U.S.A.).

5.2.12 Relations with cells and tissue

Cytological studies were only conducted with BMMV-CIAT infected *P. vulgaris* 'Nep 2' and *P. acutifolius* var. *latifolius* plants. These plants were chosen because *P. vulgaris* 'Nep 2' was used in purification experiments and *P. acutifolius* var. *latifolius* exhibited very strong mosaic symptoms.

5.2.12.1 Light microscopy

Epidermal strips, sections of infected leaves, stems and roots were prepared for light microscopy according to the method described by Christie and Edwardson (1977) using the Azure A and Luxol brilliant green calcomine orange (O-G) stains. The tissues were stained for 30 min, rinsed in absolute alcohol and treated with undiluted 2-methoxyethyl acetate for 15 min to 24 h. Leaves from which the lower epidermis was removed, were used to study the infected mesophyll cells. In the latter case the chlorophyll from the plastids was re-

moved before staining by soaking in undiluted 2-methoxyethanol for 1 h. Staining was carried out as above and all tissues were mounted in Euparal for observation.

5.2.12.2 Electron microscopy

Tissue pieces infected with BMMV-CIAT were prepared for *in situ* studies according to the method described by Jayasinghe and Dijkstra (1978). Pieces measuring approximately 1 mm^2 from infected leaves, stems and roots were fixed in Karnovsky fixative in 0.07 M cacodylate buffer pH 7.2, for 30 min at 20 mercury inches of vacuum and another 30 min at atmospheric pressure, washed six times for 10 min each in 0.1 M cacodylate buffer pH 7.2, with 0.1% $CaCl_2$ and post-fixed for 1 h in a solution of 1% osmium tetraoxide in 0.1 M cacodylate buffer containing 0.1% $CaCl_2$, pH 7.2, at room temperature. The tissue pieces were then washed twice in 0.1 M cacodylate buffer pH 7.2, with 0.1% $CaCl_2$ each for five minutes, once in 0.1 M veronal acetate buffer pH 5.1, and they were post-fixed again in 2% uranyl acetate in veronal acetate buffer pH 5.1, for 1 h. The fixed tissues were then washed twice in 0.1 M veronal acetate buffer pH 5.1, and once in distilled water for five min each. The fixed tissues were dehydrated in a graded series of acetone, infiltrated and embedded with Epoxy resin (Spurr, 1969). Ultrathin sections were cut in a Sorvall Porter Blum or LKB Ultratome III microtome using a diamond knife. The sections were collected on 200 mesh copper grids, stained with 2% aqueous uranyl acetate and later with lead citrate in 0.01 M sodium hydroxide (Reynolds, 1963) for 15 min each. The sections were examined with Jeol and Siemens Elmiskp 101 electron microscopes.

Table 8. Reactions of test plants inoculated with BMMV-CIAT (Latin names are according to Bailey, 1977; Encke et al., 1980; Maréchal et al., 1978).

Plant species, cultivar and breeding line tested	Symptoms	
	local	systemic
Amaranthus dubius Mart.	-	-
Arachis hypogaea L.	-	-
Cajanus cajan (L.) Huth.	-	-
Canavalia ensiformis (L.) DC.	-	-
Canavalia gladiata (Jacq.) DC.	L	L
Capsicum annuum L.	-	-
Capsicum frutescens L.	-	-
Chenopodium album L.	-	-
Chenopodium amaranticolor Coste & Reyn.	-	-
Chenopodium quinoa Willd.	-	-
Crotalaria juncea L.	-	-
Cucumis sativus L.		
'Ashley'	-	-
'Marketer'	-	-
Cyphomandra betacea (Cav.) Sendth.	-	-
Datura stramonium L.	-	-
Desmodium ovalifolium Guill & Perr.	-	-
Euphorbia geniculata Orteg. (= E. prunifolia)	-	-
Euphorbia repanda Sweet.	-	-
Glycine max (L.) Merr.		
'ICA Caribe'	-	MOS
'ICA Faroa'	-	MOS
'ICA Pelican'	-	MOS
'ICA Pance'	-	MOS
'ICA Tunia'	-	MOS
'Improved Pelican'	-	MOS
'ICA Linea 109'	-	MOS
'ICA Linea 121'	-	MOS
Gomphrena globosa L.	-	-
Helianthus annuus L.		
'Dursol Alta'	-	-
'Russia 1'	-	-
Hibiscus cannabinus 'Everglades'	-	-
Ipomoea batatas (L.) Lam.	-	-
Jatropha gossypifolia L.	-	-
Lablab niger Medik.	-	MOS
Lens culinaris Medik.	-	-
Lycopersicon esculentum Mill.		
'Floridale'	-	-
'Rutgers'	-	-
Macroptilium atropurpureum (Moc. & Sessé) Urb. PI 318685	L	L
Macroptilium lathyroides (L.) Urb. PI 146800	L	L
Mucuna pruriens (L.) DC. (= Stizolobium pruriens (L.) Medik.)	-	-

Table 8 (cont.)

Mucuna utilis Wall. ex Wight (= Stizolobium utile (Wall.) Piper & Tracy)	-	-
Nicandra physalodes (L.) Gaertn.	-	-
Nicotiana benthamiana Domin	-	-
Nicotiana clevelandii Gray	-	-
Nicotiana glutinosa L.	-	-
Nicotiana rustica L.	-	-
Nicotiana tabacum L.		
'Havana 425'	-	-
'Samsun NN'	-	-
'White Burley'	-	-
Oryza sativa L.		
'Mudgo'	-	-
IR - 8	-	-
IR - 22	-	-
Phaseolus aconitifolius Jacq. (= Vigna aconitifolia (Jacq.) Maréchal)	-	-
Phaseolus acutifolius var. acutifolius Gray.		
(G-40044)	-	MOS
(G-40045)	-	MOS
(G-40046)	-	MOS
(G-40047)	-	MOS
(G-40049)	-	MOS
(G-40072)	-	MOS
(G-40077)	-	MOS
Phaseolus acutifolius var. latifolius Freem.		
(G-40018)	-	MOS
(G-40030)	-	MOS
(G-40035)	-	MOS
(G-40040)	-	MOS
Phaseolus angularis (Willd.) W.F. Wight (= Vigna angularis (Willd.) Ohwi & Ohashi)	-	-
Phaseolus anisotrichus Schlect.	-	-
Phaseolus coccineus ssp. coccineus Maréchal, Marscherpa & Stainer		
(COC-105)	-	MOS
(COC-115)	-	MOT
(COC-150)	-	MOS
(COC-153)	-	MOS
Phaseolus coccineus ssp. polyanthus (Greenman) Maréchal		
(COC-02)	-	MOS
(COC-083)	-	MOS
(COC-084)	-	MOS,ST
Phaseolus filiformis Benth.	-	-
Phaseolus lunatus L.		
'Henderson Bush'	L	-
(G-25110)	L	-
(G-25145)	L	-
(G-25172)	L	-
(G-25181)	-	-
(G-25191)	-	-

Table 8 (cont.).

Phaseolus vulgaris L.		
'Actopan x Sanilac-37' (G-6368)	-	MOS
'Amanda' (G-4978)	-	MOS
'Azufrado' (G-2843)	-	MOS
'Bataaf'	-	MOS,EP,R,ST
'Bountiful' (G-5496)	-	MOS
'Brazil 2'	-	MOS
'Bush Romano-14' (G-5722)	-	MOS
'Callactlan' (G-0278)	-	MOS
'Cuilapa 72' (G-4489)	-	MOS
'Diacol Calima' (G-4494)	-	MOS
'Dubbele Witte' (G-6721)	-	MOS,EP,ST
'Exrico 23'	-	MOS
'Great Northern U.I. 31' (G-5710)	-	MOS
'Great Northern U.I. 123' (G-5487)	-	MOS,ST
'Honduras 46' (G-5448)	-	MOS
'ICA Pigao' (G-5773)	-	MOS
'Improved Tendergreen'	-	MOS
'Imuna'	-	MOS
'IVT 7214 (G-11270)	-	MOS
'IVT 7233 (G-11269)	-	MOS,ST
'Jamapa' (G-4486)	-	MOS
'Jubila'	-	MOS
'Manteigo Preto-20' (G-5731)	-	VB,MOS
'Michelite 62' (G-3942)	-	VY,MOS
'Monroe' (G-6096)	-	MOS,ST
'Namur Guroba' (G-1242)	-	VB,MOS
'Nep 2' (G-4459)	-	MOS
'Noordholandse Bruine'	-	VY,MOS,ST
'Pinto U.I. 111' (G-5046)	-	MOS
'Pinto U.I. 114' (G-4449)	-	MOS
'Pinto U.I. 650' (G-5768)	-	MOS
'Pinto Ovary' (G-6271)	-	MOS
'Pinto Olathe'	-	MOS
'Porrillo 1' (G-4481)	-	MOS
'Porrillo Sintético (G-4495)	-	VC,MOS
'Preto 897' (G-5743)	-	VC,MOS
PI 207262 (G-1320)	-	MOS
PI 313664 (G-2551)	-	VY,MOS
'Redlands Greenleaf-B' (G-5745)	-	MOS,EP
'Redlands Greenleaf-C' (G-5746)	-	MOS,ST
'Red Mexican U.I. 34' (G-5048)	-	MOS
'Red Mexican U.I. 35' (G-6384)	-	VY,MOS
'Rico Pardo 896' (G-4468)	-	VB,MOS
'Sanilac (G-4498)	-	MOS
'Stringless Green Refugee' (G-0416)	-	MOS,EP,ST
'Topcrop' (G-4014)	-	MOS,R
'Widusa' (G-4503)	-	MOS,EP,ST
Advanced breeding lines developed by CIAT, EP 1980: see Appendix		
Phaseolus vulgaris var. aborigineus (Burk.) Baudet. (G-6388)	-	-
Physalis ixocarpa Brot. ex Hornem.	-	-
Psophocarpus tetragonolobus (L.) DC.	-	-

Table 8 (cont.)

Rhynchosia minima (L.) DC.	-	MOS
Ricinus communis L.	-	-
Sesamum indicum L.	-	-
Solanum torvum Sw.	-	-
Vigna radiata (L.) R. Wilzek (AC-009)		
PI 213012	-	-
Vigna unguiculata L.		
'Antioquia Linda'	-	-
'Bush Sitao'	-	-
'Cabeata Negra'	-	-
'California Blackeye'	-	-
'Floricream'	-	-
'Hambru'	-	-
'Monteria'	-	-
PI 292889	-	-
PI 293476	-	-
PI 352885	-	-
PI 353380	-	-
Vigna unguiculata ssp. sesquipedalis (L.) Verde (= V. sesquipedalis (L.) Fruwirth.)	-	-

EP = epinasty; L = latent; MOS = mosaic; MOT = mottle; R = rugosity; ST = stunting; VB = vein banding; VC = vein clearing; VY = vein yellowing; - = no symptoms.

NOTE: The G, COC or AC numbers which follow behind the name of certain test plants are CIAT's germplasm collection accession numbers.

5.3 *Results*

5.3.1 Mechanical inoculation and host range

The virus was successfully transmitted by mechanical inoculation to beans (*P. vulgaris*). In all transmission experiments 100% infection was achieved. Though washing of the inoculated leaves was not important for infection, in certain cases such as with *P. vulgaris* 'Diacol Calima' it proved useful in preventing scorching of the inoculated leaves, which may have been due to Na_2SO_3 used in the buffer. The reactions of the test plants to inoculation with BMMV-CIAT are given in Table 8. The results show that the virus has a very narrow host range. All seven species of the genus *Vigna* tested showed immunity to BMMV-CIAT infection. Among the six species of the genus *Phaseolus* tested, only *P. anisotrichus* and *P. filiformis* showed immunity to BMMV-CIAT, whereas all the cultivars and varieties of *P. acutifolius*, *P. coccineus* and *P. vulgaris* tested were susceptible. Some cultivars of *P. lunatus* showed local latent infection while the others were immune. After inoculation of the primary leaves of *P. vulgaris* plants, the first trifoliolate leaves usually showed a very mild vein yellowing which later developed into a very mild chlorotic mosaic. The symptoms were usually slightly more severe in the second trifoliolate leaves. As the plant matured the symptom severity was reduced drastically and only a very mild chlorotic mosaic was visible. In certain cultivars, such as in *P. vulgaris* 'Widusa', the first symptom de-

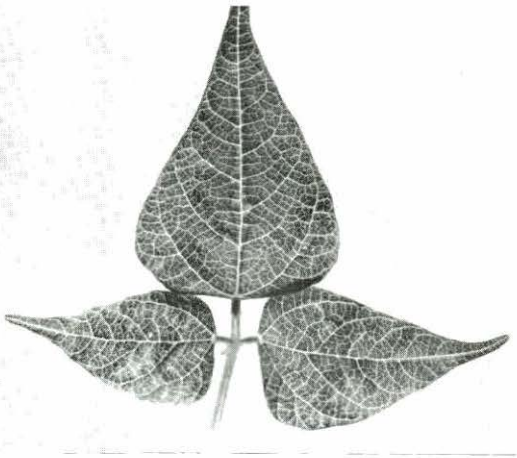


Fig. 12. P. vulgaris 'Widusa' infected with BMMV-CIAT with vein yellowing in the first trifoliolate leaf.

veloped as a mild vein yellowing at the basal part of the leaves (Fig. 12 and later the symptoms disappeared. The plant growth was not impaired and normal-looking pods were produced. In many of the *P. vulgaris* cultivars tested the symptoms were hardly visible. In *P. vulgaris* forma *regressiva* the mosaic was only visible in transmitted light. However, in certain cultivars of *P. vulgaris*, especially the CIAT advanced breeding lines (EP 1980; see Appendix) the mosaic was stronger and the plants could be used as indicators for this virus. Diagnostic mosaic developed two weeks after inoculation in *P. acutifolius* and *G. max* plants.

5.3.2 Transmission by insects and mites

Chrysomelid beetles, *Diabrotica balteata* and *Cerotoma facialis* were efficient vectors of BMMV-CIAT. Out of the six plants used in the experiment, four developed symptoms three weeks after inoculation with *D. balteata*, whereas *C. facialis* transmitted the virus to all test plants. No transmission occurred when leafhoppers (*Empoasca kraemeri*), aphids (*Aphis gossypii*) and mites (*Polyphagotarsonemus latus*) were used.

Table 9. Different cultivars of P. vulgaris tested for seed transmission of BMMV-CIAT

Cultivar	Number of seeds planted	Number of seeds germinated	Number of plants infected	% Transmission
'Amanda'	244	152	3	2.0
'Bountiful'	84	62	1	1.6
'Dubbele Witte'	183	146	2	1.4
'Nep 2'	34	34	1	2.9
'Pinto U.I. 114'	192	120	2	1.7
'Pinto U.I. 650'	84	83	3	3.6
'Porrillo Sintético'	37	18	0	0
'Redlands Greenleaf-B'	184	96	2	2.1
'Stringless Green Refugee'	296	252	3	1.2
'Topcrop'	133	72	1	1.3
'Widusa'	166	121	3	2.5

5.3.3 Transmission through seed

The seeds collected from infected plants did not show any external symptoms of infection. However, in all the bean cultivars tested transmission of BMMV-CIAT through seed occurred (Table 9). The percentage of transmission ranged from 1.2% in *P. vulgaris* 'Stringless Green Refugee' to 3.6% in *P. vulgaris* 'Pinto 650'.

5.3.4 Transmission by dodder

Cuscuta spec. transmitted BMMV-CIAT to two of the three *P. vulgaris* 'Nep 2' plants and in *P. vulgaris* 'Diacol Calima' to one out of three plants used. The infected plants developed symptoms one month after training the *Cuscuta* spec. onto the test plants, but it was possible to detect the virus six days earlier with serology.

5.3.5 Stability in sap

The virus had a dilution end-point ranging from 10^{-6} to 10^{-11}. In all experiments conducted to determine the dilution end-point, the results varied greatly. As the experiments were repeated under constant conditions, the reasons for the variations in results are not known. The virus was still infective after heating for 10 min at 84°C but non-infective after a similar time at 86°C. The longevity *in vitro* of BMMV-CIAT was between 60 and 70 days at room temperature.

5.3.6 Purification

Irrespective of the purification method used, infectious preparations of BMMV-CIAT were obtained. The virus yield ranged from 300-350 mg per kg of infected tissue. The use of a 40% (w/v) sucrose-foot in the final purification step completely eliminated the need for rate-zonal centrifugation to separate plant proteins, thus reducing the time needed for obtaining purified virus preparations.

Experiments performed indicated that large amounts of virus could be isolated from the roots and stems of infected plants, as indicated by the absorbance of the respective virus suspensions at 260 nm (Fig. 13).

After rate-zonal density gradient centrifugation only a single opalescent band was visible between 2.2 and 2.5 cm below the meniscus. When the gradients were fractionated, a single UV-absorbing region was observed (Fig. 14). Rate-zonal density gradient centrifugation of BMMV-W singly and in a mixture with BMMV-CIAT showed only one opalescent band in each of the tubes between 2.7 and 2.9 below the meniscus. When the gradients were fractionated a single UV light absorbing region was observed in each of the tubes (Fig. 15). These results indicate that BMMV-CIAT and BMMV-W have the same sedimentation coefficients.

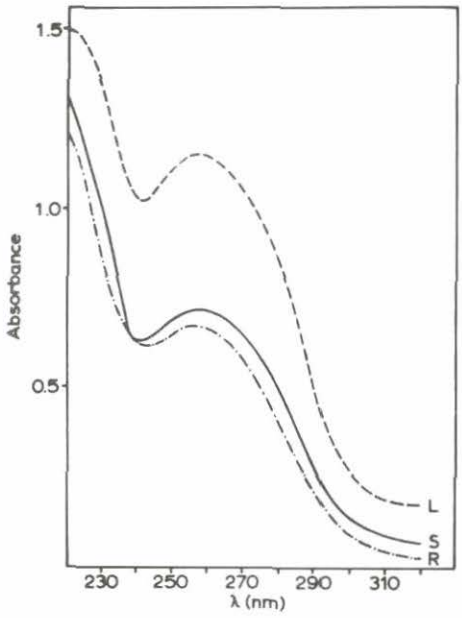


Fig. 13. UV-absorption spectrum of BMMV-CIAT isolated from leaves (L), stems (S) and roots (R).

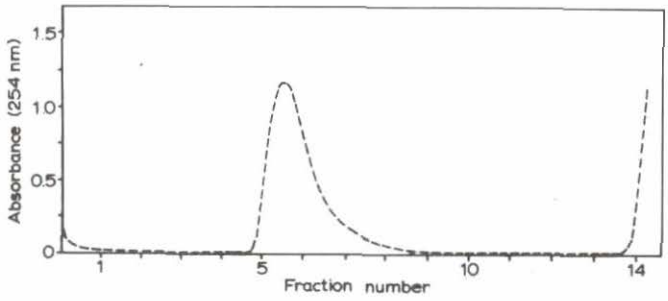


Fig. 14. UV-absorption pattern of BMMV-CIAT after rate-zonal centrifugation. Sedimentation is from left to right.

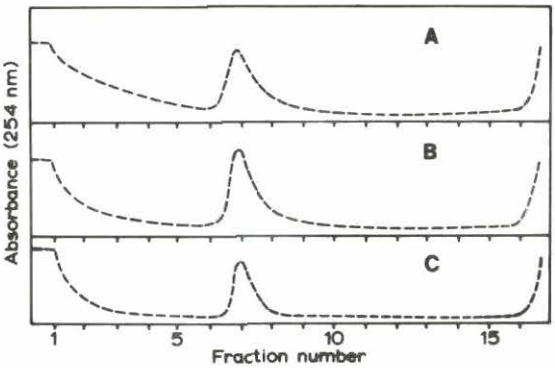


Fig. 15. UV-absorption patterns of BMMV-CIAT (A), BMMV-CIAT + BMMV-W mixture (B) and BMMV-W (C) after sucrose gradient centrifugation. Sedimentation is from left to right.

5.3.7 Properties of particles

5.3.7.1 UV-absorption spectrum

The UV-absorption spectrum of purified BMMV-CIAT was typical of a nucleoprotein (Fig. 16). The maximum absorption was at 262 nm, the minimum at 245 nm. The $E_{max/min}$ ratio was 1.54, the $E_{260/280}$ 1.52 and the $E_{260/240}$ 1.23 (all results corrected for light scattering). The $E_{260/280}$ ratio without correction for light scattering was 1.57.

5.3.7.2 Buoyant density

After centrifuging the virus suspension in caesium sulphate solution for 17½ h there was only one opalescent band. Out of the 28 fractions collected from the centrifuged suspension, only fraction 12 showed absorption at 260 nm (Fig. 17). This fraction was infective and had a density of 1.3637 g/cm^3.

5.3.7.3 Sedimentation coefficient

In the analytical ultracentrifuge the virus sedimented as a single component. The average sedimentation coefficient of the virus was 132 S.

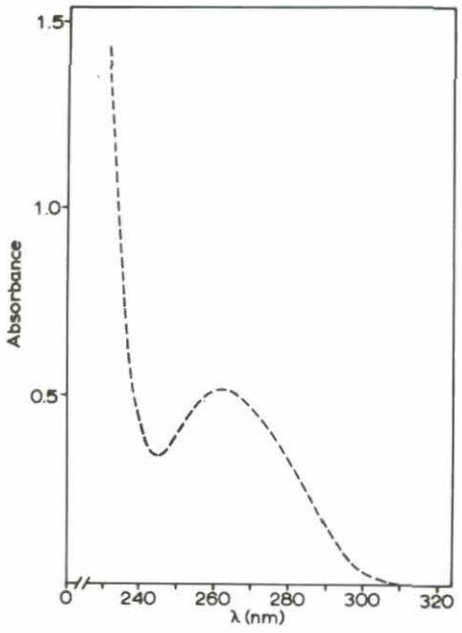


Fig. 16. UV-absorption spectrum of BMMV-CIAT in 0.02 M sodium phosphate buffer pH 7.0, corrected for light scattering.

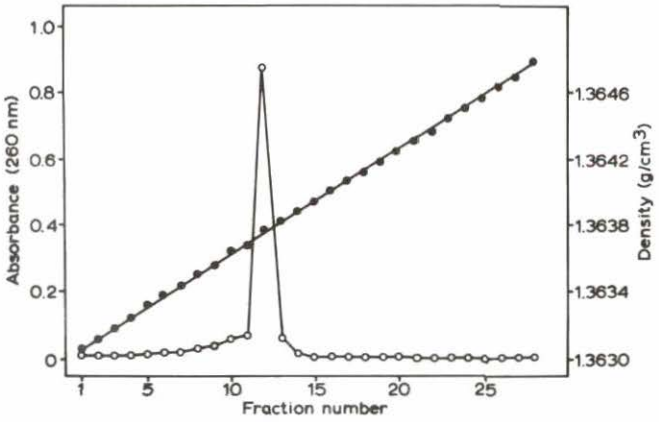


Fig. 17. UV-absorption pattern of the fractions collected after equilibrium centrifugation of BMMV-CIAT in caesium sulphate. • = density of the fractions; o = absorbance at 260 nm.

5.3.8 Particle morphology

Purified BMMV-CIAT suspensions contained many isometric particles. Some of these particles had been partially or fully penetrated by uranyl acetate stain, but the majority of them seemed intact and unaffected by the stain (Fig. 18). The average diameter of the particles was 32.2 nm (variance 1.37 and standard deviation 1.27) as compared to a length of TMV of 300 nm.

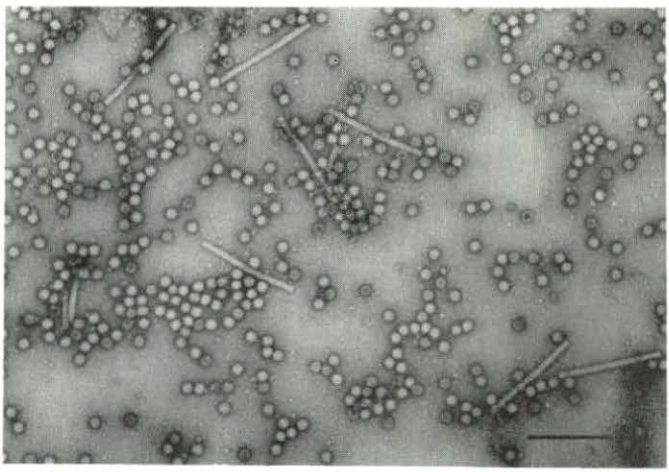


Fig. 18. Purified preparation of BMMV-CIAT stained with 2% uranyl acetate pH 4.5. The rod-shaped particles of TMV were added as an internal size standard. Bar represents 250 nm.

5.3.9 Particle composition

5.3.9.1 Nucleic acid

Approximately 30% of the nucleic acid could be isolated from BMMV-CIAT. The isolated nucleic acid was infective at a concentration of 0.01 µg/ml but not at 0.002 µg/ml when inoculated onto *P. acutifolius* var. *latifolius*.

The UV-absorption spectrum of the nucleic acid showed a maximum absorption at 256-257 nm and minimum absorption at 226-227 nm (Fig. 19). The value for $E_{260/230}$ was 2.23.

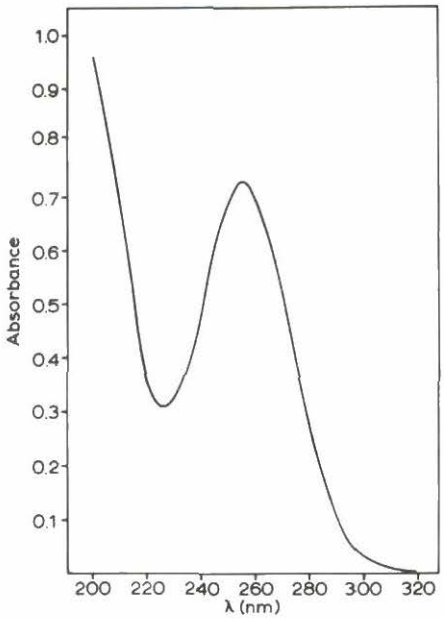


Fig. 19. UV-absorption spectrum of BMMV-CIAT nucleic acid in distilled water.

5.3.9.1.1 Type of nucleic acid

The BMMV-CIAT nucleic acid and the TMV-RNA displayed a blue colour in the orcinol reaction, typical for ribose, while the controls and DNA solutions remained yellowish. In the diphenylamine reaction the DNA solution developed a green colour typical for DNA, while the nucleic acid-solutions of BMMV-CIAT and TMV and the control solutions did not change colour.

The infectivity of all BMMV-CIAT nucleic acid preparations in buffer was abolished by treatment with pancreatic RNase-A (1 µg/ml). Treatments with DNase-1 (5 µg/ml) in the presence of 2mM Mg^{2+} had no effect.

5.3.9.1.2 Base composition

In the dried filter paper after chromatography four well-separated spots were visible under UV light. The absorption curve after eluting the four spots in 0.1 N HCl were identical to those of guanine, adenine, cytidylic acid and uridylic acid. The molar base ratios were: guanine 21.6 ± 1.2, adenine 25.5 ± 1.0, cytosine 26.4 ± 0.5 and

uracil 26.3 ± 1.3. The nucleic acid of BMMV-CIAT appeared to be relatively rich in cytosine and uracil, which together accounted for about 52% of the bases, while the guanine/adenine ratio was 0.85.

5.3.9.1.3 Phosphorus content and nucleic acid content

The phosphorus analysis of seven different virus samples gave a mean value of 1.7% ± 0.3%.

From the 260/280 ratio of 1.52 for BMMV-CIAT (section 5.3.7.1) a nucleic acid content of about 16.5% was estimated. Using the buoyant density which was 1.3637 (section 5.3.7.2) a nucleic acid content of about 23.1% was estimated. The calculated nucleic acid content of BMMV-CIAT using the base composition and phosphorus content was 18.49% ± 0.65%. From these data the percentage of nucleic acid in BMMV-CIAT was 19%.

5.3.9.1.4 Molecular weight

Only one species of RNA was detected by gel electrophoresis of BMMV-CIAT nucleic acid (Fig. 20). The molecular weight for BMMV-CIAT nucleic acid calculated from its electrophoretic mobility, relative to those of the 4 RNA species of CCMV was 1.37 x 10^6D.

Fig. 20. Polyacrylamide gel electrophoresis of BMMV-CIAT-RNA and marker RNAs. A: The four species of cowpea chlorotic mottle virus-RNA (1, 2, 3 and 4); B: BMMV-CIAT-RNA.

5.3.9.2 Coat protein

5.3.9.2.1 Molecular weight

Only one polypeptide was detected by gel electrophoresis of the BMMV-CIAT coat protein (Fig. 21). The molecular weight of this polypeptide calculated from its electrophoretic mobility, relative to the markers BSA, CA, the coat proteins of CCMV, SBMV, BCMV and the larger coat protein of CPMV, was 43,000 ± 1500. The smaller coat protein of CPMV showed an anomalous electrophoretic behaviour, possibly due to its proteolytic sensitivity being a small protein, and was therefore not used for determining the molecular weight of BMMV-CIAT. The BMMV-W coat protein co-migrated as a single band when run in the same gel along with BMMV-CIAT polypeptide, indicating that the molecular weight of the coat proteins of BMMV-CIAT and BMMV-W were the same.

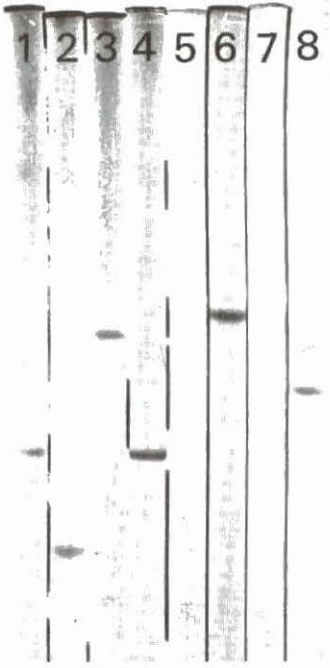


Fig. 21. Polyacrylamide gel electrophoresis of BMMV-CIAT coat protein (c.p.) as compared with marker proteins. 1. southern bean mosaic virus bean strain c.p.; 2. cowpea chlorotic mottle virus c.p.; 3. cowpea mosaic virus c.p.'s; 4. carbonic anhydrase; 5. bovine serum albumin; 6. BMMV-CIAT c.p.; 7. BMMV-W c.p.; 8. bean common mosaic virus-NL strain c.p.

5.3.10 Serology

The antiserum prepared against BMMV-CIAT had a titre of 1/256 in the agar double-diffusion test. The clearest precipitation lines were observed at a dilution of 1/32 of the bean sap containing BMMV-CIAT and 1/8-1/64 dilution of the antiserum. No reactions were noticed in tests with sap from diseased and healthy leaves against normal serum, nor in tests with sap from healthy leaves against the antiserum.

BMMV-CIAT did not give any reaction with the different antisera tested, except with its homologous antiserum and with antiserum to BMMV-W. When BMMV-CIAT and BMMV-W were placed in adjacent wells and tested against the antisera to BMMV-CIAT and BMMV-W there was fusion of precipitation lines without spur formation, indicating that the two antigens are closely related if not identical.

5.3.11 Relations with cells and tissues

5.3.11.1 Light microscopy

No inclusions of BMMV-CIAT could be found in the infected epidermal strips and in the mesophyll cells of *P. vulgaris* 'Nep 2' and *P. acutifolius* var. *latifolius* stained with Azure A and O-G stains. However, the cytoplasm of diseased mesophyll cells stained with Azure A generally dark purplish to magenta, the coloration being most intense in the cells adjacent to the veins. The veins generally stained

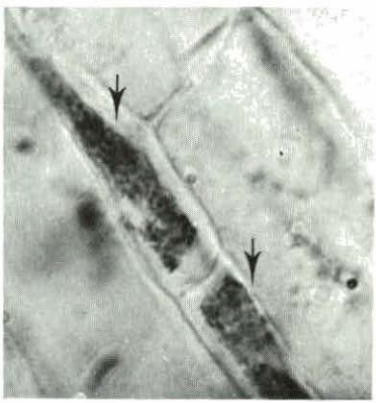


Fig. 22 Phloem cells in stems of P. vulgaris 'Nep 2' infected with BMMV-CIAT showing darkly stained granular material (arrows). Magnification: x 2520.

bluish-purple and no granulation or aggregation could be detected in these tissues. Free-hand sections of stems and roots stained with Azure A, showed very densely stained phloem and cells adjacent to it. In certain cases a darkly stained granular material completely occupying the phloem cells could be observed (Figs. 22 and 23). Such granulation was most abundant in the roots especially in *P. acutifolius* var. *latifolius*. Such structures were not found in healthy tissues.

Fig. 23. Phloem cells in roots of P. acutifolius var. latifolius infected with BMMV-CIAT showing darkly stained granular material (arrows). Magnification: x 2100.

5.3.11.2 Electron microscopy

Ultrathin sections of the mesophyll of *P. acutifolius* var. *latifolius* infected with BMMV-CIAT showed cells in a severe state of disintegration randomly distributed among healthy-looking cells (Fig. 23). These former cells had no central vacuole and the cytoplasm was aggregated to a mass filled with vacuoles and membranes. Within these vacuoles groups of virus particles were present (Fig. 24B). Ultrastructural studies of roots of *P. acutifolius* var. *latifolius* infected with BMMV-CIAT showed the presence of large numbers of virus particles in collenchyma cells of the vascular bundle (Figs. 25, 26 and 27). In some cells the virus particles were arranged in crystals (Fig. 25) while in others the particles were randomly distributed either in the whole cell space (Fig. 26) or only in a vacuole (Fig. 27). The average diameter of the virus particles was approximately 26 nm.

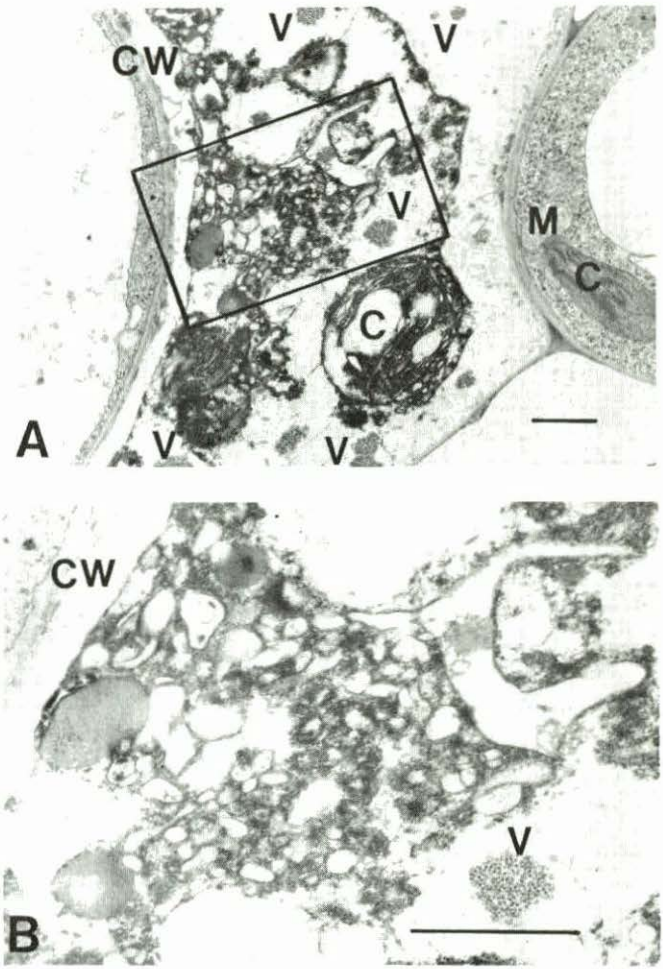


Fig. 24. Ultrathin section of the mesophyll of P. acutifolius var. latifolius infected with BMMV-CIAT showing disintegrating cells. C = chloroplast; CW = cell wall; M = mitochondrion; V = virus particles. B is an enlargement of the inset in A. Bars represent 1 µm.

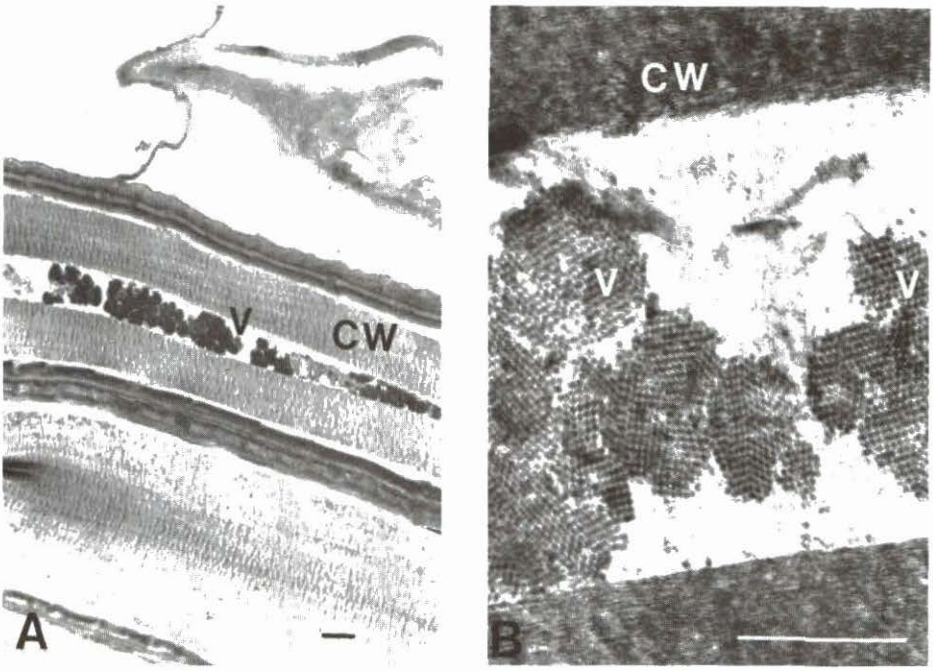


Fig. 25 A en B. Ultrathin sections of *root* cells of P. acutifolius var. latifolius infected with BMMV-CIAT showing masses of aggregated virus crystals within the collenchyma cells of the vascular bundle. B is an enlargement of such masses. CW = cell wall; V = virus crystals. Bars represent 1 µm.

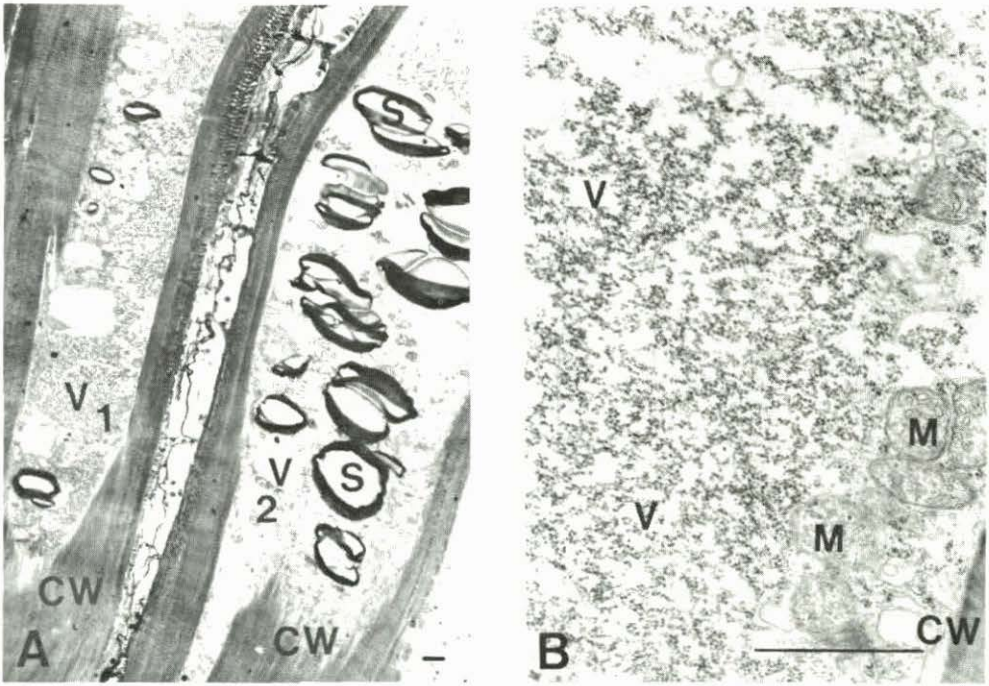
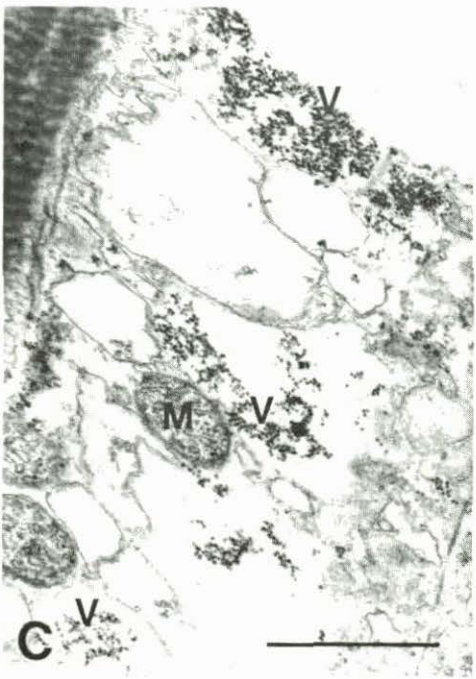


Fig. 26. Ultrathin sections of root collenchyma cells of P. acutifolius var. latifolius infected with BMMV-CIAT showing virus particles (V) scattered in the cytoplasm and in small vacuoles. B and C are enlargements of the respective regions 1 and 2 in A. CW = cell wall; M = mitochondrion; S = starch grain. Bars represent 1 µm.



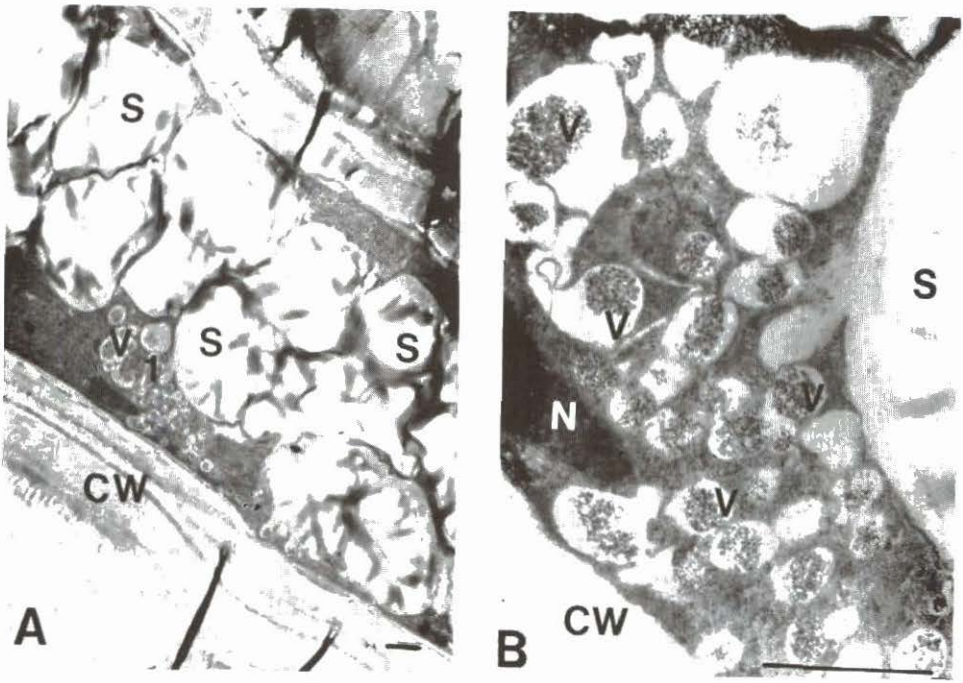


Fig. 27. Ultrathin section of a root collenchyma cell of P. acutifolius var. latifolius infected with BMMV-CIAT showing aggregated masses of virus particles (V) within vacuoles. CW = cell wall; N = nucleus; S = starch grain. B is an enlargement of region I in A. Bars represent I µm.

5.4 *Discussion and conclusions*

Bean mild mosaic virus-CIAT isolate was readily transmitted mechanically to legumes. Chrysomelid beetles *D. balteata* and *C. facialis* proved to be efficient vectors of the virus. Although the results obtained under glasshouse conditions showed *C. facialis* to be a more efficient vector than *D. balteata* this may not necessarily be the case under field conditions as well. The artificial conditions used in the glasshouse experiment may not have been optimal for *D. balteata* to be an efficient vector for the virus. Though BMMV-CIAT is readily transmitted mechanically, the inability of *E. kraemeri*, an insect with sucking mouth parts, to transmit the virus makes it likely that transmission by *D. balteata* and *C. facialis* cannot merely be explained by mechanical contact between the virus-contaminated

mouth parts and the plant. The more complex character of transmission by beetles has also been recognised for other virus diseases (Walters, 1964). Though Waterworth et al. (1977) did not use *D. balteata* and *C. facialis*, but *Epilachna varivestris* and *D. undecimpunctata* in their transmission experiments, the authors suspected *D. balteata* and *C. ruficornis* to be vectors as well.

BMMV-CIAT is seed transmitted, the percentage ranging from 1.2-3.6% in the different *P. vulgaris* cultivars tested. The percentage of seed transmission was low in comparison with another beetle transmitted virus in legumes, like for instance CPMV with a transmission percentage of 10 in *V. sinensis* (Shepherd, 1964). However the presence of beetles in the field throughout the year is sufficient to start an epidemic in field plantings. The small number of seeds tested from *P. vulgaris* 'Porrillo Sintético' may have been the cause for not detecting any seed transmission in this bean cultivar. No reports are available on the seed transmission of BMMV-W.

The host range of BMMV is very narrow. No resistant or hypersensitive host was found among the *P. vulgaris* cultivars and CIAT breeding lines tested. The symptoms expressed by most of the bean cultivars are hardly visible and in certain cases with age the virus becomes latent. In contrast to the results of Waterworth et al. (1977) BMMV-CIAT did not infect *C. quinoa* and *G. globosa*. Experiments conducted at CIAT with both BMMV-W and BMMV-CIAT did not show any infection of *C. quinoa* and *G. globosa*. The reason for these differences in susceptibility may be due to slight taxonomic differences in the above test plants (Van der Want et al., 1975). Four varieties of *P. coccineus* ssp. *coccineus* and three varieties of *P. coccineus* ssp. *polyanthus* developed mosaic symptoms two weeks after inoculation with BMMV-CIAT and BMMV-W. According to Waterworth et al. (1977) the *P. coccineus* cultivars Achievement, Harrison Tenderpod and Kelvedon Marvel were immune to BMMV-W. As no seeds of these cultivars were available they could not be tested under CIAT conditions. Similarly *P. lunatus* 'Henderson Bush' is immune to BMMV-W (Waterworth et al., 1977), but under CIAT conditions the latter virus caused local latent infection in this cultivar. These differences in results can only be explained by different environmental conditions.

No experiments were conducted to assess the economic loss in beans due to an infection with BMMV-CIAT. Though infected plants grew normally, producing healthy-looking pods, flowering and pod formation were usually delayed by about a week under glasshouse conditions.

In contrast to all other beetle-transmitted viruses (except BMMV-W and SBMV) BMMV-CIAT sediments in sucrose gradients as one particle. From Table 10 it can be seen that with respect to the physical and chemical properties there is a great similarity between BMMV-CIAT and BMMV-W except for the differences in particle size and base composition of the nucleic acid. The diameter of the particles of BMMV-CIAT is greater than that of BMMV-W. This difference may be due to the methods used for measuring the particles. In the measurements of BMMV-W, polystyrene latex spheres and diffraction-grating replica have been used as standards (Waterworth et al., 1977). Both standards are not very accurate (Bos, 1975). The diameter of the polystyrene particles varies greatly (Groenewegen, personal communication, 1981) and if the diffraction-grating replica is being used as a standard, special precautions have to be taken, because of shifting of the specimen stage of the electron microscope during changing of the grids (Elbers and Pieters, 1964). A diffraction-grating replica can be used as a standard only after calibration and measurement of the objective lens current of the electron microscope. To take a micrograph of a sample, keeping the measured objective lens current constant, focussing is done by adjusting the specimen stage. This is only possible if the electron microscope is equipped with an accessory which makes it possible to change the specimen stage in vertical direction. If these precautions are not taken, a diffraction-grating replica cannot be used as a standard (Groenewegen, personal communication, 1981). The use of TMV particles from crude virus-containing sap as an internal standard (Bos, 1975) overcomes all these possible magnification errors. Micrographs thus obtained have an error of less than 2% (Groenewegen, personal communication, 1981).

The guanine and adenine content of the nucleic acid of the two viruses are approximately similar, but there is a significant difference between the cytosine and uracil content. The results for BMMV-W are the averages of five replicates (Waterworth et al., 1977) whereas for BMMV-CIAT the results are averages of nine experiments.

Table 10. Physical and chemical properties of BMMV-CIAT (as determined in the present study) and of BMMV-W (as reported by Waterworth et al., 1977).

Physical and chemical properties	BMMV-CIAT	BMMV-W
Stability in sap		
Longevity in vitro (days)	60 to 70	42
Thermal inactivation point (°C)	84 to 86	84
Dilution end-point	10^{-6} to 10^{-11}	10^{-8}
Properties of the particles		
$A_{260/280}$	1.52	1.52
$A_{260/240}$	1.23	1.23
Sedimentation coefficient ($S^{\circ}_{20,w}$)	132	129
Buoyant density in $CsSO_4$ (g/cm^3)	1.3637	-
Particle morphology	isometric	isometric
Particle diameter (nm)	32	28
Properties of the nucleic acid		
$A_{260/230}$	2.23	2.25
Base composition (%)	G = 21.6 ± 1.2	21.7
	A = 25.5 ± 1.0	25.8
	C = 26.4 ± 0.5	31.5
	U = 26.3 ± 1.3	21.0
Nucleic acid content of the particles (%)	19.0	-
Molecular weight (daltons)	1.36×10^6	1.27×10^6
Properties of the coat protein		
Molecular weight (daltons)	43,500 ± 1500	- (*43,500)
Serological relationship	closely related to BMMV-W	- (*closely related to BMMV-CIAT)

* As determined in the present study.
- not reported

Serological studies showed that BMMV-CIAT and BMMV-W are closely related if not identical and that there is no relationship to any of the other viruses tested. As BMMV-infected bean plants in the field often show very mild symptoms or no symptoms at all, serology remains the only reliable and easiest method available to detect infected plants.

The presence of large crystalline virus aggregates in the phloem of roots of infected *P. acutifolius* var. *latifolius* plants, as seen in the electron microscope, coincided with the inclusions observed in the light microscope. As the formation of virus inclusions is mainly governed by the virus genome, they are the same in sensitive hosts and as such of diagnostic value. Therefore, the BMMV inclusions found with the light microscope can also be used for diagnostic purpose.

CHAPTER 6

CHARACTERIZATION AND FURTHER IDENTIFICATION OF CUCUMBER MOSAIC VIRUS (CMV) ISOLATED FROM BEAN PLANTS SHOWING CHLOROTIC MOTTLE

6.1 *Introduction*

Cucumber mosaic virus (CMV) was first described by Doolittle (1916) as the causal agent of a plant disease. It is distributed throughout the world (Martyn, 1968; Gibbs and Harrison, 1970; Smith, 1972). The virus has an almost unlimited host range (Gibbs and Harrison, 1970; Smith, 1972; Francki et al., 1979). Price (1940) recorded 191 susceptible species in 40 families and Smith (1972) recorded 83 species in 31 families susceptible to the common strain of CMV. The virus exists in nature in a number of strains and variants inducing a variety of symptoms, some of them very different from those of the type virus. The virus is easily transmissible by mechanical means, by more than 60 species of aphids in the non-persistent manner (Kennedy et al., 1962) and by dodder in which the virus multiplies (Schmelzer, 1956). A few reports are available on natural infection of legume crops by CMV (Whipple and Walker, 1941; Hagedorn, 1950; Doolittle and Zaumeyer, 1953; Anderson, 1955; Babovič, 1968; Marrou et al., 1969; Tsuchizaki et al., 1970; Kaiser et al., 1972; Kaiser and Danesh, 1971; Bird et al., 1974; Bos and Maat, 1974; Schmelzer and Schmidt, 1975; Provvidenti, 1976). Reports are also available where *Phaseolus vulgaris* is naturally infected by CMV (Bird et al., 1974; Bos and Maat, 1974; Kaiser et al., 1972; Marrou et al., 1969; Provvidenti, 1976; Schmelzer and Schmidt, 1975; Whipple and Walker, 1941).

The aim of this study was to partially characterize CMV isolated from bean plants showing chlorotic mottle symptoms by host range studies, serology, physical properties and its relation to cells and tissues. Henceforth, this CIAT isolate of CMV is designated CMV-CIAT.

6.2 *Materials and methods*

For a detailed description of the techniques and methods used see section 5.2.

6.2.1 Maintenance of the virus

The virus was maintained and propagated in cowpea (*Vigna unguiculata* 'California Blackeye'). For cross protection experiments Price's yellow strain of CMV, received from the Department of Virology, Agricultural University, Wageningen, was maintained in *N. glutinosa*.

6.2.2 Mechanical inoculation and host range

Systemically infected cowpea leaves were ground in 0.02 M sodium phosphate buffer pH 7.0, (1:1 w/v) in a sterilized mortar. Sap from plants which did not show any symptoms in the host range tests was back-inoculated onto *P. acutifolius* var. *latifolius* or onto *V. unguiculata* 'California blackeye', to check for latent infection.

6.2.3 Transmission by insects and mites, through seed and by dodder

In experiments with insects and mites the virus was maintained for inoculation feeding in *P. vulgaris* 'Nep 2'. *Cuscuta* spec. did not flourish well on *P. vulgaris*, therefore in experiments with *Cuscuta* spec. infected *N. glutinosa* plants were used. Back-inoculations were performed on *P. acutifolius* var. *latifolius* or on *V. unguiculata* 'California Blackeye'.

6.2.4 Stability in sap

Systemically infected leaves of *V. unguiculata* 'California Blackeye' were ground in distilled water (1:1 w/v). *Phaseolus acutifolius* var. *latifolius* was used as an assay plant. For determination of the dilution end-point a series of 10-fold dilutions ($0\text{-}10^{-8}$) was made of the 1:1 diluted sap from the systemically infected leaves of *V. unguiculata*.

6.2.5 Purification and UV-absorption spectrum

Attempts were made to purify CMV-CIAT according to the methods of Scott (1963) and Murant (1965) using infected *P. vulgaris* 'Nep 2' and *V. unguiculata* 'California Blackeye' two weeks after inoculating the primary leaves. Purification experiments were also performed at Wageningen according to the method of Murant (1965) using *N. benthamiana* plants inoculated two weeks before. As this host did not grow well under CIAT conditions, it could not be used there for purification work.

The UV-absorption spectrum of purified CMV-CIAT in 0.06 M potassium phosphate buffer pH 7.5, was measured using a Zeiss M-IV spectrophotometer. Corrections for light scattering were made.

6.2.6 Particle morphology

For electron microscopic studies specimens with purified CMV-CIAT in 0.06 M potassium phosphate buffer were prepared as follows. A drop of the virus suspension was mixed with a drop of 3% glutaraldehyde in 0.3 M potassium phosphate buffer pH 7.0, over a piece of parafilm. After 10 min a carbon-reinforced, formvar-coated copper grid (400 mesh) was floated over it for 2 min. The grid was then washed twice with distilled water, dried and stained with 2% uranyl acetate or 2% PTA and observed under a Siemens Elmiskop 101 electron microscope. Particles of TMV from *N. tabacum* 'White Burley' were used as internal size standard.

6.2.7 Cross protection

For cross protection tests with CMV-CIAT, 12 plants of *N. glutinosa* were used. Eight plants were inoculated with CMV-CIAT and 15 days later four of them (already showing symptoms in the top leaves) challenge-inoculated with the yellow strain of CMV. At the same time four healthy *N. glutinosa* plants were inoculated with the latter strain. The plants were observed for a period of one month.

6.2.8 Serology

Serological studies were performed using the Ouchterlony double-diffusion test as described by Scott (1968) for the Y-strain of CMV. The test was performed in disposable plastic petri dishes. Wells were

cut out in the gel 6 mm apart using a premolded gel cutter. The virus was tested against the following antisera obtained from the Department of Virology, University of Agriculture, Wageningen: CMV-Y (Scott, 1963), CMV-isolate from *Yucca* (Bouwen et al., 1978), CMV isolate from *Nerine* (Maat, unpublished data), CMV-B32 isolate from bean (Bos and Maat, 1974) and homologous antiserum to CMV-CIAT (prepared by Berendien Berk in the Department of Virology, University of Agriculture, Wageningen). The homologous titres of the antisera, as mentioned by the donors, were 128, 128, 128, 64 and 8 for CMV-Y, CMV-*Yucca*, CMV-*Nerine*, CMV-B32 and CMV-CIAT, respectively.

6.2.9 Relations with cells and tissues

Leaves of *P. vulgaris* 'Imuna', *V. unguiculata* 'California Blackeye' and *N. glutinosa* infected with CMV-CIAT were used for light microscopy.

An attempt was made to isolate the crystalline inclusions of CMV-CIAT using the following procedure. Twenty grams of primary leaves of *V. unguiculata* 'California Blackeye' inoculated two weeks before, were homogenized with 40 ml of 2-methoxyethanol in a Waring blender. The homogenate thus obtained was allowed to stand for 10 min and was centrifuged at 7000 *g* for 10 min. The supernatant was discarded and the pellet suspended in 20 ml of 2-methoxyethanol and centrifuged as above after 10 min. The pellet was then suspended in distilled water with 5% SDS. After 30 min the suspension was centrifuged at 7000 *g* for 10 min. The resulting pellet was suspended in 10 ml of Azure A stain, allowed to stand for 30 min and centrifuged at 7000 *g* for 5 min. The pellet obtained was suspended in 10 ml of 2-methoxyethyl acetate and centrifuged at 1000 *g* for 5 min after 15 min of standing. The resulting pellet was then again suspended in 1 ml of 2-methoxyethyl acetate and a drop from this suspension was examined in the light microscope.

For electron microscopy leaf tissue of *N. rustica* infected with CMV-CIAT was used.

Table 11. Reactions of test plants inoculated with CMV-CIAT. (Latin names are according to Bailey, 1977; Encke et al., 1980; Maréchal et al., 1978)

Plant species, cultivar and breeding line tested	Symptoms	
	local	systemic
Amaranthus dubius Mart.	-	-
Arachis hypogaea L.	-	-
Cajanus cajan (L.) Huth.	-	-
Canavalia ensiformis (L.) DC.	-	-
Canavalia gladiata (Jacq.) DC.	-	-
Capsicum annuum L.	-	-
Capsicum frutescens L.	-	-
Chenopodium album L.	CS	-
Chenopodium amaranticolor Coste & Reyn.	CS	-
Chenopodium quinoa Willd.	CS	-
Crotalaria juncea L.	-	-
Cucumis sativus L.		
'Ashley'	-	VY,MOS
'Marketer'	-	VY,MOS
Cyphomandra betacea (Cav.) Sendth.	-	-
Datura stramonium L.	CRS	-
Desmodium ovalifolium Guill & Perr.	-	-
Euphorbia geniculata Orteg. (= E. prunifolia)	-	-
Euphorbia repanda Sweet.	-	-
Glycine max (L.) Merr.	-	-
'ICA Caribe'	-	-
'ICA Faroa'	-	-
'ICA Linea 109'	-	-
'ICA Linea 121'	-	-
'ICA Pelican'	-	-
'ICA Tunia'	-	-
'Improved Pelican'	-	-
Gomphrena globosa L.	-	-
Helianthus annuus L.		
'Dursol Alta'	-	-
'Russia 1'	-	-
Hibiscus cannabinus 'Everglades'	-	-
Ipomoea batatas (L.) Lam.	-	-
Jatropha gossypifolia L.	-	-
Lablab niger Medik.	CS,VN	MOS
Lens culinaris Medik.	-	-
Lycopersicon esculentum Mill.		
'Floridale'	-	-
'Rutgers'	-	-
Macroptilium atropurpureum (Moc. & Sessé) Urb. PI 318685	-	-
Macroptilium lathyroides (L.) Urb. PI 146800	L	L
Mucuna pruriens (L.) DC. (= Stizolobium pruriens (L.) Medik.)	CS	MOS,EP,TN
Mucuna utilis Wall. ex Wight (= Stizolobium utile (Wall.) Piper & Tracy)	CS	MOS,EP,TN
Nicandra physalodes (L.) Gaertn.	-	VY,MOS

Table 11 (cont.).

Nicotiana benthamiana Domin	-	VB,MOS
Nicotiana clevelandii Gray	-	LC,ST
Nicotiana glutinosa L.	CS	VY,CS,MOS,VN
Nicotiana rustica L.	-	MOS
Nicotiana tabacum L.		
'Havana 425'	CS,NLP	MOS
'Samsun NN'	-	MOS
'White Burley'	-	MOS
Oryza sativa		
'Mudgo'	-	-
IR-8	-	-
IR-22	-	-
Phaseolus aconitifolius Jacq. (AC-013)		
(= Vigna aconitifolia (Jacq.) Maréchal)	-	MOS
Phaseolus acutifolius var. acutifolius Gray		
(G-40044)	NS	-
(G-40045)	NS	-
(G-40046)	NS	-
(G-40047)	NS	-
(G-40049)	NS	-
(G-40072)	NS	-
(G-40077)	NS	-
Phaseolus acutifolius var. *latifolius Freem.*		
(G-40018)	NS	-
(G-40030)	NS	-
(G-40035)	NS	-
(G-40040)	NS	-
Phaseolus angularis (Willd.) W.F. Wight		
(= Vigna angularis (Willd.) Ohwi & Ohashi	-	VC,MOS
Phaseolus anisotrichus Schlect.	-	-
Phaseolus coccineus ssp. coccineus		
Maréchal, Marscherpa & Stainer		
(COC-105)	-	MOS
(COC-115)	-	MOS
(COC-150)	-	MOS
(COC-153)	-	MOS
Phaseolus coccineus ssp. polyanthus		
(Greenman) Maréchal		
(COC-02)	NRS	MOS
(COC-083)	VN,NRS	MOS,ST
(COC-084)	NRS	MOS
Phaseolus filiformis Benth.	VN	MOS
Phaseolus lunatus L.		
'Henderson Bush'	-	MOS,EP
(G-25110)	-	MOS
(G-25145)	-	-
(G-25172)	-	-
(G-25181)	-	-
(G-25191)	-	-
Phaseolus vulgaris L.		
'Actopan x Sanilac-37' (G-6368)	CS,R	VB,MOS,R
'Amanda' (G-4978)	NLP	VB,MOS
'Azufrado' (G-2843)	CS	MOS,R

Table 11 (cont.)

'Bataaf'	-	MOS
'Bountiful' (G-5496)	-	MOS
'Brazil 2'	NS	MOS
'Bush Romano-14' (G-5722)	CS,NS	IVC,MOS
'Callactlan' (G-0278)	CS,NS	VC,VB,MOS
'Cuilapa 72' (G-4489)	-	VC,MOS
'Diacol Calima' (G-4494)	NS	MOS
'Dubbele Witte' (G-6721)	NS,NLP	MOS
'Exrico 23'	-	MOS
'Great Northern U.I. 31' (G-5710)	-	VC,MOS
'Great Northern U.I. 123' (G-5487)	-	MOS
'Honduras 46' (G-5448)	NS	VC,MOS
'ICA Pigao' (G-5773)	NS	MOS
'Improved Tendergreen'	-	VC,MOS
'Imuna'	-	MOS
IVT 7214 (G-11270)	NLP,VN	MOS
IVT 7233 (G-11269)	NLP,VN	MOS,VN
'Jamapa' (G-4486)	-	MOS
'Jubila'	CS,NLP	MOS
'Manteigo Preto-20' (G-5731)	CS	VC,MOS,R
'Michelite 62' (G-3942)	-	VC,MOS
'Monroe' (G-6096)	NLP,NRS	MOS,VN
'Namur Guroba' (G-1242)	-	IVC,MOS
'Nep 2' (G-4459)	VN,NLP	MOS,R,ST
'Noordhollandse Bruine'	-	MOS
'Pinto Colorado'	NS,VN	MOS,EP,VN,ST
'Pinto U.I. 111' (G-5046)	NS	VY,MOS,R
'Pinto U.I. 114' (G-4449)	CS,NS	VY,MOS
'Pinto U.I. 650' (G-5768)	NS	VY,MOS,R
'Pinto Ovary' (G-6721)	NLP	MOS,R
'Pinto Olathe'	NLP	VN,EP,MOS,ST
'Porrillo 1' (G-4481)	NLP	VB,MOS,ST
'Porrillo Sintético' (G-4495)	NS,NRS	MOS,EP
'Preto 897' (G-5743)	NS,NLP	IVC,VB,MOS
PI 207262 (G-1320)	CS	VC,MOS,R
PI 313664 (G-2551)	CS	IVC,MOS
'Puregold Wax' (G-6720)	-	VC,MOS
'Redlands Greenleaf-B' (G-5745)	-	VC,MOS,VN
'Redlands Greenleaf-C' (G-5746)	-	VC,IVC,MOS
'Red Mexican U.I. 34' (G-5048)	-	VC,MOS
'Red Mexican U.I. 35' (G-6384)	NS	MOS
'Rico Pardo 896' (G-4468)	CS	VC,IVC,MOS
'Sanilac' (G-4498)	-	VC,MOS
'Stringless Green Refugee' (G-0416)	-	VC,MOS
'Topcrop' (G-4014)	-	IVC,MOS
'Widusa' (G-4503)	-	MOS
Advanced breeding lines developed by CIAT, EP 1980: see Appendix		
Phaseolus vulgaris var. aborigineus (Burk.) Baudet (G-6388)	-	MOS
Phaseolus vulgaris forma regressiva (G-9980)	NS,VN	MOS,VN
Physalis ixocarpa Brot. ex Hornem.	CS,NS	MOS
Psophocarpus tetragonolobus (L.) DC	-	-
Rhynchosia minima (L.) DC	-	MOS
Ricinus communis L.	-	-

Table 11 (cont.).

Sesamum indicum L.	-	-
Solanum torvum Sw	-	-
Vigna radiata (L.) R. Wilzek		
PI 213012 (AC-009)	CS,NS	MOS
Vigna unguiculata L.		
'Antioquia Linda'	NS	MOS
'Bush Sitao'	VY	VY,MOS
'Cabeata Negra'	NS,NLP	MOS,R
'California Blackeye'	CS,NS	MOS,R
'Floricream'	CS	MOS,R
'Hambru'	CS,NS	MOS,R
'Monteria'	VY,NS	MOS
PI 292889	CS,NS	MOS,R
PI 293476	CS,NS	MOS,R
PI 352885	VY,CS	MOS
PI 353380	NS	MOS
Vigna unguiculata ssp. sesquipedalis (L.)		
Verde (= V. sesquipedalis (L.) Fruwirth)	NS	MOS

CRS = chlorotic ringspots, CS = chlorotic spots, EP = epinasty, IVC = interveinal chlorosis, L = latent infection, LC = leaf curling, MOS = mosaic, NLP = necrotic line pattern, NRS = necrotic ringspots, R = rugosity, ST = stunting, TN = top necrosis, VB = vein banding, VC = vein clearing, VN = veinal necrosis, VY = vein yellowing.

Note: the G, COC or AC numbers behind the names of certain test plants are CIAT's germplasm collection accession numbers.

6.3 *Results*

6.3.1 Mechanical inoculation and host range

The virus was transmitted by mechanical inoculation to a number of test plants (Table 11). In all cases over 80% of the plants inoculated became infected. In all the three species of the genus *Chenopodium* chlorotic local lesions appeared three days after inoculation. In *C. amaranticolor* these lesions became necrotic and turned reddish brown in due course of time. *Datura stramonium* developed local chlorotic ringspots without any systemic infection. All cultivars and varieties of *Phaseolus acutifolius* tested reacted with necrotic local lesions 3-5 days after inoculation (Fig. 28). All species of the genus *Nicotiana* tested showed a mild systemic mosaic two weeks after inoculation. In *N. glutinosa* the first systemic symptom consisted of a vein yellowing whereafter chlorotic ringspots began to develop from the base of the leaf. Eventually many leaves became necrotic (Fig. 29). Chlorotic local lesions were formed in *N. glutinosa* and in *N. tabacum* 'Havana 425' one week after inoculation. In the later stage of infection the latter developed a necrotic oakleaf pattern on the inoculated leaves and a mosaic in the developing leaves (Fig. 30). All the plants of the subspecies *coccineus* of *P. coccineus* developed a systemic mosaic without local symptoms, whereas those in the subspecies *polyanthus* first displayed local necrotic concentric ringspots and later a systemic mosaic. The virus caused latent infection in *Macroptilium lathyroides*. All the cultivars of *P. vulgaris* and CIAT's advanced breeding lines (EP 1980) tested were susceptible to CMV-CIAT, locally reacting with chlorotic lesions, necrotic spots or necrotic line pattern and systemically with a mosaic. The severity of the mosaic varied greatly depending on the cultivar used. However, in the bean cultivars Porrillo Sintético, Honduras 46, Porrillo 1 and Argentina 2, the mosaic was very similar to the chlorotic mottle symptoms (Fig. 31). *Phaseolus vulgaris* 'Amanda' exhibited a green vein banding (Fig. 32) two weeks after inoculation. The species of the genus *Vigna* were susceptible to the virus and developed a bright systemic mosaic. In some cultivars of *Vigna unguiculata* the first sign of infection was the development of necrotic local lesions later followed by a systemic mosaic.

Fig. 28. Primary leaves of P. acutifolius var. latifolius. Central and right leaf: small necrotic lesions after inoculation with CMV-CIAT. Left: healthy control.

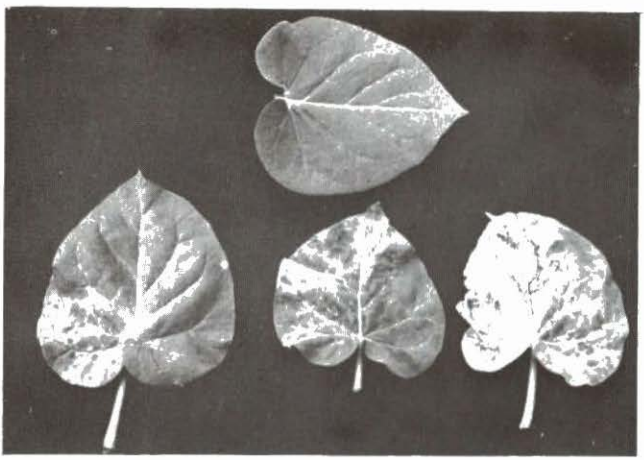


Fig. 29. Leaves of N. glutinosa systemically infected with CMV-CIAT showing chlorotic ringspots and necrosis (below). Upper leaf: healthy control.

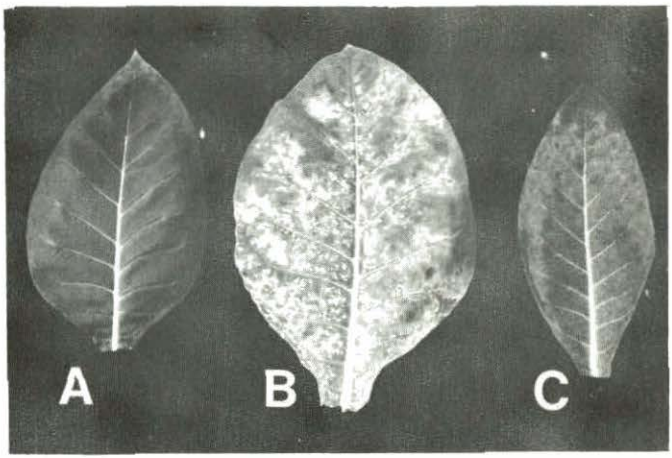


Fig. 30. Leaves of N. tabacum 'Havana 425' inoculated with CMV-CIAT, with local necrotic oakleaf pattern (B) and systemic mosaic (C). A: healthy control leaf.

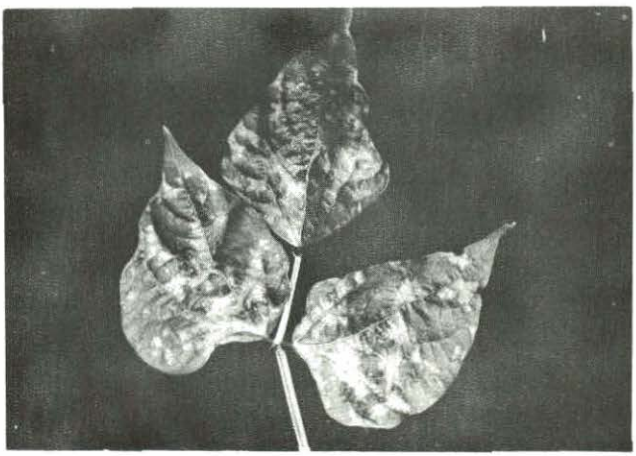


Fig. 31. Leaf of P. vulgaris 'Porrillo Sintético' systemically infected with CMV-CIAT. Note the chlorotic mottle-like symptoms.

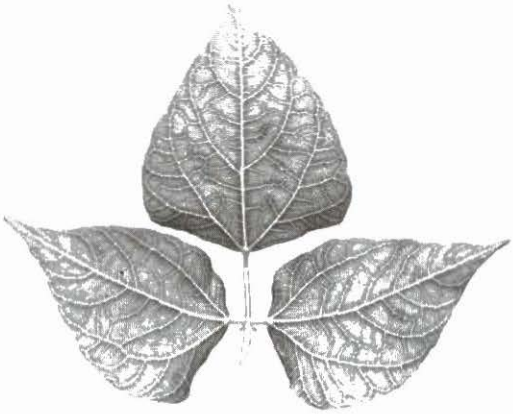


Fig. 32. Leaf of P. vulgaris 'Amanda' systemically infected with CMV-CIAT, showing vein banding.

6.3.2 Transmission by insects and mites, through seed and by dodder

Out of the six test plants used in the transmission experiments with *Aphis gossypii*, two developed symptoms after one month. No transmission occurred when chrysomelid beetles (*Diabrotica balteata* and *Cerotoma facialis*), leafhoppers (*Empoasca kraemeri*) and mites (*Polyphagotarsonemus latus*) were used in the transmission experiments.

The seeds collected from infected plants did not show any external symptoms of infection. However, in all the bean cultivars tested transmission of CMV-CIAT through seed occurred (Table 12). The percentage of transmission in beans ranged from 11.1% in *P. vulgaris* 'Imuna' to 40.2% in *P. vulgaris* 'Topcrop'. Seeds of many cultivars of *P. vulgaris* tested showed a poor germination especially 'Pinto U.I. 114' and 'Amanda', where it was less than 50%. Since no experiment was performed, it is not possible to say that the low percentage of germination in these cultivars was due to virus infection. The virus was also seed-transmitted in *V. radiata* (PI 213012) and *V. unguiculata* 'California Blackeye' (Table 12). Bean plants grown from infected seeds usually showed vein yellowing on the first trifoliolate leaves and mosaic was exhibited only on the second trifoliolate leaf.

In *V. radiata* and *V. unguiculata* 'California Blackeye' the cotyledons showed a green mosaic followed by a systemic bright yellow mosaic in the developing trifoliolate leaves.

Cuscuta spec. transmitted CMV-CIAT to one of the three *P. vulgaris* 'Nep 2' plants and in *P. vulgaris* 'Diacol Calima' to two out of three plants used. The infected plants developed symptoms three weeks after training the *Cuscuta* spec. onto test plants.

Table 12. Plants tested for seed transmission of CMV-CIAT.

Test plant	Number of seeds		Number of plants infected	% Transmission*
	planted	germinated		
P. vulgaris				
'Amanda'	218	108	30	27.7
'Dubbele Witte'	143	128	19	14.8
'Imuna'	91	90	10	11.1
'Pinto U.I. 114'	158	31	7	22.6
'Redlands Greenleaf-B'	68	50	16	32.0
'Stringless Green Refugee'	118	111	31	27.9
'Topcrop'	69	67	27	40.2
Vigna radiata	78	78	12	15.3
V. unguiculata				
'California Blackeye'	199	199	44	22.1

* The percentage (%) of transmission was calculated from the number of seeds germinated.

6.3.3 Stability in sap

The virus had a dilution end-point of 10^{-4} to 10^{-5}. It was still infective after heating for 10 min at 65°C, but non-infective after a similar time at 70°C. The longevity *in vitro* of CMV-CIAT was between 6 and 7 days at room temperature.

6.3.4 Purification and UV-absorption spectrum

The methods of both Scott (1963) and Murant (1965) failed in attempts to purify CMV-CIAT from plants of the legume species mentioned. However, using the method of Murant (1965) CMV-CIAT could be purified easily from *N. benthamiana*. The concentration of the virus was approximately 100 mg/kg of leaf tissue, calculated by using $E_{1\ cm,\ 260\ nm}^{0.1\%} = 5.0$ (Francki et al., 1966).

Purified CMV-CIAT had a typical nucleoprotein absorption spectrum. The maximum absorption was at 262 nm, the minimum at 240 nm. The $E_{260/280}$ ratio was 1.72 (corrected for light scattering).

6.3.5 Particle morphology

Samples of purified and fixed CMV-CIAT contained many isometric particles. Some of them had been partially penetrated by the respective stains used, but the majority of them seemed intact and unaffected (Fig. 33). The average diameter of the particles was 26.4 nm.

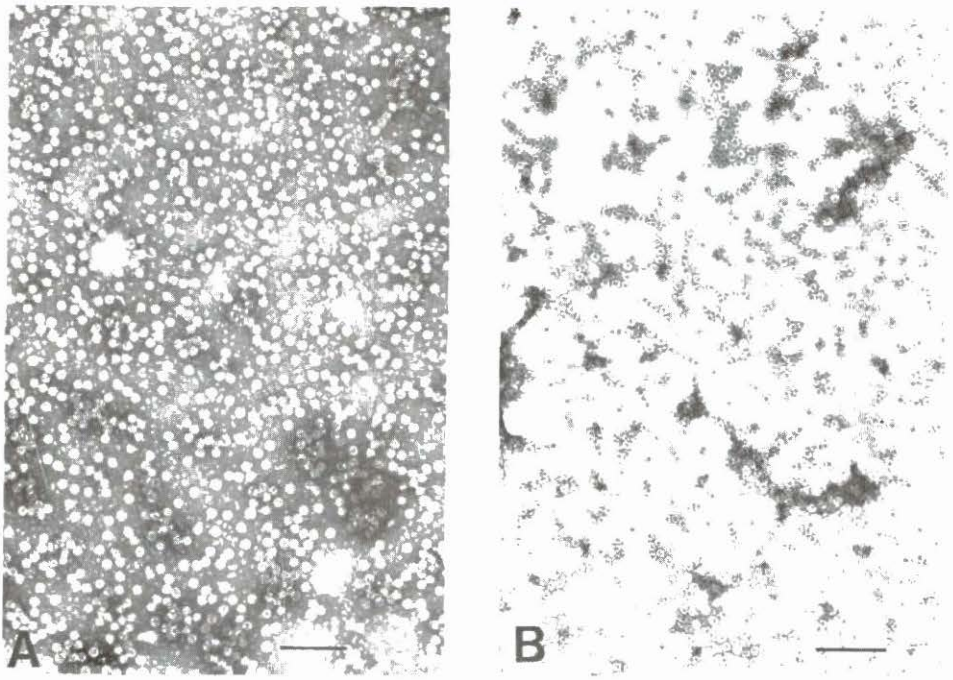


Fig. 33. Purified preparation of CMV-CIAT after glutaraldehyde fixation and stained with PTA (A) or with uranyl acetate (B). Bars represent 200 nm.

6.3.6 Cross protection

The four *N. glutinosa* plants inoculated with CMV-CIAT and those inoculated with CMV-CIAT and challenge-inoculated with the yellow strain of CMV developed a very mild systemic mosaic and necrosis, whereas the four plants inoculated with the yellow strain alone displayed the characteristic bright yellow mosaic. The results indicate that CMV-CIAT protects the plant against the effects of the yellow strain showing that CMV-CIAT is closely related to the latter strain.

6.3.7 Serology

The low homologous titre (1/8) of CMV-CIAT possibly indicates that the immunogenicity of the virus is poor. The CMV-CIAT infected plant sap or unfixed purified virus samples gave two precipitation lines with antiserum to CMV-CIAT, one close to the antigen well and the other close to the antiserum well. Only a single continuous precipitation line without spurs close to the antigen well was formed when the virus was tested against the antisera to CMV-*Yucca*, CMV-Y strain and CMV-B32, indicating close relationship of CMV-CIAT to these three viruses. No precipitation lines were formed when the antiserum to CMV-*Nerine* was tested against CMV-CIAT. There was no reaction between sap from healthy plants and antiserum to CMV-CIAT.

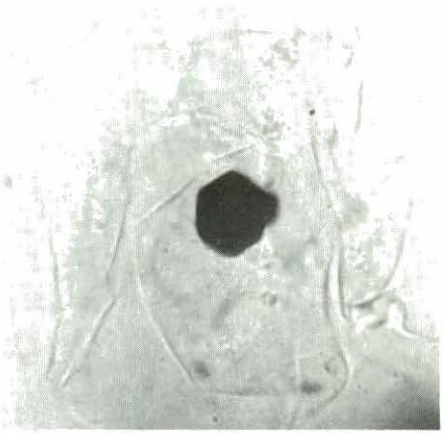


Fig. 34. Crystalline inclusions of CMV-CIAT in V. unguiculata 'California Blackeye' mesophyll cells. Magnification: x 2900.

6.3.8 Relations with cells and tissues

No inclusions could be observed in the epidermal tissues of *N. glutinosa*, *P. vulgaris* 'Imuna' and *V. unguiculata* 'California Blackeye' infected with CMV-CIAT. However, in the mesophyll cells of these infected plants deeply stained crystals were found (Fig. 34). The shape and size of them varied greatly. A majority of them had sharp angles forming rectangles, hexagons and octagons. Some amorphous or semi-circular forms were also found in the tissue. In certain cells more than one crystal could be seen apparently grown from one large crystal. Many of these crystals showed a central, less-stained area

but solid crystals were not uncommon. The largest inclusions were found in the cells adjacent to the main veins. In infected *N. glutinosa* CMV-CIAT crystals appeared in the inoculated primary leaves two weeks after inoculation; in *V. unguiculata* 'California Blackeye' 12 days after inoculation. The crystalline inclusions of CMV-CIAT in *P. vulgaris* 'Imuna' were rather small as compared with those in *N. glutinosa* and in *V. unguiculata* 'California Blackeye'. Large inclusions could only be found in the inoculated primary leaves of *P. vulgaris* 'Imuna', when the plant attained the flowering stage.

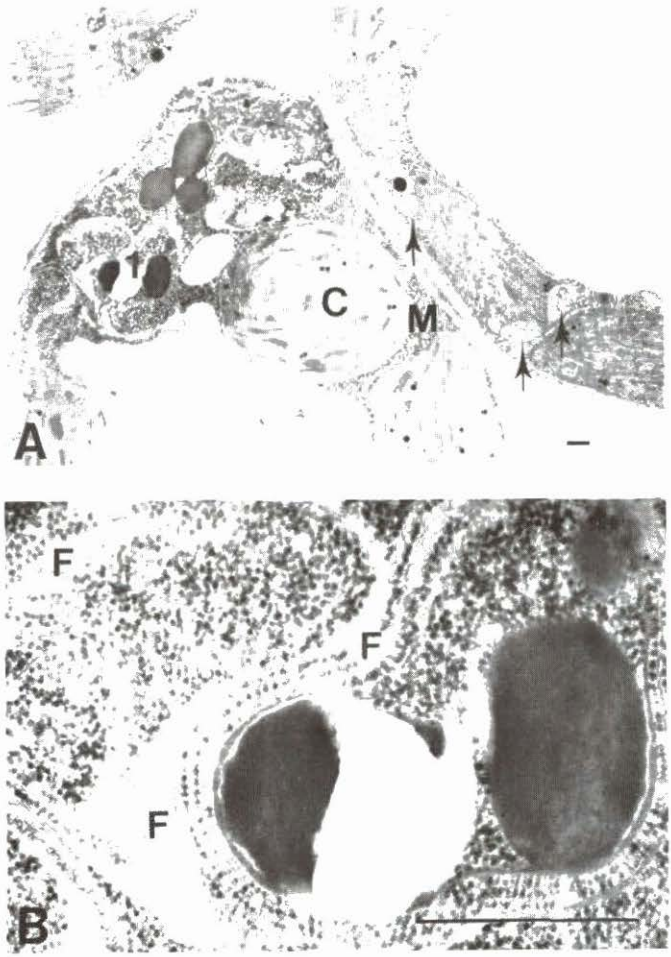


Fig. 35. Ultrathin section of mesophyll cells of N. rustica infected with CMV-CIAT showing densely stained areas in the cytoplasm, invaginations (arrows) and a disintegrated chloroplast (C). B is an enlargement of region 1 in A showing fibrillar elements (F) in the cytoplasm. M = mitochondrion. Bars represent 1 µm.

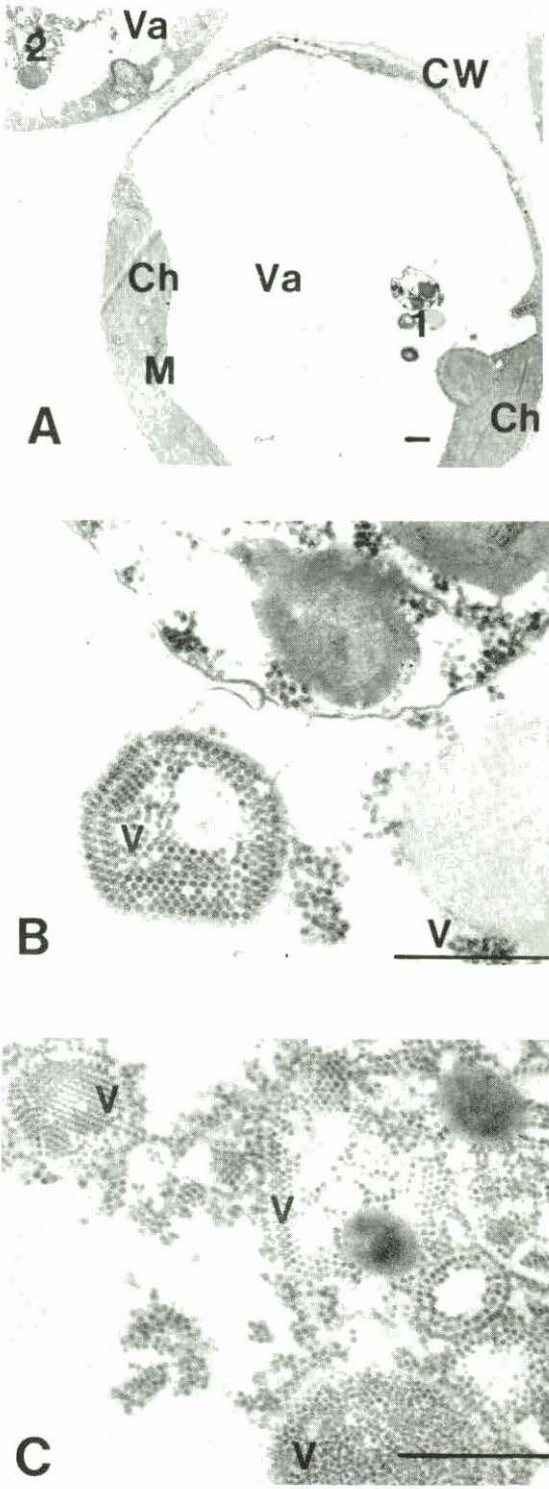


Fig. 36. Ultrathin section of mesophyll cells of N. rustica infected with CMV-CIAT showing aggregated virus particles in vacuoles. B and C are enlargements of the respective regions 1 and 2 in A. Ch = chloroplast; CW = cell wall; M = mitochondrion; V = virus particles; Va = vacuole. Bars represent 1 µm.

The suspension obtained in the experiment performed to isolate the crystalline inclusions showed a large number of the latter along with many unstained roundish bodies probably starch grains. These results indicate that the inclusions sediment at low centrifugal forces.

Ultrathin sections of the mesophyll cells of *N. rustica* infected with CMV-CIAT showed the membrane system of the chloroplast to be in a state of complete disintegration with invaginations of different shapes, sizes and forms (Fig. 35A). Certain areas of the cytoplasm in some cells stained deeply and showed a large amount of fibrillar material. It was difficult to distinguish ribosomes from virus particles in these cytoplasmic areas (Fig. 35B). The vacuoles of some cells contained aggregates of arrayed particles (Fig. 36). These particles in the crystals measured approximately 25 nm. It may be assumed that the virus-induced inclusions in the vacuole as observed in the light microscope are similar to the aggregates of arrayed particles as revealed by electron microscopical studies.

6.4 *Discussion and conclusions*

Since Whipple and Walker (1941) isolated a CMV strain from naturally infected beans in Wisconsin, U.S.A., many other similar isolates have been recovered from beans in East Germany (Schmelzer and Schmidt, 1975), France (Marrou et al., 1969), Iran (Kaiser et al., 1972), Japan (Tsuchizaki, 1973), Puerto Rico (Bird et al., 1974), Spain (Bos and Maat, 1974) and the U.S.A. (Provvidenti, 1976). In East Germany the virus frequently occurs in mixed infections with bean common mosaic virus (BCMV) and occasionally with bean yellow mosaic virus (BYMV) (Schmelzer and Schmidt, 1975). The systemic symptoms produced in certain cultivars of beans due to CMV infection are rather mild or more similar to those in BCMV or BYMV infections (Schmelzer and Schmidt, 1975; Whipple and Walker, 1941).

Serological tests, host range and cross protection prove that the virus isolated at CIAT and described here is CMV. However, many differences exist between CMV-CIAT and type CMV, the most striking being the ability of CMV-CIAT to cause systemic infection in *P. vulgaris*. The host reactions of CMV-CIAT infected plants resemble those of the CMV-B32 isolate of Bos and Maat (1974). However, differences do exist. In *N. glutinosa* for instance, CMV-CIAT caused a systemic mosaic followed by necrosis, whereas in CMV-B32 the plant completely

recovered after a mild mosaic. In *Physalis floridana* CMV-CIAT induced a bright yellow mosaic, whereas CMV-B32 was latent only. Since the CMV-B32 isolate was not available for testing under CIAT conditions, no conclusions can be drawn from the differences found. It was not possible to compare other CMV-legume isolates mentioned in the literature regarding their host range and symptomatology, because of the diversity in test plants used. Generally CMV-CIAT induced a yellow mosaic in *P. vulgaris*, but the symptoms varied greatly in different cultivars. However, in the cultivars Honduras 46 and Porrillo 1 a sharp mosaic, similar to bean chlorotic mottle was produced one month after inoculation with CMV-CIAT. None of the bean cultivars and varieties tested were found to be resistant or hypersensitive to CMV-CIAT.

The virus was transmitted by aphids (*Aphis gossypii*). However, the transmission efficiency was rather low.

Seed transmission of CMV in *P. vulgaris* has already been reported (Bird et al., 1974; Bos and Maat, 1974; Provvidenti, 1976). However, the transmission percentages mentioned in the literature are much lower than that found with CMV-CIAT in *P. vulgaris* 'Topcrop'.

Like type CMV and its strains, CMV-CIAT is a poor antigen. The antiserum produced had a very low titre. This may be due to the instability of CMV in saline when it is injected into the rabbit (Francki et al., 1966; Scott, 1968). Therefore, it might be advisable to prefix the virus in formaldehyde before injection, as has been successfully done with celery yellow vein virus by Hollings and Stone (1962). Without formaldehyde treatment the latter virus did not induce any antibody formation, but after prefixation an antiserum with a high titre could be obtained. Serological reactions showed that CMV-CIAT is closely related to CMV-B32, CMV-Y strain and CMV-*Yucca*. Scott (1968) reported that CMV-Y formed two precipitation lines in the agar double-diffusion tests with its homologous antiserum. Whether one or two precipitation lines will be formed depends on the injection method used (Scott, 1968). The CMV-CIAT antiserum was prepared by intra-muscular injections. Therefore, in agar double-diffusion tests two precipitation lines were formed - one close to the antigen well, due to the undegraded virus particles and the other close to the antiserum well, due to degraded particles. However, serology is not a very reliable method to detect CMV-CIAT in infected plants.

Many times inoculated plants failed to react even when the plants were showing very strong symptoms. This may be due to the formation of inclusions consisting of aggregates of virus particles in the host tissues. These inclusions cannot be disrupted easily to release the virus particles (Christie, personal communication, 1980). Serological testing will be successful only when the formation of inclusions has not yet taken place, so that free virus particles are still present in the cells.

Cross protection tests have proved beyond doubt that CMV-CIAT is closely related to the yellow strain of CMV. The thermal inactivation point and dilution end-point of CMV-CIAT compare well with those of the type CMV and its related strains (Gibbs and Harrison, 1970; Francki et al., 1979). The longevity *in vitro* of CMV-CIAT is much higher (7 days) than that of CMV-B32 which is 24 h (Bos and Maat, 1974). In this respect CMV-CIAT resembles the CMV-strain 17 (9 days) described by Whipple and Walker (1941).

The light microscope might be of use for diagnostic purpose. The large inclusions are of great diagnostic value (Christie and Edwardson, 1977) and can easily be detected. With light microscopy the inclusion bodies could be traced in infected plants within a couple of hours and this method proved to be much more reliable than the serological one. It was shown that in *V. unguiculata* 'California Blackeye' infected with CMV-CIAT, the inclusions are formed in the primary leaves 12 days after inoculation, which coincides with the appearance of systemic symptoms in this host. These inclusions readily sedimented when they were subjected to low centrifugal forces for a very short time. Therefore, if primary inoculated leaves are used as source for virus purification of CMV-CIAT from *V. unguiculata* 'California Blackeye' it should be performed 10 to 11 days after inoculation. The failure to purify the virus from this host as described in section 6.3.4, is most likely due to loss of aggregated virus during the early purification steps, as all the purification experiments were done 14 days after inoculation. At that time the above light microscopic data were not yet available.

Different classifications have been proposed for CMV-isolates on the basis of symptoms (Hollings et al., 1968; Marrou et al., 1975). However, no attempts were made to group CMV-CIAT according to these classification systems, as the latter are based on symptoms obtained under non-tropical conditions. How important environmental conditions

are was obvious from the results with some of the test plants used in host range studies. *Nicotiana glutinosa* plants raised from seeds obtained from the Department of Virology at Wageningen, showed a systemic mosaic followed by necrosis after inoculation with CMV-CIAT in the glasshouses at CIAT, whereas the same species exhibited a mild systemic mosaic which later disappeared when the experiment was performed in the glasshouses of the Department of Virology at Wageningen in May and June. In Europe seasonal differences in growing conditions are known to greatly affect host plant response to viruses (Hollings, 1974). This response is controlled by a combination of temperature and light intensity (Hollings and Stone, 1970). Besides these environmental factors the genotypes of the host plants play a very important role, as pointed out by Van der Want et al. (1975). Provvidenti (1976) pointed out the difficulty in categorizing the various isolates of CMV when he showed that the common CMV strain though it does not infect *P. vulgaris* yet causes a systemic mosaic in other species of *Phaseolus* and *Macroptilium*.

CHAPTER 7

CHARACTERIZATION AND FURTHER IDENTIFICATION OF SOUTHERN BEAN MOSAIC VIRUS (SBMV) ISOLATED FROM BEAN PLANTS SHOWING CHLOROTIC MOTTLE

7.1 *Introduction*

Southern bean mosaic virus (SBMV) was first observed in Louisiana, U.S.A., in 1942 and now it is reported to be widespread in the western and eastern states of the U.S.A. (Gámez, 1980), in many Central and South American countries (Costa, 1972; Murillo, 1967; Yerkes and Patiño, 1960; Zaumeyer and Thomas, 1957), in Africa (Shoyinka et al., 1979; Lamptey and Hamilton, 1974) and in France (Férault et al., 1969). The host range of SBMV is narrow, only legumes are reported to be susceptible to infection, in which it causes mosaic, mottle, systemic necrosis or local lesions (Grogan and Kimble, 1964; Shepherd and Fulton, 1962; Yerkes and Patiño, 1960; Zaumeyer and Harter, 1943a, b). In beans under experimental conditions yield losses up to 83-94% have been recorded. However, in Mexico, Colombia and Brazil the yield loss is considered to be moderate (Costa, 1972; Yerkes and Patiño, 1960). Since Zaumeyer and Harter (1943a) first described SBMV, Shepherd and Fulton (1962) reported about an SBMV strain isolated from cowpea. As the latter strain infected cowpeas but not beans, whereas the former infected beans but not cowpeas, Weintraub and Ragetli (1970) referred to these strains as SBMV-cowpea and SBMV-bean, respectively. Lamptey and Hamilton (1974) described an SBMV strain isolated in Ghana which infected both beans and cowpeas and named it SBMV-Ghana. In addition to these major strains, minor strains differing in electrophoretic mobility and buoyant density have been reported (Magdoff-Fairchild, 1967).

Southern bean mosaic virus is readily transmitted mechanically, by chrysomelid beetles and through seed (Shepherd, 1971). The virus is carried in the seed coat through which the young seedlings may

become infected (McDonald and Hamilton, 1972). The virus may also be carried in the embryo (Uyemoto and Grogan, 1977). Cheo (1955) reported that a high percentage of the immature embryos are infected, but that the virus may get completely or partly inactivated when the embryo matures.

In the present study a SBMV isolate from bean plants showing chlorotic mottle symptoms at CIAT (SBMV-CIAT) was further identified and characterized.

7.2 *Materials and methods*

For a detailed description of the techniques and methods used, see section 5.2.

7.2.1 Maintenance of the virus

The virus was maintained in *P. vulgaris* 'Bountiful' plants. For comparison a bean strain of SBMV originating from Dr. O.P. Sehgal (Missouri, U.S.A.) was used in some experiments.

7.2.2 Mechanical inoculation and host range

Systemically infected leaves of *P. vulgaris* 'Bountiful' were ground in distilled water (1:1 w/v) in a sterile mortar. Sap from plants which did not show any symptoms in the host range tests was back-inoculated onto *P. vulgaris* 'Pinto U.I. 650', to check for latent infection.

7.2.3 Transmission by insects and mites, through seed and by dodder

In experiments with the insects and mite species mentioned, the virus was maintained in *P. vulgaris* 'Bountiful'.

7.2.4 Stability in sap

Systemically infected leaves of *P. vulgaris* 'Bountiful' were ground in distilled water (1:1 w/v). Infectivity was determined by the necrotic local lesions which developed 3 to 4 days after inoculation of the primary leaves of *P. vulgaris* 'Pinto U.I. 650'.

7.2.5 Purification

The virus was purified from systemically infected *P. vulgaris* 'Bountiful' plants 20 days after inoculation using the PEG precipitation method described for BMMV-CIAT (section 5.2.7). Rate-zonal centrifugation of partially purified SBMV-CIAT was performed in linear-log sucrose gradients according to the method of Brakke and Van Pelt (1970). Rate-zonal centrifugation of SBMV-CIAT and the SBMV bean strain, singly and in a mixture, was also performed in linear-log sucrose gradients. The virus yield was calculated using $E^{0.1\%}_{1\ cm,260\ nm} = 5.85$ for SBMV (Shepherd, 1971).

7.2.6 Properties of the particles

7.2.6.1 UV-absorption spectrum

The UV-absorption spectrum of SBMV-CIAT in 0.02 M sodium phosphate buffer pH 7.0 (buffer), was measured using a Beckman DB spectrophotometer. Corrections for light scattering were made according to the optical method described by Englander and Epstein (1955).

7.2.6.2 Buoyant density

A 0.2 ml suspension of purified SBMV-CIAT (5 mg/ml) in buffer was layered over 4.8 ml of caesium chloride (density 1.3610 g/cm^3) prepared in the same buffer as the virus suspension and was centrifuged at 39,000 rpm for 18 h. After centrifugation the bottoms of the tubes were punctured with a needle and portions of three drops were collected. The density of each fraction was measured using a refractometer (Atago, Co., Ltd., Japan). Each fraction was then diluted to 1 ml with buffer, and its absorbance at 260 nm was measured using a Zeiss M IV spectrophotometer.

7.2.6.3 Sedimentation coefficient

The sedimentation coefficient of purified SBMV-CIAT (5 mg/ml) in 0.01 M sodium phosphate buffer pH 7.0, was measured by Dr. Huttinga (IPO) as described in section 5.2.8.3.

7.2.7 Particle morphology

Purified SBMV-CIAT preparations were prepared for electron microscopy as described in section 5.2.9 for BMMV-CIAT.

7.2.8 Particle composition

7.2.8.1 Nucleic acid

The procedure applied for extracting the nucleic acid of SBMV-CIAT was based on the method described by Wilcockson and Hull (1974). To 1.6 ml of SBMV-CIAT at 7.86 mg/ml in 0.025 M sodium phosphate buffer pH 7.5, 0.4 ml of 25% SDS was added and the mixture was heated to 60°C for 3 min in a cellulose nitrate centrifuge tube. To this mixture 70% (w/v) sodium perchlorate in distilled water was added, mixed and centrifuged at 1000 *g* for 5 min. The liquid phase formed was collected by puncturing the bottom of the tube with a needle. To the liquid phase 2.5 volume of cold absolute ethanol was added, and the mixture left at -20°C overnight. The precipitated nucleic acid was concentrated by centrifugation at 12,000 *g* for 10 min. The pellet was dissolved in 5 ml of sterile distilled water.

The UV-absorption spectrum of the purified SBMV-CIAT nucleic acid was measured in a Beckman DB spectrophotometer. The absorption between 320-360 nm did not give a satisfactory straight line. Therefore, corrections for light scattering could not be made. The concentration of the nucleic acid preparation was calculated taking the extinction coefficient to be 25 (Gibbs and Harrison, 1976).

In order to establish the type of nucleic acid the Mejbaum orcinol method and diphenylamine reactions were performed using purified SBMV-CIAT nucleic acid. Tobacco mosaic virus-RNA and DNA solutions were used as controls for RNA and DNA. The effect of pancreatic RNase-A and DNase-1 on SBMV-CIAT nucleic acid was studied.

Base composition, phosphorus content, nucleic acid content and molecular weight of the SBMV-CIAT nucleic acid were determined as described in section 5.2.10.1.

7.2.8.2 Coat protein

The molecular weight of the SBMV-CIAT coat protein was determined by gel electrophoresis as described in section 5.2.10.2.1.

7.2.9 Serology

An antiserum to SBMV-CIAT was prepared by administering four intramuscular injections to a white New Zealand rabbit using purified virus preparations. Serological tests were carried out with the agar double-diffusion test (Ouchterlony, 1962) using various antisera as mentioned in section 5.2.11.

7.2.10 Relations with cells and tissues

Leaves, stems and roots of *P. vulgaris* 'Imuna' infected with SBMV-CIAT were prepared for light and electron microscopy as described in section 5.2.12.

7.3 *Results*

7.3.1 Mechanical inoculation and host range

The virus was transmitted by mechanical inoculation to beans. In all transmission experiments 100% infection was achieved. The reactions of the test plants to inoculation with SBMV-CIAT are given in Table 13. The results show that the virus has a narrow host range which is not completely restricted to legumes. Approximately 80% of *Cucumis sativus* 'Ashley' plants inoculated exhibited a mild vein yellowing in the developing leaves two weeks after inoculation and later turned brown. All the cultivars and varieties of *P. acutifolius* tested were susceptible. Depending on the cultivar or variety the host reactions ranged from chlorotic to necrotic local lesions (Fig. 37) followed by systemic mosaic or top necrosis respectively (Fig. 38). All cultivars of *P. lunatus* tested gave necrotic local lesions 6-7 days after inoculation except for the breeding lines (G-25181)* and (G-25110) which were immune. All plants of *P. vulgaris* 'Pinto' gave necrotic local lesions, three to four days after inoculation (Fig. 39), while the majority of the *P. vulgaris* cultivars and breeding lines showed a systemic mosaic with severely deformed leaves, vein yellowing, rugosity, blistering and leaf curling. In certain cultivars as in *P. vulgaris* 'Monroe' and 'Topcrop' the severely deformed leaves showed vein mosaic, blistering and leaf curling (Fig. 40). Some plants of the cultivar *P. vulgaris* 'Porrillo Sintético', which is resistant to bean common mosaic virus (BCMV), showed necrotic local lesions without any systemic symptoms while the others displayed a mosaic without any local lesions. *Rhynchosia minima* reacted with a systemic mosaic. All five cultivars and two breeding lines of *G. max* showed chlorotic local lesions (Fig. 41) five to six days after inoculation followed by a strong yellow systemic mosaic (Fig. 42).

*The G number is CIAT germplasm collection accession number.

Mucuna pruriens and *M. utilis* developed pinpoint necrotic local lesions five to six days after inoculation. All cultivars of *V. unguiculata* tested showed immunity to SBMV-CIAT infection. All plants of *P. coccineus* and *P. filiformis* tested were also immune to SBMV-CIAT infection.

Table 13. Reactions of test plants inoculated with SBMV-CIAT. (Latin names are according to Bailey, 1977; Encke et al., 1980; Maréchal et al., 1978).

Plant species, cultivar and breeding line tested	Symptoms	
	local	systemic
Aeschynomene spec.	-	-
Amaranthus dubius Mart.	-	-
Arachis hypogaea L.	-	-
Boerhaavia erecta L.	-	-
Brassica juncea (L.) Czern.	-	-
Cajanus cajan (L.) Huth.	-	-
Calopogonium mucunoides Desv. (= Calapogonium mucunoides)	-	-
Canavalia gladiata (Jacq.) DC.	-	-
Capsicum frutescens L.	-	-
Cassia occidentalis L.	-	-
Chenopodium murale L.	-	-
Cucumis sativus 'Ashley'	-	VY,MOS,YEL
Cyphomandra betacea (Cav.) Sendt.	-	-
Datura stramonium L.	-	-
Datura stramonium var. tatula (L.) Torr.	-	-
Desmodium ovalifolium Guill & Perr.	-	-
Euphorbia geniculata Orteg. (= E. prunifolia)	-	-
Euphorbia repanda Sweet.	-	-
Glycine max (L.) Merr.		
'ICA Caribe'	CS	MOS
'ICA Faroa'	CS	MOS
'ICA Pance'	CS	MOS
'ICA Pelican'	CS	MOS
'ICA Tunia'	CS	MOS
'Improved Pelican'	CS	MOS
ICA Linea 109	CS	MOS
ICA Linea 121	CS	MOS
Gomphrena globosa L.	-	-
Gossypium hirsutum L.	-	-
Helianthus annuus L.		
'Dursol Alta'	-	-
'Dursol Alta Especial'	-	-
'Russia 1'	-	-
Hibiscus cannabinus 'Everglades'	-	-
Hibiscus spec.	-	-
Ipomoea batatas (L.). Lam.	-	-
Jatropha gossypifolia L.	-	-
Lactuca spec.	-	-
Lens culinaris Medik.	-	-

Tabel 13 (continued).

Lycopersicon esculentum Mill.		
'Floridale'	-	-
'Rutgers'	-	-
Macroptilium atropurpureum (Moc. & Sessé) Urb.		
PI 318685	-	-
Macroptilium lathyroides (L.) Urb.		
PI 146800	-	-
Merremia quinquefolia (L.) Hallier (= Ipomoea quinquefolia)	-	-
Momordica balsamina L.	-	-
Mucuna pruriens (L.) DC.		
(= Stizolobium pruriens (L.) Medik.)	NS	-
Mucuna utilis Wall. ex Wight.		
(= Stizolobium utile (Wall.) Piper & Tracy)	NS	-
Nicandra physalodes (L.) Gaertn.	-	-
Nicotiana glutinosa L.	-	-
Nicotiana rustica L.	-	-
Oryza sativa L.		
'Mudgo'	-	-
IR - 8	-	-
IR - 22	-	-
Phaseolus acutifolius var. acutifolius Gray.		
(G-40044)	-	MOS
(G-40045)	CS	MOS
(G-40046)	NS	MOS
(G-40047)	-	MOS
(G-40049)	NS	MOS
(G-40072)	NS,VN	VN,TN
(G-40077)	NS,VN	VN,TN
Phaseolus acutifolius var. latifolius Freem.		
(G-40018)	NS,VN	VN,TN
(G-40030)	VN,NS	VN,EP,TN
(G-40035)	NS,VN	MOS,VN
(G-40040)	NS	MOS,TN
Phaseolus coccineus ssp. coccineus Maréchal, Marscherpa & Stainer		
(COC-105)	-	-
(COC-115)	-	-
(COC-150)	-	-
(COC-153)	-	-
Phaseolus coccineus ssp. polyanthus (Greenman) Maréchal		
(COC-02)	-	-
(COC-083)	-	-
(COC-084)	-	-
Phaseolus filiformis Benth.	-	-
Phaseolus lunatus L.		
'Henderson Bush'	NS	-
(G-25110)	-	-
(G-25145)	NS	-
(G-25172)	NS	-
(G-25181)	NS	-
(G-25191)	NS	-

Tabel 13 (continued).

Phaseolus vulgaris L.		
'Actopan x Sanilac-37' (G-6368)	-	VY,MOT
'Amanda' (G-4978)	NS	-
'Azufrado' (G-2843)	-	VY,MOS,ST
'Bataaf'	-	MOS,R,ST
'Bountiful' (G-5496)	CS	MOS,EP,ST
'Bush Romano-14' (G-5722)	-	MOS,EP
'Callactlan' (G-0278)	-	MOT,ST
'Cuilapa 72' (G-4489)	-	MOS,EP,ST
'Diacol Calima' (G-4494)	CS	MOS,ST
'Dubbele Witte' (G-6721)	-	MOS,R,EP
'Great Northern U.I. 31' (G-5710)	NS	-
'Great Northern U.I. 123' (G-5487)	NS	-
'Honduras 46' (G-5448)	-	VY,MOS,ST
'Improved Tendergreen'	-	MOS,EP,ST
'Imuna'	-	MOS,EP,ST
IVT 7214 (G-11270)	NS	-
IVT 7233 (G-11269)	NS	-
'Jamapa' (G-4486)	-	MOS
'Jubila'	-	MOS,EP,ST
'Michelite 62' (G-3942)	-	MOS,ST
'Monroe' (G-6096)	-	MOS,ST
'Namur Guroba' (G-1242)	-	MOS,EP,ST
'Nep 2' (G-4459)	-	MOS,ST
'Noordhollandse Bruine'	-	MOS,EP,ST
'Pinto Colorado'	NS	-
'Pinto Olathe'	NS	-
'Pinto Ovary' (G-6271)	NS	-
'Pinto U.I. 111' (G-5046)	NS	-
'Pinto U.I. 114' (G-4449)	NS	-
'Pinto U.I. 650' (G-5768)	NS	-
'Porrillo 1' (G-4481)	-	MOS,ST
'Porrillo Sintético'* (G-4495)	NS	MOS,ST
'Preto 897' (G-5743)	-	MOS
PI 207262 (G-1320)	NS	-
PI 313664 (G-2551)	-	MOS
'Puregold Wax' (G-6720)	-	MOS,R,EP,ST
'Redlands Greenleaf-B' (G-5745)	-	MOS,EP,ST
'Redlands Greenleaf-C' (G-5746)	-	MOS,EP
'Red Mexican U.I. 34' (G-5048)	NS	-
'Red Mexican U.I. 35' (G-6384)	NS	-
'Rico Pardo 896' (G-4468)	-	VY,MOS,ST
'Sanilac' (G-4498)	-	MOS,R,ST
'Stringless Green Refugee' (G-0416)	-	MOS
'Topcrop' (G-4014)	VY	MOS,EP,ST
'Widusa' (G-4503)	-	MOS,EP,ST
*Advanced breeding lines developed by CIAT, EP 1980: see Appendix.		
Physalis ixocarpa Brot. ex Hornem.	-	-
Rhynchosia minima (L.) DC.	-	MOS
Solanum torvum Sw.	-	-
Vigna radiata (L.) R. Wilzek. (AC-009)		
PI 213012	-	-

Table 13 (continued)

Vigna unguiculata L.		
'Antioquia Linda'	-	-
'Bush Sitao'	-	-
'Cabeata Negra'	-	-
'California Blackeye'	-	-
'Floricream'	-	-
'Hambru'	-	-
'Monteria'	-	-
PI 292889	-	-
PI 293476	-	-
PI 352885	-	-
PI 353380	-	-

CS = chlorotic spots, EP = epinasty, MOS = mosaic, MOT = mottle, NS = necrotic spots, R = rugosity, ST = stunting, TN = top necrosis, VN = veinal necrosis, VY = vein yellowing, - = no symptoms, * = genetically heterogeneous population for SBMV-CIAT infection where certain plants reacted only with necrotic spots without any systemic symptoms, whereas the others reacted only with a systemic mosaic.

Note: The G, COC or AC numbers behind the names of certain test plants are CIAT's germplasm collection accession numbers.

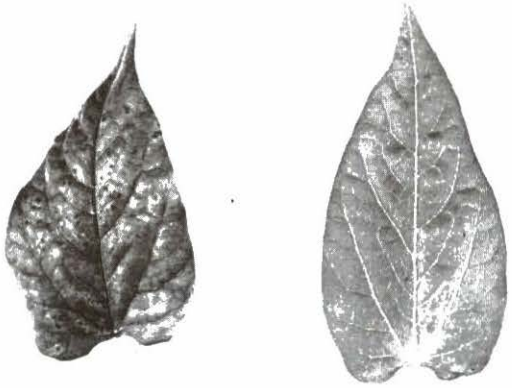


Fig. 37 Primary leaves of P. acutifolius var. latifolius. Left leaf: necrotic lesions and veinal necrosis after inoculation with SBMV-CIAT. Right leaf: healthy control.

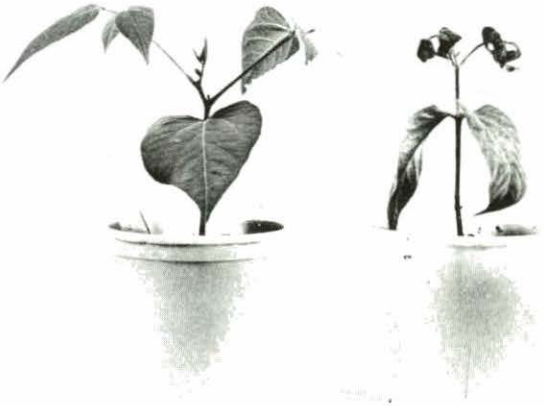


Fig. 38 P. acutifolius var. latifolius plants. Right plant: top necrosis and epinasty two weeks after inoculation with SBMV-CIAT. Left plant: healthy control.

Fig. 39 Primary leaves of P. vulgaris 'Pinto 650'. Right leaf: necrotic lesions three days after inoculation with SBMV-CIAT. Left leaf: healthy control.

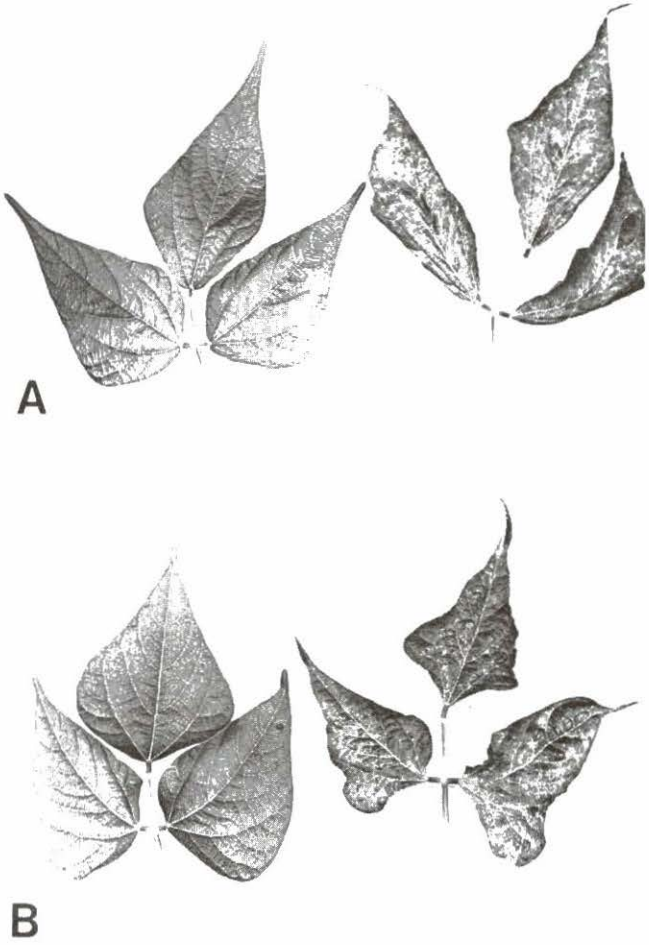


Fig. 40 Leaves of P. vulgaris 'Monroe' (A) and 'Topcrop' (B) systemically infected with SBMV-CIAT showing severe leaf deformation, vein mosaic, blistering and leaf curling. Healthy controls are on the left.

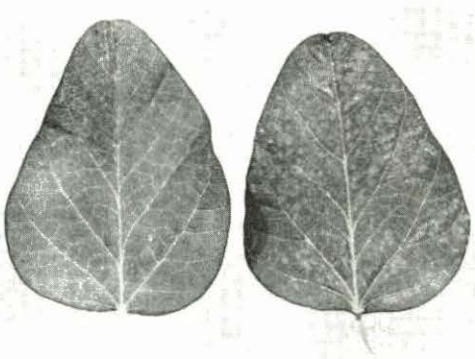


Fig. 41 Primary leaves of G. max (breeding line ICA 121). Right leaf: chlorotic lesions five days after inoculation with SBMV-CIAT. Left leaf: healthy control.

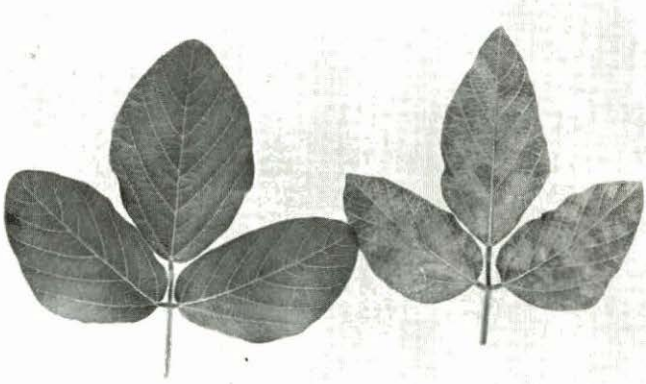


Fig. 42 Leaves of G. max (breeding line ICA 121). Right leaf: systemic mosaic three weeks after inoculation with SBMV-CIAT. Left leaf: healthy control.

7.3.2 Transmission by insects and mites, through seed and by dodder

Out of the six test plants used in the experiments three developed symptoms 17 days after *Cerotoma facialis* had fed on them, whereas *Diabrotica balteata* transmitted the virus to five out of the six test plants. In the latter case the symptoms developed 15 days after inoculation feeding. No transmission occurred when leafhoppers (*Empoasca kraemeri*), aphids (*Aphis gossypii*) and mites (*Polyphagotarsonemus latus*) had fed on test plants.

The seeds harvested from infected plants did not show any external symptoms of infection. However, in all the bean cultivars tested, transmission of SBMV-CIAT through seed occurred (Table 14). The percentages of transmission in beans ranged from 3.6 in *P. vulgaris* 'Dubbele Witte' to 33.6 in *P. vulgaris* 'Redlands Greenleaf-B'. Even one month after sowing the seeds from infected plants, the plants grown from them did not show any symptoms except *P. vulgaris* 'Dubbele Witte'. However, the presence of latent infection could be demonstrated serologically in the cultivars tested three weeks after planting. No latent infection could be established in *P. vulgaris* 'Dubbele Witte'.

Table 14. Cultivars of P. vulgaris tested for seed transmission of SBMV-CIAT

Cultivar	Number of seeds		Number of infected plants established by		% Transmission
	planted	germinated	serology	symptoms	
'Dubbele Witte'	228	192	7	7	3.6
'Improved Tendergreen'	67	65	18	0	27.7
'Imuna'	238	185	33	0	17.8
'Jubila'	138	107	19	0	17.8
'Monroe'	165	149	20	0	13.4
'Puregold Wax'	152	109	27	0	24.8
'Redlands Greenleaf-B'	299	283	95	0	33.6
'Topcrop'	152	119	11	0	9.2

Cuscuta spec. transmitted SBMV-CIAT to one out of the three *P. vulgaris* 'Nep 2' plants and two out of the three *P. vulgaris* 'Diacol Calima' plants used. The infected plants developed symptoms three weeks after training the shoots of *Cuscuta* spec.

7.3.3 Stability in sap

The virus had a dilution end-point of 10^{-5} to 10^{-6} and was still infective after incubating at room temperature for 96 days but not after 103 days. The virus was infective after heating at 90°C but non-infective after heating at 95°C.

7.3.4 Purification

Using the purification method adopted, infectious preparations of SBMV-CIAT could be obtained. The virus yield ranged from 250-325 mg per kg of infected tissue. Following rate-zonal centrifugation only

a single opalescent band was visible 2.15 to 2.40 cm below the meniscus. When the gradients were fractionated a single UV light absorbing region was observed. Rate-zonal centrifugation of SBMV-CIAT singly and in a mixture with SBMV bean strain showed only one opalescent band in each of the tubes 1.5 to 1.9 cm below the meniscus. When the gradients were fractionated, a single UV absorbing region was observed in each of the tubes (Fig. 43).

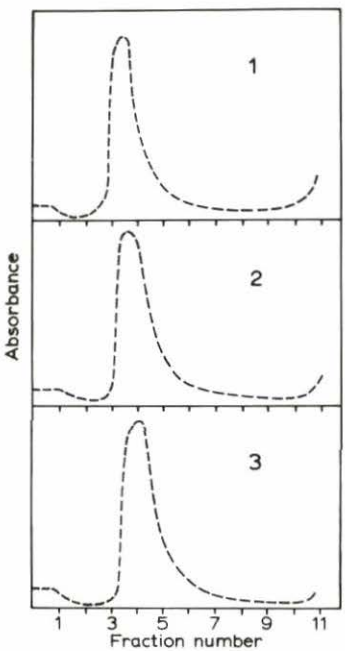


Fig. 43 UV-absorption profiles of centrifuged linear-log sucrose gradients containing SBMV-CIAT (1), a mixture of SBMV-CIAT and SBMV bean strain (2), SBMV bean strain (3). Sedimentation is from left to right.

7.3.5 Properties of the particles

7.3.5.1 UV-absorption spectrum

The UV-absorption spectrum of purified SBMV-CIAT gave a curve typical of a nucleoprotein (Fig. 44). The maximum absorption was at 262 nm and the minimum at 243 nm with $E_{max/min}$ = 1.40. The 260/280

ratio was 1.61, the 260/240 ratio 1.29. The 260/280 ratio without corrections for light scattering was 1.63.

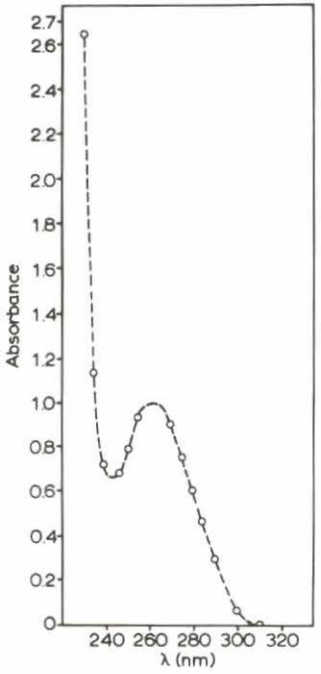


Fig. 44 UV-absorption spectrum of purified SBMV-CIAT in 0.02 M sodium phosphate buffer pH 7.0.

7.3.5.2 Buoyant density

After centrifugating the virus suspension in caesium chloride solution for 18 h, there was only one opalescent band. Only one fraction collected from the centrifuged tube showed absorption at 260 nm. This fraction had a density of 1.3651 g/cm^3.

7.3.5.3 Sedimentation coefficient

In the analytical centrifuge the virus sedimented as a single component. The average sedimentation coefficient of the virus was 112 S.

7.3.6 Particle morphology

Samples of purified SBMV-CIAT suspensions contained many isometric particles. Some of them had been partially or fully penetrated by the uranyl acetate stain, but the majority of them seemed intact and unaffected by the stain (Fig. 45). The average diameter of the particles was 28.2 nm.

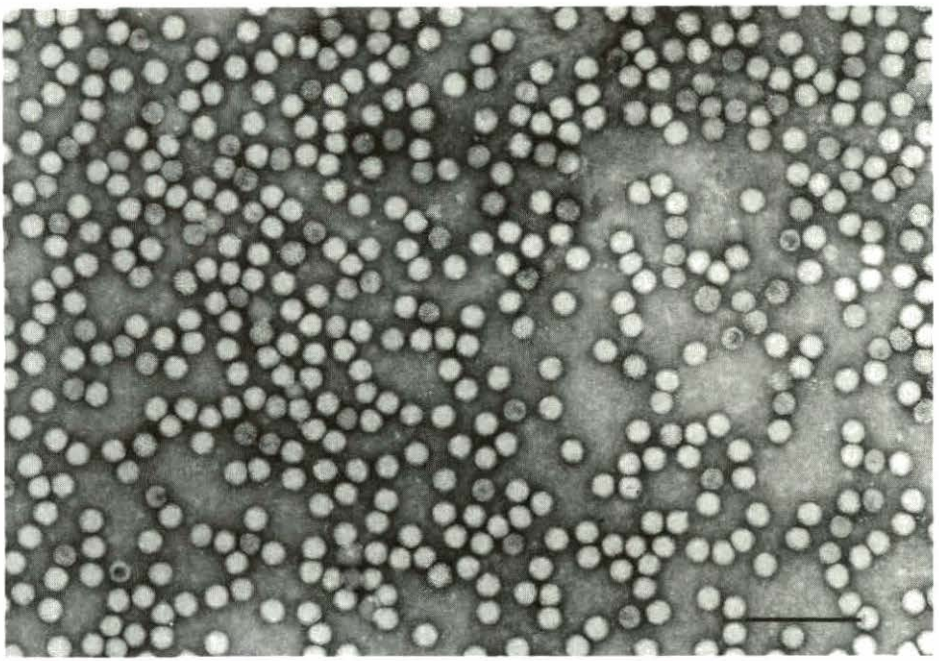


Fig. 45 Purified preparation of SBMV-CIAT stained with 2% uranyl acetate pH 4.5. The electron micrograph was taken after calibrating the lens current of the electron microscope to a magnification of x 40,000. Bar represents 125 nm.

7.3.7 Particle composition

7.3.7.1 Nucleic acid

With the above method approximately 65% of the nucleic acid could be isolated from SBMV-CIAT. The isolated nucleic acid was infective at a concentration of 0.06 µg/ml, but not at 0.006 µg/ml, when inoculated onto *P. vulgaris* 'Pinto U.I. 650'.

The UV-absorption spectrum of the nucleic acid showed a maximum at 259 nm and a minimum at 233 nm (Fig. 46). The $E_{max/min}$ ratio was 2.1, and the $E_{260/230}$ was 2.04.

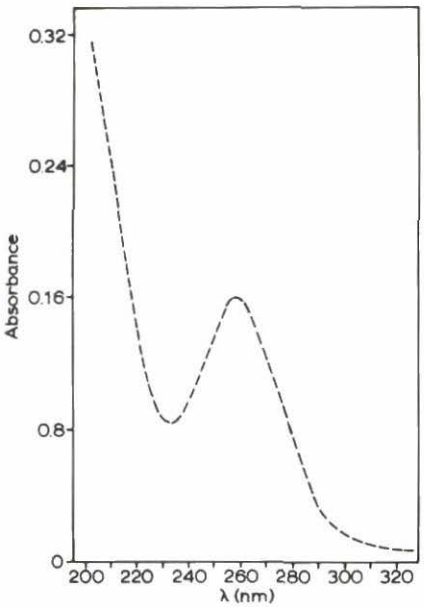


Fig. 46 UV-absorption spectrum of SBMV-CIAT nucleic acid in distilled water.

Both SBMV-CIAT nucleic acid and TMV-RNA developed a blue colour in the orcinol reaction, typical for ribose, while controls and the DNA solution remained yellowish. In the diphenylamine reaction the DNA solution developed a green colour typical for DNA, while SBMV-CIAT nucleic acid, TMV-RNA and the control solutions did not change colour.

The infectivity of all the SBMV-CIAT nucleic acid preparations in 0.02 M sodium phosphate buffer pH 7.0, was abolished by treatment with pancreatic RNase-A but not by DNase-1.

Four well-separated spots were visible under UV light on the filter paper upon chromatography of the hydrolysed SBMV-RNA. The absorption curve after eluting the four spots in 0.1 N HCl were identical to those of guanine, adenine, cytidylic acid and uridylic acid. The molar base ratio of SBMV-CIAT nucleic acid is given in Table 15.

The nucleic acid of SBMV-CIAT appears to be relatively rich in uracil and the other three bases appear to be approximately in equal concentrations.

Table 15. Molar base ratio of SBMV-CIAT. The numbers are averages of determinations made on 9 different virus preparations. Values are given as moles (%).

Guanine	Adenine	Cytosine	Uracil
24.8 ± 1.0	24.4 ± 0.5	22.4 ± 1.3	28.3 ± 1.3

The average phosphorus content of the SBMV-CIAT nucleic acid was 1.46% ± 0.055%. From the $E_{260/280}$ ratio of 1.61 for SBMV-CIAT as given in section 7.3.5.1 a nucleic acid content of 20.2% is estimated. The calculated nucleic acid content of SBMV-CIAT using the base composition and phosphorus content was 20.8%.

Only one species of RNA was detected by gel electrophoresis of SBMV-CIAT nucleic acid. The molecular weight of the SBMV-CIAT nucleic acid calculated from its electrophoretic mobility, relative to those of four RNA species of CCMV was 1.5 x 10^6 D ± 0.15 x 10^6 (Fig. 47).

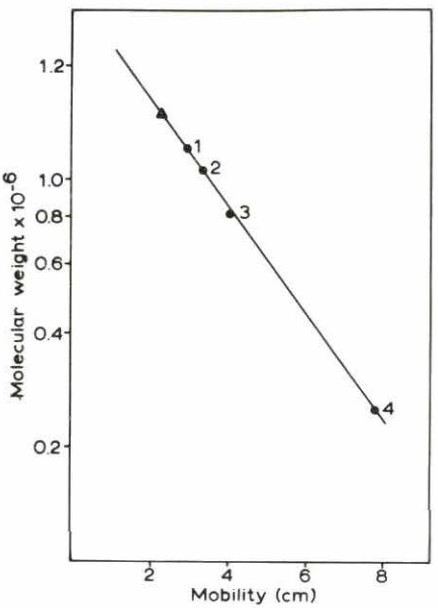


Fig. 47 The relation between the electrophoretic mobility and log molecular weight of marker RNAs (1, 2, 3, 4: the four RNA species of cowpea chlorotic mottle virus) and the RNA of SBMV-CIAT.

7.3.7.2 Coat protein

Only one polypeptide was detected by gel electrophoresis of the SBMV-CIAT coat protein (Fig. 48). The molecular weight of this polypeptide calculated from its electrophoretic mobility relative to the markers used (see section 5.3.9.2.1) was 30,000 ± 1500. The SBMV bean strain coat protein co-migrated as a single band in the same gel along with the SBMV-CIAT polypeptide.

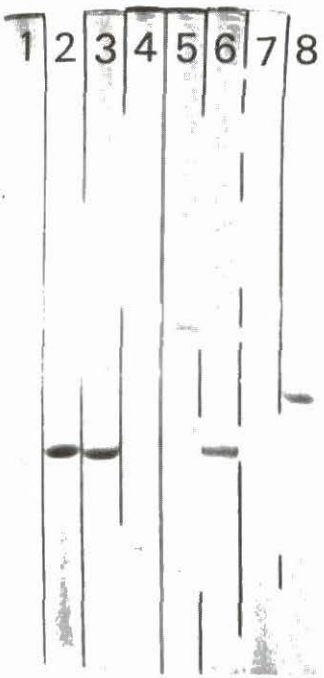


Fig. 48. Polyacrylamide gel electrophoresis of SBMV-CIAT coat protein (c.p.) as compared with marker proteins. 1. SBMV bean strain c.p.; 2. SBMV-CIAT c.p.; 3. SBMV-CIAT c.p. + SBMV bean strain c.p.; 4. cowpea chlorotic mottle virus c.p.; 5. cowpea mosaic virus c.p.'s; *6. carbonic anhydrase*; 7. bovine serum albumin; 8. bean common mosaic virus-NL strain c.p.

7.3.8 Serology

The antiserum prepared against SBMV-CIAT had a titre of 1/512 in the agar double-diffusion test. With sap from diseased leaf material the clearest precipitation lines were observed at a sap dilution of 1/8 and undiluted antiserum or with sap diluted to 1/2 and the anti-

serum diluted to 1/64. No reactions were observed in tests with sap from diseased and healthy leaves against normal serum, nor in tests with sap from healthy leaves against the antiserum. The virus did not give any reactions with the various antisera tested except with its homologous antiserum, antiserum to SBMV bean strain and severe SBMV. When SBMV-CIAT and SBMV bean strain were placed in adjacent wells and tested against their respective antisera, there was fusion of precipitation lines without spur formation, indicating that the two antigens are closely related if not identical. When SBMV-CIAT and severe SBMV were placed in adjacent wells and tested against their respective antisera, there was spur formation in the precipitation lines indicating the two viruses to be closely related but not identical.

7.3.9 Relations with cells and tissues

No inclusions of SBMV-CIAT could be observed in the infected epidermal strips of *P. vulgaris* 'Imuna' stained with Azure A and O-G stains. The mesophyll cells of both infected and healthy leaves generally stained purplish to magenta. However, the stain was intense in some mesophyll cells of the diseased trifoliolate leaves. In such cells the vacuole showed stained granular material (Fig. 49). With Azure A the veins generally had a purplish colour and in them the xylem vessels were most intensely stained. Free-hand sections of stems and roots did not show any inclusions of SBMV-CIAT. Only the xylem vessels in stems of diseased plants were slightly stained as compared with those of healthy controls.

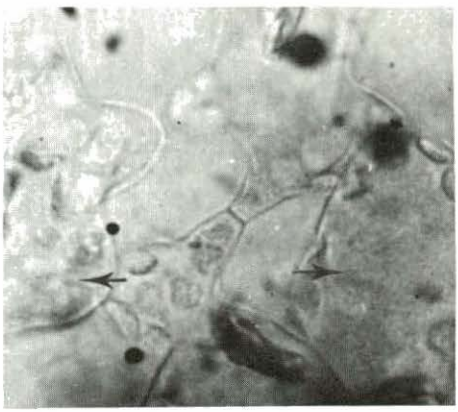


Fig. 49 Mesophyll cells of P. vulgaris 'Imuna' infected with SBMV-CIAT showing granular material in vacuoles (arrows). Magnification: x 2520.

Ultrathin sections of the mesophyll cells of *P. vulgaris* 'Imuna' infected with SBMV-CIAT did not clearly show cytopathic effects. The chloroplasts, mitochondria and nuclei of the cells all appeared normal. Being an isometric particle SBMV-CIAT could not easily be distinguished from ribosomes if they were located within the cytoplasm. The central vacuoles in many cells were normal but others contained much granular material and isometric particles (Fig. 50A and B). On the basis of their shape and size they were considered to be SBMV-CIAT particles. No such particles were found in cells of the healthy controls. In certain cells there was no central vacuole but many smaller vesicles were randomly distributed within the cell. Many of them contained SBMV-like particles. No structural deviations could be seen in the vascular tissues of the veins, stems and roots. The findings corroborate the light microscopic observations where no darkly stained material was present in the vascular tissues but only in the vacuoles of the mesophyll cells.

7.4 *Discussion and conclusions*

The virus was readily transmitted mechanically to host plants. Chrysomelid beetles, *D. balteata* and *C. facialis* were efficient vectors of SBMV-CIAT. In the literature only *C. trifurcata* is reported to transmit the bean strain of SBMV after an acquisition feeding period of more than 48 h (Walters, 1964). *E. kraemeri* and *A. gossypii* were not vectors of the virus.

The percentage of seed transmission varied from 3.6% in *P. vulgaris* 'Dubbele Witte' to 33.6% in *P. vulgaris* 'Redlands Greenleaf-B'. The plants infected through the seeds (as established by serology) did not show any symptoms, except those of the cultivar Dubbele Witte, which displayed a mosaic. However, all the cultivars used in the seed transmission experiment developed mosaic symptoms upon mechanical inoculation with SBMV-CIAT. It is a question whether this discrepancy in results from experiments in which the virus had been introduced into the plant in two different ways, could be explained by a variation in concentration of active virus in seeds of different bean cultivars as observed by Cheo (1955).

The host range of SBMV-CIAT is narrow. In the literature few data are available about the host range of type SBMV and its bean and cowpea strains and in many cases only legumes have been tested (Groga and Kimble, 1964; Shepherd and Fulton, 1962; Zaumeyer and Harter,

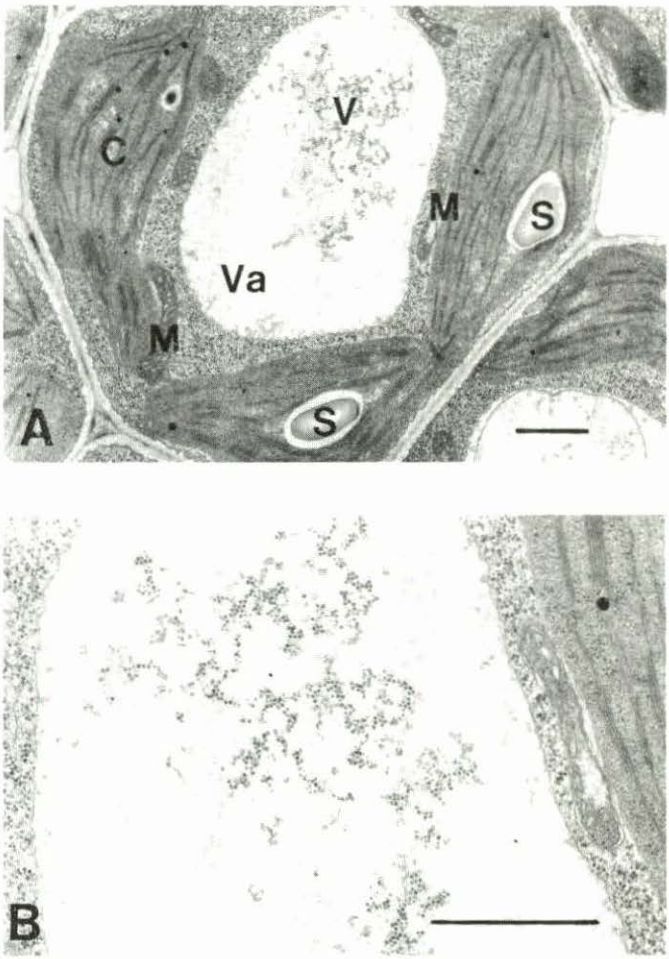


Fig. 50 Ultrathin section ot mesophyll cells of P. vulgaris 'Imuna' infected with SBMV-CIAT. Virus particles (V) within a vacuole (Va). B is an enlargement of the vacuole area in A. C = chloroplast; M = mitochondrion; S = starch grain. Bars represent 1µm.

1943b). The virus infected *C. sativus* 'Ashley', causing mild vein yellowing followed by browning of the leaves. This is the first time that cucumber has been found to be susceptible to SBMV.

All seven cultivars of *G. max* tested produced chlorotic local lesions followed by a bright yellow mosaic after inoculation with SBMV-CIAT. However, inoculations conducted with SBMV bean strain caused only latent infection in all the above seven cultivars. Shepherd (1971) reported, that SBMV bean strain provoked chlorotic local lesions and systemic mosaic in *G. max* 'Kent'. As seeds of this cultivar were not available, the latter could not be used in comparative inoculation experiments with SBMV-CIAT and SBMV bean strain under CIAT conditions. Among the ten cultivars and varieties of *P. acutifolius* tested, SBMV-CIAT caused chlorotic and necrotic local lesions followed by a systemic mosaic or top necrosis. However, with SBMV bean strain no local lesions were formed but only a systemic top necrosis. All other plant species and cultivars tested reacted similarly to infection with both SBMV-CIAT and SBMV bean strain.

As an assay host for SBMV bean strain usually *P. vulgaris* 'Pinto' is used (Sehgal, 1973). Besides the advantage of this assay plant (big necrotic local lesions, formed 3 to 4 days after inoculation) there is the disadvantage that at high concentrations of SBMV the large local lesions coalesce and the whole leaf surface becomes necrotic, making lesion counting impossible. In the course of this study other suitable assay hosts were found, viz. *M. pruriens* and *M. utilis* which reacted with pin-point necrotic lesions to SBMV. These *Mucuna* species proved to be more susceptible to SBMV than 'Pinto' beans, but they had the disadvantage that germination of their seeds took 12 to 13 days (in contrast to 8 days in the case of 'Pinto') and appearance of the pin-point local lesions (which could only be seen in transmitted light) six days.

The *P. vulgaris* 'Porrillo Sintético' used, proved to be a genetically heterogeneous population with regard to SBMV-CIAT infection. Among the plants tested some reacted with necrotic local lesions only, others only systemically with a mosaic. Two similarly heterogeneous populations were also found in CIAT breeding lines (EP, 1980: see Appendix). These breeding lines are resistant to BCMV. By isolating the breeding lines hypersensitive to SBMV from these two populations, valuable material may be obtained for future breeding work.

From Table 16 it can be seen that there is a great similarity between the physical and chemical properties of both SBMV-CIAT and SBMV bean strain. The only two major differences found were with respect to the particle size and the base composition of the nucleic acid. In the literature there is much discrepancy as to the size of SBMV particles. The diameter of particles packed in crystals is reported to be 25 nm (Price et al., 1946), 35 nm (Shepherd and Fulton, 1962), 33 nm (Kuhn, 1963) and 26 nm (Grogan and Kimble, 1964). None of the above data are very reliable because of the measuring method used. Price et al. (1946) did not use any internal standard for measuring the particle diameter. Shepherd and Fulton (1962) applied polystyrene latex spheres as an internal standard with all the drawbacks (as discussed in section 5.4) involved. The use of purified TMV preparations as an internal standard, as done by Grogan and Kimble (1964) is not very accurate since TMV particles can easily be disrupted or aggregated during the purification procedures. The molar percentage of guanine in SBMV bean strain is higher than that of SBMV-CIAT. This difference is unlikely to be due to an experimental error, as all the other physical and chemical properties were the same or similar. Therefore, it is conceivable that the rather low percentage of guanine is a characteristic property of SBMV-CIAT. One might even speculate that this property is linked up with the differences in host reactions observed between SBMV-CIAT and SBMV bean strain.

Serological studies showed that SBMV-CIAT and SBMV bean strain are closely related if not identical. SBMV-CIAT, like SBMV bean strain, is not serologically identical to severe SBMV.

No diagnostic virus inclusions could be seen in the infected tissues using the light microscope. The cells of infected leaves stained only slightly with Azure A stain and the vacuoles showed granular material. This is rather surprising when the concentration of SBMV in infected plant parts is taken into account. One might have expected that at such high concentrations in the cells the virus particles would arrange themselves into inclusions, but no such structures could be found in the light or electron microscope. The presence of so many free virus particles in the cells may be the reason that large amounts of virus could be isolated from the infected tissues even two months after inoculation (Christie, personal communication, 1980). In ultrathin sections of the infected mesophyll cells of *P.*

Table 16. A comparison of the physical and chemical properties of SBMV-CIAT (as determined in the present study) with those of SBMV bean strain (as reported in literature).

Physical and chemical properties	SBMV-CIAT	SBMV bean strain
Stability in sap		
Longevity in vitro (days)	94-103	165 (a)
Thermal inactivation point (°C)	90-95	90-95 (a)
Dilution end-point	10^{-5}-10^{-6}	10^{-5}-10^{-6} (b)
Properties of the particles		
A 260/280	1.61	1.6 (c)
A max/min	1.4	-
Sedimentation coefficient (S°_{20w}) (S)	112	115 (d)
Buoyant density in CsCl (g/cm^3)	1.3645	1.3545 (e)
Particle morphology	isometric	isometric (f)
Particle size	29.5	26.3 (f)
Properties of the nucleic acid		
A max/min	2.1	1.98 (g)
A 260/230	2.04	-
Base composition (% moles)	G 24.8	27 (h)
	A 24.1	23.5
	C 22.4	22.5
	U 28.3	27.0
Nucleic acid content (%)	20.8	21 (i)
Molecular weight (daltons)	1.5×10^6	1.4×10^6 (i)
Properties of the coat protein		
Molecular weight (daltons)	30,000	29,000 (j)
Serology	closely related to SBMV bean strain	* closely related to SBMV-CIAT

a = Zaumeyer and Harter (1943a); b = Zaumeyer and Harter (1943b); c = Shepherd (1971); d = Miller and Price (1946); e = Magdoff-Fairchild (1967); f = Grogan and Kimble (1964); g = Wilcockson and Hull (1974); h = Ghabrial et al. (1967); i = Diener (1965); j = Tremaine (1966).
* Results obtained in the present study.

vulgaris 'Imuna', particles were found in the vacuole that were tentatively identified as virus particles, mainly on the basis of size, perfect isometric structure and their regular arrangement. Regularly arranged particles in virus infected cells are generally considered to be virus particles, but there are reports of regular arrangements of ribosomes (24 nm in diameter) in *Entamoeba invadens* (Morgan and Uzman, 1966) and in chick embryo cells (Byers, 1967). As the diameter of the plant ribosomes is not very different from that of the SBMV particles, the results obtained in light and electron microscopy have to be interpreted with extreme caution.

THE COMBINED EFFECT OF BMMV-CIAT, CMV-CIAT AND SBMV-CIAT ON BEAN PLANTS

8.1 *Introduction*

As mentioned in Chapter 4, BMMV, CMV and SBMV could consistently be isolated from bean plants with chlorotic mottle. The effect of each of the above-mentioned viruses alone and in combination with each other on bean plants was investigated. In the first place this was done with a view to reproduce the chlorotic mottle symptoms observed on beans in the field. In the second place it might give an insight into possible other symptoms mentioned in literature in connection with bean chlorotic mottle such as dwarfing, stunting and witches' broom-like phenomena. To this end mechanical inoculation was performed both in the glasshouse and in the field.

8.2 *Materials and methods*

8.2.1 Virus isolates

The three virus isolates used in the experiment were BMMV-CIAT (Chapter 5), CMV-CIAT (Chapter 6) and SBMV-CIAT (Chapter 7) isolated from bean plants in the field showing chlorotic mottle symptoms.

8.2.2 Test plants

Phaseolus vulgaris 'Argentina 2' was used as a test plant. Under natural conditions this bean cultivar often shows symptoms of chlorotic mottle (Castaño, personal communication, 1980).

8.2.3 Growing of the plants

8.2.3.1 Glasshouse experiment

The seeds were planted in plastic pots of 12 cm diameter (one seed/pot) in sterilized soil. Abnormal-looking plants were discarded and five healthy-looking plants per treatment were used.

8.2.3.2 Field experiment

This experiment was conducted in a netted cage of 8 x 4 x 4 m^3 placed in the CIAT experimental bean fields. To the soil inside the cage was added Furadan (FMC. Corp., of Mobay Chem. Corp., U.S.A.) at a concentration of 30 kg/ha to eliminate larvae of chrysomelid beetles, one week before planting. Seeds used were surface sterilized with Terracoat, SD-205 (Clin. Corp. Arkansas, U.S.A.) seed dressing and planted in groups of 8 seeds at an approximate distance of 3 cm. The distance between the groups was 60 cm. After germination each group was thinned to 4 plants each just before inoculation.

8.2.4 Inoculum

Inocula of BMMV-CIAT and SBMV-CIAT were prepared as described in Chapter 5 and 7, respectively, and diluted with 0.02 M sodium phosphate buffer pH 7.0, to 10^{-3} just before inoculation. Inoculum of CMV-CIAT was prepared as in Chapter 6 and diluted with distilled water to 10^{-2}.

8.2.5 Inoculation procedure

The plants were inoculated at different growth stages with different combinations of the inocula according to the outline given in Table 17. Seven days after planting (in the field nine days) only the primary leaves were inoculated (Table 17, treatment 1), while 14 and 19 days after planting (in the field 16 and 21 days, respectively) only the fully expanded trifoliolate leaves (usually the first and the second) were used (Table 17, treatments 2 and 3, respectively). When two inocula had to be applied to the same plant at the same time, one leaf-half was inoculated with one inoculum and the other half with the other. When three inocula had to be applied at the same time on the same plant, each of the three different leaflets of a trifoliolate leaf were inoculated with each of the three inocula.

Table 17. Symptoms in P. vulgaris 'Argentina 2' inoculated with BMMV-CIAT, CMV-CIAT and SBMV-CIAT at different times after planting of seeds.

Treatment [a]			Symptoms [b] observed (days after planting)				General development of the plant [c]
			20	27	49		
(1)	(2)	(3)	leaves	leaves	older leaves	younger leaves	
-	-	-	-	-	-	-	P
-	-	B	-	VY	MOS	MOS	P
-	-	C	-	VY	-	Y	ST,P
-	-	S	-	VY	MOS	SL,MOS	ST,F
-	-	SC	-	VY,MOS	MOS,EP	VC	ST,DP
-	-	SB	-	VY,MOS	EP,N	MOS	ST,DP
-	-	CB	-	VY	-	MOS,EP	DP
-	-	CBS	-	MOS	-	EP,R	ST,DP
-	S	-	-	MOS	EP	SL,MOS	ST
-	S	B	-	MOS	EP,MOS,N	MOS,EP	ST,DP
-	S	CB	-	MOS,EP	EP	SL,MOS	ST,DP
-	C	-	-	MOS	MOS*	SL,MOS	ST,F
-	C	S	-	MOS	MOS*	SL,MOS,VN	ST,F
-	C	B	-	MOS	MOS*,NS	SL,MOS,VN	ST,F
-	C	SB	-	MOS	MOS,EP,VN	SL,MOS	ST,DP
-	B	-	-	MOS	-	MOS?	ST,P
-	B	S	-	MOS	MOS,R	MOS,SL,R	ST
-	B	C	-	MOS	-	MOS?	ST
-	B	SC	-	MOS	R	MOS,R?	ST
-	SC	-	-	MOS	SL,MOS*	SL,MOS,R	ST,DP
-	SC	B	-	MOS	MOS	SL,MOS,R	ST
-	SB	-	-	MOT	MOS,EP	MOS,R	ST
-	SB	C	-	MOT,EP	EP	SL,MOS	WB,DP
-	CB	-	-	VY,EP	MOS*,EP	SL,MOS,R	ST
-	CB	S	-	VY	MOS,EP	MOS,EP	ST
-	CBS	-	-	VY	MOS*	SL,MOS,R	ST,DP
S	-	-	VY	MOS,R,EP	MOS	SL,MOS,R	ST,DP
S	-	C	VY	MOS	MOS*	MOS*,EP,R	ST
S	-	B	VY	MOS	MOS	SL,MOS,R	ST,DP
S	-	CB	VY	MOS	MOS*	MOS*	ST
S	C	-	VY	MOS	MOS	VY	ST
S	C	B	VY	MOS	MOS*	MOS*,R	ST
S	B	-	VY	MOS	MOS,EP	Y	ST,F
S	B	C	VY	MOS	Y	Y	ST
S	CB	-	VY	MOS	DL,MO,EP	MOS,VN,R	ST
C	-	-	VY,MOS	MOS	MOS*	MOS,EP	ST
C	-	S	VY	MOS	SL,MOS*	SL,MOS,EP,R	ST
C	-	B	VY	MOS	MOS*	MOS	P
C	-	SB	VY	MOS	Y,EP,NS	MOS,EP,NS	ST,DP
C	S	-	VY	MOS	SL,MOS,R	MOS*,EP	ST,F
C	B	S	VY?	MOS	SL	SL,MOS*,R	ST
C	B	-	VY,MOS	MOS	MOS	-	ST
C	SB	-	VY,MOS	MOS	-	MOS,EP	ST,WB
B	-	-	MOS	MOS	-	MOS?	F
B	-	S	MOS	MOS,EP	DL,Y	SP	ST
B	-	C	MOS	MOS	MOS,EP,R	MOS,DL,R	ST

Table 17. Continued.

B	-	SC	-	MOS,EP	MOS,DL,EP	MOS,Y,R	ST,DP
B	S	-	VY	MOS,EP	MOS,DL	MOS	ST
B	S	-	VY	MOS,EP	MOS,VN	MOS,R	ST,F
B	S	C	VY	MOS,EP	MOS*,VN	MOS*,R	ST,F
B	C	-	VY	MOS,EP	MOS*,EP	MOS,VN	ST,F
B	C	S	VY	MOS,EP	MOS*,EP	MOS,R,VN	ST
B	SC	-	MOS	MOS	MOS,NS,EP	SL,VY,MOS	ST
SC	-	-	MOS	MOS	MOS	SL,MOS,EP	ST,WB
SC	-	B	MOS	MOS	Y,EP	SL,MOS	ST
SC	B	-	MOS	MOS	Y,EP	SL,MOS	ST
SB	-	-	MOS	MOS,R	SL,MOS	SL,DL,MOS	ST,WB
SB	-	C	MOS	MOS	MOS	SL,DL,MOS	ST,WB,F
SB	C	-	VY	MOS	MOS	SL,DL,MOS	ST,WB,F
CB	S	-	MOS	MOS	MOS*	SL,MOS,R	ST
CB	-	-	MOS	MOS	MOS	MOS,EP	ST
CB	-	S	MOS	MOS,EP	MOS,EP,R,NS	MOS	ST
CBS	-	-	MOS	MOS	MOS,EP,VN	DL,MOS	ST,WB

a) Plants inoculated at 7 (1), 13 (2) and 19 (3) days after planting. B = BMMV-CIAT, C = CMV-CIAT, S = SBMV-CIAT, - = not inoculated.

b) DL = deformed leaves, DP = deformed pods, EP = epinasty, MOS = mosaic, MOS* = mosaic resembling bean chlorotic mottle, MOT = mottle, N = necrosis, NS = necrotic spots, R = rugosity, SL = small leaves, ST = stunting, VN = veinal necrosis, VY = vein yellowing, WB = witches' broom-like symptoms, Y = yellowing, - = no visible symptoms, ? = doubtful symptoms.

c) F = normal flowers, P = normal pods, ST = stunted growth, WB = witches' broom-like growth.

8.2.6 Recording of the results

The symptoms in the inoculated plants were assessed 13, 20, 27 and 49 days after planting. In the last case the symptoms were divided into two categories, viz. those in the older leaves (usually the first four trifoliolate leaves) and those in the younger leaves. This arbitrary division was necessary because of the differences in symptoms on older and younger leaves.

8.2.7 Detection of the viruses

Agar double-diffusion tests were performed with sap from the diseased plants 49 days after planting to detect the three viruses. As the serological test often failed in case of CMV-CIAT, the presence of the latter was demonstrated by bio-assay on *P. acutifolius* var. *latifolius*.

8.3 *Results*

8.3.1 Glasshouse experiment

The results of the experiment are recorded in Table 17. Symptoms resembling chlorotic mottle were produced in 18 out of the 62 treatments performed. The symptoms produced, however, were not exactly those of chlorotic mottle as described in Chapter 3. The type of mosaic was similar but the colour of the mosaic was slightly less pronounced than that exhibited by field plants with chlorotic mottle. As was stated before (section 6.4) CMV-CIAT alone could produce chlorotic mottle-like symptoms in beans. However, this was only the case when CMV-CIAT was inoculated onto the primary leaves and the first trifoliolate leaves of the bean plant (Fig. 51). From Table 17 it can be seen that CMV-CIAT inoculated onto the second trifoliolate leaves brought about only general yellowing of the leaves without any mosaic. In five treatments (Table 17) all the leaves of the infected plants showed chlorotic mottle symptoms. The rest showed such a mosaic only in the lower trifoliolate leaves (1st - 4th), whereas the younger leaves exhibited a mosaic with rugosity, epinasty, deformation of leaves and veinal necrosis depending on the treatment. These symptoms were similar to those observed in some of the bean plants in the field showing chlorotic mottle. Witches' broom-like symptoms were produced in plants inoculated on the primary leaves with a mixture SBMV-CIAT + CMV-CIAT, SBMV-CIAT + BMMV-CIAT, and CMV-CIAT + SBMV-CIAT + BMMV-CIAT or on the first trifoliolate leaves with a mixture of SBMV-CIAT + BMMV-CIAT followed by inoculation with CMV-CIAT or BMMV-CIAT one week later. These results indicate that the presence of SBMV-CIAT is essential for the production of witches' broom-like symptoms. Among the diseased plants only those inoculated with BMMV-CIAT onto the second trifoliolate leaves produced pods comparable to the healthy controls. Already single virus infections, resulting from inoculations on the first trifoliolate leaves (14 days after planting of seeds), increased the time to flower.

8.3.1.1 Detection of the viruses

In all the plants inoculated with BMMV-CIAT and SBMV-CIAT the virus could be detected by serological tests. However, with CMV-CIAT the virus could not be detected by serology in the following treat-

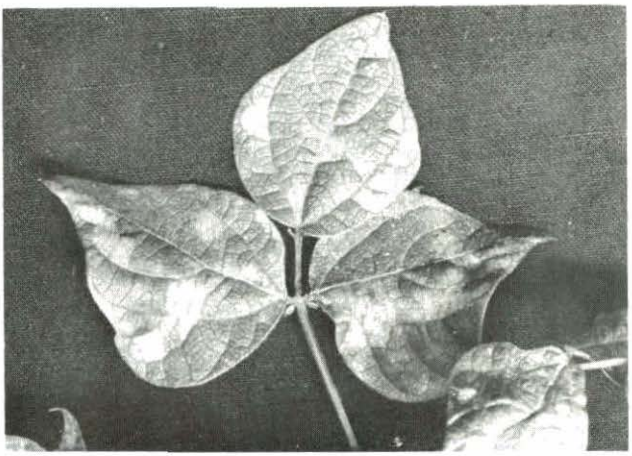


Fig. 51. One of the trifoliolate leaves of P. vulgaris 'Argentina 2' showing chlorotic mottle-like symptoms after inoculation of the primary leaves with CMV-CIAT.

ments: (see footnote of Table 17) - CBS - ; - - CBS; - C S ; - SC B ; C - - ; C - S ; C - B ; C - SB ; C B S. In these treatments the presence of virus could be demonstrated by assaying on *P. acutifolius* var. *latifolius*.

8.3.2 Field experiment

The field experiment performed failed due to the development of "Problem X" symptoms in the plants 29 days after planting. "Problem X" (for details see CIAT Annual Report, 1976) is a disease in dry beans already reported in the Cauca Valley of Colombia in 1953. However, since 1974 this disease, the etiology of which so far is not known, has become significant at CIAT. The symptoms appear in the first trifoliolate leaves and become more severe in the younger leaves. They consist of vein clearing, malformation of the leaves and witches' broom-like phenomena. The disease is soil-borne and is generally believed to be caused by residual herbicides in soils with a high organic matter content and high pH (Schwartz et al., 1978).

8.4 *Discussion and conclusions*

The experiment conducted proved that symptoms resembling those of bean chlorotic mottle could be reproduced in bean plants by mechanically inoculating CMV-CIAT alone or in combination with the other two viruses. As pointed out in Chapter 3 chlorotic mottle-showing bean plants in the field sometimes exhibited a vast range of other symptoms. The latter could also be reproduced by mechanically inoculating the viruses in different combinations at different times after planting the seeds.

Witches' broom-like symptoms reported to be a part of the bean chlorotic mottle syndrome were produced in plants inoculated on the primary leaves with a mixture of all the three viruses or with a mixture containing SBMV-CIAT with BMMV-CIAT or CMV-CIAT. Under field conditions some of these situations may occur, especially as the three viruses studied are seed transmitted. In a situation where bean plants have grown from BMMV-CIAT or SBMV-CIAT infected seeds, chrysomelid beetles may transmit these viruses, thus introducing a second virus into the already infected plants. When viruliferous aphids visit these plants they may introduce CMV-CIAT. The resulting infections may lead to the development of witches' broom-like symptoms. It has been shown that whiteflies (*B. tabaci*) can acquire simultaneously, two different viruses and later transmit them during feeding (Flores and Silberschmidt, 1963). Nothing is known about a similar ability of chrysomelid beetles.

The experiment was conducted in a glasshouse with only one bean cultivar and under experimental conditions which certainly differed from those prevalent in the bean fields. The reason for not reproducing the bright mosaic, characteristic of bean chlorotic mottle, under experimental conditions may be due to the above mentioned factors. It is known from observations that bean chlorotic mottle symptoms greatly vary according to the different bean genotypes. The colour of the mosaic ranged from yellow, chlorotic to pale-green. It is quite possible that another bean cultivar would have shown the exact bean chlorotic mottle symptoms. However, 'Argentina 2' was the only cultivar of which seeds were available in the very large number needed for these experiments.

From this study no indications could be obtained that besides the three viruses mentioned, another pathogen was present in bean chlorotic mottle-affected beans as well. The majority of the symptoms recorded in the literature for chlorotic mottle-affected bean plants could be attributed to the three mechanically transmissible viruses isolated from these plants.

CHAPTER 9

GENERAL DISCUSSION AND CONCLUSIONS

On the basis of symptoms and its supposedly whitefly-borne character, bean chlorotic mottle was grouped along with other whitefly-transmitted diseases of plants showing variegation (Gálvez and Cárdenas, 1980; Schwartz et al., 1978). However, no experimental evidence was available for categorizing bean chlorotic mottle among the whitefly-borne virus diseases of malvaceous plants.

According to the literature the symptoms described for field infected bean plants with chlorotic mottle ranged from mosaic to severe dwarfing, bunchy, or rosette type of plant development and witches' broom-like symptoms. In general the symptoms described above can be brought about by many pathogens and even pests. Thus it was difficult to recognise in the field bean plants affected with bean chlorotic mottle. Therefore, a characteristic symptom common to all allegedly bean chlorotic mottle infected plants was necessary for diagnosis in the field. This characteristic symptom was a sharp mosaic where the colour ranged from mild greenish-yellow to bright yellow. Therefore, only when this characteristic symptom was present in at least one of the trifoliolate leaves, a bean plant was considered to be affected with chlorotic mottle. Affected field plants thus selected showed besides the above-mentioned sharp mosaic in at least one of the leaves, a tremendous variation in symptoms, ranging from mild mottle to rugosity, leaf curl, malformation, proliferation of buds and witches' broom-like growth. Though the name bean chlorotic mottle suggests the symptoms to be a mottle, the characteristic symptom of the disease is a sharp mosaic. The name chlorotic mosaic would have been more appropriate. However, we refrain from introducing a new name, because of the already existing chaos of names for this disease.

Although bean chlorotic mottle was supposed to be caused by a virus transmitted by *B. tabaci* (Gálvez and Cárdenas, 1980), transmis-

sion experiments with whiteflies were negative. However, three sap transmissible viruses were repeatedly isolated from bean plants with chlorotic mottle, viz. BMMV-CIAT, CMV-CIAT and SBMV-CIAT.

This is the first report indicating the natural occurrence of BMMV in South America. The presence of SBMV has already been reported (Costa, 1972). However, the results indicated that SBMV-CIAT is not exactly identical to the bean strain of SBMV.

The host range studies conducted showed that the symptoms produced by BMMV-CIAT in beans are hardly visible and the plants grow normally producing healthy-looking pods. Therefore, BMMV-CIAT-infected plants may easily be overlooked in the field. Though no noticeable damage is caused in beans due to BMMV-CIAT infection, in mixed infections with SBMV-CIAT or CMV-CIAT the consequences are devastating as the production of pods is practically nil in these plants. The severity of symptoms caused by SBMV-CIAT is usually less in beans than in *P. acutifolius* in which the virus brings about systemic top necrosis. Already two experiments with *P. acutifolius* have had to be abandoned in CIAT fields due to an epidemic of SBMV-CIAT.

From the present study it is clear that bean chlorotic mottle, as described in the literature, is a composite disease. Infection with CMV-CIAT alone or in combination with SBMV-CIAT and BMMV-CIAT in bean cultivars Porrillo 1, Porrillo Sintético, Honduras 46 and Argentina 2 produced a sharp mosaic very much similar to the characteristic symptom of bean chlorotic mottle. Inoculation of the three viruses in different combinations and at different times onto bean plants sometimes gave rise to a number of other symptoms besides the sharp mosaic, such as malformation, dwarfing, rugosity, leaf curl and witches' broom-like phenomena described in literature as a part of the bean chlorotic mottle syndrome.

Efficient vectors for the three viruses viz. BMMV-CIAT, CMV-CIAT and SBMV-CIAT are present in the field. Though data are not available for the population distribution of aphids, chrysomelid beetles are present throughout the year in CIAT experimental fields in Palmira (Cardona, personal communication, 1980). Weeds such as *Rhynchosia minima* may harbour the three viruses from which bean plants in the field become infected via the vectors. *Macroptilium* spec., a common weed, is rarely seen in nature without aphids. In glasshouse experiments this leguminous plant proved to be a symptomless carrier of

CMV-CIAT. However, the seed transmission of all the above three viruses constitutes the greatest danger to the health of bean plantings. Seed transmission is of considerable epidemiological significance. Infected seeds are a vehicle for long-distance transport and survival from one season to another, especially for those such as BMMV-CIAT and SBMV-CIAT with a rather narrow host range. Plants infected through their seeds are usually not in optimal condition. Besides that, they act as randomly scattered inoculum in the field plantings to trigger a large scale epidemic, especially when the virus involved is readily transmitted by sap and by vectors omnipresent in the fields, as is the case with BMMV-CIAT and SBMV-CIAT.

As commonly, no direct measures against a virus in an affected plant can be applied, indirect measures must rather be used. The latter consist of prevention of infection by eliminating or reducing the number of infection sources and of limiting the spread of viruses.

At present it is not known which weeds act as sources for the three above-mentioned viruses. Many leguminous, cucurbitaceous and malvaceous plants are found growing along the roads and drainage bunds. However, such data will not be of much value since their elimination will be infeasible and uneconomical. Volunteer beans, common in the CIAT fields, may constitute an even greater danger since they can easily act as a source of infection. Therefore, measures should be taken to prevent their growth.

Roguing of infected plants from a crop has been recommended for controlling many virus diseases (Smith, 1972). This is effective only if the virus invades the crop rapidly from an outside source or if it spreads relatively slowly from a source within the crop. Use of virus-free seed might be recommended in those localities where the crop can be grown in isolation from outside sources of infection.

A rotation programme during which no plants susceptible to these viruses are grown for a certain period may provide adequate control. Such a programme has already proved effective in case of onion yellow dwarf virus in Christchurch, New Zealand (Smith, 1972).

One of the most effective ways to control a virus disease in plants is breeding for field resistance or immunity. Hypersensitive reactions without systemic spread have been obtained with SBMV-CIAT in some of the *P. vulgaris* CIAT advanced breeding lines (EP 1980) and in certain commercial cultivars such as 'Amanda' and 'Pinto Ovary'. Some of these cultivars and breeding lines are resistant to both

bean common mosaic virus and SBMV-CIAT. However, all the cultivars and breeding lines of *P. vulgaris* did not show any resistance to BMMV-CIAT and CMV-CIAT. Sources of resistance to the latter two viruses are available in other *Phaseolus* spp. which could therefore be used for interspecific crosses. *Phaseolus acutifolius* and *P. polyanthus* have been crossed with *P. vulgaris* with some success in controlling the CMV-B strain (Honma, 1956; Lorz, 1954; Provvidenti, 1976).

Another important aspect is the control of long distance spread of the viruses, both within the country and between countries. In a country like Colombia where high mountains act as natural barriers, man plays an important role in spreading these viruses by transporting infected seeds. The seeds infected with BMMV-CIAT, CMV-CIAT and SBMV-CIAT, unfortunately, do not show any external symptoms so that they cannot be detected in seed lots.

No data are available as to the location of virus in seeds infected with BMMV-CIAT and CMV-CIAT, but according to the literature the bean strain of SBMV is transmitted in the seed coat and endosperm of bean seeds (Crowley, 1959; McDonald and Hamilton, 1972, 1973).

In many cases international transfer of genetic resources is responsible for long distance spread of viruses (Bos, 1978). The best way to avoid such a spread is to make sure that the seeds involved are virus-free.

SUMMARY

For the past years there have been outbreaks of a disease of bean (*Phaseolus vulgaris* L.) in Colombia called bean chlorotic mottle. The etiology of bean chlorotic mottle was not known, but the disease was generally believed to be incited by the same whitefly-transmitted virus that causes variegation in malvaceous plants. The aim of this study was to identify and further characterize the causal agent of bean chlorotic mottle.

As from the literature it was not clear which symptoms were characteristic of the disease, firstly a symptom had to be established common to all allegedly bean chlorotic mottle-affected bean plants. This symptom, a sharp mosaic in which the discoloured areas ranged from pale greenish-yellow to bright yellow, was of diagnostic value to detect infected plants in the field. In addition to this sharp mosaic, chlorotic mottle-affected bean plants in the field often showed a host of other symptoms, such as mild mottle, rugosity, leaf curling, malformation, dwarfing, proliferation and witches' broom-like growth. In the same affected population some bean plants showed only the sharp mosaic, whereas others showed additional witches' broom-like growth.

Mechanical inoculation experiments conducted with crude sap and partially purified preparations from bean plants exhibiting chlorotic mottle in the field, showed three viruses to be present, viz. bean mild mosaic virus, cucumber mosaic virus and southern bean mosaic virus. By means of differential hosts these three viruses could be separated. Isolates of these viruses were designated BMMV-CIAT, CMV-CIAT and SBMV-CIAT, respectively. Experiments conducted with whiteflies did not reveal the presence of any whitefly-transmitted virus in chlorotic mottle-affected bean plants.

BMMV-CIAT is an isometric particle, 32 nm in diameter, with a single-stranded RNA molecule. The virus is readily transmitted mechanically and its host range is restricted to legumes, viz. *Glycine max*, *Macroptilium* spp., *Phaseolus* spp. and *Rhynchosia minima*. In *P. vulgaris* the virus usually causes a mild mosaic which is hardly visible, and in certain cultivars with age, the virus becomes latent. Though affected bean plants grew normally producing healthy-looking pods, flowering and pod formation were usually delayed by about a

week under glasshouse conditions. The chrysomelid beetles *Diabrotica balteata* and *Cerotoma facialis* were efficient vectors of BMMV-CIAT. The virus is seed-transmitted in beans, percentages ranging from 1.2 to 3.6 in the different cultivars tested. In contrast to many other beetle-transmitted viruses, BMMV-CIAT sediments in sucrose gradients as one particle. The physical and chemical properties of BMMV-CIAT are similar or equal to those of BMMV described by Waterworth et al. (1977). The darkly stained granular material in root cells occasionally observed in the light microscope, proved to be aggregates of virus particles.

Cross protection and serological tests showed that CMV-CIAT is related to the CMV-type strain. However, many differences exist between them, the most striking being the ability of CMV-CIAT to cause systemic mosaic in *P. vulgaris*. The host reactions of CMV-CIAT resembled those of CMV-B 32, an isolate from bean in Spain (Bos and Maat, 1974), but the former had a longer longevity *in vitro*. Generally, CMV-CIAT induced a yellow mosaic in *P. vulgaris*, but the symptoms varied greatly in different cultivars. In the cultivars Honduras 46 and Porrillo 1 a sharp mosaic, similar to chlorotic mottle, was produced one month after inoculation with CMV-CIAT. None of the bean cultivars and CIAT advanced breeding lines tested were found to be resistant or hypersensitive to CMV-CIAT. The virus is transmitted by *Aphis gossypii* and through seeds of *P. vulgaris*, *Vigna radiata* and *V. unguiculata* 'California Blackeye'. It has poor antigenic properties and is serologically related to CMV-B 32, to a CMV isolate from *Yucca* (Bouwen et al., 1978) and to the Y-strain of CMV (Scott, 1968). Large inclusions in plant tissues infected with CMV-CIAT could easily be detected in the light microscope.

The third virus, SBMV-CIAT, is readily transmitted mechanically. Though the host plant range included mostly legumes, the virus also infected *Cucumis sativus* 'Ashley'. The virus was more harmful to *P. acutifolius*, in which it caused severe top necrosis, than to *P. vulgaris*. Among the cultivars and CIAT advanced breeding lines of *P. vulgaris*, hypersensitive hosts were found. The virus induced a bright yellow mosaic in *G. max*. Besides *P. vulgaris* 'Pinto U.I. 650' other suitable assay hosts for SBMV-CIAT were found viz. *Mucuna pruriens* and *M. utilis* which reacted with pin-point necrotic local lesions to the virus. SBMV-CIAT is seed transmitted, the percentages ranging

from 3.6 to 33.6 depending on the bean cultivar. The physical and chemical properties of SBMV-CIAT are similar or equal to the SBMV bean strain, except for the guanine content of the nucleic acid.

An experiment conducted to find out the effect of the above-mentioned viruses alone or in combination with each other on bean plants, indicated that symptoms resembling chlorotic mottle could be reproduced in bean plants by mechanical inoculation with CMV-CIAT alone or in combination with the other two viruses. The vast range of other symptoms sometimes exhibited by bean plants with chlorotic mottle in the field, could also be reproduced by mechanically inoculating the viruses in different combinations at different times after planting of seeds. Witches' broom-like symptoms were produced in bean plants inoculated on their primary leaves with a mixture containing SBMV-CIAT with BMMV-CIAT or with CMV-CIAT.

From the present study it is clear that bean chlorotic mottle, as described in literature, is a composite disease caused by a combination of BMMV-CIAT, CMV-CIAT and SBMV-CIAT, in which CMV-CIAT is responsible for the sharp mosaic and the other two viruses together are responsible for the additional symptoms like rugosity, leaf curling, witches' broom-like growth etc. Efficient vectors of these viruses are present in the bean fields. Though data are not available for the population distribution of aphids, chrysomelid beetles are present throughout the year in the CIAT fields. Seed transmission of the virus is of considerable epidemiological significance. Infected seeds are vehicles for long distance transport and survival from one season to another, especially in case of BMMV-CIAT and SMBV-CIAT, which have a narrow host range. The diseases caused by the three viruses might be controlled by reducing the number of infection sources and by limiting the spread of the viruses.

SAMENVATTING

De afgelopen jaren is in Colombia herhaalde malen een ziekte bij boon, 'bean chlorotic mottle' genaamd, opgetreden. De veroorzaker van deze ziekte was niet bekend, maar men nam algemeen aan, dat 'bean chlorotic mottle' door hetzelfde, door wittevliegen overgedragen virus, wordt veroorzaakt als de bekende bontheid bij Malvaceae. Het doel van de onderhavige studie was het identificeren en nader karakteriseren van de veroorzaker van het 'bean chlorotic mottle'.

Aangezien uit de literatuur niet duidelijk bleek welke van de vele genoemde symptomen karakteristiek geacht werden voor de ziekte, moest eerst naar een symptoom worden gezocht dat in alle publikaties over 'bean chlorotic mottle' werd vermeld. Van dit symptoom, een scherp mozaïek waarin de verkleurde gedeelten van het boneblad varieerden van bleek groengeel tot heldergeel, werd gebruik gemaakt bij het diagnostiseren van de ziekte in het veld. Behalve dit scherpe mozaïek vertoonden boneplanten in het veld vaak een groot aantal andere symptomen, zoals lichte vlekkerigheid, diepnervigheid, bladkrulling en heksenbezemachtige groei. In dezelfde aangetaste populatie vertoonden sommige boneplanten alleen het scherpe mozaïek, andere daarentegen tevens heksenbezemachtige groei.

Inoculatieproeven met ruw sap en gedeeltelijk gezuiverde preparaten van boneplanten die in het veld 'chlorotic mottle' vertoonden, wezen uit, dat drie virussen in deze planten aanwezig waren, nl. 'bean mild mosaic virus', komkommermozaïekvirus en 'southern bean mosaic virus'. Met behulp van differentiële waardplanten konden deze drie virussen worden gescheiden. Isolaten van deze virussen werden respectievelijk aangeduid als BMMV-CIAT, CMV-CIAT en SBMV-CIAT. Overdrachtsproeven met wittevliegen wezen niet in de richting van de aanwezigheid van een virus dat door deze insekten kon worden overgebracht.

Het BMMV-CIAT heeft een isometrisch deeltje met een middellijn van 32 nm en bezit een enkelstrengig RNA-molecuul. Het virus kan gemakkelijk mechanisch worden overgebracht; zijn waardplantenreeks is beperkt tot vertegenwoordigers uit de familie der Leguminosae, nl. *Glycine max*, *Macroptilium* spp.,*Phaseolus* spp. en *Rhynchosia minima*. Bij *P. vulgaris* veroorzaakt het virus gewoonlijk een zwak mozaïek, dat nauwelijks zichtbaar is, en bij oudere infecties wordt het virus

in bepaalde cultivars latent. Hoewel de groei van aangetaste boneplanten normaal was en gezond-uitziende peulen werden geproduceerd, trad onder kas-omstandigheden in de bloei en peul-vorming meestal een vertraging van ongeveer een week op. *Diabrotica balteata* en *Cerotoma facialis*, behorende tot de keverfamilie der bladhaantjes, waren efficiënte vectoren van BMMV-CIAT. Het virus gaat in bonen met zaad over, in percentages die variëren van 1,2 tot 3,6%, afhankelijk van de cultivar. In tegenstelling tot veel andere virussen die door kevers worden overgedragen, sedimenteert BMMV-CIAT in suikergradiënten als één deeltje. De fysische en chemische eigenschappen van BMMV-CIAT vertonen veel overeenkomst met, of zijn identiek aan die welke door Waterworth c.s. (1977) werden beschreven voor BMMV. Het donker gekleurde, korrelige materiaal dat af en toe in lichtmicroscopische coupes van wortelcellen werd waargenomen in het collenchym, bleek te bestaan uit aggregaten van virusdeeltjes.

Uit premunitieproeven en serologische toetsingen bleek, dat CMV-CIAT verwant is aan de 'type strain' van het komkommermozaïekvirus. In tegenstelling tot laatstgenoemd virus was CMV-CIAT echter in staat bij *P. vulgaris* een systemisch mozaïek te veroorzaken. De reacties van andere waardplanten van CMV-CIAT leken veel op die welke beschreven zijn voor infectie met CMV-B 32, een komkommermozaïekvirus geïsoleerd uit bonen in Spanje (Bos en Maat, 1974), maar CMV-CIAT heeft een langere houdbaarheid *in vitro*. Meestal veroorzaakte CMV-CIAT een geelachtig mozaïek in *P. vulgaris*, maar de symptomen varieerden sterk bij de verschillende cultivars. 'Honduras 46' en Porrillo 1' vertoonden een maand na inoculatie met CMV-CIAT een scherp mozaïek, dat veel overeenkomst vertoonde met dat van 'chlorotic mottle'. Geen enkele getoetste cultivar of CIAT-veredelingslijn van boon bleek resistent of overgevoelig te zijn voor CMV-CIAT. Het virus wordt overgebracht door *Aphis gossypii* en via zaad bij *P. vulgaris*, *Vigna radiata* en *V. unguiculata* 'California Blackeye'. Het CMV-CIAT bleek slechte antigene eigenschappen te bezitten en serologisch verwant te zijn met CMV-B 32, een isolaat van komkommermozaïekvirus uit *Yucca* (Bouwen c.s., 1978) en de Y-stam van komkommermozaïekvirus (Scott, 1968). In lichtmicroscopische preparaten van *N. glutinosa*, *P. vulgaris* en *V. unguiculata* geïnfecteerd met CMV-CIAT werden grote, kristalachtige insluitsels gevonden in het mesofyl.

Het derde virus, SBMV-CIAT, wordt eveneens gemakkelijk mechanisch overgebracht. Hoewel de waardplanten hoofdzakelijk vertegenwoordigers uit de familie der Leguminosae waren, infecteerde het virus ook *Cucumis sativus* 'Ashley'. Het ziektebeeld bij *P. acutifolius* was heviger (ernstige topnecrose) dan bij *P. vulgaris*. Onder de cultivars en CIAT-veredelingslijnen van *P. vulgaris* werden overgevoelige waardplanten gevonden. Het virus veroorzaakte een heldergeel mozaïek in *G. max*. Behalve *P. vulgaris* 'Pinto U.I. 650' bleken *Mucuna pruriens* en *M. utilis* geschikte toetsplanten te zijn, die met speldeknopgrote necrotische lokale lesies op infectie met het virus reageerden. Het SBMV-CIAT gaat met zaad over in percentages die varieerden van 3,6 tot 33,6%, afhankelijk van de cultivar. Met uitzondering van het guaninegehalte van het nucleïnezuur vertonen de fysische en chemische eigenschappen van het SBMV-CIAT veel overeenkomst met, of zijn identiek aan die van de bonestam van SBMV.

Een proef werd opgezet om na te gaan wat het effect van de drie bovengenoemde virussen, alleen of in combinatie met elkaar, op boneplanten was. Symptomen die sterk geleken op die van 'chlorotic mottle' werden verkregen in boneplanten die waren geïnoculeerd met CMV-CIAT alleen, of in combinatie met de twee andere virussen. De grote verscheidenheid aan andere symptomen die boneplanten met 'chlorotic mottle' soms in het veld vertonen, konden eveneens worden gereproduceerd door inoculatie van de drie virussen in verschillende combinaties op verschillende tijdstippen na het zaaien. Heksenbezemachtige symptomen kwamen voor bij boneplanten die op hun primaire bladeren waren geïnoculeerd met een mengsel bestaande uit SBMV-CIAT met BMMV-CIAT of CMV-CIAT.

Uit het huidige onderzoek is duidelijk geworden dat 'bean chlorotic mottle' zoals het in de literatuur is beschreven, een complexe ziekte is, veroorzaakt door een combinatie van BMMV-CIAT, CMV-CIAT en SBMV-CIAT, waarin CMV-CIAT verantwoordelijk is voor het scherpe mozaïek en de andere twee virussen voor de bijkomende symptomen, zoals diepnervigheid, bladkrulling, heksenbezemachtige groei etc. Efficiënte vectoren van deze virussen zijn in de bonevelden aanwezig. Hoewel geen gegevens beschikbaar zijn over de populatieverdeling van bladluizen, is het wel bekend dat bladhaantjes het hele jaar door voorkomen in de bonevelden van het CIAT. Zaadoverdracht is van grote epidemiologische betekenis. Geïnfecteerde zaden zijn middelen voor transport over grote afstand en voor het overblijven van het virus gedurende de verschillende seizoenen. Dit is speciaal van be-

tekenis in het geval van BMMV-CIAT en SBMV-CIAT die een kleine waardplantenreeks hebben. De ziekten veroorzaakt door de drie virussen kunnen mogelijk worden bestreden door het aantal infectiebronnen en de verspreiding van de virussen te beperken.

RESUMEN

En los últimos años ha habido en Colombia brotes de una enfermedad del fríjol (*Phaseolus vulgaris* L.) llamada moteado clorótico del fríjol. La etiología del moteado clorótico del fríjol no era conocida pero se creía generalmente que la enfermedad era incitada por el mismo virus transmitido por la mosca blanca qua causa variegación en las plantas malváceas. El ánimo de este estudio fue identificar y caracterizar aún más el agente causal del moteado clorótico del fríjol.

Debido a que en la literatura no era claro cuáles síntomas eran característicos de la enfermedad, se debía establecer primero un síntoma común a todas las plantas de fríjol que parecieran estar afectadas por el moteado clorótico. Este síntoma, un mosaico definido en el cual las áreas decoloradas varían entre el amarillo verdoso pálido y el amarillo brillante fue de valor diagnóstico para detectar las plantas infectadas en el campo. Además de este mosaico definido, las plantas de fríjol afectadas por el moteado clorótico en el campo mostraban a menudo otros síntomas tales como un moteado suave, rugosidad, enrollamiento de las hojas, malformación, enanismo, proliferación y crecimiento en escoba de bruja o blastomania. En la misma población afectada algunas plantas de fríjol mostraron únicamente el mosaico definido, mientras que otras mostraban crecimiento en escoba de bruja adicional.

Experimentos de inoculación mecánica conducidos con savia cruda y preparaciones parcialmente purificadas de plantas de fríjol que exhibían el moteado clorótico en el campo, mostraron que había tres virus presentes a saber: el virus del mosaico suave del fríjol, el virus del pepino y el virus del mosaico sureño del fríjol. Por medio de húespedes diferenciales se pudieron separar estos tres virus. Los aislamientos de estos virus fueron designados como BMMV-CIAT, CMV-CIAT y SBMV-CIAT respectivamente. Los experimentos conducidos con mosca blanca no revelaron la presencia de ningún virus transmitido por mosca blanca en las plantas de fríjol afectadas por el moteado clorótico.

El BMMV-CIAT es una partícula isométrica de 32 nm de diámetro con una sóla molécula de RNA. El virus se transmite mecánicamente muy fácil y el rango de sus huéspedes está restringido a las legum-

inosas, por ejemplo *Glycine max*, *Macroptilium* spp., *Phaseolus* spp. y *Rhynchosia minima*. En *P. vulgaris* el virus causa usualmente un mosaico leve el cual es difícilmente visible y en algunos cultivares con la edad el virus se convierte en latente. Aunque las plantas de fríjol afectadas crecen normalmente y producen vainas con una aparienca saludable, la floración y la formación de las vainas se retardaba usualmente cerca de una semana bajo condiciones de invernadero. Los crisomélidos *Diabrotica balteata* y *Cerotoma facialis* fueron vectores eficientes del BMMV-CIAT. Este virus se transmite por la semilla en fríjoles, en un porcentaje que varía entre 1.2 y 3.6 en los diferentes cultivares probados. En contraste con muchos otros virus transmitidos por cucarrones, el BMMV-CIAT se sedimenta en gradientes de sucrosa como una partícula. Las propiedades físicas y químicas del BMMV-CIAT son similares ó iguales a aquellas del BMMV descrito por Waterworth et al. (1977). El material granular oscuramente oxidado en las células de las raíces observado ocasionalmente en el microscopio de luz resultó ser agregados de partículas de virus.

La protección cruzada y las pruebas serológicas mostraron que el CMV-CIAT está relacionado con la cepa del tipo CMV. Sin embargo, existen muchas diferencias entre ellas, siendo la más sobresaliente la habilidad del CMV-CIAT para ocasionar un mosaico sistémico en *P. vulgaris*. Las reacciones del húesped de CMV-CIAT se parecen a aquellas del CMV-B32, un aislamiento de fríjol en España (Bos y Maat, 1974), pero el último tenía una longevidad más larga en vitro. Generalmente, el CMV-CIAT indujo un mosaico amarillo en *P. vulgaris* pero los síntomas variaron grandemente en diferentes cultivares. En los cultivares Honduras 46 y Porrillo 1 un mosaico definido, similar al moteado clorótico, se produjo un mes después de la inoculación con CMV-CIAT. Ninguno de los cultivares de fríjol ó líneas avanzadas de mejoramiento de CIAT probadas, se encontraron resistentes ó supersensitivas al CMV-CIAT. El virus se transmite por *Aphis gossypii* y a través de semillas de *P. vulgaris*, *Vigna radiata* y *V. unguiculata* 'California Blackeye'. Tiene propiedades antigénicas pobres y serológicamente está relacionado con el CMV-B32, con un aislamiento de CMV obtenido de *Yucca* (Bouwen et al., 1978) y con una cepa Y del CMV (Scott, 1968). Grandes inclusiones en tejidos de plantas infectadas con CMV-CIAT se pudieron detectar fácilmente en el microscopio de luz.

El tercer virus SBMV-CIAT es fácilmente transmitido mecánicamente. Aunque el rango de plantas huéspedes incluye la mayoría de las leguminosas, el virus infectó *Cucumis sativus* 'Ashley'. El virus fue más peligroso para *P. acutifolius*, en el cual causó una severa necrosis superior que para *P. vulgaris*. Entre los cultivares y líneas avanzadas del CIAT de *P. vulgaris* se encontraron húespedes supersensitivos. El virus indujo un mosaico amarillo brillante en *G. max*. Además del *P. vulgaris* 'Pinto U:I: 650' se encontraron otros húespedes utilizables para el SBMV-CIAT, por ejemplo el *Mucuna pruriens* y *M. utilis* los cuales reaccionaron al virus con lesiones necróticas locales. El SBMV-CIAT se transmite por semilla, con un porcentaje que varía entre el 3.6 y 33.6 dependiendo del cultivar. Las propiedades físicas y químicas del SBMV-CIAT son similares ó iguales a las de cepa de SBMV para fríjol excepto en el contenido de guanina del ácido nucléico.

Un experimento realizado para encontrar el efecto de los virus arriba mencionados solos ó en combinación con cada uno de los otros en plantas de fríjol, indicó que los síntomas que parecían el moteado clorótico podían reproducirse en plantas de fríjol por inoculación mecánica con CMV-CIAT sólo ó en combinación con los otros dos virus. El amplio rango de otros síntomas algunas veces exhibido por las plantas de fríjol con moteado clorótico en el campo, se puede reproducir también por inoculación mecánica de los virus en diferentes combinaciones y en diferentes tiempos después de la siembra de las semillas. Los síntomas de crecimiento en escoba de bruja fueron producidos en plantas de fríjol inoculadas en sus hojas primarias con una mezcla que contenía SBMV-CIAT con BMMV-CIAT ó con CMV-CIAT.

A partir del presente estudio es claro que el moteado clorótico del fríjol, como se describe en la literatura, es una enfermedad compuesta causada por una combinación de BMMV-CIAT, CMV-CIAT, y SBMV-CIAT en la cual el CMV-CIAT es el responsable del mosaico definido y los otros dos virus en conjunto con los síntomas adicionales tales como la rugosidad, el enroscamiento de las hojas y el crecimiento en escoba de bruja, etc. Vectores eficientes de estos virus están presentes en los campos de fríjol. Aunque no hay datos disponibles para la distribución de la población de áfidos los crisomélidos están presentes a través de todo el año en los campos de CIAT. La transmisión del virus por semilla es de una considerable significancia epidem-

iológica. Las semillas infectadas son vehículos para el transporte a gran distancia y supervivencia de una estación a otra especialmente en el caso del BMMV-CIAT y del SBMV-CIAT los cuales tienen un rango de hospedería estrecho. Las enfermedades causadas por los tres virus pueden controlarse reduciendo el número de fuentes de infección y limitando el esparcimiento de las mismas.

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REFERENCES

Agudelo, S.F., 1978. Revisión de trabajos hechos en Latinoamérica sobre virus de la habichuela (*Phaseolus vulgaris* L.) y su relación con el mosaico dorado de este cultivo en la República Dominicana. Investigación 6: 43-46.

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: 345-348.

Babovič, M., 1968. Virus diseases of alfalfa in Yugoslavia (Yugosl. with Engl. summ.). Zast. Bilja 102: 345-348.

Bailey, L.H., 1977. Manual of cultivated plants. The MacMillan Company, New York, 1116 pp.

Bancroft, J.B., 1971. Cowpea chlorotic mottle virus. C.M.I./A.A.B. Descriptions of plant viruses. No. 94: 4 pp.

Bancroft, J.B. & I.H. Flack, 1972. The behaviour of cowpea chlorotic mottle virus in CsCl. J. gen. Virol. 15: 247-251.

Bird, J. & J.H. López-Rosa, 1973. Whitefly and aphid-borne viruses of bean in Puerto Rico. In: Proceedings of the first IITA Grain Legume Improvement Workshop. IITA, Ibadan, Nigeria: 276-278.

Bird, J. & J. Sánchez, 1971. Whitefly-transmitted viruses in Puerto Rico. J. Agric. Univ. Puerto Rico 55: 461-466.

Bird, J., J. Sánchez & J.H. López-Rosa, 1970. Whitefly-transmitted viruses in Puerto Rico. Phytopathology 60: 1539 (Abstr.).

Bird, J., J. Sánchez, R.L. Rodríguez & F.J. Juliá, 1975. Rugaceous (whitefly-transmitted) viruses in Puerto Rico. In: J. Bird and K. Maramorosch (Eds.), Tropical diseases of legumes. Academic Press, New York: 3-25.

Bird, J., J. Sánchez, R.L. Rodríguez, A. Cortés-Monllor & W.J. Kaiser, 1974. A mosaic of beans (*Phaseolus vulgaris* L.) caused by a strain of common cucumber mosaic virus. J. Agric. Univ. Puerto Rico 53: 151-161.

Bishop, D.H.L., J.R. Claybrook & S. Spiegelman, 1967. Electrophoretic separation of viral nucleic acids on polyacrylamide gels. J. mol. Biol. 26: 373-387.

Bos, L., 1975. The application of TMV particles as an internal magnification standard for determining virus particle sizes with the electron microscope. Neth. J. Pl. Path. 81: 168-175.

Bos, L., 1978. Seed-borne viruses. In: W.B. Hewitt and L. Chiarappa (Eds.), Plant health and quarantine in international transfer of genetic resources. Chemical Rubber Company Press, Inc.: 39-69.

Bos, L. & D.Z. Maat, 1974. A strain of cucumber mosaic virus, seed-transmitted in beans. Neth. J. Pl. Path. 80: 113-123.

Bouwen, I., A.J. Wit & J. Dijkstra, 1978. Cucumber mosaic virus isolated from *Yucca flaccida*. Neth. J. Pl. Path. 84: 174-183.

Brakke, M.K. & N. van Pelt, 1970. Linear-log sucrose gradient for estimating sedimentation coefficients of plant viruses and nucleic acid. Anal. Biochem. 38: 56-64.

Bruna de Tosso, A. & C. Urbina de Vidal, 1976. Presencia del virus del mosaico amarillo en Chile. Agric. Tech. (Chile) 36: 19-25.

Buol, S.W., P.A. Sánchez, R.B. Cate & M.A. Granger, 1975. Soil fertility capability classification. In: E. Bornemisa and A. Alvarado (Eds.), Soil management in Tropical America. North Carolina State Univ.: 126-141.

Burga, M.C. & M.R. Scheffer, 1974. Estudios de transmisión por semilla del virus del mosaico común del fríjol y su efecto en el rendimiento. Resúmenes 2° Congreso Nal. Investigadores Agric. del Perú (II CONIAP), Lima, 42-43.

Burton, K., 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. 62: 315-323.

Byers, B., 1967. Structure and formation of ribosome crystals in hypothermic chick embryo cells. J. mol. Biol. 26: 155-167.

Cartree, R.L. & R.J. Hanks, 1974. Effect of ridging and early season cultivation on bean yield. Agron. J. 66: 632-635.

Cheo, P.C., 1955. Effect of seed maturation on inhibition of southern bean mosaic virus in bean. Phytopathology 45: 17-21.

Christie, R.G. & J.R. Edwardson, 1977. Light and electron microscopy of plant virus inclusions. Fla. Agric. Exp. Stns. Monogr. Series 9: 150 pp.

CIAT, 1973. Bean Production Program, Ann. Rept. Centro Internacional de Agricultura Tropical, Cali, Colombia: 146-184.

CIAT, 1974. Bean Production Program, Ann. Rept. Centro Internacional de Agricultura Tropical, Cali, Colombia: 52.

CIAT, 1975. Bean Production Program, Ann. Rept. Centro Internacional de Agricultura Tropical, Cali, Colombia: 58.

CIAT, 1976. Bean Production Program, Ann. Rept. Centro Internacional de Agricultura Tropical, Cali, Colombia: 83.

CIAT, 1977. Bean Production Program, Ann. Rept. Centro Internacional de Agricultura Tropical, Cali, Colombia: 84.

CIAT, 1978a. Bean Producion Program, Ann. Rept. Centro Internacional de Agricultura Tropical, Cali, Colombia: 81.

CIAT, 1978b. International Bean Rust Nurseries. Series 20 EB-1: 22.

CIAT, 1979. Bean Production Program, Ann. Rept. Centro Internacional de Agricultura Tropical, Cali, Colombia: 111.

Costa, A.S., 1955. Studies on *Abutilon* mosaic in Brazil. Phytopath. Z. 24: 97-112.

Costa, A.S., 1965. Three whitefly-transmitted virus diseases of beans in São Paulo, Brazil. F.A.O. Plant Prot. Bull. 13: 1-12.

Costa, A.S., 1972. Investigaçoes sobre moléstias do feijoeiro no Brasil. In: Anais Do I Simpósio Brasileiro de Feijão. Univ. Federal Vicosa, Minas Gerais, Brazil: 338-342.

Costa, A.S., 1975. Increase in the populational density of *Bemisia tabaci*, a threat of widespread virus infection of legume crops in Brazil. In: J. Bird and K. Maramorosch (Eds.), Tropical diseases of legumes. Academic Press, New York: 27-49.

Crowley, N.C., 1959. Studies on the time of embryo infection by seed-transmitted viruses. Virology 8: 116-123.

Díaz-Romeu, R., F. Balerdi & H.W. Fassbender, 1970. Contenido de materia orgánica y nitrogeno en suelos de América Central. Turrialba 20: 185-192.

Diener, T.O., 1965. Isolation of infectious ribonucleic acid from southern bean mosaic virus. Virology 27: 425-429.

Doolittle, S.P., 1916. A new infectious mosaic disease of cucumber. Phytopathology 6: 145-147.

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

Döbereiner, J., 1966. Manganese toxicity effects on nodulation and nitrogen fixation of beans (*Phaseolus vulgaris* L.) in acid soils. Plant and Soil 24: 153-166.

Elbers, P.F. & J. Pieters, 1964. Accurate determination of magnification in the electron microscope. J. Ultrastruct. Res. 11: 25-32.

Encke, F.H.C., G. Buchheim, & S. Seybold, 1980. Zander Handwörterbuch der Pflanzennamen 12th ed. Verlag Eugen Ulmer, Stuttgart, 844 pp.

Englander, S.W. & H.T. Epstein, 1955. Optical method for measuring nucleoprotein and nucleic acid concentrations. Arch. Biochem. Biophys. 68: 144-149.

F.A.O., 1975. Production year book, volume 29. Food and Agriculture Organisation, Rome: 523 pp.

F.A.O., 1976. Production year book, volume 30. Food and Agriculture Organisation, Rome: 290 pp.

F.A.O., 1977. Production year book, volume 31. Food and Agriculture Organisation, Rome: 286 pp.

Fassbender, H.W., L. Muller & F. Balerdi, 1968. Estudio del fósforo en suelos de América Central II. Formas y su relación con las plantas. Turrialba 18: 333-347.

Férault, A.C., D. Spire, H. Bannerot, J.J. Bertrandy & T. le Tan, 1969. Identification dans la région parisienne d'une marbrure du haricot comparable au bean southern mosaic virus (Zaumeyer et Harter). Ann. Phytopathol. 1: 619-626.

Flores, E. & K. Silberschmidt, 1958. Relations between insect and host plant in transmission experiments with infectious chlorosis of Malvaceae. Ann. Acad. Bras. Ciênc. 30: 535-560.

Flores, E. & K. Silberschmidt, 1963. Ability of single whiteflies to transmit concomitantly a strain of infectious chlorosis of Malvaceae and of *Leonurus* mosaic virus. Phytopathology 53: 238.

Flores, E., K. Silberschmidt & M. Kramer, 1960. Observações de 'clorose infecciosa' das malváceas em tomateiros do campo. O Biológico 26: 65-69.

Francki, R.I.B., D.W. Mossop & T. Hatta, 1979. Cucumber mosaic virus. C.M.I./A.A.B. Descriptions of plant viruses No. 213: 6 pp.

Francki, R.I.B., J.W. Randles, T.C. Chambers & S.B. Wilson, 1966. Some properties of purified cucumber mosaic virus (Q strain). Virology 28: 729-741.

Franco, A.A., 1977. Nutritional restraints for tropical grain legume symbiosis. In: J.M. Vincent (Ed.), Exploiting the legume-*Rhizobium* symbiosis in tropical agriculture. Univ. of Hawaii, Coll. of Trop. Agric. Misc. Pub. 145: 237-252.

Gálvez, G.E., 1977. Enfermedades virales del fríjol y su control. CIAT, Cali, Colombia: 33 pp.

Gálvez, G.E., 1980. Aphid-transmitted viruses. In: H.F. Schwartz and G.E. Gálvez (Eds.), Bean production problems. CIAT Pub. Series 09EB-1: 211-238.

Gálvez, G.E. & M.R. Cárdenas, 1974. Pérdidas económicas causadas por el virus del mosaico común (BCMV) en 4 variedades de fríjol. Proc. Amer. Phytopath. Soc. 1: 121-122.

Gálvez, G.E. & M.R. Cárdenas, 1980. Whitefly-transmitted viruses. In: H.F. Schwartz and G.E. Gálvez (Eds.). Bean production problems. CIAT Pub. Series 09EB-1: 261-289.

Gálvez, G.E., M.J. Castaño & S. Belalcázar, 1975. Presencia de los virus del mosaico dorado y del moteado clorótico del fríjol en Colombia. Ascolfi Informa (Colombia) 1: 3-4.

Gámez, R., 1969a. Los virus del fríjol en Costa Rica. III. Moteado clorótico. In: C.L. Arias (Ed.), XV Reunión Anual Programa Cooperativo Centroamericano para el Mejoramiento de Cultivos Alimenticios, San Salvador, El Salvador, IICA Publicación Miscelánea 68: 29.

Gámez, R., 1969b. Estudios preliminares sobre virus del fríjol transmitidos por moscas blancas (Aleroididae) en El Salvador. In: C.L. Arias (Ed.), XV Reunión Anual del Programa Cooperativo Centroamericano para el Mejoramiento de Cultivos Alimenticios, San Salvador, El Salvador: 32-33.

Gámez, R., 1970. El virus del moteado amarillo del fríjol. Plantas hospederas y efecto en producción. In: C.L. Arias (Ed.), XVI Reunión Anual del Programa Cooperativo Centroamericano para el Mejoramiento de Cultivos Alimenticios, Antigua, Guatemala: 44-48.

Gámez, R., 1971. Los virus del fríjol en Centroamérica. I. Transmisión por moscas blancas (*Bemisia tabaci* Gen.), y plantas hospedantes del virus del mosaico dorado. Turrialba 21: 22-27.

Gámez, R., 1972. Reacción de variedades de fríjol a diversos virus de importancia en Centro América. In: R.V. Fernando and M. Heleodoro (Eds.), XVIII Reunión Anual del Programa Cooperativo Centroamericano para el Mejoramiento de Cultivos Alimenticios, Managua, Nicaragua: 108-109.

Gámez, R., 1976. Los virus del fríjol en Centroamérica. IV. Algunas propiedades y transmisión por insectos crisomélidos del virus del moteado amarillo del fríjol. Turrialba 26: 160-166.

Gámez, R., 1980. Beetle-transmitted viruses. In: H.F. Schwartz and G.E. Gálvez (Eds.), Bean production problems. CIAT Pub. Series 09EB-1: 241-259.

Ghabrial, S.A., R.J. Shepherd & R.G. Grogan, 1967. Chemical properties of three strains of southern bean mosaic virus. Virology 33: 17-25.

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

Gibbs, A.J. & B.D. Harrison, 1976. Plant virology; the principles. Edward Arnold (Publishers) Ltd., London: 292 pp.

Gould, H.J., J.C. Pinder & H.R. Matthews, 1969. Fractionation of low molecular weight fragments of ribosomal and viral RNA by polyacrylamide gel electrophoresis. Anal. Biochem. 29: 1-21.

Graham, P.H., 1978. Problems and potentials of field beans (*Phaseolus vulgaris* L.) in Latin America. Field Crops Res. 1: 295-317.

Grogan, R.G. & K.A. Kimble, 1964. The relationship of severe bean mosaic virus from Mexico to southern bean mosaic virus and its related strain in cowpea. Phytopathology 54: 75-78.

Gutiérrez, U., M.A. Infante & A.M. Pinchinat, 1975. Situación del cultivo de fríjol en América Latina, Centro Internacional de Agricultura Tropical, CIAT Series ES-19: 33 pp.

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

Hearst, J.E. & J. Vinograd, 1961. The net hydration of T-4 bacteriophage deoxyribonucleic acid and the effect of hydration on buoyant behavior in a density gradient at equilibrium in the ultracentrifuge. Proc. Nat. Acad. Sci. U.S. 47: 1005-1014.

Hollings, M., 1974. Recent advances in virus detection and identification by bioassay and serological tests. Acta Horticul. 36: 23-34.

Hollings, M. & O.M. Stone, 1962. Use of formalin-treated antigen in the production of antiserum to a plant virus. Nature (London) 194: 607.

Hollings, M. & O.M. Stone, 1970. Attempts to eliminate chrysanthemum stunt from chrysanthemum by meristem-tip culture after heat-treatment. Ann. appl. Biol. 65: 311-315.

Hollings, M., O.M. Stone & A.A. Brunt, 1968. Cucumber mosaic virus. Rep. Glasshouse Crops Res. Inst. 1967: 95-98.

Honma, S., 1956. A bean interspecific hybrid. J. Hered. 47: 217-220.
Jayasinghe, U. & J. Dijkstra, 1979. *Hippeastrum* mosaic virus and another filamentous virus in *Eucharis grandiflora*. Neth. J. Pl. Path. 85: 47-65.
Kaiser, W.J. & D. Danesh, 1971. Etiology of virus-induced wilt of *Cicer arietinum*. Phytopathology 61: 453-457.
Kaiser, W.J., D. Danesh, M. Okhovvat & G.H. Mossahebi, 1972. Virus diseases of pulse crops in Iran. Bull., Faculty of Agriculture, Univ. of Tehran, Karaj, Iran: 111 pp.
Kennedy, J.S., M.F. Day & V.F. Eastop, 1962. A conspectus of aphids as vectors of plant viruses. London, Commonwealth Institute of Entomology: 114 pp.
Kitajima, E.W. & A.S. Costa, 1974. Microscopia electronica de tecidos foliares de plantas afetadas por virus transmitidos por mosca-branca. VII Reuniao Anual Soc. Bras. Fitopat. 9: 54-55.
Knight, C.A., 1963. Chemistry of viruses. Protoplasmatologia, IV (2). Springer Verlag, Wien: 80-82.
Knight, C.A., 1975. Chemistry of viruses. Springer Verlag, Wien: 93-94.
Kuhn, C.W., 1963. Field occurrence and properties of the cowpea strain of southern bean mosaic virus. Phytopathology 53: 732-733.
Lamptey, P.N.L. & R.I. Hamilton, 1974. A new cowpea strain of southern bean mosaic virus from Ghana. Phytopathology 64: 1100-1104.
Lane, L.C. & P. Kaesberg, 1971. Multiple genetic components in bromegrass mosaic virus. Nature New Biology 232: 40-43.
Loening, U.E. & J. Ingle, 1967. Diversity of RNA components in green plant tissues. Nature (London) 215: 363-367.
Lorz, A.P., 1954. The development of new varieties of table legumes for production in Florida. Proc. Soil Sci. Soc. Florida 13: 64-70.
Magdoff-Fairchild, B.S., 1967. Electrophoretic and buoyant density variants of southern bean mosaic virus. Virology 31: 142-153.
Maizel, J.V., 1966. Acrylamide-gel electropherograms by mechanical fractionation: Radio active adenovirus proteins. Science 151: 988-990.
Maizel, J.V., 1971. Polyacrylamide gel electrophoresis of viral proteins. In: K. Maramorosch and H. Koprowski (Eds.), Methods in Virology. Academic Press. New York/London: 179-246.

Mancía, J.E. & M.R. Cortez, 1975. Lista de insectos clasificados encontrados en el cultivo del fríjol, *Phaseolus vulgaris* L. SIADES 4: 120-136.

Maréchal, R., J.M. Marscherpa & F. Stainer, 1978. Etude taxonomique d'un groupe complexe d'espèces des genres *Phaseolus* et *Vigna* (Papilionaceae) sur la base de données morphologiques et polliniques, traitées par l'analyse informatique. Boissiera Volume 28: 272.

Markham, R., 1960. A graphical method for the rapid determination of sedimentation coefficients. Biochem. J. 77: 516:519.

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

Marrou, J., J.B. Quiot, G. Marchoux & M. Duteil, 1975. Caractérisation par la symptomatologie de quatorze souches du virus de la mosaïque du concombre et de deux autres cucumovirus: Tentative de classification. Meded. Fac. Landbouwwet. Rijksuniv. Gent. 40: 107-121.

Martyn, E.B., 1968. Plant virus names. An annotated list of names and synonyms of plant viruses and diseases. Commonwealth Mycol. Inst., Surrey, England: 204 pp.

McDonald, J.G. & R.I. Hamilton, 1972. Distribution of southern bean mosaic in the seed of *Phaseolus vulgaris*. Phytopathology 62: 387-389.

McDonald, J.G. & R.I. Hamilton, 1973. Analytical density-gradient centrifugation of southern bean mosaic virus from seedcoats of *Phaseolus vulgaris*. Virology 56: 181-188.

Meiners, J.P., H.E. Waterworth, R.H. Lawson & F.F. Smith, 1977. Curly dwarf mosaic disease of beans from El Salvador. Phytopathology 67: 163-168.

Miller, G.L. & W.C. Price, 1946. Physical and chemical studies on southern bean mosaic virus II. Crystallization by dialysis. Arch. Biochem. 11: 329-335.

Morales, F.J., 1979. Purification and serology of bean common mosaic virus. Turrialba 24: 320-323.

Morgan, R.S. & B.G. Uzman, 1966. Nature of the packing of ribosomes within chromatoid bodies. Science 152: 214-216.

Morrison, W.R., 1964. A fast, simple and reliable method for the micro-determination of phosphorus in biological materials. Analyt. Biochem. 7: 218-224.

Muller, L., F. Balerdi & R. Díaz-Romeu, 1968. Estudio del fósforo en suelos de América Central I. Ubicación, características físicas y químicas de los suelos estudiados. Turrialba 18: 319-332.

Murant, A.F., 1965. The morphology of cucumber mosaic virus. Virology 26: 538-544.

Murillo, J.I., 1967. Estudio sobre dos aislamientos virosos de fríjol en Costa Rica. In: C.L. Arias (Ed.), XIII Reunión Anual del Programa Cooperativo Centroamericano para el Mejoramiento de Cultivos Alimenticios, San José, Costa Rica: 52-55.

Ouchterlony, O., 1962. Diffusion-in-gel methods for immunological analysis. II. In: Progress in Allergy 6: 30-154.

Paul, H.L., 1959. Die Bestimmung des Nucleinsäuregehaltes pflanzlicher Viren mit Hilfe einer spektrophotometrischen Methode. Z. Naturf. 14B: 427-432.

Paz, S., R. Reyna & A. Martinez, 1975. El rendimiento del fríjol en funcion del grado daño de la conchuela, *Epilachna varivestris* Muls. Agrociencia (Mexico) 22: 25-38.

Peacock, A.C. & C.W. Dingman, 1967. Resolution of multiple ribonucleic acid species by polyacrylamide gel electrophoresis. Biochemistry 6: 1818-1827.

Peacock, A.C. & C.W. Dingman, 1968. Molecular weight estimation and separation of ribonucleic acid by electrophoresis in agarose-acrylamide composite gels. Biochemistry 7: 668-674.

Price, W.C., 1940. Comparative host ranges of six plant viruses. Am. J. Bot. 27: 530-541.

Price, W.C., R.C. Williams & R.W.G. Wyckoff, 1946. Electron micrographs of crystalline plant viruses. Arch. of Biochem. 9: 175-185.

Provvidenti, R. 1976. Relation of *Phaseolus* and *Macroptilium* species to a strain of cucumber mosaic virus. Pl. Dis. Reptr. 60: 289-293.

Purcifull, D.E. & D.L. Batchelor, 1977. Immunodiffusion tests with sodium dodecyl sulfate (SDS)-treated plant viruses and plant viral inclusions. Univ. of Florida, Agric. Expt. Statns. bull. 788: 1-39.

Putman, E.W., 1957. Paper chromatography of the sugars. In: S.P. Colowick and N.O. Kaplan (Eds.), Methods in enzymology III. Academic Press, New York: 62-77.

Reynolds, E.S., 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17: 208-212

Ruiz de Londoño, N. & P. Pinstrup-Andersen, 1978. Barreras a los incrementos de productividad de fríjol a nivel de finca en Colombia. CIAT, Cali, Colombia: 28 pp.

Ruppel, R.F. & E. Idrobo, 1962. Lista preliminar de insectos y otros animales que dañan fríjoles en América. Agric. Trop. (Colombia) 18: 651-679.

Rushizky, G.W. & C.A. Knight, 1959. Ribonuclease-sensitive infectious units from tomato bushy stunt virus. Virology 8: 448-455.

Sanders, J.H. & P.C. Alvarez, 1978. Evolución de la producción del fríjol en América Latina durante la última década. CIAT series 06SB-1: 48 pp.

Schmelzer, K., 1956. Beiträge zur Kenntnis der Übertragbarkeit von Viren durch *Cuscuta*-Arten. Phytopath. Z. 28: 1-56.

Schmelzer, K. & H.E. Schmidt, 1975. Systemischer Befall durch das Gurkenmosaik-Virus an der Gartenbohne (*Phaseolus vulgaris* L.) in der DDR. Zbl. Bakt. Abt. II. 130: 234-237.

Schwartz, H.F., G.E. Gálvez, A. van Schoonhoven, R.H. Howeler, P.H. Graham & C. Flor, 1978. Field problems of beans in Latin America. Centro Internacional de Agricultura Tropical, Series GE19: 136 pp.

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

Scott, H.A., 1968. Serological behavior of cucumber mosaic virus (strain Y) and the virus protein. Virology 34: 79-90.

Sehgal, O.P., 1973. Inactivation of southern bean mosaic virus and its ribonucleic acid by nitrous acid and ultraviolet light. J. gen. Virol. 18: 1-10.

Sehgal, O.P., J. Jong-Ho, R.B. Bhalla, M.M. Soong & G.F. Krause, 1970. Correlation between buoyant density and ribonucleic acid content in viruses. Phytopathology 60: 1778-1784.

Shepherd, R.J., 1964. Properties of a mosaic virus of cowpea and its relationship to the bean pod mottle virus. Phytopathology 54: 466-473.

Shepherd, R.J., 1971. Southern bean mosaic virus. C.M.I./A.A.B. Descriptions of plant viruses. No. 57: 4 pp.

Shepherd, R.J. & R.W. Fulton, 1962. Identity of a seed-borne virus of cowpea. Phytopathology 52: 489-493.

Shoyinka, S.A., R.F. Bozart, J. Reese & B.O. Okusanya, 1979. Field occurrence and identification of southern bean mosaic virus (Cowpea strain) in Nigeria. Turrialba 29: 111-116.

Silberschmidt, K. & L.R. Tommasi, 1955. Observações e estudos sôbre espécies de plantas sucetíveis à Clorose Infecciosa das Malváceas. Ann. Acad. Bras. Ciênc. 27: 195-214.

Silberschmidt, K. & L.R. Tommasi, 1956. A solanaceous host of the virus of 'infectious chlorosis' of Malvaceae. Ann. appl. Biol. 44: 161-165.

Smith, K.M., 1972. A textbook of plant virus diseases. Longman Group Ltd. London: 684 pp.

Sober, H.A., 1970. Handbook of Biochemistry. Chemical Rubber Company, Cleveland.

Spurr, A.R., 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26. 31-43.

Summer, W.C., 1970. A simple method for extraction of RNA from *E. coli* utilizing diethylpyrocarbonate. Anal. Biochem. 33: 459-468.

Tremaine, J.H., 1966. The amino acid and nucleotide composition of the bean and cowpea strains of southern bean mosaic virus. Virology 30: 348-354.

Tsuchizaki, T., 1973. Peanut stunt virus isolated from beans in Japan. Ann. Phytopath. Soc. Japan 39: 67-72.

Tsuchizaki T., K. Yora & H. Asuyama, 1970. The viruses causing mosaic of cowpeas and Adzuki beans and their transmissibility through seeds. Ann. Phytopath. Soc. Japan 36.: 112-120.

Uyemoto, J.K. & R.G. Grogan, 1977. Southern bean mosaic virus evidence for seed transmission in bean embryos. Phytopathology 67: 1190-1196.

Van der Want, J.P.H., M.L. Boerjan & D. Peters, 1975. Variability of some plant species from different origins and their suitability for virus work. Neth. J. Pl. Path. 81: 205-216.

Van Kammen, A. & C.P. de Jager, 1978. Cowpea mosaic virus. C.M.I./ A.A.B. Descriptions of plant viruses. No. 197: 6 pp.

Van Schoonhoven, A., 1974. Insects associated with beans in Latin America. Int. Symp. on Pests of Grain Legumes. IITA, Ibadan, Nigeria.

Verduin, B.J.M., 1978. Characterization of cowpea chlorotic mottle virus and its assembly. PhD. Thesis, Agric. Univ., Wageningen, The Netherlands: 1-134.

Von der Phalen, A., 1962. El mosaico amarillo del poroto, *Phaseolus* virus 2 (Pierce) Smith en cultivos de haba, arveja y poroto en los alrededores de Buenos Aires. Revista de Invest. Agrícolas 16: 18-92.

Walters, H.J., 1964. Transmission of southern bean mosaic virus by the bean leaf beetle. Pl. Dis. Reptr. 48: 935.

Waterworth, H.E., J.P. Meiners, R.H. Lawson & F.F. Smith, 1977. Purification and properties of a virus from El Salvador that causes mild mosaic in bean cultivars. Phytopathology 67: 169-173.

Weber, K. & M. Osborne, 1969. The reliability of molecular weight determinations by dodecyl sulphate polyacrylamide gel electrophoresis. J. biol. Chem. 244: 4406-4412.

Weintraub, M. & H.W.J. Ragetli, 1970. Electron microscopy of the bean and cowpea strains of southern bean mosaic virus within leaf cells. J. Ultrastruct. Res. 32: 167-189.

Wellmann, F.L., 1968. More diseases on crops of the tropics than in the temperate zone. Ceiba 14: 17-28.

Whipple, O.C. & J.C. Walker, 1941. Strains of cucumber mosaic virus pathogenic on bean and pea. J. agric. Res. 62: 27-60.

Wilcockson, J. & R. Hull, 1974. The rapid isolation of plant virus RNAs using sodium perchlorate. J. gen. Virol. 23: 107-111.

Yerkes, W.D. & G. Patiño, 1960. The severe bean mosaic virus, a new bean virus from Mexico. Phytopathology 50: 334-338.

Zaumeyer, W.J. & L.L. Harter, 1943a. Two new virus diseases of beans. J. agric. Res. 67: 305-327.

Zaumeyer, W.J. & L.L. Harter, 1943b. Inheritance of symptom expression of bean mosaic virus 4. J. agric. Res. 67: 287-292.

Zaumeyer, W.J. & H.R. Thomas, 1950. Yellow stipple, a virus disease of beans. Phytopathology 40: 847-859.

Zaumeyer, W.J. & H.R. Thomas, 1957. A monographic study of bean diseases and methods for their control. U.S.D.A. Agr. Tech. Bull. 868: 255 pp.

APPENDIX

Reactions of advanced breeding lines of dry bean developed by CIAT (EP 1980 = ensayo preliminar 1980 = preliminary trial 1980) inoculated with BMMV-CIAT, CMV-CIAT and SBMV-CIAT.

Breeding line	BMMV-CIAT		CMV-CIAT		SBMV-CIAT	
	Local	Systemic	Local	Systemic	Local	Systemic
A 24	-	MOS,EP	-	MOS	-	MOS
A 40	-	MOS**	-	MOS	-	MOS
A 43	-	MOS	-	MOS	-	MOS
A 48	-	MOS	-	MOS	-	MOS
A 51	-	MOS,EP	NS	MOS	-	MOS
A 52	-	MOS	-	MOS	-	MOS
A 54	-	MOS	-	MOS	-	MOS
BAT 37	-	MOS	-	MOS	-	MOS
BAT 122	-	MOS	-	MOS	-	MOS
BAT 137	-	MOS	-	MOS	-	MOS
BAT 148	-	MOS	-	MOS	-	MOS
BAT 235	-	VC,MOS	-	MOS	-	MOS
BAT 331	-	MOS	-	MOS	-	MOS
BAT 337	-	MOS	-	MOS	-	MOS
BAT 339	-	MOS	-	MOS	-	MOS
BAT 341	-	MOS	-	MOS	-	MOS
BAT 447	-	VC,MOS,EP	NS	MOS	-	MOS
BAT 451	-	VC,MOS	-	MOS	-	MOS
BAT 527	-	MOS	-	MOS	-	MOS
BAT 589	-	MOS	-	MOS	NS	MOS(1)
BAT 731	-	MOS	-	MOS	-	MOS
BAT 790	-	MOS	NS	MOS	-	MOS
BAT 791	-	MOS,EP	-	MOS	-	MOS
BAT 792	-	MOS	NS	MOS	-	MOS
BAT 794	-	MOS	NS,NLP	MOS	-	MOS
BAT 795	-	MOS	-	MOS	-	MOS
BAT 799	-	MOS	-	MOS	NS	MOS(1)
BAT 804	-	MOS	-	VC,MOS	-	MOS
BAT 805	-	MOS	-	MOS	NS	-
BAT 828	-	MOS	-	VC,MOS	-	MOS
BAT 832	-	MOS	-	VC,MOS	-	MOS
BAT 838	-	MOS	-	MOS	-	MOS
BAT 839	-	MOS	-	MOS	-	MOS
BAT 841	-	MOS	-	MOS,TN	-	MOS
BAT 847	-	VC,MOS	-	VC,MOS	-	MOS
BAT 858	-	MOS	-	MOS	NS	-
BAT 867	-	MOS	-	MOS	-	MOS
BAT 868	-	MOS	-	MOS	-	MOS
BAT 871	-	MOS	-	VC,MOS	-	MOS
BAT 873	-	MOS	-	VC,MOS	NOT TESTED	
BAT 874	-	MOS	-	MOS	-	MOS
BAT 876	-	MOS,EP	-	MOS	NS	-
BAT 885	-	MOS	-	MOS	-	MOS
BAT 906	-	MOS	-	VC,MOS	-	MOS

BAT 910	-	MOS	-	MOS	-	MOS
BAT 912	-	MOS	-	MOS	-	MOS
BAT 913	-	MOS	-	MOS	-	MOS
BAT 919	-	MOS	-	MOS	-	MOS
BAT 923	-	MOS	-	MOS	-	MOS
BAT 940	-	MOS	-	MOS	-	MOS
BAT 945	-	MOS	-	MOS	-	MOS
BAT 947	-	MOS	NS	MOS	-	MOS
BAT 950	-	IVC,EP	-	MOS,VN	-	MOS
BAT 958	-	MOS,EP	-	MOS	NOT TESTED	
BAT 963	-	IVC	-	MOS	-	MOS
BAT 964	-	MOS	-	MOS	-	MOS
BAT 965	-	MOS	-	MOS	-	MOS
BAT 966	-	MOS	-	MOS	-	MOS
BAT 971	-	MOS	GS,NS	VC,MOS	-	MOS
BAT 972	-	MOS	-	MOS	-	MOS
BAT 986	-	MOS	-	MOS	-	MOS
BAT 995	-	MOS	-	MOS	-	MOS
BAT 1037	-	MOS	-	MOS	-	MOS
BAT 1056	-	MOS	-	MOS	-	MOS
BAT 1057	-	MOS	-	MOS	-	MOS
BAT 1060	-	MOS	-	MOS	-	MOS
BAT 1061	-	MOS	-	MOS	-	MOS
BAT 1079	-	MOS	-	MOS	-	MOS
BAT 1081	-	MOS	-	MOS	-	MOS
BAT 1088	-	MOS	-	MOS	-	MOS
BAT 1090	-	MOS	-	MOS	-	MOS
BAT 1103	-	MOS	-	MOS	-	MOS
BAT 1105	-	MOS	-	MOS	-	MOS
BAT 1113	-	MOS	-	MOS	-	MOS
BAT 1127	-	MOS	-	MOS	-	MOS
BAT 1129	-	MOS	-	MOS	-	MOS
BAT 1136	-	MOS	-	MOS	-	MOS
BAT 1139	-	MOS	-	MOS	-	MOS
BAT 1145	-	MOS	-	MOS	-	MOS
BAT 1163	-	MOS	-	MOS	-	MOS
BAT 1170	-	MOS**	-	MOS	-	MOS
CEMA 164-1	-	MOS	-	MOS	-	MOS
CEMA 164-2	-	MOS,EP	-	TN	-	MOS
E 00926	-	MOS	-	MOS	-	MOS
G 04489-1-CM	-	MOS	-	MOS	-	MOS
G 3658	-	MOS	-	MOS	-	MOS
G 6520	-	MOS,EP	-	MOS	-	MOS
IPAR-RAI-54	-	MOS	-	MOS	-	MOS
PRETO 132	-	MOS	-	MOS	-	MOS
TE 1	-	MOS	-	MOS	-	MOS
TE 2	-	MOS	-	MOS	-	MOS
TE 3	-	MOS	-	MOS	-	MOS
TE 4	-	MOS	-	MOS	-	MOS
TE 5	-	MOS	-	MOS	-	MOS
TE 6	-	MOS	-	MOS	-	MOS
TE 7	-	MOS,EP	-	MOS	-	MOS
TE 8	-	MOS	-	MOS	-	MOS
TE 9	-	MOS	-	MOS	-	MOS

TE 11	-	MOS	-	MOS	-	MOS
TE 12	-	MOS	-	MOS	NS	MOS(1)
TE 13	-	MOS	NS	MOS	-	MOS
TE 14	-	MOS	NS	MOS	-	MOS
TE 15	-	MOS	-	MOS	-	MOS
TE 16	-	MOS	NS	MOS	-	MOS
V 799	-	MOS	-	MOS	-	MOS
V 7059	-	MOS	-	MOS	-	MOS
V 7913	-	MOS	-	MOS	-	MOS
V 7917	-	MOS	-	MOS	-	MOS
V 7918	-	MOS	-	MOS	-	MOS
V 7920	-	MOS	-	MOS	-	MOS
V 7921	-	MOS	NS	MOS	-	MOS
V 7923	-	MOS,EP,**	-	MOS	-	MOS
V 7936	-	MOS,VN	-	MOS	-	MOS
V 7939	-	MOS	-	MOS	-	MOS
V 7944	-	MOS	-	MOS	-	MOS
V 7945	-	MOS	-	MOS	-	MOS
V 7949	-	MOS	-	MOS	-	MOS
79-0327	-	MOS	NS	MOS	-	MOS
78-0374	-	MOS,EP	-	MOS	-	MOS

CS = chlorotic spots; EP = epinasty; IVC = interveinal chlorosis; MOS = mosaic; NLP = necrotic line pattern; NS = necrotic spots; TN = top necrosis; VC = vein clearing; - = no visible reaction.

(1) = Genetically heterogeneous population for SBMV-CIAT infection where certain plants reacted only with necrotic spots without any systemic symptom, whereas others reacted only with a systemic mosaic.

** = strong mosaic.

Curriculum vitae

W. Upali Jayasinghe was born on the 20th of March 1948 at Halgranoya, Sri Lanka. After completing the general certificate of education at Kingswood College, Kandy, he left for Pakistan in 1969 to follow a course in agriculture at the University of Agriculture, Mymensingh, and in 1971 migrated to the Agriculture University, Lyallpur, where he completed B.Sc. with honours in Plant Pathology. In 1975 he joined the American University of Beiruth, Lebanon, to continue his studies as a post-graduate student. Due to civil war in Lebanon, in 1976 he joined the Agricultural University at Wageningen. In 1979 he completed his "ingenieurs" degree majoring in Plant Virology with Plant Pathology and Tropical Crops Science as supporting subjects. In 1979 he joined the bean program of the Centro International de Agricultura Tropical, Cali, Colombia, as a visiting research associate to investigate chlorotic mottle of bean. At present he is working in the CIAT cassava program as a post-doctoral fellow.