ANNUAL REPORT

VIROLOGY RESEARCH UNIT

December 1989

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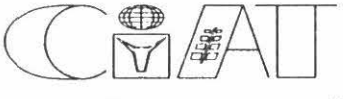

Centro Internacional de Agricultura Tropical

The Virology Research Unit was officially created in 1988, to provide centralized service to the four CIAT Commodity Programs in relation to research on specific virus-related problems of CIAT-mandated species.

TABLE OF CONTENTS

ANNUAL REPORT
1989

VIROLOGY RESEARCH UNIT

Introduction

1989 has been an exciting and productive year for the Virology Research Unit (VRU), despite the major re-organization that took place within the Unit in response to the additional responsibilities created by the initiation of research on the viruses that affect tropical forage legumes.

As announced last year, and following the arrival of the second Senior Staff virologist, the VRU organized its support personnel to conduct research across commodities but in specialized areas of plant virology, such as immunology, electron microscopy, and molecular virology. The two Senior Staff virologists also shared research responsibilities, but maintained selective contact with individual Commodity Programs to better define research priorities, and to ensure proper interaction and feedback.

The following sections present the results of research conducted by the VRU for the four Commodity Programs of CIAT (in alphabetical order), during 1989.

APHID-TRANSMITTED VIRUSES

Bean Common Mosaic Virus

Present situation in Latin America and research strategy: The rapid incorporation of the hypersensitive I gene in the majority of improved bean cultivars released in Latin America has effectively diminished the distribution and economic impact of bean common mosaic virus (BCMV) in this region. The genetic linkage problem involving the recessive alleles of the I gene and some bean seed colors has also been overcome to a large extent. Recombinant genes are now being deployed in local varieties by back-crossing, particularly in Central America and Peru, where red- and yellow-seeded grain types, respectively, are highly prized. At present, 23 land races, all susceptible to BCMV, have passed through the backcross program to be purified for the dominant I gene. Many of these materials have already been distributed to interested national program collaborators, to be actively used in crossing programs to improve land races for a variety of desirable traits.

The incidence of BCMV, however, seems to be increasing in mid-to-high altitude bean production areas where climbing

cultivars are commonly cultivated. In past years, these regions were relatively free of BCMV and, therefore, emphasis was placed on the incorporation of resistance to the more limiting fungal diseases, such as anthracnose and angular leaf spot, prevalent in the Andean highlands. Whether the increasing incidence of BCMV in climbing bean cultivars is due to environmental changes favoring an increase in aphid vector populations, or to some other epidemiological factors, is not known at present. However, it is evident that climbing beans will have to follow the breeding and screening schemes in place at CIAT to incorporate resistance to BCMV in bush bean lines.

Present situation in Africa and research strategy: The high incidence of necrosis (black root)-inducing strains of BCMV in E. Africa has been well documented. Fortunately, the implementation of different strategies, such as the combination of the dominant I gene with strain-specific recessive genes (bc 1^2, 2^2), or the incorporation of a recessive gene (bc-3) conferring immunity, has resulted in a steady flow of mosaic- and black root-resistant bean lines to CIAT's regional projects in E. Africa.

Also, the original sources of resistance to black root-inducing strains were predominantly found in European materials and, consequently, their adaptation to tropical conditions was poor. Through the backross breeding program,

which initially began as a collaborative project with IVT-Wageningen and later continued as one of CIAT's special breeding projects for Africa, resistance to black root-inducing strains has been incorporated into tropically-adapted beans in a variety of grain types and growth habits (Table 1). Many of these breeding lines are also resistant to other important bean diseases.

During 1989, over 160 backrosses were made for the BCMV project, and 115 advanced lines from previous backrosses were selected as VEF candidates for 1990. Black root resistance is now incorporated in large-seeded bush and climbing beans, and these new lines will soon be available for evaluation. These BCMV-resistant genotypes can now be deployed under mono-culture or as components in seed mixtures, even in the presence of BCMV-susceptible cultivars which act as reservoirs of necrosis-inducing strains of BCMV.

Bean Yellow Mosaic Virus

Present situation in the world and research strategy

Bean yellow mosaic virus (BYMV) is a constraint to bean production in most semi-tropical and temperate countries of

Table 1. Blackroot resistant breeding lines from the African breeding project, Palmira 1988-1989.

Breeding line	Growth habit	Seed color	100 seed wt (g)	Rust	CBB	Anthracnose leaves	Anthracnose pods	Angular L.S. leaves	Angular L.S. pods	Phoma blight	BCMV	Yield (kg/ha)	Grain type
VEF 88													
MCM 3030	2B	1	18	I	S	S	I	S	S	I	O	2085	Navy
MCM 3031	2B	1	19	I	I	I	R	S	I	I	O	2577	Navy
MCM 5001	3	3R4	23	S	S	R	R	S	I	I	O	3289	Pinto
MCM 2001	3	6	22	S	S	I	I	S	I	S	O	2290	Red Mexican
MCM 2002	3	6	22	S	S	S	S	S	I	I	O	2021	Red Mexican
MCM 1014	3	8	19	S	I	S	I	S	I	I	O	1847	Black
MCM 1015	2B	8	17	S	I	S	R	S	R	I	O	2444	Black
MCM 1016	2B	8	22	S	I	S	I	S	S	I	O	2479	Black
VEF 89 (data not complete)													
MCM 5005	2B	2R4	-	I	I	I	R	R	R	-	O	-	Carioca
MCM 5006	2B	2R8	-	I	I	I	R	R	R	-	O	-	Carioca
MCM 5003	2B	2R8	-	I	I	I	R	R	R	-	O	-	Carioca
MCM 5004	2B	2R8	-	I	S	I	R	R	R	-	O-N	-	Other
MCM 5002	2A	3	-	S	S	R	R	S	R	-		I.	
MCM 1021	2B	8	-	I	I	R	R	I	R	-	O-N	-	Black
MCM 1018	2B	8	-	S	S	S	S	S	I	-	O	-	Black

BCMV: O = no symptoms, L = local lesions, N = necrosis

the world. In Latin America, BYMV is found predominantly in Chile and, to a lesser extent, in Uruguay. Although BYMV has not shown the destructive potential of other mosaic-inducing viruses, it can reach epidemic proportions in certain years, causing significant yield losses. BYMV is also endemic to the Mediterranean countries of Europe, Africa and Asia.

Research on BYMV is conducted through a cooperative project with the Institute of Applied Plant Virology, in Torino, Italy. The principal investigator, Dr. Vittoria Lisa, has been characterizing BYMV isolates from Latin America, Europe and Africa, with emphasis on pathogenic variabity. Curiously, the most pathogenic BYMV isolate found so far was collected in Chile. No immunity has been found to this virus isolate in numerous cultivated and wild P. vulgaris accessions, or in several P. coccineous accessions screened to date.

The majority of the BYMV isolates collected, however, seem to belong to patho-groups for which resistant bean genotypes have been identified. This is the case of the latest group of BYMV patho-types from Turkey and Iran, detected by Dr. Lisa in Italy. A detailed progress report for the second fiscal year April 1988-1989 can be found in the appendix.

Future plans include the characterization of other geographical BYMV isolates, preferably from China, and the cultivation of BYMV-tolerant bean cultivars to control the more pathogenic strains, while the search for a suitable source of genetic resistance continues.

Other Aphid-Transmitted Viruses Infecting Beans

Various other plant viruses can be transmitted by aphids from several crops, particularly legumes, to beans. The most common group of legume viruses sporadically found infecting P. vulgaris, both in Africa and Latin America, is the potyvirus group. These filamentous viruses can induce systemic infection of various bean genotypes and most of them can challenge the I gene inducing black root-like symptoms. Fortunately, their incidence in commercial bean plantings has been negligible so far, with few exceptions in countries, such as Brazil, where soybean mosaic virus has occasionally caused some damage in bean commercial plantings.

There is also an isometric (round) aphid-transmitted plant virus, the legume strain of cucumber mosaic virus (CMV), which is becoming widely distributed in the main bean-producing continents of the world. CMV was first reported in Europe, and, now, it has been detected in the American, Asian and African continents affecting beans. In

Latin America and Africa, however, CMV seems to be a virus of quarantine rather than economic importance, since there have been no reports of a major epidemic of CMV in these continents. The current world-wide distribution of CMV is most likely associated to its transmission via infected bean seed.

BEETLE-TRANSMITTED VIRUSES

Current Situation and Research Strategies

Bean severe mosaic virus

With the exception of Venezuela, where bean severe mosaic virus (bean curly dwarf mosaic virus) seems to be significantly transmitted by chrysomelid beetles, no other country reported major epidemics of this or other beetle transmitted viruses in 1989. A bean severe mosaic survey will be conducted in Venezuela in 1990.

Bean southern mosaic virus

Bean southern mosaic virus (BSMV) is a well known seed-borne pathogen of P. vulgaris, often found affecting beans in single or mixed infections around the world. BSMV is the virus most often detected by the VRU in samples taken in the

main bean-producing regions of Latin America. Despite the mild symptoms induced by BSMV in most bean genotypes, studies previously conducted at CIAT have demonstrated the occurrence of significant yield losses in BSMV-infected bean cultivars. Also, BSMV acts synergistically with other bean viruses causing severe symptoms.

The main research strategy at CIAT has been the production of BSMV-free seed. Finally, should the need arise, the VRU has already identified adequate sources of genetic resistance.

Viruses Affecting Beans in E. Africa

A survey of plant viruses affecting P. vulgaris in Eastern Africa was undertaken last year (CIAT Bean Program Annual Report, 1988) through a cooperative project with the W. German Institute of Plant Virology, in Brawnschweig. The main investigator, Dr. J. Vetten, has made several collection trips in countries, such as Burundi, Malawi, Rwanda, Tanzania, Uganda, Zambia and Zimbabwe, with the cooperation of the SADCC/CIAT regional coodinator, Dr. D. Allen.

This year, most of the samples collected in 1988 have been processed and the viruses identified. In summary, Dr. Vetten's findings agree with preliminary observations

suggesting that bean common mosaic virus (BCMV) is by far the main viral pathogen in the E. African countries surveyed. Most of the other viruses found affecting beans, only occur in low incidence wherever other legume crops, such as cowpea or peanuts, are cultivated near bean fields. An exception to the above may be the recent detection of the legume (bean) strain of cucumber mosaic virus (CMV). The VRU produced an antiserum to detect this CMV strain, which is reaching a world-wide distribution, and has a considerable pathogenic potential.

WHITEFLY-TRANSMITTED VIRUSES

Bean Golden Mosaic Virus

Current situation and research strategy in South America

As reported last year, the VRU made available 188 bean genotypes selected for various bean golden mosaic resistance traits, to all national and international breeding programs currently working on the control of this devastating virus disease.

This year, the Brazilian National Center for Rice and Bean Research (CNPAF) organized a national meeting, with the participation of CIAT. The unanimous agreement of all

participants was that the disease continues to spread in Brazil, north of the major bean-producing States of Sao Paulo and Minas Gerais, as far as the States of Alagoas and Pernambuco. Among the main reasons for the increasing incidence of BGMV in Brazil, we could cite the expansion of crops, such as soybean, which act as suitable breeding hosts for the whitefly vector, and the lack of non-black seeded sources of BGMV resistance in Brazil. Thus, the meeting, was a timely opportunity to present the new sources of BGMV resistance and to explain the different defense mechanisms associated with these resistant or tolerant genotypes. Some of these sources of BGMV resistance have already been used in crosses (Table 2) by the bean breeder of CNPAF, Dr. M.J. Zimmermann, to obtain some very promising and vigorous progenies to be tested under BGMV pressure next year.

Efforts have also been made at CIAT to incorporate the new resistance genes into tropically-adapted genotypes. Temperate-adapted sources such as Blanco INIA, Pinto 114 and Red Mexican 35 have been used extensively for the improvement of small-seeded cultivars, especially for Brazil. A group of F_4 families, combining these and other sources, were planted in Brazil this year in two sites, Capinopolis (CNPAF) and Rio Verde (EMGOPA), in the State of Goias. From the Rio Verde nursery, individual plants were selected within the best families for future testing. In addition, the same sources are being incorporated into

Table 2. Crosses for bean golden mosaic virus resistance made at CNPAF, Brazil, using the most recent sources of genetic resistance

Cultivar		Source of Resistance
Carioca	x	Red Mexican UI 35
Carioca	x	Pinto 114
Carioca	x	Great Northern 31
Carioca	x	GN 31
LM 30630	x	Pinto 114
LM 30630	x	Pinto 114
CNF 178	x	GN 31
G 3714 (Turrialba 2)	x	Red Mexican UI 35
G 3714	x	Pinto 114
A 295	x	GN 31
A 295	x	Red Mexican UI 35
A 295	x	GN 31
A 176	x	Red Mexican UI 35
A 176	x	GN 31
MD 478	x	Red Mexican UI 35
MD 478	x	Pinto 114
MD 478		

crosses for selection, in the State of Parana (IAPAR), Brazil.

Argentina is perhaps the bean-producing country in Latin America with the most severe whitefly-transmitted virus problems. As in Brazil, the expansion of soybean in the north-western Provinces, where beans have been traditionally produced, has aggravated the incidence of these viruses. Despite the high geminivirus pressure, Argentina has been following an integrated virus control approach, whose main component is the use of BGMV-tolerant bean cultivars introduced by CIAT (Table 3) and selected by Argentinian bean breeders.

It is interesting to note that the three red-seeded lines selected are red-kidney genotypes derived from some of the new sources of resistance recently identified by the VRU, particularly 'Royal Red' (G04450). The average yield of ZAA-2 (Royal Red x Kabanima) under whitefly-transmitted virus pressure in the locality of Ceibalito (Anta), Argentina, was 1.249 kg/ha, as compared to the yield of the local cultivar, Chaucha Colorada, which was 674 kg/ha (data for 1987 taken by Ing. Raul Ricci of Argentina). The white-seeded lines released in Argentina have been derived from multiple crosses including BGMV-tolerant black and white-seeded genotypes.

Table 3. Bean cultivars with resistance/tolerance to whitefly-borne bean viruses, introduced in Argentina from CIAT.

Black-Seeded Genotypes
DOR 41
DOR 157
BAT 304
XAN 112
Red-Seeded Genotypes
PVA 1111
ZAA 2
AFR 180
White-Seeded Genotypes
Tuc 122
Tuc 56

The above results clearly show that the new sources of BGMV resistance can be effectively utilized by breeders to produce commercial lines with high yield potential. The successful deployment of BGMV-resistant genotypes in Argentina has not been the result of costly chemical inputs, since these costs do not amount to 3% of the crop's value in the case of BGMV-resistant cultivars. Rather, it is the result of the rapid adoption of resistant/tolerant genotypes and disease control practices by commercial growers. Obviously, this process will take longer in traditional bean-producing countries where BGMV-susceptible cultivars have to be crossed with the new sources of resistance, to select resistant lines possessing the commercial characteristics of the original cultivar.

Current situation and research strategy in Central America and the Caribbean

The incidence of bean golden mosaic has also been increasing in Central America and the Caribbean due to enviromental factors and the large-scale introduction of commercial crops, such as eggplant, tomatoes, tobacco, and cotton, which support the development of large populations of the whitefly vector Bemisia tabaci in traditional bean-growing areas.

Several populations of crosses made between the new sources of BGMV-resistance and Central American materials, were also made and shipped for planting in Guatemala in September 1989.

Resistance to BGMV is also important for medium large-seeded cultivars of Andean origin which are grown in the Caribbean area and on the Pacific coast of Mexico. Populations involving local cultivars and new sources of resistance were shipped in 1989 to the Dominican Republic and to Sinaloa, Mexico, for selection by national program scientists. However, the improvement of these cultivars with race D sources, such as Pinto 114, is difficult due to the genetic distance between the cultivars and the sources of resistance. Therefore, in the crosses to improve the Dominican cultivar Pompadour, the race D sources were used in triple crosses in which the resistant parents represented one quarter of the genetic contribution. Preliminary observations of these same populations planted in Guatemala suggested that progress can be made with these sources in the improvement of medium large-seeded cultivars.

Cooperative Projects on Whitefly-Transmitted Bean Viruses

1. Bean/Cowpea CRSP: **Molecular Approaches to Control of Bean Golden Mosaic Virus.** University of Wisconsin -

Madison/Dominican Republic CRSP Project - in cooperation with - CIAT - University of Puerto Rico.

The objective of this project is to investigate the degree of pathogenic variability of BGMV in the Dominican Republic (DR), in order to design suitable breeding/screening strategies.

As reported last year, full-length DNA clones of BGMV-DR were obtained, and DNA probes developed to detect the virus in cultivated and wild hosts. The objectives for 1989-1990 were to sequence BGMV-DR clones and to produce strain-specific DNA probes. This year several samples were taken in the Dominican Republic and tested in Wisconsin for the presence of BGMV, using the BGMV-Guatemalan (GA) isolate probes developed last year. In this test, only golden mosaic-affected P. vulgaris and P. lunatus samples gave positive hybridization signals with the BGMV-GA probe at high stringency. This indicates a significant degree of genomic relatedness between the Dominican Republic and Guatemalan isolates of BGMV.

At low stringency, that is, under less demanding test conditions for hybridization to occur between distantly related nucleic acid molecules, moderate levels of hybridization were obtained with mosaic-affected Sida, Euphorbia, Rhynchosia and Jatropha sp. samples also

collected in the Dominican Republic. Another weed, Malva sp. gave an intense positive reaction at low stringency but did not hybridize under high strigency conditions. Interestingly no hybridization was obtained with Macroptilium sp. affected by a bright yellow mosaic, considering that Macroptilium spp. have been considered major reservoirs of BGMV in Latin America.

These preliminary results seem to confirm previous observations made by national program scientists in the DR concerning the apparent major role that BGMV-infected beans play in the epidemiology of the virus, in contrast with the generally accepted notion of BGMV being mainly transmitted by whiteflies from weeds to beans.

The complete Bean/Cowpea CRSP 1989 Annual Report can be found in the appendix of the Bean Virology section.

2. AID PSFC Project **"Use of Cloned Viral DNAs in Characterization, Epidemiology and Control of Bean Golden Mosaic Virus".** Institute for Molecular Virology and Department of Plant Pathology, University of Wisconsin-Madison, and Centro Internacional de Agricultura Tropical (CIAT).

   Principal Investigators: Ahlquist, P., Gilbertson, R., Maxwell, (Univ. Wisconsin)D., Morales, F. (CIAT)

Overall aim and Specific Objetives:

1. Isolate and clone the DNA of selected Latin America isolates.

2. Develop universal and isolate-specific BGMV DNA probes and optimize protocols for affective use of these probes under field conditions.

3. Use the specific BGMV DNA-probes in extensive field surveys to define the actual geographical distribution and relative importance of distinct BGMV genotypes, and to identify unrecognized plant reservoir of BGMV.

Although this project was recently approved, considerable research has been already conducted at the University of Wisconsin with the BGMV isolates provided by CIAT. So far, full-length DNA clones of BGMV-BZ, GA, and DR have been produced and partially sequenced.

Bean Dwarf Mosaic Virus

The characterization of bean dwarf mosaic virus (BDMV) was completed this year at the VRU. BDMV is now recognized as a new bean geminivirus (Figure 1), transmitted by the whitefly Bemisia tabaci. 'Bean dwarf mosaic' is the current name

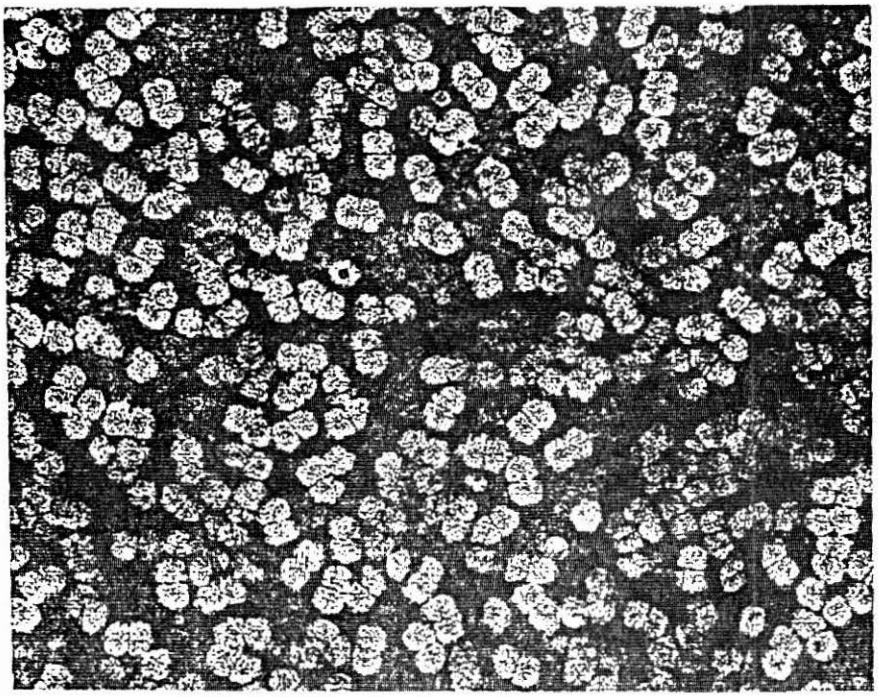

Figure 1. Electron micrograph of purified bean dwarf mosaic particles.

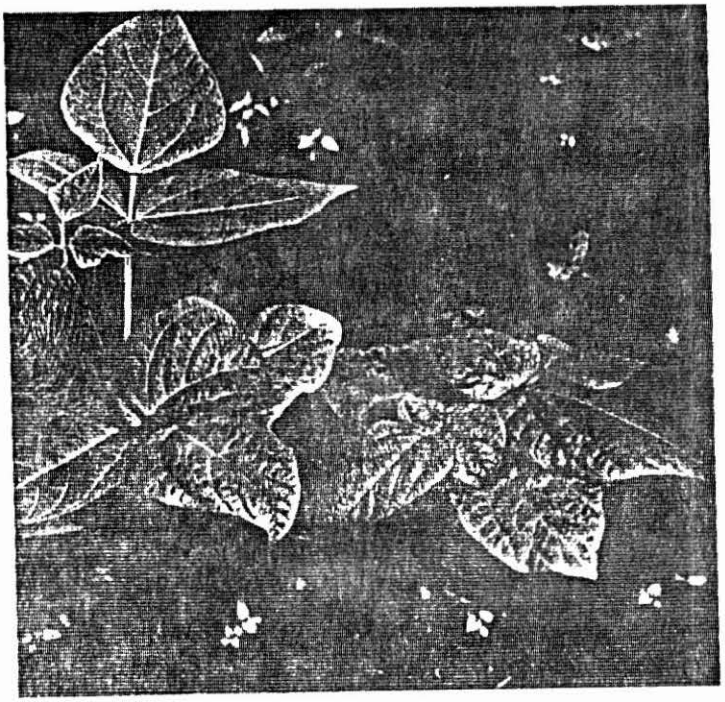

Figure 2. Plant dwarfing inducing by bean dwarf mosaic virus.

given to the disease previously referred to as "bean chlorotic mottle". The main reasons for changing the name of this disease were to better describe the most characteristic symptom (Figure 2) induced in beans by this virus, which consists of severe dwarfing ('achaparramiento'' in spanish). Also, the name bean chlorotic mottle has been previously associated with other bean viruses transmitted by different vectors. Finally, this disease was first described in Brazil, in 1975, as 'mosaico anao do feijoeiro', the portuguese translation for 'bean dwarf mosaic'.

As, reported in earlier CIAT annual reports, BDMV has caused significant economic losses in various bean-producing regions of Latin America, particularly in NW Argentina, where over a hundred thousand hectares of beans have been lost in the past decade. As a result of these epidemics, the BDMV-resistant bean cultivar DOR 41, previously bred for Central America by ICTA/CIAT, was introduced in Argentina, where it rapidly replaced the susceptible local cultivars in more than 100,000 has. To date, the VRU has identified five new sources of BDMV resistance (CIAT-Bean Program Annual Report 1987) in Pinto, Great Northern, Red Mexican, and black-seeded grain types.

The following are the results of the BDMV investigation conducted by the VRU in the period 1987-1989.

Mechanical transmission. BDMV was sap-transmitted from and to Topcrop bean plants with up to 100% efficiency. Most of the systemically infected Topcrop bean plants developed characteristic dwarf mosaic symptoms within 8 days after inoculation. The efficiency of mechanical transmission, however, varied considerably among the different bean cultivars tested, and some could not be infected in repeated tests (Table 4). None of the other mechanically inoculated plant species tested expressed symptoms. The bean cultivar Alubia proved highly susceptible in both the whitefly and mechanical transmission tests, and plants inoculated at an early stage of development exhibited severe dwarfing.

Whitefly-transmission tests. BDMV was efficiently transmitted by B. tabaci from and to Topcrop bean plants (up to 100%). BDMV was also transmitted by B. tabaci from infected Topcrop bean plants to Sida spinosa, and from S. spinosa to Topcrop bean and S. rhombifolia plants. A bright golden mosaic developed in systemically-infected Sida spp. plants within a month after inoculation. Topcrop bean plants exhibited characteristic dwarf mosaic symptoms within 10 days following their exposure to viruliferous whiteflies. BDMV was transmitted by B. tabaci to all the bean cultivars shown to be susceptible by mechanical inoculation, as well as to eight other cultivars which could not be infected (serologically negative) in the mechanical transmission tests (Table 4). Three of these eight cultivars (ICTA-Quetzal, Porrillo Sintetico, and Red Mexican 34)

Table 4. Reaction of selected bean, cowpea, and soybean cultivars to a filamentous virus isolated from mosaic-affected Centrosema macrocarpum

Bean		Cowpea		Soybean	
Cultivar	Reaction	Cultivar	Reaction	Cultivar	Reaction
Dubbele Witte	S	293-476	-	Clark	S
Stringless Green Refugee	L,S	2143 Peru 7	-	Rampage	S
Redlands Greenleaf C	L	5006 ICA	-	Davis	-
Puregold Wax	L	Blackeye	S	York	-
Imuna	-	Bush sitao	-	Marshall	-
Redlands Greenleaf B	L	Monteria	-	Ogden	-
Great Northern 123	-	Red Ripper	-	Kwanggyo	-
Sanilac	-			Buffalo	-
Michelite 61	L			ICA Lili	S
Red Mexican 34	L,S			ICA line 109	S
Pinto 114	-			ICA line 1211	S
Monroe	L,S			ICA Mandarin	S
Great Northern 31	-			ICA Taroa	S
Red Mexican 35	-			ICA Tunia	-
Widusa	L,N,S			Improved Pelican	S
Black Turtle Soup	L,N			Williams	S
Jubila	L,N				
Topcrop	-				
Improved Tendergreen	L,N				
Amanda	-				
Bountiful	S				

Reaction = L = local lesions in inoculated leaves; N = vein necrosis in inoculated leaves; S = systemic infection; - = no infection as determined by absence of symptoms, electron microscopy, and serology.

reacted with only a few localized chlorotic leaf spots (1-3/plant). Plants exhibiting these chlorotic lesions were shown by ISEM to contain isometric virus-like particles in the local lesions but not in symptomless leaves.

Physico-chemical properties. The purification procedure described here produced a concentrated suspension of quasi-isometric virus-like particles about 20 nm in diameter, found predominantly in pairs forming bisegmented structures measuring about 20 x 33 nm (Figure 1). Spectrophotometric analyses of these purified preparations revealed an ultraviolet light-absorbing spectrum characteristic of a nucleoprotein, with an average A260/280 value of 1.4. These purified preparations were infectious to mechanically inoculated Topcrop bean plants, and induced characteristic dwarf mosaic symptoms. The yield of the nucleoprotein isolated varied between 6.4 and 11.4 mg/kg of infected bean tissue (uncorrected for light scattering). The estimated buoyant densities of the virion and viral nucleic acid were 1.2999 and 1.7095 g/cm^3, respectively.

Cytology. Electron microscopy of ultrathin sections of BDMV-infected bean tissue revealed the occurrence of distinct cytopathological abnormalities in the nuclei of phloem parenchyma cells. The most noticeable cellular alterations were the presence of fibrilar bodies and virus-like particle aggregates in the nucleus of phloem parenchyma cells (Figure 3). Similarly, ultrathin sections of BDMV-infected Sida spinosa leaf tissue were shown by

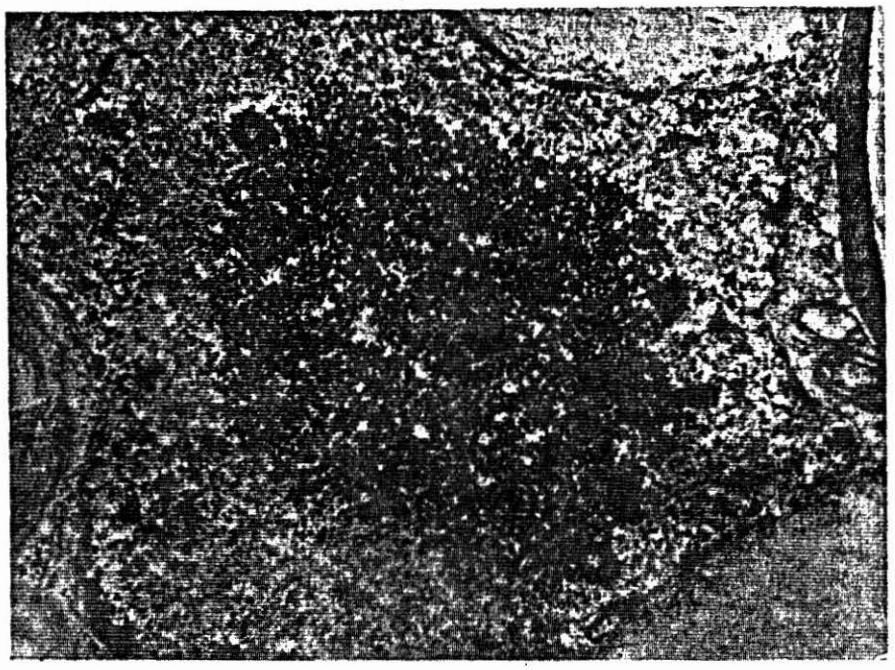

Fig.3 Ultra-thin section of bean dwarf mosaic virus-infected bean leaf tissue showing large virus inclusion in the nucleus of an infected phloem parenchyma cell.

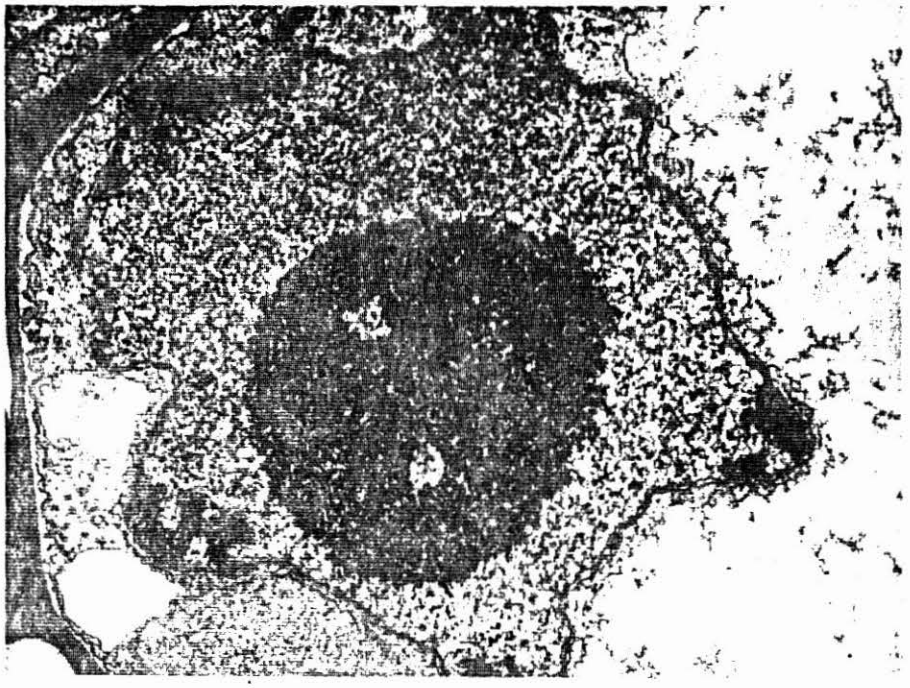

Fig. 4 Ultra-thin section of bean dwarf mosaic virus-infected Sida spinosa leaf tissue showing large virus inclusion in the nucleus of an infected phloem parenchyma cell.

electron microscopy to contain virus-like particle aggregates in the nucleus of phloem parenchyma cells (Figure 4).

Protein and nucleic acid analyses. A single protein species of apparent molecular weight 27,500 daltons was detected by SDS-PAGE of purified BDMV preparations. A single band was observed in agarose gels of nucleic acid extracts from purified BDMV, this band co-migrated with the nucleic acid fraction obtained from purified BGMV (Figure 5A), which consists of two ssDNA molecules each about 2.6 kb. The nucleic acid band extracted from purified BGMV specifically hybridized with the BGMV DNA-1 probe (Figure 5B). The BDMV nucleic acid was resistant to RNase but sensitive to degradation by DNase I and nuclease S1 (Figure 6), indicating the presence of single-stranded DNA.

Serology. In gel diffusion tests, positive precipitation reactions were observed between wells containing antiserum to BDMV and those containing purified BDMV preparations obtained from infected Topcrop bean plants. In reciprocal gel diffusion tests, BDMV was antigenically indistinguishable (no spur formation) from BGMV. In ISEM tests, however, coating of grids with BDMV antiserum produced a 46-fold and 17-fold increase in the number of BDMV and BGMV particles trapped, respectively, as compared to non-treated control grids. Similarly, when BGMV antiserum was used in ISEM tests, 118-fold and 16-fold increases were recorded for BGMV and BDMV, respectively.

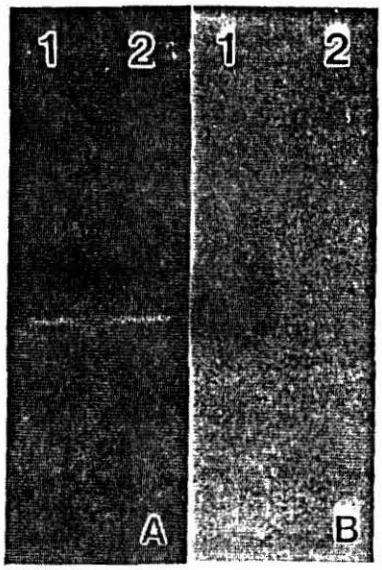

Fig. 5 Agarose gel electrophoresis (A) and southern blot hybridization analysis (B) of nucleic acids isolated from purified bean golden and bean dwarf mosaic viruses (Lanes 1 and 2, respectively).

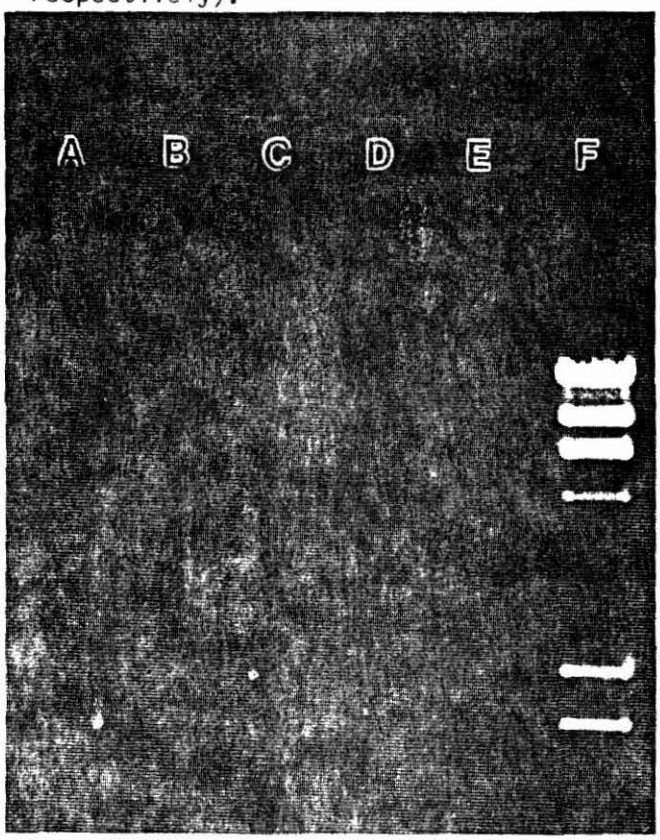

Fig. 6 Agarose gel electrophoresis of bean dwarf mosaic virus nucleic acid stained with ethidium bromide Lane A: untreated; B: DNase I-treated; C:RNase-treated; D: nuclease S1-treated (25 units); E: nuclease S1-treated (50 units); F:lambda DNA Hind III fragments used as markers.

The other three geminivirus antisera tested, also gave increases of 3-fold or more in the number of BDMV particles trapped, which is considered as evidence of serological relationship.

Relevance of BDMV research to genetic engineering approaches for bean geminivirus resistance

The isolation and characterization of BDMV led to the realization that BDMV was closely related to BGMV and that the P. vulgaris sources of resistance identified at CIAT for these two viruses, were in most cases adequate. Therefore, BDMV was included in the University of Wisconsin-CIAT cooperative project on the molecular characterization of selected Latin American BGMV isolates.

This year BDMV was cloned at the University of Wisconsin and, then, it was possible to infect Nicotiana benthamiana with these clones. This is a major breakthrough since this tobacco species should be suitable for genetic engineering manipulation to study the feasibility of controlling whitefly-transmitted bean geminiviruses by transforming plants with the coat protein gene of BDMV. If the protein capsid-mediated plant resistance approach gave positive results with the tobacco system, renewed efforts to regenerate P. vulgaris or to transform related Phaseolus

species, which could later be crossed with P. vulgaris, should be worth pursuing in the near future.

CASSAVA VIROLOGY

It has been a year of some fresh new looks at several of the major problems of cassava virology. Areas of intensive research include the viral-like diseases of cassava, continued characterization of the potexviruses of cassava, and a detailed study of the ds-RNAs found in cassava infected with viruses. The scanning electron microscope was used to differentiate between species of potential whitefly vectors and has proven a useful addition in the studies to look at whiteflies that colonize cassava. The most intensive area of research has been the work with the viral-like diseases of cassava. Cytological studies using the transmission electron microscope of healthy versus diseased cassava plants were made. Interesting results include the finding of virus-like particles, proteins, and nucleic acids associated with cassava plants affected by the Caribbean mosaic disease (CMD).

Potexviruses in Cassava

Additional characterization of both cassava common mosaic virus (CCMV) and cassava X virus (CsXV) were made. The coat proteins of these two viruses are shown in Figure 1. The relative mass (M_r) of the coat protein of CCMV is 28,000 and the coat protein of CsXV has a M_r 26,000. The nucleic acid

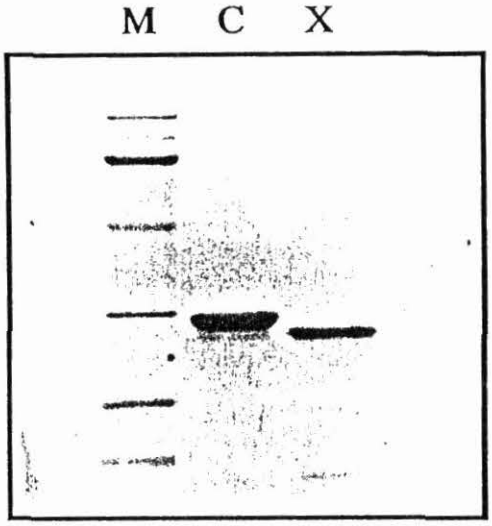

Figure 1. Lane M contains protein markers of 98, 68, 43, 29, 18, and 14 kilodaltons. Lane C contains the coat protein of cassava common mosaic virus and lane X contains the coat protein of cassava X virus.

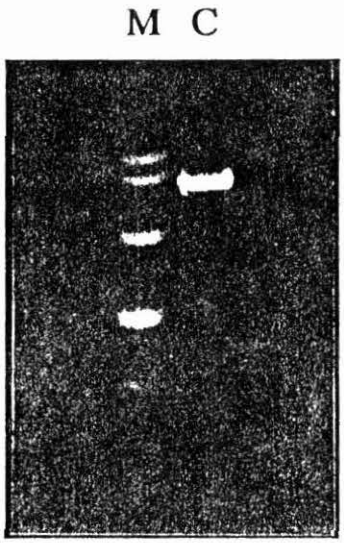

Figure 2. Lane M contains ss-RNA markers of 9.5, 7.5, 4.4, 2.4, and 1.4 kb. Lane C contains CCMV RNA.

of CCMV was analyzed in a formaldehyde agarose gel and the ss-RNA species is approximately 6,500 bases in length (Figure 2). Complementary DNA clones were prepared to the type strain of CCMV. These clones represent approximate 75% of the size of the genome, and most of the characterization has been on the cDNA clone, designated pCCMV28 (Figure 3), which is 3400 bases in length and represents over 50% of the CCMV genome. This cDNA clone contains a poly A tail of a least 60 bases in length which corresponds to the 3' end of the viral genome. Approximately 1500 bases of this clone has been sequenced, including the area of the clone which contains the coat protein of CCMV. The molecular characterization of CCMV is proceeding as part of the collaborative Cassava Trans Project whose ultimate goal is to produce transgenic cassava clones that are resistant to CCMV and African cassava mosaic virus (ACMV).

The cDNA clone of pCCMV28 was used as a hybridization probe to detect plants infected with CCMV. The virus was readily detected from plants grown in the greenhouse for both the Type and Colombia strains (Figure 4). Additional experiments are needed to test the efficacy of using dot blot hybridization on field materials. The advantage of this type of test is that the membranes can be prepared very rapidly with very simple equipment and the results analyzed in a central facility. The membranes can be sent through the mail, and there are no problems with quarantine regulations.

Cassava Common Mosaic Virus

cDNA Restriction Map

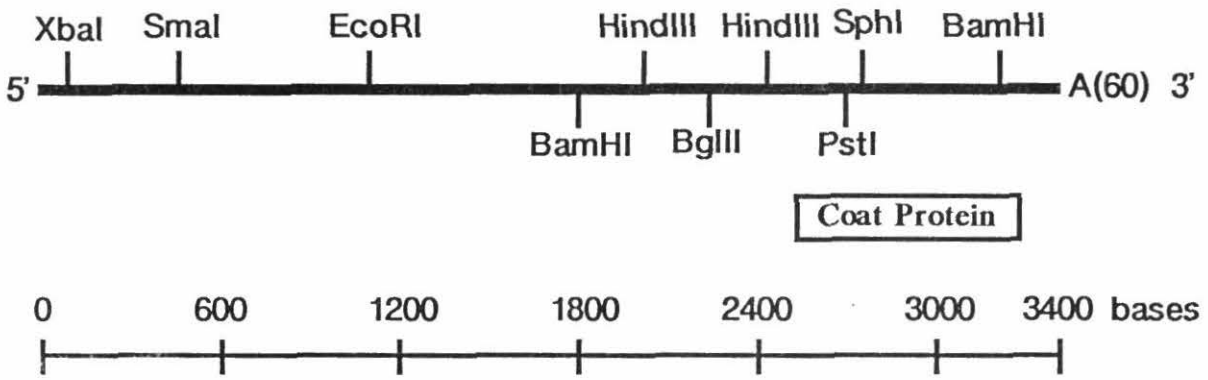

Figure 3. This cDNA clone is approximately 3,400 bases in length and represents 60% of the genome of CCMV. The 3' end of this clone contains a polyA tail and the 3' terminal gene is the capsid protein.

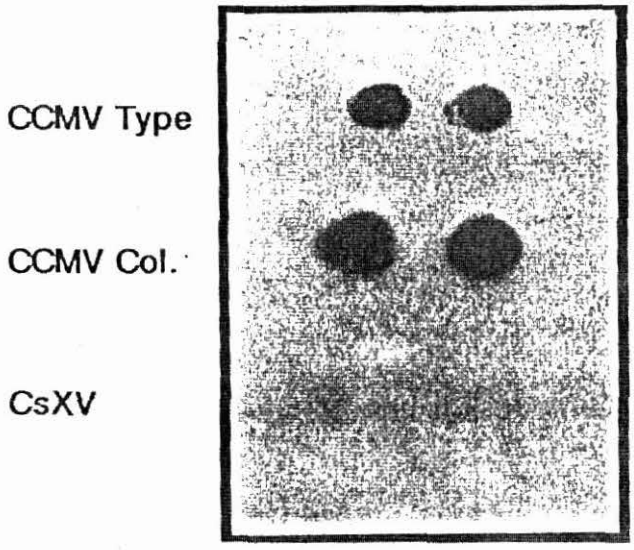

Figure 4. An autoradiogram of a dot blot of cassava common mosaic virus strains, cassava X virus, and healthy plant material. The probe was a cDNA clone representing the type strain of CCMV which was radiolabelled in a nick translation reaction.

Figure 5. A transmission electron micrograph of an inclusion body found in cassava plants infected with cassava common mosaic virus. The inclusion body consists primarily of CCMV viral particles.

A cytological study, using the transmission electron microscope (TEM), of plants infected with CCMV or CsXV was made, and inclusion bodies were found in the parenchyma cells of infected leaves and consisted of viral particles (Figure 5). These inclusions are typical of inclusion bodies reported for other potexviruses. Inclusion bodies have not been found in cassava infected with CsXV, which probably reflects the low titer of CsXV in cassava.

Serologically specific electron microscopy (SSEM) was used to detect CCMV and CsXV. Both viruses could be detected by their homologous antisera, and this may be an alternate test to ELISA when only small sample volumes are available (Figure 6).

Characterization of Nucleic Acid Bands Found in ds-RNA Extractions

Double stranded RNA (ds-RNA) analysis as a means to detect cassava viruses has been used for several years in the indexing program to assure that cassava is virus-free. This technique, however, like serology works best when one has positive and negative controls and knows the origin and size of the bands of the virus being detected. When setting up the procedure, there are several positive controls which are needed, including plants singly inoculated with each virus

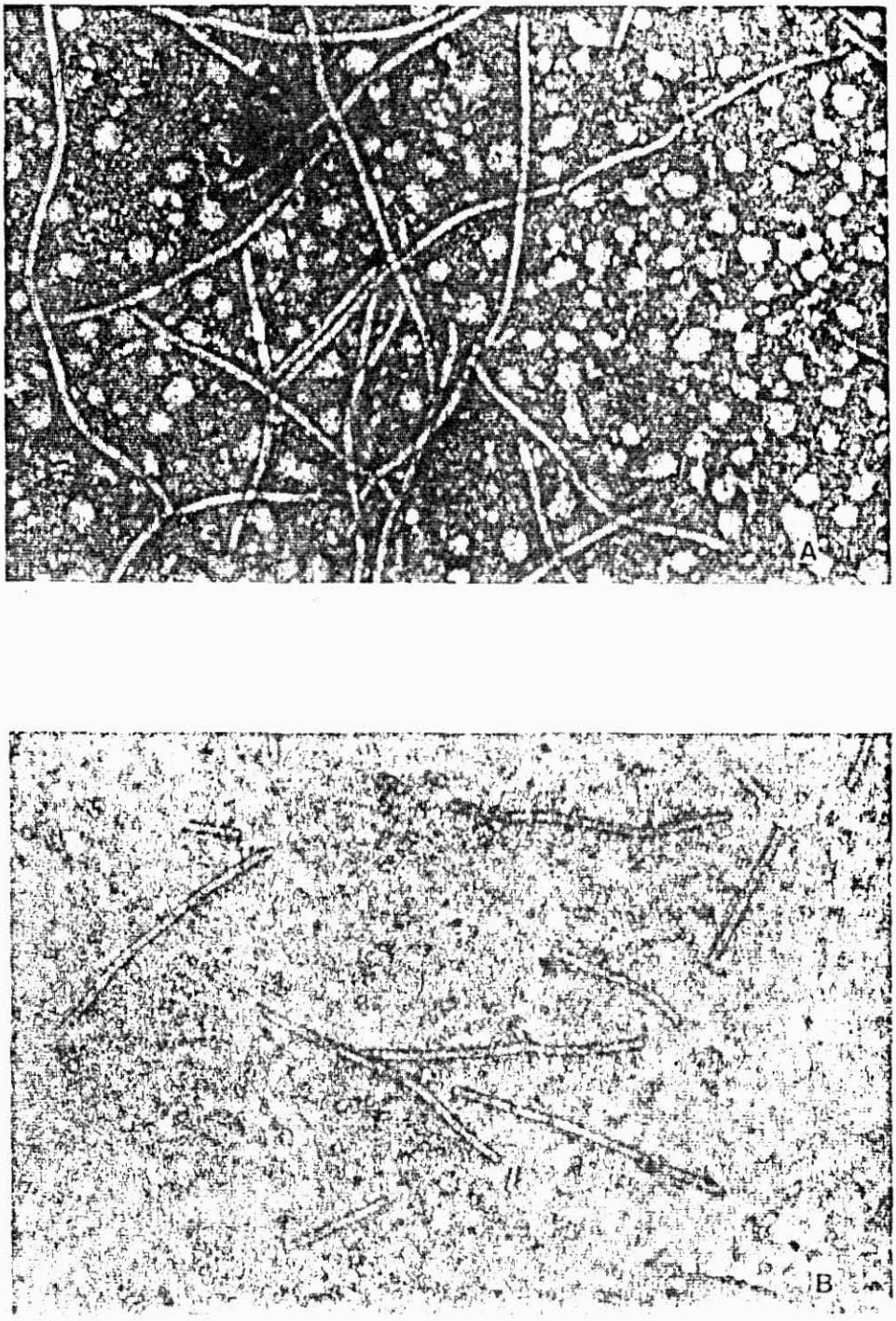

Figure 6. *Serologically specific electron micrographs of cassava common mosaic* virus (A) and cassava X virus (B) The viruses were reacted with antisera specific to their respective coat proteins.

being tested, a sample of DNA to test the DNase I treatment, and ss-RNAs and ds-RNAs to test the treatment of RNase A in presence of high and low salt. The necessity of the enzyme treatments is illustrated in Figure 7. This is a picture of an agarose gel of the nucleic acids present after the ds-RNA extraction procedure of N. benthamiana infected with CCMV, and there are two bands of high molecular weight. The top band (T) is DNA, since it is digested with RQ1 DNase but is not digested with RNase A treatments. This band is found in healthy N. benthamiana (data not shown) and is probably genomic DNA. The band labelled M is approximately 7000 bases in size and was only digested by the RNase A treatment in low salt proving that it is ds-RNA. This band is not found in healthy N. benthamiana and corresponds to the expected size of the replicative form of CCMV.

One band of ds-RNA, approximately 10,000 bases in length, was found in some apparently healthy cassava plants. This band was shown to be graft-transmissible (Figure 8), and is larger than the size expected for the replicative form of a potexvirus. While there are no symptoms in Secundina or other cassava clones containing this ds-RNA band, it is considered to be of probable viral origin because it is graft-transmissible.

Although the ds-RNA technique is useful in identifying plants that are infected with viruses that have RNA genomes,

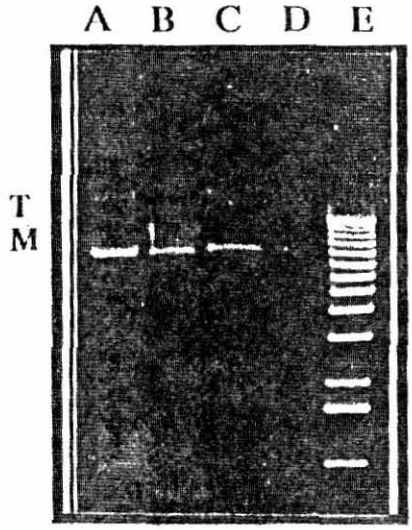

Figure 7. The nucleic acid pattern using the ds-RNA extraction procedure of plants infected with cassava common mosaic virus. Lane A is untreated; lane B is treated with DNase; lane C is treated with RNase in high salt; lane D is treated with RNase in low salt; lane E are DNA size markers

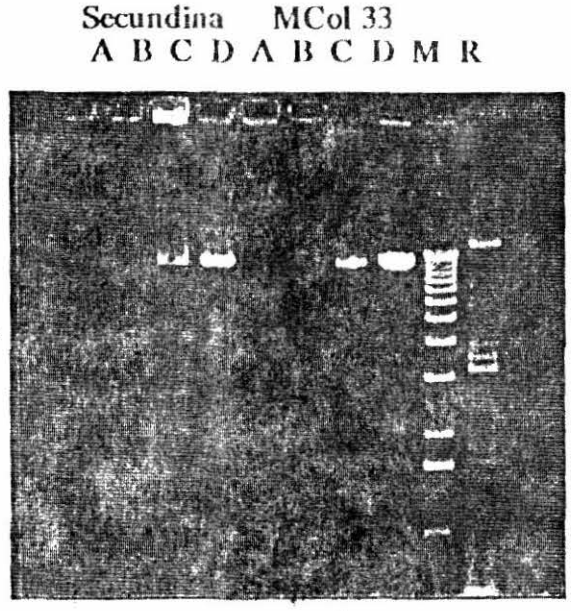

Figure 8. Double-stranded-RNA bands of approximately 10,000 base were found in appearently healthy plants of Secundina and MCol 33. Lane A is a healthy plant without bands; lane B is a healthy plant grafted onto a healtly plant; lane C is the mother plant with the ds-RNA band; lane D is a healthy plant that was grafted onto the mother plant containing the ds-RNA band. The scion was remove from the rootstock to prove that the ds-RNA band was not being translocated to the scion. Lane M contains ds-DNA markers and lane R contains ds-RNA markers.

this assay is only partly reliable, as demonstrated by the negative results obtained with some plants known to be infected with CCMV or CsXV. One gram is currently the minimum leaf tissue that is used in the ds-RNA assays. The most consistent results, using the ds-RNA extraction procedure, were the identification of plants containing a 10 kb ds-RNA band, therefore the VRU continues to assay plants for ds-RNAs in order to screen for latent viruses.

Screening Elite Germplasm for Viruses

The VRU is continuing to maintain designated virus-free elite cassava clones for international shipment. (Table 1). These cassava clones are indexed for the presence of viruses twice a year, and before shipment to national and international collaborating institutions or programs. The following tests are performed:

1. Enzyme-linked immuno-sorbent assays (ELISA) for CCMV and CsXV.

2. The indicator cassava clone Secundina is grafted onto the plants to be assayed, and the plants are placed in an area where temperature does not exceed 28-30C. This is the only reliable test for the Caribbean mosaic and frogskin diseases. This test should also detect ACMV.

Table 1. List of cassava clones that are currently certified as virus-free.

Cassava Clone	Grafting on secundina	CCMV	CsXV	dsRNA
CG 5-79	-	-	-	-
CG 7-64	-	-	-	-
CG 915-1	-	-	-	-
CM 523-7	-	-	-	-
CM 723-3	-	-	-	-
CM 955-2	-	-	-	-
CM 1335-4	-	-	-	-
CM 2766-5	-	-	-	-
CM 3281-4	-	-	-	-
CM 3306-4	-	-	-	-
CM 3306-9	-	-	-	-
CM 3372-4	-	-	-	-
CM 3435-5	-	-	-	-
CM 3555-6	-	-	-	-
CG 107-35	-	-	-	-
CG 455-1	-	-	-	-
MCol 33	-	-	-	-
Secundina	-	-	-	-

3. The cassava plants are screened for the presence of ds-RNAs.

The VRU is in the process of testing an antiserum to a latent potexvirus tentatively designated as cassava Colombian symptomless virus by Dr. Brian Harrison (SCRI, Scottland). Since this virus is latent, a study is needed to determine the geographical distribution of the virus. There is also the need to set up a cooperative project with Brazil for the production of an antiserum to cassava vein mosaic virus, which is a caulimovirus of common occurrence in that country. This will facilitate the exchange of germplasm with Brazil.

Virus-Like Diseases of Cassava

Considerable work has been done on the detection of the virus-like agents affecting cassava. Areas of investigation have included cytological studies of the diseased plants, immunological and hybridization assays with antisera and cDNAs of known viruses, and purification protocols with each step monitored by transmission electron microscopy, protein and nucleic acid analyses.

Cytological studies of both healthy and diseased cassava plants were made using the transmission electron microscope. Multiple dark staining areas were found in the nuclei of

phloem companion cells of plants affected with CMD (Figure 9). These inclusion-like bodies are similar but not identical to the fibrilar rings of the inclusion bodies found in the phloem companion cells of plants infected with geminiviruses. Similar inclusion-like bodies were found in the nuclei of cassava plants affected with FSD (Figure 10), but they were found not only in the nuclei of phloem companion cells but also in nuclei of epidermal and parenchyma cells.

Since there are several lines of evidence that suggest that CMD and FSD might be caused by geminiviruses, both cDNA hybridization and SSEM analyses of plants affected with CMD or FSD were performed. Since most whitefly-transmitted geminiviruses cross react serologically, the antisera to bean golden mosaic (BGMV), bean dwarf mosaic (BDMV), and ACMV were used in SSEM analyses in an attempt to detect a geminivirus in cassava. Geminivirus particles were easily found in bean plants infected with BGMV, which were used as controls, but not in CMD or FSD affected cassava. The second type of test consisted of total genomic DNA extractions from both healthy and diseased plants which were run on gels and transferred to nitrocellulose membranes. BGMV-infected plants were used as positive controls, and the probe was cDNA clones of BGMV, but the results were again negative. A cDNA clone for ACMV has been obtained and will be used to confirm these results, but at this time there is

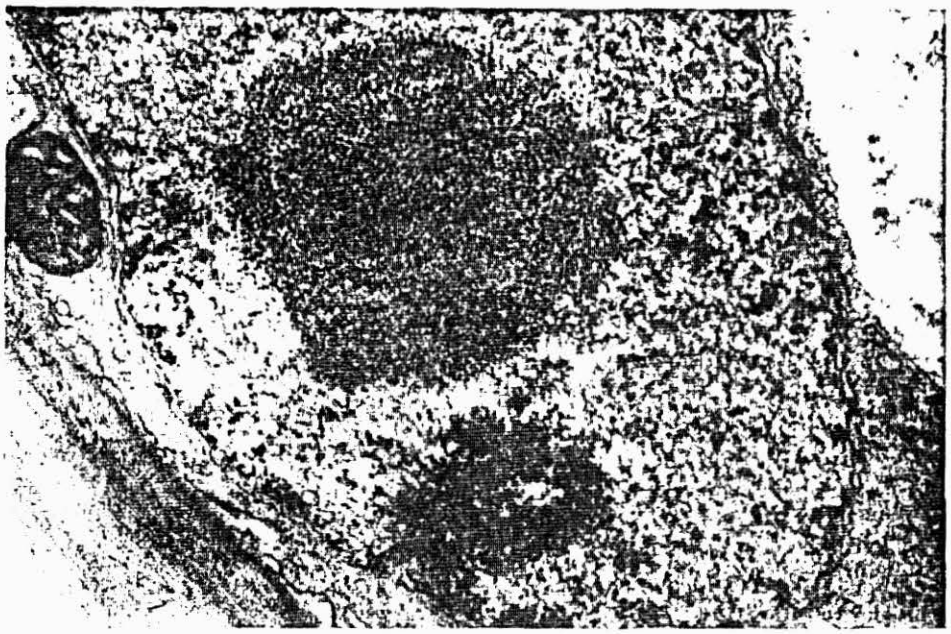

Figure 9. Inclusion-like bodies found in the nuclei of phloem companion cells of in cassava plants affected by Caribbean Mosaic Disease. These nuclear inclusion-like bodies are similar but not identical to the fibrilar rings found in the phloem companion cells of plants infected with geminiviruses.

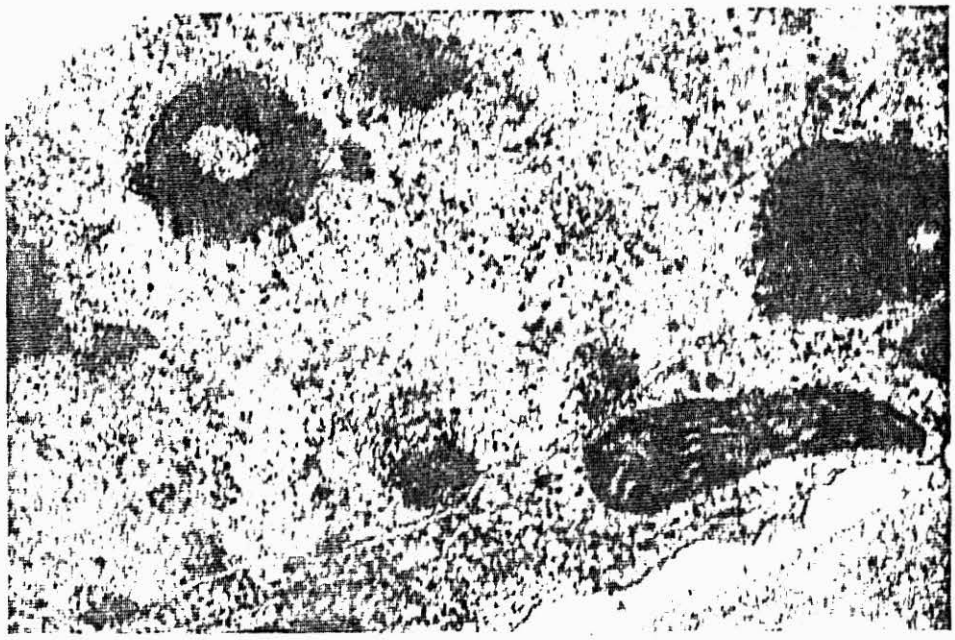

Figure 10. Inclusion-like bodies found in the nuclei of several tissue types including phloem companion, epidermal, and parenchyma cells in plants affected with Frogskin Disease.

still no firm evidence of a geminivirus infecting cassava in tropical America.

Most of the experiments to isolate the causal agents of the cassava diseases of unknown etiology have concentrated on CMD. This reflects the success in finding differences between healthy and CMD-affected plants with the virus purification protocols so far attempted. The growth conditions appear to be very important, and to obtain consistent results the CMD affected plants are transferred to a growth room with 14 hours of light and temperatures below 26 C for two weeks before harvesting the leaf tissue.

The purification procedure for CMD consists of grinding the tissue, a chloroform clarification, a PEG precipitation, followed by centrifugation to concentrate the preparation. The resuspended preparations are analyzed for proteins and nucleic acids by electrophoretic methods, and samples are taken for analysis with the TEM. To further purify the virus-like agent, preparations have been run on cesium chloride or cesium sulphate gradients. Similar results have been obtained using three different isolates of CMD.

The most consistent result has been the presence of a protein band of M_r 60,000 (Figure 11). Protein bands of similar size are sometimes found in healthy plants but always in very low concentrations. This band is larger than

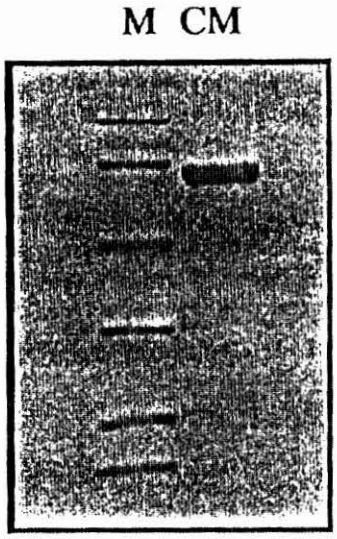

Figure 11. Lane M contains protein markers of 98, 68, 43, 29, 18, 14 kilodaltons. Lane CM contains a protein purified from plants affected with Caribbean Mosaic Disease.

Figure 12. A transmission electron micrograph of virus-like particles that are purified from plants affected with Caribbean Mosaic Disease.

expected for a capsid protein of a virus, but the protein is highly purified after collecting the virus-like band from a cesium gradient. The presence of viral-like particles have also been found both before and after the cesium gradient. These are rod shaped particles that appear to have a central core (Figure 12). They are of many different lengths and are very labile. Additional experiments are needed to find more stable conditions for these particles.

Nucleic acids have been associated with the preparations before the cesium gradients. When liquid nitrogen is used to grind the tissue, there is a band of DNA in the preparation, which is probably genomic DNA that is precipitated by the PEG along with the virus-like particles. There also appear two species of RNA, but these are easily degraded. These results would be expected of a labile ss-RNA virus.

The M_r 60,000 protein is being used as an antigen to produce an antiserum in rabbits. This antiserum will be tested for its efficacy in detecting plants affected with CMD and should clarify the question of whether this protein is a viral coat protein or a host protein that is in higher concentrations in CMD affected plants.

Whiteflies of Cassava

The VRU and Cassava Entomology have a collaborative project with Drs. David Wool and Dan Gerling of Tel Aviv University titled: **Identification and Characterization of Genetic Strains in Whiteflies of the genus** Bemisia. The project is using isozyme analysis to distinguish species of Bemisia and possible biotypes. The same tests have been used to distinguish various species of other whiteflies that colonize cassava.

Using samples collected in Ecuador, Colombia, and Venezuela, the race-specific electrophoretic patterns within B. tabaci indicate the existence of six races in Colombia, one in Western Ecuador and two in Venezuela (Figure 13). These races were named for the geographical location in which they predominate (Figure 14), and tend to occur in areas that are geologically isolated by mountain ranges. There is little evidence to support race differences that reflect host plant preference.

Five species of whiteflies have been found colonizing cassava in Colombia. Three of these species Aleurotrachelus socialis, Trialeurodes variabilis, and Bemisia tuberculata have been previously reported on cassava, but Aleurothrirux sp. and Paraleyrodes sp. were found in the Amazon region of

Figure 13. **DISTRIBUCION GEOGRAFICA DE RAZAS DE *Bemisia tabaci* EN EL NORESTE DE SURAMERICA**

Race specific isozyme patterns of the whitefly Bemisia tabaci

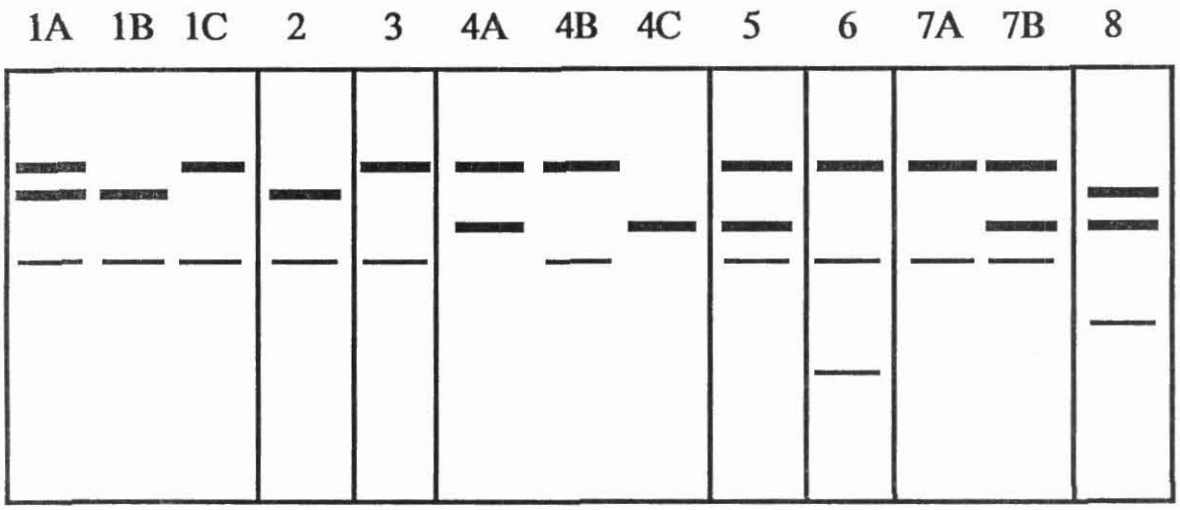

Figure 14. Esterase isozyme banding patterns of eight Bemisia tabaci races from Colombia, Ecuador, and Venezuela. The numbers correspond to the following geographic regions: 1. Valle 2. Cucuta 3. Cauca 4. Tolima 5. Caribe 6. Villavicencio 7. Manabi 8. Maracaibo. Three regions appear to contain heterogenous populations of B. tabaci as marked by the letters. The races tend to occur in areas that are geographically isolated by mountain ranges.

Colombia and represent the first report of these whiteflies colonizing cassava.

The scanning electron microscope (SEM) has been used to identify adult whiteflies. The two Bemisia species, tuberculata and tabaci can be distinguished by the number of ommatidia separating their compound eyes (Figure 15). The main advantage of using the SEM to identify species of whiteflies is that specimens are easily preserved in alcohol. This contrasts with the liquid nitrogen treatment that must be used to preserve whiteflies for isozyme analysis.

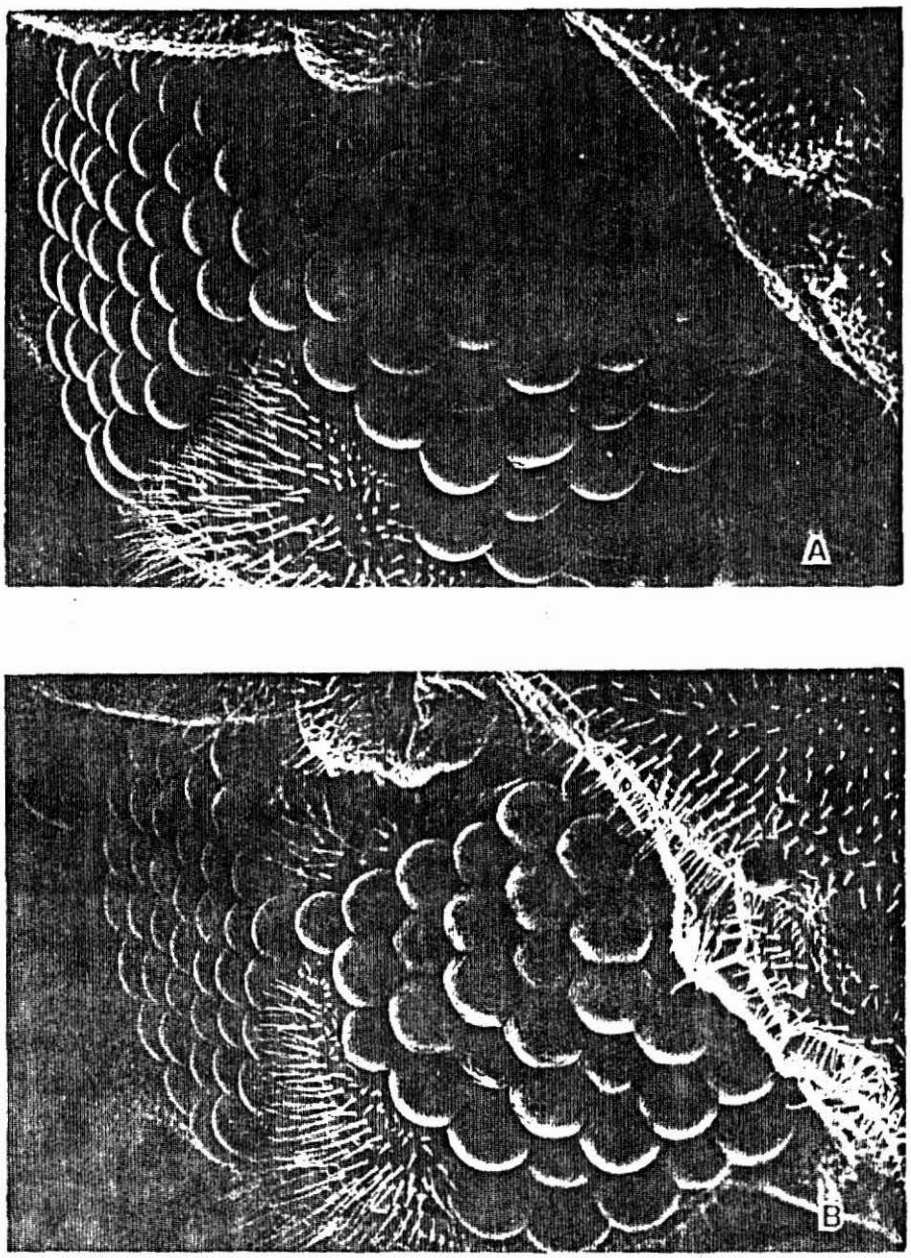

Figure 15. A. Scanning electron micrograph of the eye of the whitefly Bemisia tuberculata. B. Scanning electron micrograph of the eye of the whitefly Bemisia tabaci. The adults of these whiteflies can be distinguished by the number of ommatidia separating the sections of their compound eyes. Bemisia tabaci has only one ommatidium separating the sections while Bemisia tuberculata has two.

RICE VIROLOGY

Current Situation and Research Strategy

Rice hoja blanca virus (RHBV) is still the only viral pathogen of rice recognized in the American Continent. Nevertheless, this virus has induced major epidemics in the main rice-producing countries of Latin America and the Caribbean, causing significant yield losses since 1935. Consequently, the CIAT Rice Program (RP) has been breeding for hoja blanca resistance from its beginnings.

The isolation and partial characterization of RHBV at CIAT (Figure 1), in 1982, led to the implementation of improved screening methodologies. Once a highly specific and sensitive serological technique (ELISA) was developed for RHBV, it became possible to detect the presence of the virus in viruliferous planthoppers and, thus, create highly viruliferous vector colonies for screening breeding lines under field conditions. The VRU regularly provides the RP with the serological materials needed to continue this important germplasm screening activity.

The RP and VRU, however, feel that it is necessary to gain a better understanding of both the epidemiology of the virus and the genetic basis of resistance to RHBV. It is already

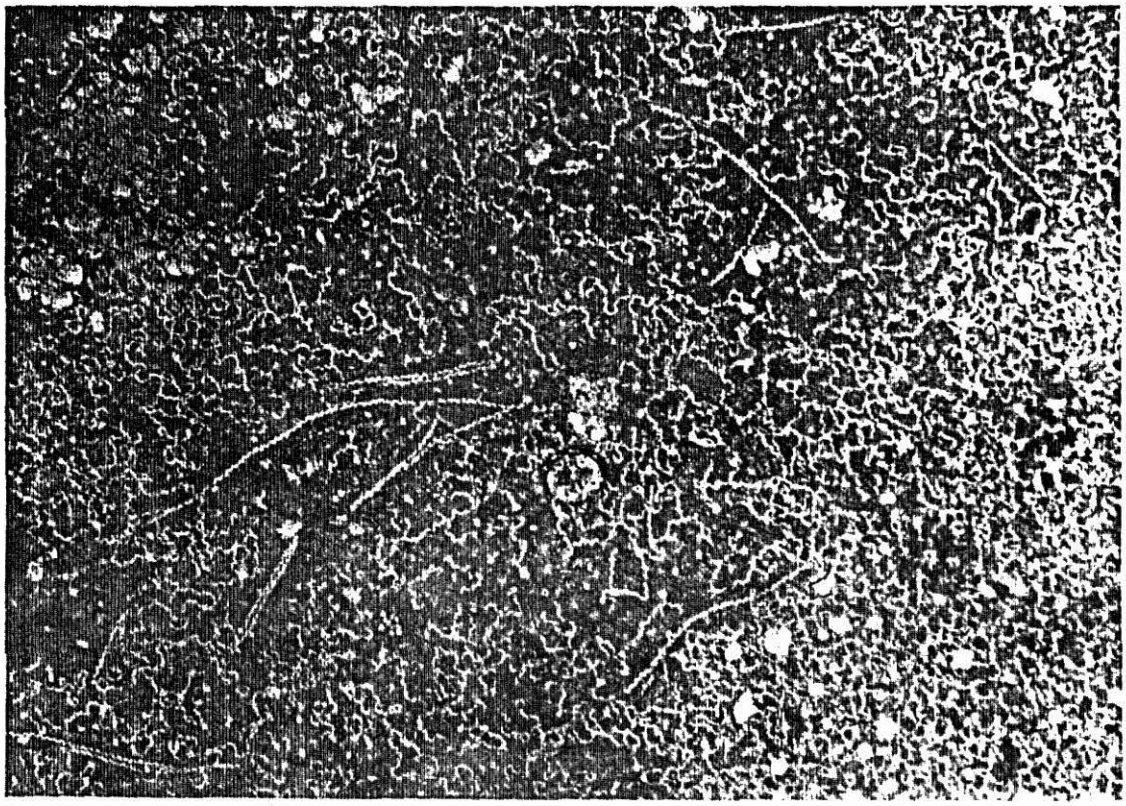

Figure 1. Rice hoja blanca virus particles showing characteristic polymorphism.

known that RHBV epidemics are cyclical in nature, causing significant yield losses for some years before the incidence of the virus starts decreasing for a variable number of years, till the next outbreak. Little is known about the factors which either trigger or slow down RHBV epidemics. Regarding the genetics of resistance to RHBV, it is evident that the current sources of resistance are scarce and not well characterized.

To study the epidemiology of RHBV, particularly the dynamics of planthopper vector populations, the ELISA technique can be readily utilized to monitor the proportion of viruliferous vectors in rice production regions with a past history of RHBV epidemics. The ELISA technique can also be used to detect the presence of RHBV in potential weed reservoirs commonly found in these production regions. With respect to plant resistance sources and mechanisms of resistance, we need to generate more information on the genetic interaction between RHBV and the main rice genotypes cultivated in Latin America. An important aspect of this work is the study of pathogenic variability.

Such a study, however, necessitates a more thorough characterization of RHBV at the molecular level. Consequently, the characterization of the RHBV genome was recently initiated at the VRU. So far, six species of ribonucleic acid (RNA) have been isolated from purified

virus particles, suggesting the existence of a multicomponent viral genome. The size estimate for the six RNA species detected are 10, 6, 4, 3.5, 2, and 1.6 kb. The four smaller species are in higher concentrations than the two high molecular weight species.

A library of complementary DNA (cDNA) clones will be prepared to a Colombian strain of RHBV, to study the genetic diversity of virus isolates from other Latin American countries. This cDNA library can also be used in the molecular characterization of RHBV. Initial efforts will be directed at locating and sequencing the coat protein gene. The availability of such a clone would open up the possibility of genetically engineer rice for coat protein-mediated resistance to RHBV.

TROPICAL PASTURES VIROLOGY

The VRU officially initiated research this year on the detection and characterization of viruses of economic or quarantine importance to the Tropical Pastures Program (TPP). Particular emphasis was given to tropical forage legumes, considering the well documented susceptibility of most legume species to plant viruses, and the absence of noticeable virus problems in grasses.

Research Priorities and Strategy

The VRU held various meetings with the Leader, agronomists, and past and present pathologists of the TPP to define research priorities for 1989. The consensus was that three of the most promising forage legume genera, namely, Centrosema spp., Stylosanthes spp., and Arachis sp. should be the initial research priorities of the VRU during 1989.

The main strategy followed was the examination of symptomatic and symptomless plant samples of some of the main species of the three genera selected by the TPP. Samples were taken from the three main CIAT experiment stations, Palmira, Santander de Quilichao, and Carimagua, utilized by the TPP. In the case of A. pintoi, plant samples were also obtained from farmers' fields.

All plant samples were initially processed for electron microscopy, and the viruses detected maintained in suitable host plants for characterization. The main objective of this characterization work, however, was to develop reliable virus detection tests for diagnostic and quarantine purposes. The following is a progress report of the research conducted by the VRU with plant viruses detected up to November 1989, in selected species of Centrosema, Arachis, and Stylosanthes.

VIRAL DISEASES OF CENTROSEMA SPP.

As reported last year (VRU Ann. Rept. 1988), several accessions of Centrosema spp. surveyed at three CIAT experiment stations, Palmira, Santander de Quilichao, and Carimagua, were noticeably affected by severe mosaic and foliar distortion symptoms of probable viral etiology. The virus isolated was characterized this year as described in the following extract of a scientific report on the causal agent.

Results

Filamentous, flexuous particles ca. 715 nm long and 12 nm wide were observed in negatively stained leaf extracts of a mosaic-affected C. macrocarpum plant selected as the source

of inoculum for this study. The virus was mechanically transmitted from C. macrocarpum to five Centrosema species, and to susceptible G. max, P. vulgaris and V. unguiculata cultivars (Table 1). None of the other manually inoculated legume species tested showed symptoms or was observed to harbor CeV by electron microscopy or serology.

Symptom expression in Centrosema spp. varied according to the species inoculated. Characteristic leaf distortion and mosaic symptoms (Figure 1) were only observed in C. macrocarpum, approximately 45 days after inoculation. Less conspicuous mosaic and chlorosis symptoms were observed in C. brasilianum, C. pubescens and C. pascuorum plants. Local chlorotic lesions were found on the inoculated primary leaves of C. pascuorum.

The virus systemically infected nine of the 16 soybean cultivars tested (Table 1) inducing a yellow leaf vein clearing. Local and/or systemic symptoms were observed in 13 of the 21 bean cultivars inoculated (Table 1).

Bean cultivars Bountiful and Dubbele Witte were the most susceptible, showing severe mosaic and leaf malformation symptoms. All inoculated bean Dubbele Witte plants eventually died. Cultivars Stringless Green Refugee, Michelite, Red Mexican 34, and Monroe, reacted with ring-shaped local lesions on the manually inoculated primary

Table 1. Reaction of selected bean, cowpea, and soybean cultivars to a filamentous virus isolated from mosaic-affected *Centrosema macrocarpum*

Bean		Cowpea		Soybean	
Cultivar	Reaction	Cultivar	Reaction	Cultivar	Reaction
Dubbele Witte	S	293-476	-	Clark	S
Stringless Green Refugee	L,S	2143 Peru 7	-	Rampage	S
Redlands Greenleaf C	L	5006 ICA	-	Davis	-
Puregold Wax	L	Blackeye	S	York	-
Imuna	-	Bush sitao	-	Marshall	-
Redlands Greenleaf B	L	Monteria	-	Ogden	-
Great Northern 123	-	Red Ripper	-	Kwanggyo	-
Sanilac	-			Buffalo	-
Michelite 61	L			ICA Lili	S
Red Mexican 34	L,S			ICA line 109	S
Pinto 114	-			ICA line 1211	S
Monroe	L,S			ICA Mandarin	S
Great Northern 31	-			ICA Taroa	S
Red Mexican 35	-			ICA Tunia	-
Widusa	L,N,S			Improved Pelican	S
Black Turtle Soup	L,N			Williams	S
Jubila	L,N				
Topcrop	-				
Improved Tendergreen	L,N				
Amanda	-				
Bountiful	S				

Reaction = L = local lesions in inoculated leaves; N = vein necrosis in inoculated leaves; S = systemic infection; - = no infection as determined by absence of symptoms, electron microscopy, and serology.

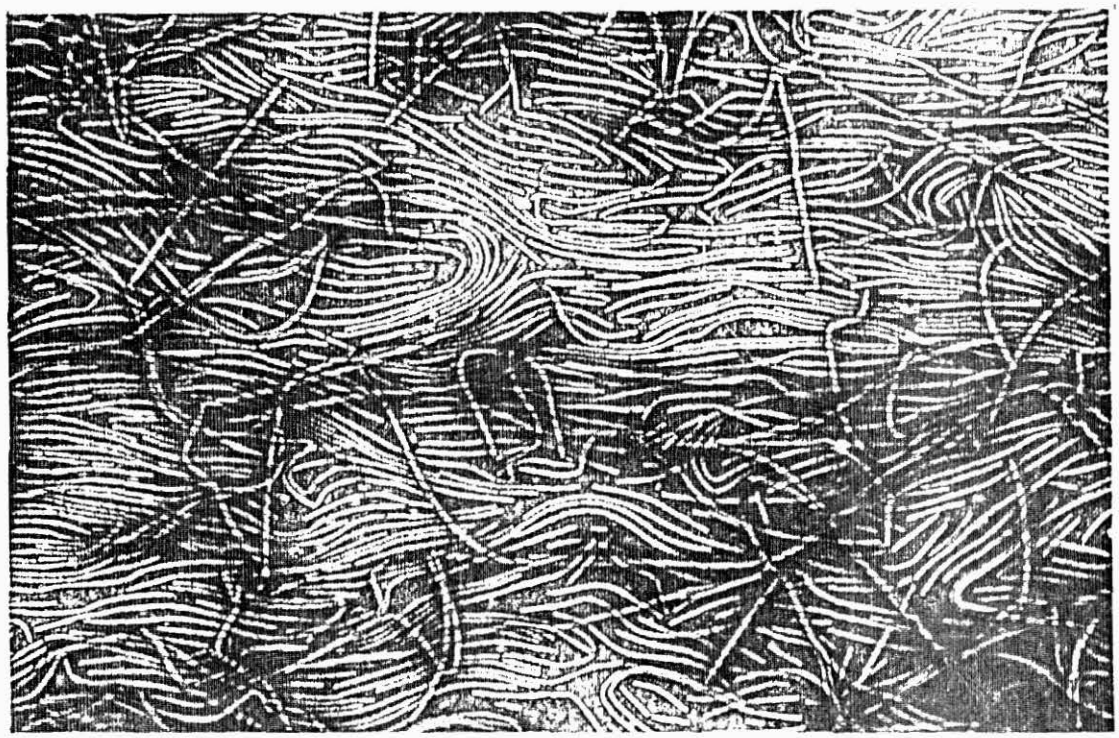

Figure 1. Purified particles of a filamentous virus (SMV-CE) isolated from Centrosema macrocarpum.

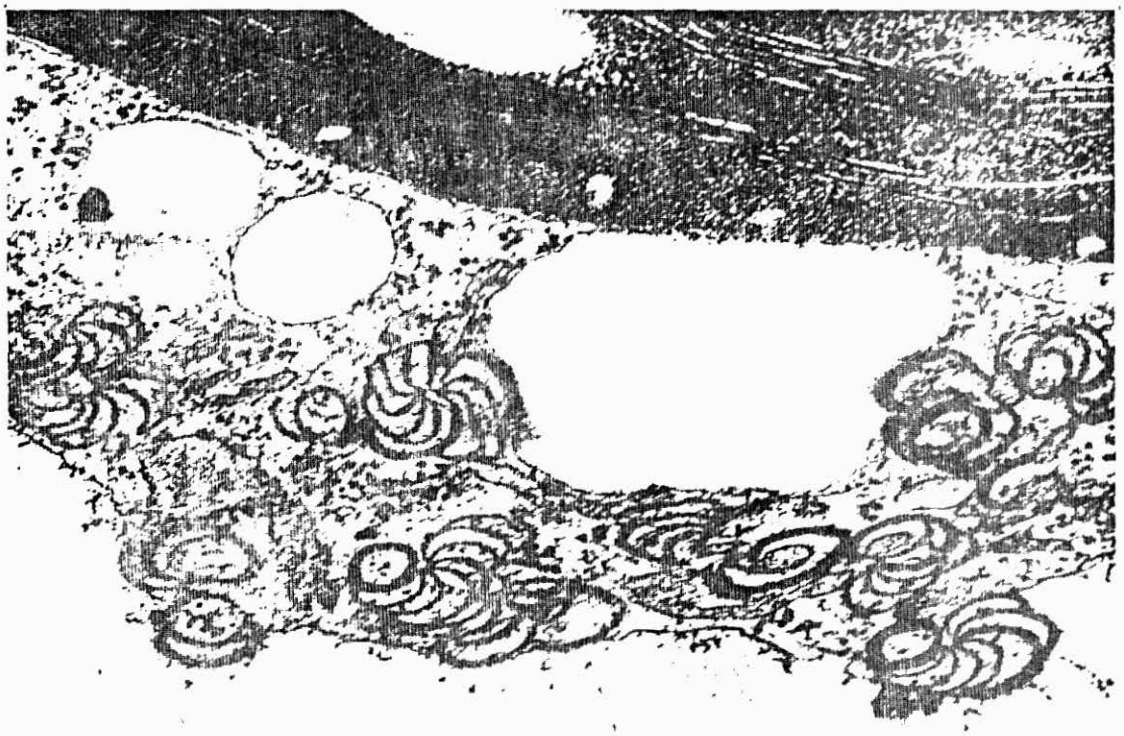

Figure 2. Characteristic cytoplasmic inclusions induced by a potyvirus in infected Centrosema macrocarpum.

leaves. Improved Tendergreen, Black Turtle Soup, Jubila, and Widusa, reacted with vein necrosis on the inoculated primary leaves. One Widusa test plant died from systemic necrosis. Cultivars Puregold Wax and Redlands Greenleaf B and C, developed chlorotic local lesions on the inoculated primary leaves. Only one of the seven inoculated cowpea cultivars (Blackeye), became systemically infected and developed mosaic symptoms (Table 1).

The virus was transmitted by the aphid Myzus persicae from Centrosema macrocarpum and bean Dubbele Witte to all test Dubbele Witte and Bountiful bean plants.

The virus was transmitted via the seed of the four C. pubescens accessions (Nos. 438, 5144, 5634, and 15149) and bean cultivar (Dubbele Witte) tested, in percentages of 1.5 (6/400 seeds), 1.4 (11/770 seeds), 2.5 (10/400 seeds), 1.6 (6/380 seeds),and 6% (3/50), respectively.

This virus was isolated with a high degree of purity (Figure 1) following the purification procedure outlined above. Virus yields were 0.5 mg/100 g of bean tissue despite the noticeable virus aggregation problems observed throughout the purification procedure. Purified virus preparations exhibited an A 260/280 ratio of 1.13 and the SDS-PAGE analysis of its coat protein revealed the presence of a single subunit of M_r 32,500. A single nucleic acid molecule of approximately 9 kb was detected in agarose gels.

The examination of ultrathin sections of mosaic-affected C. macrocarpum leaf tissue, revealed the presence of cylindrical inclusions, consisting of pinwheels and scrolls (Figure 2), in the cytoplasm of infected cells.

The virus was serologically indistinguishable from soybean mosaic virus in reciprocal Ouchterlony, ELISA, and SSEM (1,000 to 1,100 particles/1,000 μm^2) tests, and was related to bean common mosaic and watermelon mosaic-2 viruses (weak serological reactions in Ouchterlony tests). The virus did not react with antiserum to blackeye cowpea mosaic virus or passionfruit woodiness virus in SSEM tests.

Discussion

The virus isolated in this study from C. macrocarpum was determined to be a member of the potyvirus group based on its morphology, particle length, aphid transmissibility in a non-persistent manner, presence of 'pinwheel' inclusions in infected plants cells, molecular weight of its capsid protein subunit and nucleic acid genome, ultraviolet spectrum of the viral nucleoprotein, and serological relationship with known members of the potyvirus group.

The close serological relationship of this virus to soybean mosaic virus (SMV), and its pathogenic behavior in soybean

and bean cultivars previously used to differentiate SMV strains, indicate that it belongs to the G1 group of soybean mosaic virus strains.

Although SMV is known to infect some 16 different species in the Leguminosae, the high incidence of this virus in most Centrosema spp. evaluation nurseries established in various geographically-isolated regions of Colombia, and the detection of the virus in the Caribbean area, suggest that SMV is a major viral pathogen of this important tropical forage legume. Also, SMV must be considered a potential constraint to the establishment of mixed grass-legume pastures in the Tropics, particularly in Latin America and Africa, where several forage legume species are being collected and utilized for this purpose. Among the tropical forage legumes currently maintained by the Genetic Resources Unit of CIAT, the plant genera Astragalus, Canavalia, Cassia, Crotalaria, Galactia, Lablab, Lespedeza, and Sesbania are reported hosts of SMV.

Finally, the demonstration of the seed transmissibility of SMV in Centrosema spp. further supports the consideration of this virus as a pathogen of considerable economic importance. We propose the designation SMV-CE for this strain.

As can be concluded from this investigation, the mosaic and leaf distortion of Centrosema spp. are caused by a previously known and ubiquitous legume pathogen, soybean mosaic virus. This finding has significant implications for the future of Centrosema spp. as a tropical forage legume considering the rapid expansion of soybean plantings in Latin America. In South America alone, the area planted to soybean increased from 1,433,000 has in 1970, to 13,985,000 has in 1987 (FAO data), in response to an increasing demand for soybean products in the international market.

Detection of other Viruses in Centrosema spp.

Several Centrosema spp. plants affected by virus-like symptoms similar to those described above for the Centrosema strain of soybean mosaic virus (SMV-CE), were serologically tested with the antiserum to SMV-CE. Unexpectedly, some samples did not react with this antiserum, which suggets the presence of other viruses. An electron microscopy examination of these samples, demonstrated the presence of filamentous particles similar to those of SMV-CE (Figure 3).

This finding indicates that there are either different centrosema (SMV) virus strains or different potyviruses infecting Centrosema spp.

While the new centrosema viruses detected are characterized, the VRU attempted to implement a detection method for Centrosema spp. viruses, using a commercial (Agdia) monoclonal antibody produced to detect all members and strains of the potyvirus group. Table 2 shows the results of the test performed with the anti-potyvirus monoclonal antibody using an immuno-enzymatic test (ELISA). As shown, four of the six centrosema potyvirus isolates tested were detected by the anti-poty monoclonal antibody. The SQ-25 and CP-1 isolates, however, were not detected in this test.

Consequently, the CP-1 isolate was increased in Phaseolus vulgaris and purified to obtain enough antigen for production of an antiserum. The SQ-25 isolate was serologically related to other potyviruses described later on in this report and, therefore, there was no need to produce another antiserum. The CP-1 isolate was successfully purified and a specific antiserum produced this year.

In conclusion, it is evident that at least three different potyviruses can infect Centrosema spp. in Colombia. However, the TPP is now in capacity to detect these potyviruses in infected plants and sexual seed.

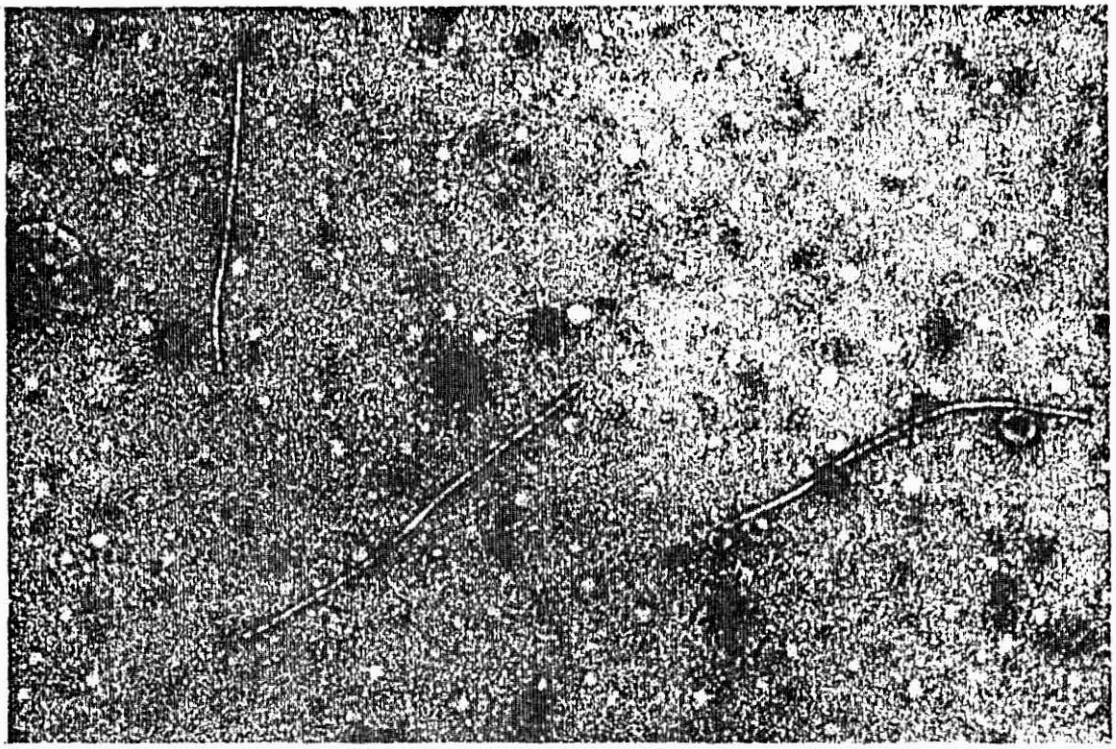

Figure 3. Filamentous virus particles detected in Centrosema spp. plants found free of the SMV-CE potyvirus by serological assays.

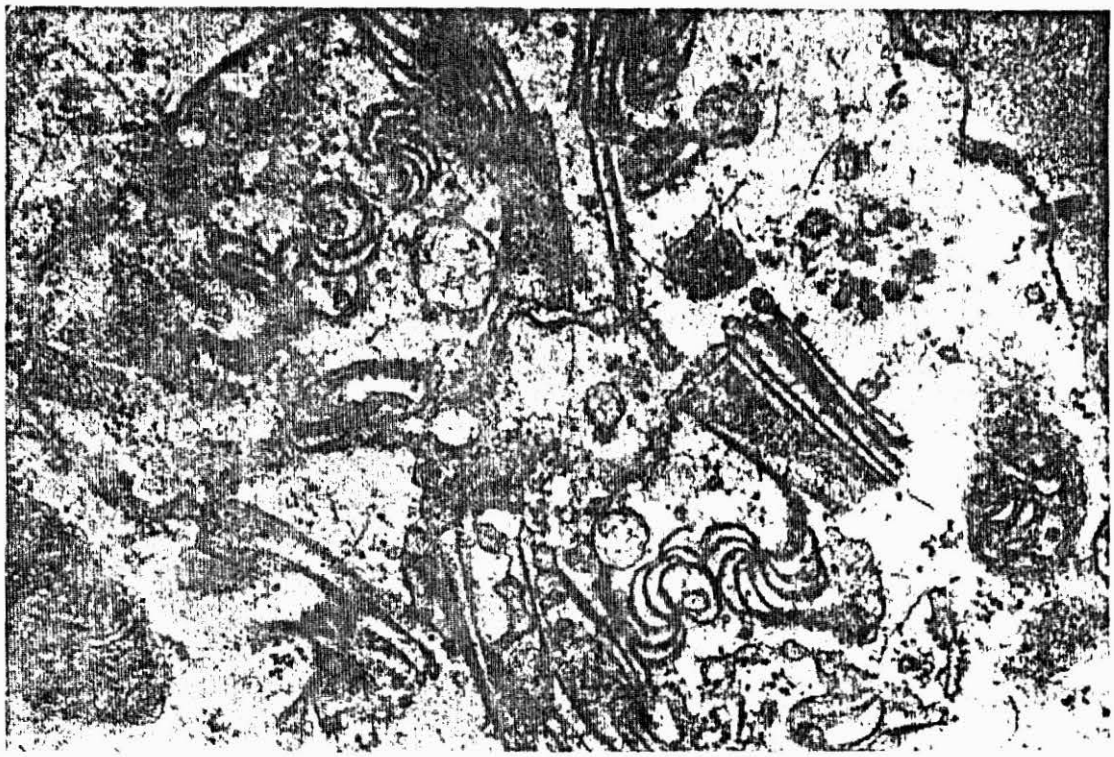

Figure 4. Cytoplasmic inclusions induced by a potyvirus in infected cells of Arachis pintoi.

Table 2. ELISA (OD_{405}) reaction of different potyviruses isolated from Centrosema spp. to a monoclonal antibody specific for the potyvirus group.

Centrosema isolate[a]	Incubation time 30 min	60 min
SMV-CE	2.9	2.9
CP-1	0.0	0.0
CP-2	2.8	2.8
CP-3	2.8	2.8
SQ-15	1.5	2.8
SQ.25	0.0	0.0
SMV	2.8	2.8

a SMV-CE = centrosema strain of soybean mosaic virus; CP = CIAT-Palmira isolate No.; SQ = Santander de Quilichao isolate No.; SMV = soybean mosaic virus.

VIRAL DISEASE(S) OF ARACHIS PINTOI

As reported last year, Arachis pintoi plants with ringspot and yellow mottling were collected in the locality of Ginebra, Valle. These plants were brought to the VRU and shown by electron microscopy to contain a filamentous virus. These plants were used as virus sources for all subsequent characterization work.

The virus was mechanically transmissible to bean (Phaseolus vulgaris), cowpea (Vigna unguiculata), pea (Pisum sativum), soybean (Glycine max), and peanut (Arachis hypogaea), inducing systemic infection in all of these legumes. An electron microscopy study of Arachis pintoi plants affected by the ringspot/mottling disease revealed the presence of 'pinwheel' inclusions (Figure 4) characteristic of the potyvirus group.

The virus was subjected to a purification procedure suitable for plant viruses of the potyvirus group, to obtain a purified preparation (Figure 5). The purified virus was injected in a New Zealand white rabbit to produce an antiserum for diagnostic purposes. After a series of four injections at weekly intervals, the rabbit was bled to obtain the antiserum. The antiserum was successfully used in double immuno-diffusion tests to specifically detect the virus in A. pintoi and in a susceptible pea cultivar.

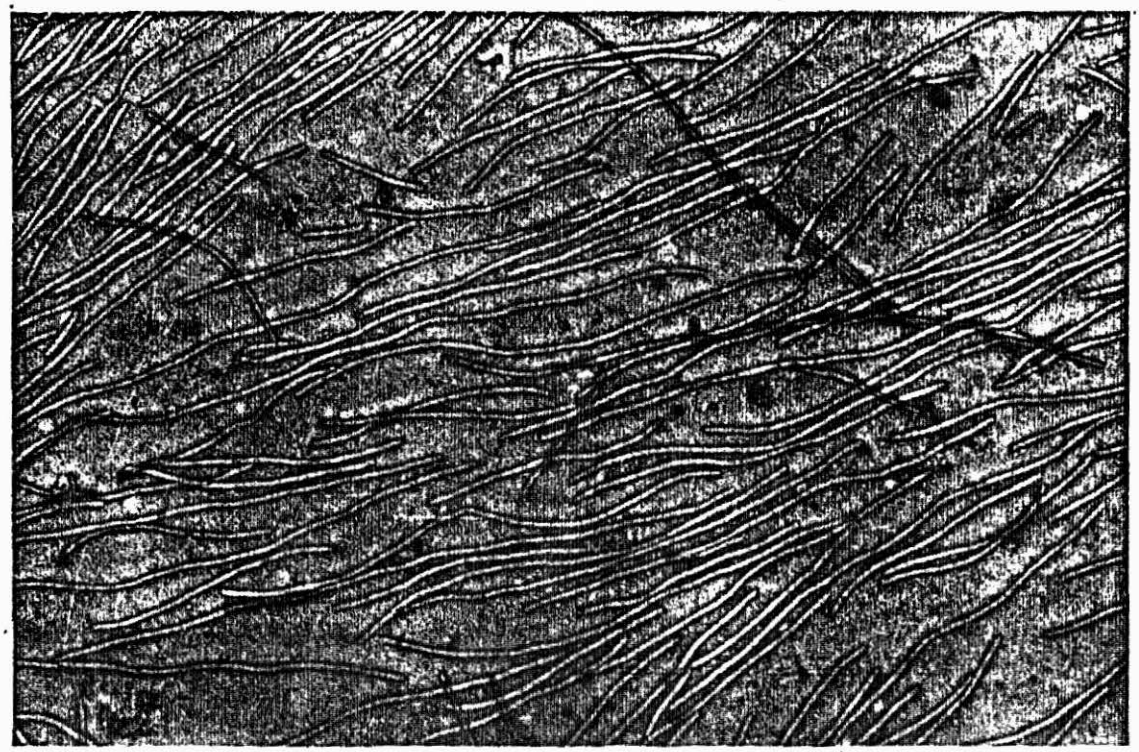

Figure 5. Purified particles of a potyvirus isolated from Arachis pintoi.

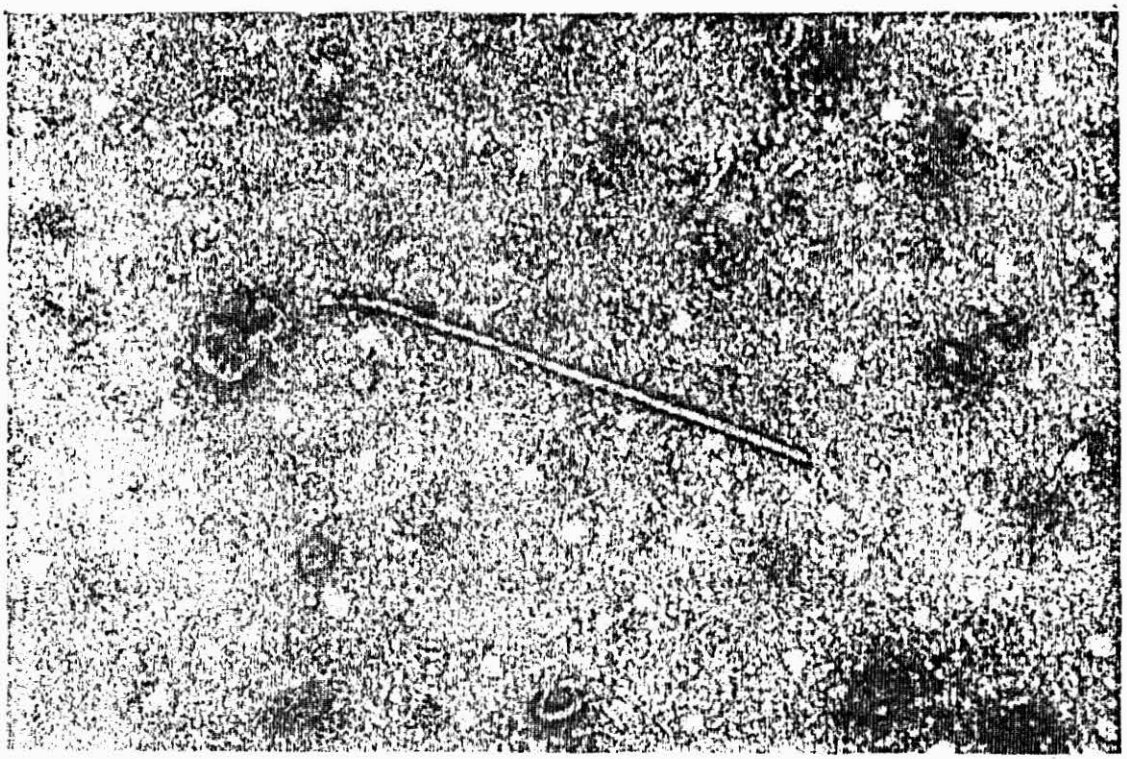

Figure 6. Filamentous virus detected by electron microscopy in leaf extracts of symptomless Stylosanthes macrocephala plants.

The antiserum was also further purified to obtain the globulin fraction to implement an ELISA test suitable to detect the virus in sexual seed of A. pintoi. For this test, 540 A. pintoi seeds were assayed in groups of 2 and 10 seeds per sample, with negative results, indicating that the seed provided by the TPP was free of this potyvirus. We will be testing seed from virus-infected A. pintoi plants to investigate the possibility of seed transmission of this virus.

Characterization of the A. pintoi potyvirus.

Given the considerable expertise of the Plant Pathology Department of the University of Florida with potyviruses and forage legumes in general, the VRU contacted Dr. F.W. Zetter, a virologist in that Deparment, to request antisera to some of the potyviruses known to infect Arachis spp. This informal cooperation quickly led to the demonstration that the potyvirus isolated from A. pintoi was serologically identical to peanut mottle virus, an ubiquitous potyvirus of world-wide distribution. Work is now in progress to fully characterize the A. pintoi potyvirus as a possible peanut mottle virus strain.

VIRAL DISEASE(S) OF STYLOSANTHES SPP.

A virus survey conducted this year to detect the presence of viruses in Stylosanthes capitata, S. guianensis, and S. macrocephala in two locations, CIAT-Palmira and CIAT-Santander de Quilichao, did not reveal any apparent virus-like diseases. Nevertheless, leaf samples were taken to the VRU for electron microscopy to examine the plants for latent virus infections.

The observation of these samples with the electron microscope revealed the presence of a filamentous virus (Figure 6) in S. macrocephala but not in S. capitata or S. guianensis. The filamentous virus isolated had a mean particle length and morphology similar to those of potyviruses. The virus was mechanically transmitted to beans, causing both mosaic and necrosis, and to Nicotina benthamiana.

In order to establish the possible relationship between this potyvirus and those isolated from Centrosema spp. and Arachis pintoi, various serological tests were performed. The results of these tests clearly showed serological relationships between the Stylosanthes macrocephala, Arachis pintoi, and Centrosema SQ-25 potyviruses. These findings suggest that we are in the presence of a peanut mottle virus

strain(s) capable of attacking Arachis, Centrosema, and Stylosanthes species.

Conclusions and Future Research Strategy

The relatively high incidence of potyviruses in species of Arachis, Centrosema and Stylosanthes, is not surprising considering the broad pathogenicity range of potyviruses in the Leguminosae, and the fact that potyviruses are the largest group of plant viruses known. Also, potyviruses are ubiquitous in legumes due to their transmission by several aphid species and, more important, in the seed of many susceptible legume species.

In the case of Arachis, Centrosema, and Stylosanthes, at least three distinct potyviruses have been already detected. All of these viruses can now be detected and identified by serological means at CIAT, both in infected plants and seed. The quarantine significance of these potyviruses, however, is relatively low, considering that soybean mosaic and peanut mottle viruses are widely distributed in the world. Nevertheless, the VRU is already producing virus-free seed of these forage legumes to meet international standards for the exchange of plant germplasm.

It is evident that the virus characterization work must be continued to determine the extent of pathogenic variability

and pathogenicity of these potyviruses in the Centrosema and Stylosanthes species currently evaluated by the Tropical Pastures Program. Also, a more thorough indexing of these species must be conducted to exclude the possible existence of viruses not readily detected by conventional electron microscopy techniques.

PUBLICATIONS

In Refereed Journals

Morales, F., Niessen, A., Ramirez, B.C., Castaño, M., 1989. Isolation and partial characterization of a geminivirus causing bean dwarf mosaic. Phytopathology 79:in press.

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Morales, F.J., and Gamez, R. 1989. Beetle-transmitted viruses. in Bean Production Problems in the Tropics. 2nd ed. CIAT, Cali, Colombia. pp 363-378.

Morales, F.J., and Galvez, G.E. 1989. Additional viruses. in Bean Production Problems in the Tropics. 2nd ed. CIAT, Cali, Colombia. pp 407-412.

Galvez, G.E., and Morales, F.J. 1989. Aphidetransmitted viruses. in Bean Production Problems in the Tropics. 2nd ed. CIAT, Cali, Colombia. pp. 33-362.

Galvez, G.E., and Morales, F.J. 1989. Whitefly-transmitted viruses. in Bean Production Problemsin hte Tropics. 2nd ed. CIAT, Cali, Colombia. pp. 379-406.

Schwartz, H.F., and Morales F.J. 1989. Seed Pathology. in Bean Production Problems in the Tropics. 2nd ed. CIAT, Cali, Colombia. pp. 413-432.

Submitted in 1989 to Refereed Journals

Morales, F. J., Niessen, A.J., Castaño, M., and Calvert L.A., Detection of a strain of soybean mosaic virus affecting tropical forage species of Centrosema. Plant Disease. Accepted for publication. October 2, 1989.

Scientific Papers Presented by VRU staff in 1989

Ramirez, B.C., Morales, F.J., and Calvert, L. A., Differentiation of closely related bean geminiviruses with cloned DNA probes. II International Plant with cloned DNA probes. II International Plant Pathology Congress. CIAT, Cali, Colombia.

Cuervo, M., Nolt, B.L., Pineda B., and Velasco, A.C. Caracterizacion de los acidos ribonucleicos de cadena doble (ARN-cd) asociados a enfermedades virales en yuca.

II. International Plant Pathology Congress. CIAT, Cali, Colombia.

Castaño, M., Niessen, A., and Morales, F.J. Caracteristicas adicionales del virus de la macana o rayadilla del fique (Furcraea spp.) II International Plant Pathology Congress. CIAT, Cali, Colombia.

Morales, F.J., and Niessen, A.I. Isolation of a potyvirus from mosaic-affected Centrosema spp. in Colombia. II International Plant Pathology Congress, CIAT, Cali, Colombia.

Acosta, O., Alegria, A. Lastra, R. and Morales. F.J. Partial characterization of a Colombian isolate of bean mild mosaic virus from Phaselus vulgaris. II International Plant Pathology Congress, CIAT, Cali, Colombia,.

Zettler, F.W., Stansly, P.A., Elliot, M., Peralta A., Carranza, C., and Morales, F.J. Bean pod mottle virus (BDMV) in Ecuador and its transmission by Cerotoma facialis maculata. II International Plant Pathology Congress. CIAT, Cali, Colombia.

Pineda, B., Velasco, A.C., Angel, J.C., and Nolt, B. Detección del virus del mosaico común de la yuca (CCMV) en frutos y semillas sexuales del clon CM 976-15. II

International Plant Pathology Congress. CIAT, Cali, Colombia.

Velasco, a., Nolt, B. and Pineda L. Comparación de tres métodos de la técnica inmunoenzimatica ELISA para el diagnóstico del virus del mosaico común de la yuca. II. International Plant Pathology Congress. CIAT, Cali, Colombia.

Calvert, L.A., and Nolt, B. Cassava Frogskin disease. IV International Plant Virus Epidemiol Congress. Montpellier, France.

Morales, F.J., New approach to breeding for bean golden mosaic resistance. IV International Plant Virus Epidemiol Congress. Montpellier, France.

Audiovisuals and Publications for Training Purposes

Audiovisual: "Principales enfermedades virales del frijol en America Latina y su control.

Publication: "Bean Common Mosaic: research methodology and control measures. English edition.

CURRENT COOPERATIVE RESEARCH PROJECTS WITH ADVANCE RESEARCH INSTITUTIONS

IVP/CIAT/BMZ

TITLE: "Distribution and Importance of Viruses Naturally Infecting Phaseolus vulgaris and its Relatives in Africa"

Institutions: Institute of Plant Virology (Institute fur Viruskrankheiten der Pflazen). Braunschweig, W. Germany/CIAT.

Principal Investigators: Drs. J. Vetten (IVP), D. Allen (CIAT), F.J. Morales (CIAT).

CIAT/IFVA/Bean/BYMV Project

TITLE: "Characterization of the Main Bean Yellow Mosaic Virus Strains that Limit Bean Production in Northen Africa, West Asia, and China.

Institutions: Institute of Applied Plant Virology (Instituto di Fitovirologia Applicata). Torino, Italy/CIAT).

Principal Investigators: Drs. Vittoria Lisa (IFVA); Francisco J. Morales (CIAT).

CDR-AID Grant # C7-077

TITLE: "Identification and Characterization of Genetic Strains in Whiteflies".

Institutions: Tel Aviv University, Israel/VRU-CIAT.

Principal Investigators: Drs. D. Wool, D. Gerling (TAU), L. Calvert, A. Belloti, F. Morales (CIAT).

Bean/Cowpea

TITLE: "Molecular Approaches to Control of Bean Golden Mosaic Virus.

Institutions: University of Wisconsin, Univ.of Nebraska and Puerto Rico; CESDA (D. Rep), VRU-CIAT

Principal Investigators: D. Maxwell, R. Gilbertson, P. Ahlquist, S. Hanson (U. Wisconsin); Morales F.. Calvert, L. (CIAT); F. Saladin, T. Martinez (Dominican Rep.)

AID/PSTC Project No. 9.175

TITLE: USE OF CLONE VIRAL DNAs IN CHARACTERIZATION, EPIDEMIOLOGY AND CONTROL OF BEAN GOLDEN MOSAIC VIRUS.

Institutions: Institute for Molecular Virology and Department of Plant Pathology University of Wisconsin/VRU-CIAT.

Principal Investigators: P. Ahlquist, D. Maxwell, R. Gilbertson, F. Morales.

ICTP (International Cassava-Trans Project)

TITLE: CONTROL OF CASSAVA VIRUSES BY IN VITRO GENETIC RECOMBINATION.

Institutions: ORSTOM, Washington University, CIAT - VRU.

Principal Investigators: C. Fauquet (ORSTOM), R. N. Beachy (Washington University), L. Calvert (CIAT).

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Maria Consuelo Martínez	Technician III
Rosa Marina Jiménez	Worker II
Cesar Tulio Rodríguez	Worker II
Apolinar Umaña	Worker II
Gloria López	Secretary V
Yolanda Rincón (*)	Secretary V

(*) Left 1989

A P P E N D I X

89crsp/10-24-89

BEAN/COWPEA CRSP FY 89 Annual Report
Maxwell/Wisconsin-Saladin/Dominican Republic-Morales/CIAT

TITLE: Molecular Approaches for the Control of Bean Golden Mosaic Virus

October 1, 1988- September 30, 1989

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1989 ANNUAL REPORT

TABLE OF CONTENTS

TITLE: MOLECULAR APPROACHES FOR THE CONTROL OF BEAN GOLDEN MOSAIC VIRUS

EXECUTIVE SUMMARY

Dominican Republic/University of Wisconsin/CIAT
Saladin/Maxwell/Morales

Bean golden mosaic is a major factor reducing yields of beans in many areas of the subtropical and tropical Americas. Some fields experience 100% losses. In the host country of the Dominican Republic, the severity of bean golden mosaic is increasing because of changing agricultural practices, which favor increases in the population of the whitefly insect vector, Bemisia tabaci, of bean golden mosaic virus (BGMV).

The virus causing bean golden mosaic in Brazil and Argentina (BGMV) is not mechanically transmitted, whereas the BGMV isolates from Central America and the Caribbean can be transmitted mechanically. This observation has led researchers to suggest that there might be genetic diversity among isolates of BGMV from different regions and that this diversity may complicate attempts to develop resistant cultivars by standard breeding approaches.

This project was undertaken to characterize the virus(es) causing bean golden mosaic and related viruses that infect beans. Isolates of BGMV thought to represent the greatest genetic diversity, were obtained from Brazil (BGMV-BZ), the Dominican Republic (BGMV-DR), and Guatemala (BGMV-GA). Each of these virus isolates was shown to have a genome composed of two distinct DNA components, component A and component B. Subsequently, these cloned DNA components were sequenced and comparisons of sequence similarity made by computer analysis. Similar genome organizations were found for these three isolates. Each isolate had four open reading frames (ORFs, genes) on component A and two ORFs on component B. This genome organization is identical to that described for other bipartite geminiviruses such as tomato golden mosaic virus and African cassava mosaic virus. Sequence similarity between BGMV-BZ, which is not mechanically transmissible, and the two mechanically transmitted isolates, BGMV-DR and BGMV-GA, was about 70-85%, which is the percent sequence similarity between BGMV-BZ and tomato golden mosaic virus. The sequence similarity between BGMV-DR, BGMV-GA, and a BGMV isolate from Puerto Rico (sequenced by R. Goodman and associates, University of Illinois) was greater than 90%. Therefore, we conclude that at least two distinct strains of BGMV exist. One strain is designated the nonmechanically transmissible strain, BGMV-NMT strain, and is located in South America, e.g. Argentina and Brazil; and the other strain is designated the mechanically transmissible strain, BGMV-MT strain, and is located in Central America and the Caribbean. The sequence divergence of these two groups is great enough that they could be considered separate viruses, but to avoid confusion, they have been designated strains of BGMV. Full-length, infectious clones of bean dwarf mosaic virus were obtained, and this geminivirus was determined to be a distinct virus

from BGMV by DNA hybridization and sequence comparisons. Also, partial clones of the B component of bean calico mosaic virus, which is a bean-infecting geminivirus from Mexico, were isolated.

Nucleic acid spot hybridization test with BGMV-DNA probes were continued with samples from Puerto Rico, Brazil, Honduras, Rwanda, and Dominican Republic. The technique was effective in detecting viral nucleic acid in all bean samples that had typical golden mosaic symptoms. Two weeds with geminivirus-like symptoms also gave strong reactions with the BGMV-DNA probe. Identification of such weed hosts is an important step towards designing practices to eliminate or reduce natural reservoirs of the virus. After an analysis of the sequence data, BGMV-strain specific DNA probes will be designed as well as probes to detect other bean-infecting geminiviruses.

Training included an M.Sc. student in Brazil, a visiting scientist from Dominican Republic, a graduate student from Indonesia, a graduate student from USA, a senior scientist from Brazil, an Assistant Scientist from USA, three undergraduate students, a research specialist, and the US-PI. US and Brazilian scientists participated in scientific meetings and reported on the molecular characterization of the BGMV-BZ and BGMV-GA isolates. Also, a workshop on application of DNA probes for detection of plant pathogens was given.

Through the joint efforts of a particularly hard working team of scientists, this year has brought important successes on a number of fronts. The researchers are now poised at a position of exciting opportunities, which promise to make major contributions to the goals of the CRSP global plan

1989 ANNUAL REPORT: Molecular approaches for the control of bean golden mosaic virus

Saladin/Maxwell/Morales-Dominican Republic/Wisconsin/CIAT

I. PROGRESS

A. Specific Research Contributions

1. Research in progress in HC and USA

BEAN GOLDEN MOSAIC VIRUS:

Bean golden mosaic is a serious constraint to bean production in major agricultural regions of Brazil, Argentina, Central America, and the Caribbean. In some situations, it causes total loss of the bean crop. In the San Juan de la Maguana area of the Dominican Republic in March 1988, bean golden mosaic was extremely serious. Additionally, the whitefly insect vector, Bemisia tabaci, has also been increasing in this area due to planting of crops that are excellent hosts for this insect. Future profitable bean production in this area will require the development of resistant cultivars to bean golden mosaic virus (BGMV).

CONSTRAINT: Lack of resistant cultivars of beans for bean golden mosaic virus in the Dominican Republic.

FIELD EVALUATION OF GERMPLASM FOR RESISTANCE TO BGMV IN THE DOMINICAN REPUBLIC: Dr. F. Morales from CIAT supplied 188 germplasm accessions, which had been selected for their tolerance to BGMV in five countries (Agrentina, Brazil, Guatemala, Mexcio, and El Salvador). Difficulty was encountered in the field evaluation of this material; however, 41 non-black-seeded accessions were selected for future evaluation. Currently, seed increases of F_2 populations of triple crosses between bean common mosaic virus-resistant lines of the Pompadour types and of F_1 populations of crosses involving some of the new sources of BGMV resistance selected from advanced DOR lines (breed for BGMV resistance) are being completed at Constanza. This seed will be evaluated at San Juan de la Maguana.

CONSTRAINT: Lack of understanding of the complex of geminiviruses that cause bean golden mosaic and bean dwarf diseases.

MOLECULAR CHARACTERIZATION OF BGMV ISOLATES: Previous research by Dr. R. Goodman and Associates at the University of Illinois resulted in the sequencing of a geminivirus from Phaseolus lunatus (lima bean) collected in Puerto Rico. This virus was known originally as bean golden yellow mosaic virus, and because it is capable of causing the bean golden mosaic syndrome, it has become the "standard" BGMV isolate (BGMV-PR).

Last year we reported the sequence of the Brazilian isolate of bean golden mosaic virus (BGMV-BZ). From the sequence comparison of BGMV-BZ to BGMV-PR and tomato golden mosaic virus (a geminivirus of tomatoes in Brazil), it was concluded that BGMV-BZ might be considered a distinct geminivirus. At the very least, BGMV-BZ should be designated as a distinct strain of BGMV.

This year scientists at the University of Wisconsin have cloned and sequenced the DNAs from isolates of BGMV collected from beans in the Caribbean and Central America. Dr. Francisco J. Morales, CIAT, provided an isolate from Guatemala (BGMV-GA) and the Dominican Republic (BGMV-DR). These two isolates, as well as BGMV-PR, can be mechanically transmitted to beans, whereas the BGMV-BZ isolate can not be mechanically transmitted. The BGMV-GA isolate was selected because it is the standard isolate used at CIAT and represents the Central American BGMV types. The BGMV-DR isolate was included in this study because it appears to be more aggressive on BGMV-tolerant germplasm developed by CIAT, and it causes more severe symptoms than BGMV-GA. Additionally, another bean-infecting geminivirus, bean dwarf mosaic virus (BDMV), from Colombia was supplied by Dr. Morales. This geminivirus was thought to be closely related to BGMV.

Cloning of the viral DNA involved the extraction of the circular double-stranded replicative form (RF) DNA from infected beans. These DNA preparations were digested with suitable restriction endonucleases and the resulting fragments cloned into plasmid vectors. After testing a number of restriction endonucleases with each virus, partial and full-length clones of both DNA components were obtained for all three bean-infecting geminiviruses. Full-length clones of BGMV-GA for DNA A and DNA B components were designated pBGGARG5 (EcoR I insert) and pBGGBRG2 (BamH I insert), respectively. For BGMV-DR, the DNA A and DNA B components were denoted pBGDAHind3 (Hind III insert) and pBGDBHind304 (Hind III insert), respectively. With BDMV, the full-length inserts were called pBDCABgl635 (Bgl II insert) and pBDCBBam40 (BamH I insert), respectively.

These BGMV-GA and BGMV-DR clones were sequenced by first creating libraries of nested deletions using Exonuclease III. These subclones were then sequenced by the dideoxy chain termination technique from single-stranded DNA generated by the helper phage M13K07. To assure accuracy, both DNA strands were sequenced in all cases. Independent clones were used to sequence through the cloning sites to confirm that sequenced clones were full-length. DNA component A and B of BGMV-GA have 2647 bp (nucleotide base pairs) and 2595 bp, respectively; and the DNA component A and B of BGMV-DR have 2641 bp and 2608 bp, respectively. These complete sequences are available from D. P. Maxwell (Appendix).

Computer-assisted analysis of the sequenced DNA clones showed that the gene (open reading frames, ORFs) organization of the components of BGMV-GA and BGMV-DR were similar to the gene organization for BGMV-BZ and other bipartite geminiviruses, e.g. tomato golden mosaic virus. The DNA A components have four ORFs and the DNA B components have two ORFs (Fig. 1). A segment, designated the common region, of nearly 100% sequence identity exists between each of the two components (DNA A and DNA B) for each isolate. The sequence of this common region is different among different geminiviruses (Table 1).

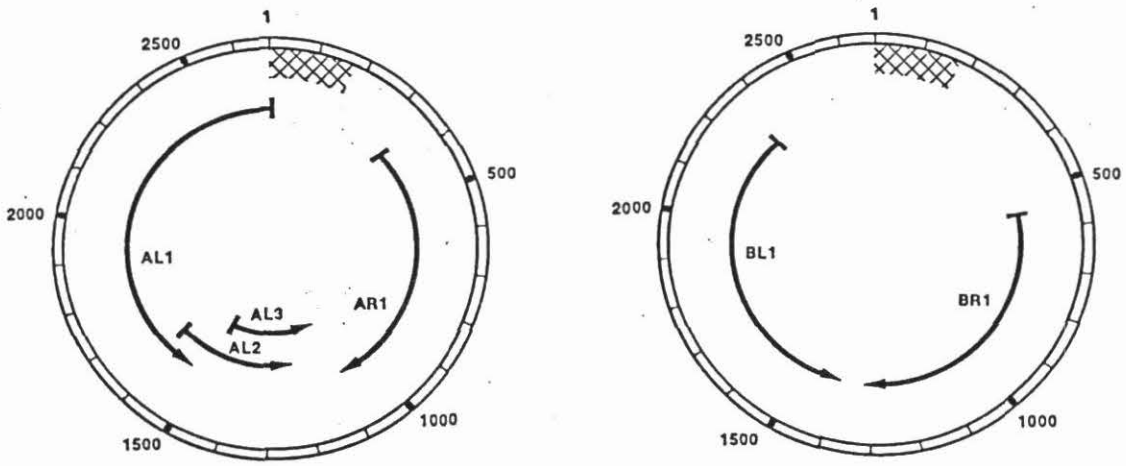

Fig. 1. Schematic map showing common region (cross hatched region starting in position 1), open reading frames (genes, e.g. AR1), for the replicative forms (component A, left circular DNA, and component B, right circular DNA) of bean golden mosaic virus isolate from Guatemala. This map is based on computer assisted analysis of sequence data.

Table 1. Nucleotide homologies (percent identity) for the common region between whitefly-transmitted geminiviruses.

	BGMV-GA	BGMV-DR	BGMV-BZ	TGMV	ACMV
BGMV-PR	97%	94%	59%	48%	51%
BGMV-GA		96%	69%	48%	
BGMV-DR			70%	66%	
BGMV-BZ				68%	49%
TGMV					48%

BGMV-PR, bean golden mosaic virus isolate from Puerto Rico; BGMV-GA, BGMV isolate from Guatemala; BGMV-DR, BGMV isolate from Dominican Republic; BGMV-BZ, BGMV isolate from Brazil; TGMV, tomato golden mosaic virus from Brazil; ACMV, African cassava mosaic virus from Africa.

The following characteristics of the various ORFs encoded by BGMV-GA and BGMV-DR have been identified (Fig. 1):

ORF (gene)	Map position in nucleotides from start of common region	
	BGMV-GA	BGMV-DR
COMPONENT DNA A		
AR1	340-1125	344-1123
AL1	6-1,2647-1592	10-1,2641-1590
AL2	1785-1267	1783-1265
AL3	1520-1122	1518-1120
COMPONENT DNA B		
BR1	503-1273	512-1282
BL1	2187-1306	2197-1316

The three characters in the name for each ORF, e.g. AR1, denote the relevant DNA component (A or B), right or left (R or L) direction of transcription, and gene order (1,2, etc.), respectively. Between BGMV-GA and BGMV-DR, the gene lengths and putative protein products are nearly identical (93 % or greater identity for amino acids). When comparisons are extended to BGMV-PR, BGMV-BZ, and tomato golden mosaic virus (TGMV) (Table 2), it is evident from protein identity comparisons that there are three distinct groups of viruses. BGMV-GA, BGMV-DR, and BGMV-PR are one group with amino acid identities of 93% or greater for each gene or ORF. BGMV-BZ and TGMV are separate and distinct from this group. The greatest difference in amino acid identities for gene comparisons is 69% for BR1 of BGMV-GA compared to BR1 of TGMV. AR1, the coat protein gene, is the most highly conserved gene with a 89% or greater amino acid identity for all pairwise comparisons. This indicates that serological techniques may not be able to distinquish these three groups.

Table 2. Sequence homologies of the possible proteins encoded by some whitefly transmitted geminivirues.

ORFs	Isolates Compared							
	GA to BZ	DR to BZ	GA to TGMV	DR to TGMV	BZ to TGMV	GA to PR	DR to PR	GA to DR
AR1	96/90*	95/89	97/93	96/92	95/91	99/97	98/96	98/98
AL1	82/73	83/74	82/73	83/74	88/77	96/94	96/94	97/97
Al2	78/69	77/67	81/70	81/67	83/71	99/96	98/97	99/98
Al3	89/79	89/79	86/78	86/77	86/78	98/97	98/95	98/97
BR1	81/70	82/71	79/69	80/70	81/70	95/93	96/94	94/93
BL1	92/81	92/81	95/89	93/89	90/82	98/93	99/95	99/98

* Data is for % similarity/% identity. GA = BGMV-GA; BZ = BGMV-BZ; DR = BGMV-DR; TGMV = tomato golden mosaic virus.

Based on the amino acid identities, it is proposed that at least two distinct strains of BGMV exist. The mechanically transmissible strain (BGMV-MT strain) and the nonmechanically transmissible strain (BGMV-NMT strain). Isolates of BGMV from Central America and the Caribbean are in the BGMV-MT strain. It is expected that the BGMV isolates from Argentina and Brazil would be in the BGMV-NMT strain. Thus, future breeding efforts should recognize the presence of these two distinct strains.

MOLECULAR CHARACTERIZATION OF BEAN DWARF MOSAIC VIRUS: A cooperative effort was initiated with Agracetus Corporation, Middleton, WI in September 1989 to sequence the full-length clones of BDMV. From limited sequence data, it is evident that this bean-infecting geminivirus has DNA A and B components. Also, because of the sequence divergence, this virus should be distinguished from BGMV-MT and BGMV-NMT strains. Restriction endonuclease maps of the cloned DNAs of BDMV also supports its being considered a separate virus.

CONSTRAINT: Lack of a rapid method to identify geminiviruses that infect bean and weeds.

DEVELOPMENT OF DNA PROBES FOR DETECTION OF BGMV: The molecular characterization of the BGMV isolates allows the design of both general- and isolate/strain-specific DNA probes, which can be used for the detection and identification of BGMV in beans and other plants. In FY 90, emphasis will be placed on the construction of isolate/strain-specific probes for the identification of the BGMV-MT strain and BGMV-NMT strain as well as a specific probe for BDMV. The available sequence data indicates that it will be possible to design and construct these probes (Table 3).

Table 3. Sequence homologies for the 3' end of the DNA B component of selected whitefly transmitted geminiviruses.

	BGMV-GA	BGMV-DR	BGMV-PR	BGMV-BZ
BGMV-DR	86%			
BGMV-PR	70%	80%		
BGMV-BZ	43%	46%	52%	
TGMV	39%	44%	39%	38%

BGMV-GA, bean golden mosaic virus isolate from Guatemala; BGMV-DR, BGMV isolate from Dominican Republic; BGMV-BZ, BGMV isolate from Brazil; TGMV, tomato golden mosaic virus from Brazil. These sequence comparisons are made between the 3' ends (about 350 nucleotides) of the DNA B component which represents the most divergent regions between the different viruses and isolates.

This year, the general DNA probes (full-length clones of BGMV) were used to test for geminiviruses in beans and weeds from Puerto Rico, Honduras, Brazil, Dominican Republic, Rwanda, and Idaho. Strong hybridization with viral DNA probes occurred with a sample from Desmodium sp. collected in Puerto Rico, and a weed collected in a BGMV-infected bean field in the Dominican Republic. Most surprisingly, Macroptilium lathyroides, a leguminous weed, with typical golden mosaic symptoms collected in the Dominican Republic did not hydridize with the BGMV DNA probe. This weed was thought to be a major inoculum source of BGMV, but this does not seem to be the case. The important BGMV reservoirs are thus likely to be found in other weeds and/or crops. In Brazil, soybean samples with typical golden mosaic symptoms hybridized strongly to the BGMV-BZ-DNA probe. Samples of suspected BGMV-infected beans collected in Honduras gave strong signals with our BGMV-DNA probes. Plants without golden mosaic symptoms always gave negative hybridization responses in our tests.

CONSTRAINT: Lack of standard breeding methods to produce beans with high levels of resistance to bean golden mosaic.

VIRUS-DERIVED RESISTANCE SCHEMES: Efforts to develop a virus-derived resistance scheme would be enhanced by an understanding of the functions of the genes (ORFs) associated with the two components of bean-infecting geminiviruses. Thus, it is essential that the cloned viral DNAs be tested for biological activity. Many experiments involving mechanical inoculation of beans with cloned-DNAs of BGMV-GA in various constructions, e.g. *monomers* (full-length inserts at various restriction endonuclease sites) or dimers (two full-length inserts connected head to tail) were all unsuccessful. Mechanical inoculation of natural BGMV-GA DNAs also failed to produce infection, indicating that alternate DNA introduction techniques are needed. Currently, experiments are underway with scientists at Agracetus to test the use of an electrical discharge particle accelerator (particle gun) for inoculation of beans with cloned-BGMV DNAs.

Scientists with Monsanto Co. (Elmer et al. 1988. Plant Mol. Biol. 10:225-234.) have reported the successful infection of Nicotiana benthamiana, a species of tobacco, with cloned-TGMV DNA. N. benthamiana was not a host for the BGMV-GA or BGMV-DR; however, it was infected by BDMV. Thus, once full-length clones of BDMV were obtained, these were tested on this plant. Monomers (full-length inserts) and dimers (two full-length inserts in a head to tail construction) were found to be infectious by mechanical inoculation of N. benthamiana but not of beans. Sap prepared from infected N. benthamiana inoculated with cloned-DNAs was infectious on beans. This indicates that viral particles are likely formed in the N. benthamiana plants inoculated with cloned-viral DNA. Once these two DNA components for BDMV have been sequenced, we will be ready to start to determine the function of the genes associated with the viral DNA components. The coat protein gene and those genes associated with viral DNA replication will be targets for use in the formation of transgenic plants, which will be tested for resistance to BGMV.

2. Research results disseminated and currently in use in HC and US.

Our project is presently concerned with the development of a research base for understanding the extent of genetic variability in bean-infecting geminiviruses, with the development of DNA probes for detection of geminiviruses, and with the evaluation of a virus-derived scheme for resistance. We have used our DNA probe technology to confirm the presence of bean-infecting geminiviruses for researchers from Honduras, Puerto Rico, Brazil, and Dominican Republic. Additionally, we have detected geminiviruses in two weeds. This year we will be field evaluating strain-specific DNA probes for the BGMV-NMT strain and the BGMV-MT strain.

We have assisted Dr. Judy Brown, University of Arizona, in the determination that a geminivirus, which she isolated from beans in Sonora, Mexico, is different than bean dwarf mosaic virus and strains of BGMV. She has designated this new bean-infecting geminivirus as bean calico mosaic virus. We will clone and sequence part of this virus and develop a specific DNA probe for its detection. Dr. R. Forester, extension plant pathologist, at the University of Idaho had some interest in the diagnosis of a potential bean virus and we probed samples which he collected. Likewise, Dr. Robert Lambe, a private consultant, is using our nucleic acid probe technology this fall for the detection of viruses of peas and beans in Guatemala. Thus, we are encouraged by the interest in the nucleic acid probe methods by field pathologists and will continue to cooperate with them in the future.

Members of our project had various opportunities to present the findings from our research efforts and to participate in workshops. Drs. Gilbertson and Faria presented research reports at the annual meeting of the American Phytopathological Society in November 1988 on the molecular characterization of the Brazilian and Guatemala isolates of BGMV. In December, Dr. Maxwell reported on our geminivirus research at the Western Regional meeting of bean researchers held in Puerto Rico. In February, Dr. Ahlquist reported our results to a group of colleagues at the University of Nebraska. Dr. Faria attended a special conference on bean golden mosaic virus in Campinas, Sao Paulo, Brazil in April 1989. In July, Dr. Maxwell presented an invited paper on the application of DNA probes for the detection of plant pathogens at the Meeting of the Latin American Plant Pathologist at Cali, Colombia. Dr. Gilbertson presented a research seminar to the Department of Plant Pathology, University of Wisconsin in July. At the annual meetings of the American Phytopathological Society in Richmond, VA, Drs. Gilbertson and Maxwell presented a workshop on the use of DNA probes for detection of plant pathogens, and Dr. Gilbertson presented an invited symposium paper on the use of geminiviruses as gene vectors.

B. Institutional Development and Training

1. Changes since 1988: With the additional $5,000 that was added to our project, we brought Ms. R. Teresa Martinez from the Dominican Republic to our laboratory in June 1989. She has taken

courses in English and is currently enrolled in introductory plant pathology. She is learning technology associated with the application of DNA probes for viral detection.

2. Over the life of the project: This is our first year in the Dominican Republic and our project has not had enough funds to provide a training component for HC personnel. In July 1989, we received a USAID/PSTC grant, which will allow us to continue the training of Ms. Martinez. If funds were available, we would have space in our program for one more graduate student.

Currently, two M.Sc. students are in our reseach program. Ms. Elizabeth Hendrastuti from Indonesia is supported by a World Bank Fellowship and Ms. Denise R. Smith from Wisconsin is on a departmental research assistantship. Ms. Amy Loniello, a freshman, has been working on our project for several months as well as a senior, Mr. Mark Wegmann, who has worked for us for several years. Ms. S. Johnson, a sophomore, also assisted us in the lab for several months.

Dr. J. Faria from CNPAF, Goiânia, Brazil has received training in molecular virology in our laboratory since January 1988. He returns to Brazil in December 1989. He was supported by funds from the Brazilian government and the Graduate School, Univ. of Wisconsin.

Dr. Robert L. Gilbertson, an Assistant Scientist, has been with the project since 1985; and because of Dr. Maxwell's responsibilities as Department Chairman, he works closely with the graduate students and technical support staff, Mr. Stephen F. Hanson.

3. In prospect: It is important that our project provide training for the CESDA staff in the Dominican Republic. This will be achieved by offering them opportunities to come to Madison as visiting scientists for periods up to one year. Ms. Aridia Figueroa is the next person who plans to come.

4. Training completed by 1992: Training for Teresa Martinez and Aridia Figueroa from the Dominican Republic will be completed. Two M.Sc. graduate students will finish their degrees. One student from either Costa Rica or Colombia will start a Ph. D. program. One visiting scientist (Eunize Zambolim) from Brazil will complete a years training in molecular virology. Dr. J. Faria from Brazil will have received two years training in molecular virology and is expected to return to Brazil as a scientist in a biotechnology laboratory for EMBRAPA. Dr. Robert L. Gilbertson and Mr. S. F. Hanson will have had excellent experience in molecular virology and international agriculture. At least four undergraduate students will work on this project and become exposed to the excitment of science and the importance of international agriculture.

C. Progress achieved in relation to the log frame

Output: CHARACTERIZATION OF STRAINS OF BEAN GOLDEN MOSAIC VIRUS: The Brazilian isolate of BGMV was cloned and sequenced in

1987-1988. This year, the Guatemalan and Dominican Republic isolates of BGMV were cloned and sequenced and computer analysis initiated for these three complete viral sequences (about 17,000 bp). Also, bean dwarf mosaic virus was cloned and cloning efforts were started on bean calico mosaic virus. Infectivity tests were successfully completed for the BDMV clones.

Output: DEVELOPMENT OF TECHNIQUES TO IDENTIFY STRAINS OF BGMV: The nucleic acid hydridization method, which involves squashing leaf disks on a special DNA binding membrane, was further tested using general DNA probes for BGMV. Samples were collected in Puerto Rico and Dominican Republic. Two potential weed hosts of BGMV were detected. Samples were also received from Brazil and Honduras; and geminivirus DNA was detected in beans, soybean, and a weed.

Output: VIRUS-DERIVED SCHEME FOR RESISTANCE: All mechanical inoculations of beans with cloned viral DNA of BGMV-GA and of tobacco, Nicotiana benthamiana, with BGMV-BZ DNA were negative. N. benthamiana plants were infected with virions, crude isolated viral DNA, and cloned-DNA of bean dwarf mosaic virus.

Output: EVALUATION OF BEANS FOR RESISTANCE TO BGMV IN DOMINICAN REPUBLIC: Forty-one non-black-seeded accessions were selected for additional evaluation for tolerance to BGMV.

2. Estimates of time frame for achieving objectives:

Objective I: Cloning and Sequencing of BGMV: This was to be completed by Oct. 1989. This has been done. Other bean-infecting geminiviruses would be characterized in the second year.

Objective II: DNA probes: The isolate specific probes are to be developed in year two and this is on schedule.

Objective III: Virus-derived scheme for resistance: This part of project will be started in year two and continue for several years. We have obtained infectious clones of bean dwarf mosaic virus so this research is on schedule.

Objective IV: Evaluation of germplasm in Dominican Republic: Progress is expected to be very slow.

3. Estimated time required to complete objectives: We are on schedule for the reserach in the US. However, major goal is to develop transgenic beans with resistance to BGMV, which is a formidable tak for any research laboratory. So far no one has been able to develop a transformed bean plant. Because of the excellent cooperation with scientists at Agracetus, we believe that we can accomplish this objective in 2-4 years.

4. Relation of the US research to research elsewhere: Our effort is the major project in the world concerned with the molecular characterization of bean-infecting geminiviruses. We coordinate activities very closely with CIAT. Scientists at CIAT have

agreed to assist in the evaluation of infectious, cloned-DNAs of BDMV, e.g. whitefly transmission, and to develop the general/universal DNA probe for geminiviruses. This probe will be useful for detecting geminiviruses of plants other than beans. Also, we are working with Dr. Judy Brown on the molecular characterization of bean-infecting geminiviruses, which she is detecting in Mexico. Dr. Roger Beachy and associates at Washington University are doing similar research to ours with African cassava mosaic virus, another economically important geminivirus. This group is currently involved in the production of transgenic cassava plants, which are resistant to this geminivirus. Several countries are involved in the evaluation of bean germplasm for resistance to BGMV and this is coordinated by scientists at CIAT, e.g. Dr. Steve Beebe. Thus, our project is making a unique contribution to the characterization of bean-infecting geminiviruses and application of biotechnology to bean improvement.

5. Contributions of research: It is expected that our research will provide data on the extent of genetic diversity among bean-infecting geminiviruses. This will be used to design logical breeding strategies for disease resistance for the various strains of BGMV and the other bean-infecting geminiviruses. The strain specific DNA probes will allow detection and identification of bean-infecting geminiviruses in beans, other crop plants, weeds, and whiteflies. Thus, these probes can be used in epidemiological studies, which can provide essential information for design of control strategies. For example, the identification of a weed host might allow its elimination as one source of inoculum. This practice would delay infection of beans and result in increased yields. In the US, there is interest in the development of rapid and accurate diagnostic nucleic acid probes for pathogens, which our project is doing. Most importantly, our project will devote effort to the development of technology for the transformation of beans. When we are successful, this will have a major impact on many other aspects of bean impovement besides resistance to geminiviruses. This could include studies on insect resistance, changes in protein content of seeds, etc.

D. Biological/social sciences integration

1. Issues relevant to WID: Training of women scientist has received considerable attention. Currently, four women are receiving training in our laboratory: M.Sc. degree students, Elisabeth Hendrastuti from Indonesia and Denise Smith from Wisconsin; Teresa Martinez from Dominican Republic; and Amy Loniello, an undergraduate student, are working on independent research projects. Ms. Glaucia de Figueiredo, who was supported by our Project, is finishing her M.Sc. degree at Universidade Federal de Vicosa, Brazil in the fall of 1989. Ms. Eunize Zambolim from Brazil will join our project in January 1990 for a years training in molecular virology. Ms. Aridia Figueroa from the Dominican Republic will spend two months at the University of Wisconsin in 1990.

E. Collaboration with other groups

Universidade Federal de Viçosa, Brazil: Dr. Acelino Alfenas visited us in Sept. 1989 to discuss the research of Ms. Figueiredo. Ms. Maria Christina del Pelosa, Chair of Plant Pathology Dept., started her Ph.D. research in our Deptartment. Two other scientists from this Univeristy will join our Department in January 1990, and one, Eunize Zambolim, will work with us.

CIAT, bean pathology program: Plans are being made for Ms. Mercedes Otoya, who is a specialist with Dr. M. A. Pastor-Corrales, to work in our laboratory in the summer of 1990.

University of Nebraska/University of Puerto Rico: We coordinate all activities in the Dominican Republic with Drs. Beaver, Coyne and Steadman. Dr. Beaver serves as a consultant on our PSTC/USAID grant.

Agracetus Corporation: This is a plant biotechnology company near Madison, WI. In September 1989, we started working with Drs. David Russell and Dennis McCabe on improved inoculation techniques for evaluation of infectivity of the cloned viral DNA by the use of electrical discharge particle acceleration. They are also cooperating with us on the construction of plasmid vectors for Agrobacterium-mediated infection and transformation, on the sequencing of bean dwarf mosaic virus, on construction of a coat protein transformation vector, and on development of techniques for transformation of beans.

II. FUNDING/FISCAL MANAGEMENT IN 1989

A. Audits

There was not a special audit requested for this project since all funds are processed through the University of Wisconsin-Madison Office for Research Administration. This project has been handled by Ms. B. Keenan and Mr. R. Lux.

B. Adequacy of funding to accomplish objectives from:

1. AID: CRSP invested about $54,000 in direct costs for this project for last year.

2. Dominican Republic: They contributed resources toward the screening of beans for resistance to BGMV in field plots.

3. US, nonCRSP funds (direct costs):

Salaries of researchers	$139,240
Supplies	$12,000
Travel	$2,500
TOTAL	$153,740

*****THUS, IT IS EVIDENT THAT FOR EVERY DOLLAR FROM CRSP THREE DOLLARS FROM OTHER SOURCES ARE SPENT.

C. Problems: Additional funds are needed to allow us to meet

our objectives for the third year (virus-derived resistance). These funds are necessary because future research will require increased expenditures for growth chambers (The experiments with transgenic plants and the genetic analysis of the infectious clones will require special containment facilities which we must rent.), supplies, service contract on equipment, rental charges for electron microscopy, publication of research, and some additional travel for students on the project. Also, salary increases occur for personnel. These costs could be covered by an addition of $8,000 in direct costs.

D. Adequancy of current management: Things are satisfactory.

E. Activities toward other funds: In direct costs, this project spent over $153,740 of nonCRSP funds last year. These funds came from the University of Wisconsin, The Graduate School Grant program of the University of Wisconsin, The World Bank, Gift Funds, Federal Hatch funds, and the Brazilian Government. We were also successful in obtaining a three year grant from the PSTC/USAID program, which started in July 1989. This grant will add a training component to our program. Also, Agracetus Corporation is not contributing funds directly to the University for our program, but their activities represent a significant contribution.

III. PLANNING

A. Review of 1989 work plan during 1989

Dr. Maxwell devoted his research leave to this project and the MO personnel were notified. No changes were made in the work plan.

B. Plans for 1990

1. Research in HC and US

Research in the Dominican Republic:

Continuation of the evaluation of the DNA hybridization probes: The squash blot method will be used to analyze samples from field beans and weeds collected in the major bean growing areas in March 1990. This will involve the use of a BGMV-DR derived probe, which we expect will detect isolates of the mechanically transmissible strain of BGMV.

Continuation of the field evaluation of bean germplasm: This will involve testing germplasm selected by Drs. J. Beaver and S. Beebe in discussions with Ing. F. Saladin. These beans will be evaluated for components of resistance to BGMV, which might be combined for better resistance. Ing. A. Sanchez will be studying at the Univeristy of Puerto Rico and someone else will have responsibility for this field work.

Research in the US:

Molecular characterization of bean-infecting geminiviruses: Two manuscripts will be prepared from the sequence analysis of the three BGMV isolates sequenced in FY89. The infectious, full-length clones of bean dwarf mosaic virus will be sequenced. The partial clones of bean calico mosaic virus will characterized by preparing restriction endonuclease site maps.

Isolate/strain specific DNA probes: From the sequence data, it will be possible to design isolate specific DNA probes for the detection of the nonmechanically transmissible strain of BGMV (the Brazilian isolate) and the mechanically transmissible strain of BGMV (the Guatemala and the Dominican Republic isolates). These probes will be evaluated on samples collected in the Dominican Republic and Brazil.

Virus-derived resistance schemes: Cloned DNAs from the BGMV isolates will be tested for infectivity by direct mechanical inoculation of beans and by Agrobacterium-mediated infection. Additionally, other approaches involving the particle accelerator of Agracetus Corporation will be tried. If these experiments are successful, then experiments to test various methods to obtain transgenic Nicotiana benthamiana and beans will be initiated. If difficulty is encountered in obtaining transgenic beans, then research can proceed with N. benthamiana, which can be readily transformed. It is necessary to develop a viral replication system with cloned viral DNAs so that individual viral genes can be evaluated as potential targets for development of virus-derived resistance in beans.

2. Expected changes from 1989: Several personnel changes will occur, Dr. Maxwell returned to his responsibilities as Departmental Chair on Sept. 1, 1989, Dr. J. Faria will return to Brazil in Dec. 1989. Ms. T. Martinez will return to the Dominican Republic in August 1990. Ms. Eunize Zambolim from Brazil will join the group in Jan. 1990 for one year. Short term training will be provided for Ms. Aridia Figueroa, Dominican Republic, and Ms. Mercedes Otoya, CIAT, Colombia.

IV. STATUS IN 1989

A. Appropriateness of activities to goals of Global Plan

The Global Plan addresses the need to improve the living conditions of small farm producers in LDC's and to increase the availability of low cost nutritious foodstuffs for rural and urban poor in LDC's. Our effort involves the collaboration of scientists from Brazil, Dominican Republic, CIAT, Agracetus Corporation, Univerisity of Puerto Rico, University of Nebraska, and University of Wisconsin. The focus of the project is on the molecular characterization of the geminiviral pathogens causing diseases of bean. From our sequence data, it is evident that two different strains of BGMV occur in Latin America: a mechanically transmissible

strain and a nonmechanically transmissible strain. This would indicate that breeding programs for resistance need to be continued for both strains. Since these strains differ considerably at the nucleotide sequence level, it is likely that breeding material resistant to one strain may not be resistant to the other strain. Our efforts will continue to define the extent of variability among bean-infecting geminiviruses and to develop diagnostic kits for the detection of different strains and viruses. These DNA probe diagnostic kits will then be used to determine the weed hosts, and they can be used to detect geminiviruses of other commerically important plants.

Major effort will be devoted to using recombinant DNA technology to develop beans resistant to bean golden mosaic virus. Some of the approaches which will be tested will be similar to the work with RNA plant viruses such as tobacco mosaic virus. If successful, these methods could dramatically increase the availability of adapted bean lines with resistance to a major constraint to production in Latin America.

Through the efforts of CRSP/USAID, PSTC/USAID, EMBRAPA-Brazil, CESDA-Dominican Republic, CIAT, and the University of Wisconsin-Madison, the largest research program devoted to basic studies on this bean golden mosaic viral complex has been organized and will lead to new understandings of this disease and improvements in disease control.

B. Balance between research and training

If the funds for Dr. Robert Gilbertson, an Assistant Scientist, are assigned to training, then 80% of the expenditures are for training. Our project has not had adequate funds to provide training for scientists from the Dominican Republic until we received a $5,000 addition for FY89. Ms. Teresa Martinez from the Dominican Republic joined our group in June and is now funded on the PSTC/USAID grant. Two M.Sc. students and one postgraduate from Brazil are receiving training on this project. These people are supported by nonCRSP funds.

C. Balance of US vs. overseas activities

Because of the highly technical nature of the research, which involves techniques associated with molecular biology, the major research effort has been at the University of Wisconsin. It will be possible for CIAT to do some of the future research on the characterization of the infectious clones. The field research for evaluation of germplasm is conducted in the Dominican Republic.

D. Level of cooperation between US and HC personnel

This was our first official year in the Dominican Republic, and efforts are being made to increase collaboration between US and Dominican Republic scientists. This project was developed with most

activities and funds committed to basic research. This next year will involve considerable field collections of samples in the Dominican Republic and a planning sessions is organized for Nov. 6, 1989 at the Bean Improvement Conference meeting. Scientists from the Dominican Republic, Univ. of Puerto Rico, Univ. of Nebraska, and Univ. of Wisconsin will participate in this planning session. Efforts have been made to increase training for Dominican Republic scientists and Freddy Saladin participated in the selection process. It is expected that joint publications will come from the research on the applications of DNA probes for detection of bean-infecting geminiviruses in beans and weeds in the Dominican Republic.

Cooperation between CRSP and CIAT scientists has been excellent. This is essential for the continuation of all aspects of this project. Drs. F. J. Morales, Silvio Hugo Orozco, and S. Beebe from CIAT met with Drs. J. Beaver, J. Steadman, and D. Maxwell and with Dominican Republic scientists in the Dominican Republic in March 1989. This provided an excellent opportunity for these scientists to interact. Additionally, Dr. Maxwell went to Puerto Rico in Dec. 1988 and to CIAT in July 1989. These trips are essential for the continued communication.

E. Relative contributions of collaborating institutions and individuals towards accomplishment of objectives

Because of the highly technical nature of the research for this year, which involved cloning and sequencing viral DNA, most of the research was completed at the University of Wisconsin. Germ plasm evaluation was conducted in the Dominican Republic. CIAT scientists contributed information on geminiviral isolates, seeds, and antisera to BGMV.

1. Contributions for 1989

USAID:

Direct costs (Funds for an Assistant Scientist, supplies, visiting scientist (3 mo), and travel)	$54,000
Indirect costs	$24,000
TOTAL	$78,000

University of Wisconsin (includes State Funds, Hatch Funds, Graduate School grant, gift funds, Brazilian government funds, and World Bank funds):

Salaries (Funds for PI and Co-PIs, research specialist, two graduate students, visiting scientist)	$139,240
Supplies	$12,000
Travel	$2,500
TOTAL	$153,740

Agracetus Corporation, Middleton, WI

Their most important contribution has been cooperating scientists, equipment, and technology, which is not available at the University of Wisconsin-Madison.

CIAT:

(approximate values)	
Salaries	$20,000
Supplies	$3,000
Travel	$2,600
TOTAL	$25,600

Dominican Republic

(approximate values)	
Salaries	$2,000

2. Other contributions by HC

Most of the research in the Dominican Republic is field research and experimental land and equipment is provided by HC.

3. Other contributions by US instutituions

Major contributions include 500 SQ.FT. of laboratory space, secretarial staff, and the use of over $200,000 of equipment purchased on nonCRSP funds.

4. Relate contributions from AID, US institution, and HC institution to levels of activities:

Expenditures for this project are mainly at the University of Wisconsin-Madison, and the scientists there have made the major contributions toward the objectives. Next year, there will be an increase in activity in the Dominican Republic by US scientists as greater effort is placed on field testing of the DNA probes for geminiviruses. This will also involve additional effort by the scientists in the Dominican Republic.

F. Interest and support from the USAID mission in Dominican Republic

In March, the CRSP scientists visited the USAID mission and have always received full support from AID personnel. There has been an excellent working relationship established by the University of Nebraska and Univeristy of Puerto Rico scientists and the USAID mission. Additionally, on this trip our group met with the Secretary for Agriculture about the increasing whitefly problem. This was related to the serious epidemic of bean golden mosaic virus in San Juan area.

G. Cost effectiveness, especially regarding level of activity vs. USAID funding

A conservative estimate is that for each dollar CRSP/USAID contributes toward this project there is at least THREE additional dollars spent from other sources. The molecular characterization of the bean-infecting geminiviruses is very expensive research. One person requires about $5,000 in supplies per year to maintain a full-time research effort in molecular biology. This year there were 4.5 person years devoted to this project at the University of Wisconsin, and many of these scientists would easily spend 65 hours per week at the research bench. (This is because the research went very well and everyone wanted to make contributions. It was a great year for our GEMINIVIRUS TEAM!!!)

H. Evidence of institutionalization in Dominican Republic and in US

1. Faculty recognition for internatinal activities

The Agricultural Deans have been fully supportive of the PI's activities and allowed Dr. Maxwell to complete his research leave on this project at the University of Wisconsin.

2. Integration of commodity research programs with CRSP projects

Dr. Maxwell is in the process of preparing a grant for Hatch and College research support in the area of virus-derived resistance schemes for viruses of bean.

3. Internal project management and institutinal management support

This has been excellent at the University of Wisconsin-Madison. The CRSP projects in the Dominican Republic are an essential component of the national bean program and receive administrative encouragement.

4. Student/professor interactions

Dr. Maxwell in cooperation with Dr. Gilbertson has responsibility for the two M.Sc. students on this project and the one visiting scientist from the Dominican Republic. This year Dr. Maxwell was on a research leave and had considerable time to devote to the direct training of these individuals. Because of his Departmental Chair position for FY90, more of the bench training will be provided by Dr. Gilbertson, Assistant Scientist, and Mr. Steve Hanson, Research Specialist. They are both competent scientists and good teachers. Dr. Maxwell reviews all research data and provides suggestions for future experiments. He also organizes a literature review group for these individuals.

I. Other comments

The PI has had excellent encouragment from his University Administrators, faculty of Plant Pathology, MO personnel and scientists from CIAT. However, the most important factor has been the hard working and team spirit of the research group in Madison. Without all of this support, this project would not be making its current level of contributions.

V. PUBLICATIONS IN 1989

Presentations:

1. Gilbertson, R. L., J. C. Faria, E. Hiebert, and D. P. Maxwell. 1988. Properties and cytopathology of bean golden mosaic from Brazil. Phytopathology 78:1567-1568. Abstract of oral presentation given at the Annual Meeting of the American Phytopathological Society, San Diego CA, Nov. 1988.

2. Gilbertson, R. L., J. C. Faria, F. Morales, S. A. Leong, D. P. Maxwell, and P. G. Ahlquist. 1988. Molecular characterization of geminiviruses causing bean golden mosaic. Phytopathology 78:1568. Abstract of the oral paper given at the Annual Meeting of the American Phytopathological Society, San Diego CA, Nov. 1988.

3. Maxwell, D. P. 1988. Overview of the CRSP project on the molecular approaches to control of bean golden mosaic virus. Annual meeting of W150. Mayagez, Puerto Rico, Dec. 1988.

4. Faria, J. C. 1989. Characterization of bean-infecting geminiviruses. Brazilian conference on bean golden mosaic virus, San Paulo, Brazil, February 1989.

5. Gilbertson, R. L. 1989. Molecular characterization of bean golden mosaic virus from Brazil and application of DNA probes for detection of geminiviruses. Department of Plant Pathology, University of Wisconsisn-Madison, July 1989.

6. Maxwell, D. P. 1989. Application of DNA probes for the detection of plant pathogens. Invited symposium presentation at the joint meeting of the Caribbean Division of the American Phytopathology Society, the Association of Colombian Plant Pathologists, and the Latin American Plant Pathologists, Cali, Colombia, July 1989.

7. Gilbertson, R. L. 1989. Geminiviruses as vectors. Invited symposium talk at the American Phytopathological Society meetings, Richmond, VA, August 1989.

8. Gilbertson, R. L., J. C. Faria, E. Hendrastuti, and D. P. Maxwell. 1989. Detection of bean geminiviruses by nucleic acid squash and dot blot hybridization methods. Workshop at the Annual Meeting of the American Phytopathological Society, Richmond, VA, August 1989.

Publications:

1. de Faria, J. C. 1988. Inoculação seqüencial para avaliação da resistência do feijoeiro (Phaseolus vulgaris) a quatro doenças (Sequential inoculation for evaluating resistance of common beans (Phaseolus vulgaris) to four diseases.) Fitopathol. Bras. 13:269-273.

2. de Faria, J. C., and P. E. de Melo. 1989. Inoculação do feijoeiro com Xanthomonas campestris pv. phaseoli em condições de campo (Inoculation of common beans with Xanthomonas campestris pv. phaseoli under field conditions). Pesq. agropec. bras., Brasília 24:987-990

3. Gilbertson, R. L., D. J. Hagedorn, D. P. Maxwell, and S. A. Leong. 1989. Development and application of DNA probes for detection of bacteria causing common bacterial blight of beans. Phytopathology 79:518-525.

4. Gilbertson, R. L., D. J. Hagedorn, D. P. Maxwell, and S. A. Leong. 1989. Detection of Xanthomonas campestris pv. phaseoli using a plasmid DNA probe. Ann. Reptr. of the Bean Improvement Cooperative 32:94-95.

5. Gilbertson, R. L., J. C. Faria, S. A. Leong, P. G. Ahlquist, and D. P. Maxwell. 1989. Molecular characterization of a Brazilian isolate of bean golden mosaic virus. Ann. Reptr. of the Bean Improvement Cooperative 32:92-93.

Journal articles submitted for review but not yet accepted:

1. Gilbertson, R. L., R. E. Rand, and D. J. Hagedorn. 1990. Survival of Xanthomonas campestris pv. phaseoli and pectolytic strains of X. campestris in bean debris. Plant Disease

2. Gilbertson, R. L., J. C. Faria, S. A. Leong, F. Morales, and D. P. Maxwell. 1990. Properties of a Brazilian isolate of bean golden mosaic virus and detection of bean-infecting geminiviruses by nucleic acid hybridization. Plant Disease

Other articles accepted for publication:

1. Haber, S., D. P. Maxwell, and R. L. Gilbertson. 1990. Bean golden mosaic. Compendium of Bean Diseases, APS Press.

Journal articles in preparation:

1. Faria, J. C., R. L. Gilbertson, S. F. Hanson, P. G. Ahlquist, F. J. Morales, and D. P. Maxwell. 1990. Sequence of full-length, cloned-DNAs of mechanically transmissible isolates of bean golden mosaic virus from Guatemala and Dominican Republic. Virology

2. Gilbertson, R. L., J. C. Faria, S. A. Leong, D. P. Maxwell, and P. G. Ahlquist. 1990. Sequence of full-length, cloned DNAs of a Brazilian isolate of bean golden mosaic virus. Virology

3. Gilbertson, R. L., J. C. Faria, S. F. Hanson, P. G. Ahlquist, D. Russell, and D. P. Maxwell. 1990. Electrical particle accelerator facilitates evaluation of geminiviral cloned DNA. Molecular Plant Microbe Interaction

APPENDIX

Fig. 1. Complete nucleotide sequence of bean golden mosaic virus isolate from Guatemala. A, DNA A component; B, DNA B component. The common region (CR) is underlined and extends from nucleotide 1 through 204. Bold letters in the CR indicate a direct repeat (18 through 32 and 40 through 54) and a hairpin (stem loop) structure (149 through 184). Start (ATG) and stop (TAA, TAG) codons are double underlined. Arrows by the ORFs (genes) indicate the direction of transcription, e.g. < AL1 ORF is transcribed from the negative strand and thus is read from the 5' to the 3' end of the ORF. AR1 represents the coat protein gene. In tomato golden mosaic virus, the AL1 gene product is essential for replication of viral DNA.

```
   1 TGGCATATTTGTAAATATGCGAGTGTCTCCAAATGAGTTTGCGAGTGTCTCCAATTGAGGCTCCTCAAACTCTCGCTATGCAATTGGAGACTGGAGTACA
                < AL1
 101 ATATATACTAGTACCCTCAATCTCGTGAATTATCAGATTCACACACGTGGCGGCCATCCGATATAATATTACCGGATGGCCGCCCGCGCCCCTTTATTTC

 201 CGTACGCCTCCACGTGTCGCTTTAATTTAAATTAAAGATGTCTATTTTTGACTGACCAATGCTTTTGCATGTGAGAAGCTTAGATATTTGTGTAAAACTT

 301 GGTGACTAAGTTTTACCCTCGTTTATAAATTTAAATTAAATGTATGCCCATTTTACGTGTAAGTCCAGAATGCCTAAGCGTGATGCGCCGTGGCGTCATA
                                                 AR1 >
 401 TGGCGGGAACCTCCAAGGTTTCCCGTTCTGGCAATTATTCTCCGGGTGGTGGAATGGGCTCAAAATCCAACAAGGCCAATGCATGGGTCAACAGGCCCAT

 501 GTATAGAAAGCCAAGGATATATCGGATGTACAGAAGCCCAGACGTGCCCAAGGGATGTGAAGGACCTTGCAAGGTCCAATCATATGAACAACGCCATGAT

 601 ATATCTCATGTTGGTAAGGTTATGTGTATATCCGATGTCACACGTGGGAATGGTATTACCCACCGTGTTGGTAAACGTTTTTGTGTGAAGTCTGTGTACA

 701 TTTTAGGGAAGATATGGATGGATGAAAACATCAAGCTTAAGAACCATACGAATAGTGTTATTTTTTGGTTGGTCCGTGACCGTAGACCATATGGAACCCC

 801 TATGGATTTTGGACAAGTTTTTAATATGTTTGACAATGAACCCAGTACTGCTACGGTGAAGAACGATCTTCGTGATCGTTATCAAGTTATGCATAGGTTC

 901 AATGCAAAGGTGACTGGTGGTCAATATGCAAGCAACGAACAAGCCTTGGTAAGGCGATTTTGGAAGGTGAACAACCATGTCGTCTATAACCACCAGGAAG

1001 CAGGAAAGTATGAGAATCATACGGAGAATGCGTTATTGTTGTATATGGCATGTACACATGCCTCTAACCCTGTATATGCGACATTGAAAATTCGGATCTA

1101 TTTCTATGATTCGATAACCAATTAATAAAATTTATATTTTATTTCATGATTCTCAAGCACAGTATTTACATATAATTTGTCTGTTGCGAACTGAACAGCT
                  AR1 >      < AL3
1201 CGAATTACATTGTTTATTCCTATTACGCCTAACCTATGTAAATACAATAAAACCAAATGTCTAAATCTATTTAAATATGTCGTCCCAGAAGCTTGAATCG
                                                          < AL2
1301 ATGTCGTCCAGACTTGGAAGTTCAGGAATGCTTTGTGGAGATCCAGTGCTTTCCTGAGGTTGTGATTGAACCTGATTTGGATGTGGTATATCCTTGTCCG

1401 TGTGTATGCTGGATCTTCCACCCGCATTATCTTGAAATAAAGGGGATTTGGTACCTCCCAAATAAAAACGGAATTCTCTGCCTGATGCGCAGTGATGTTC

1501 TCCCCTGTGCGTGAATCCATGATCTGCGCACTTGATATGGTAAAATATGGAACAGCCGCAGTTCAAGTCAATGCGTCGTCGACGAATGGCTCTACGTTTG
           < AL3                                                                                  < AL1
1601 GCAATCCTGTGTTGTGCTTTGATAGAGGGGGGCTGTGAGGGTGACGAAGATCGCATTATGAATAGTCCAGTTGTGTAAAGCTCGGTTTTCTTCTTTGTCG

1701 AGGAAGTCTTTATAACTGGAACCCTCACCTGGATTGCACAACACGATTGATGGTATTCCTCCTTTAATTTGAACCGGCTTTCCATATTTACAGTTAGATT
                                                                  < AL2
1801 GCCAGTCCTTTTGTGCCCCAATTAGTTCTTTCCAGTGCTTTAACTTCAAATAATTGGGGCTTATGTCATCAATGACGTTGTATTCCACTGCGTTGGAATA

1901 GACACGTGAATTAAAGTCCAAATGACCGCTCAAATAATTATGTGGTCCTAATGCACGAGCCCACATTGTCTTTCCGGTTCGTGAATCACCTTCGACGATG

2001 ATACTAATAGGTCTTTCCGGCCGCGCAGCGGAACCCCTTCCGAAATAGTCGTCAACCCATTCTTGCATAACAACCGGAACATTGATGAATGATGACAACG

2101 GAAATGGAGGAACCCATGGTTCCGGCACTTTGACGAAGATCCGTTCGAGATTAGAACGGATGTTGTGATGTTGAAGGACGTAATCTTTCGGTTGTTCTTC

2201 CTTCAATATTGTCAAGGCAGATTCAATTGAATCTGCGTTTAATGCCTTTGCATATGAGTCGTTGGCAGACTGCTGACCTCCTCTTGCAGATCTGCCGTCG

2301 ACTTGGAATTGTCCCCATTCGATTGTGACTCCATCTTTGTCGATGTATGCCTTGACGTCTGAACTTGATTTAGCTCCCTGAATGTTCGGATGGAAAGGTG

2401 CTGACCTGGTTGAGGATACCAGGTCGAACAATCTTTTATTTGTGCAGACGAATTTACCTTCGAATTGAATAAGCGCATGAAGATGAGGTTCACCATTCTC

2501 GTGACGTTCCTCACAGACTTTGATGAATTTTTTATTCGTGGCTGTATGAATCTTCTGAAGTTGCGAAAGAACTTCTTCTTTCGGTATAGGGCAACGAGGA

2601 TAAGTGAGGAAATAGTTTTTCGACTGAACTCTAAATCTTTGAGGTGG 2647
                                                  < AL1
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Fig. 1A

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   1 TGGCATATTTGTAAATATGCGAGTGTCTCCAAATGAGTTTGCGAGTGTCTCCAATTGAGGCTCCTCAAACTCTCGCTATGCAATTGGAGACTGGAGTACA
 101 ATATATACTAGTACCCTCAATCTCGTGAATTATCAGATTCACACACGTGGCGGCCATCCGATATAATATTACCGGATGGCCGCGCGCCCCCCCTCTTTCC
 201 GTACACCGCATTTCGATACCAGAAATGCCCTTCCCACGCTTTATACACGGTATTCTATACCGTTGGATAAAGCTTATCGCACTCTATCTTGAATTTTGAA
 301 TTATTGGCTTTGCCTTTGTGTTTAGATATTTATGGATATGTCTGACACATATCTGATTTTGTACGACTCGACCAATCAAAATTCAATATAGAGGTCTAGT
 401 TAATTCGTGTGTTAATATGTGATTTGTATAAATAAGCAGTTTTGATAAAGGAAAACGATCACCGTTTTGTCTATGTTTTTATTTTAGTCACACGAATTAA
 501 TAATGTATGCGTCTAAGTATAAACGTGGTTCGTCTAAGTATCAACGTCGAGGATATTCACGTACTCAAGGTTGTCGACAAACGGCAAGTGTTAAGCGTTA
                BR1 >
 601 TGATGGTAATCGTCGACAACATCTATCCAGTAAGTCTAATGAAGATCCTAAATTGTTGGTGCAGTGTATACGTGAAAATCAGTTTGGTCCTGATTTTGTT
 701 ATGTCTCATAATACTGCCATATCCACGTTTATTAATTATCCCCAATTGGGTAAGATTGAGCCTAATCGATGTAGGTCGTATATCAAGTTGAAACGATTGC
 801 GTTTCAAAGGGACGGTTAAAATTGAACGTATACATACGGATGTGAACATGGATGGATTATCTCCTAAGATTGAAGGCGTATTTAGTATTGTTATTGTGGT
 901 TGATCGGAAACCACACCTAAGTCCAAGTGGATGCCTTCACACATTTGATGAACTATTTGGAGCAAGAATTCATAGCCATGGAAATCTAGCTGTGGTGCCT
1001 TCTTTGAAAGACCGATTTTACATACGCCACGTTTTGAAACGTGTTTTATCGGTAGATAAAGACACAACCATGATAGACGTTGAGGGTTCCACTTTGTTGT
1101 CTAATAAGCGTTATAATATGTGGTCTACATTTAATGATTTTGATCATGATTCATGTAATGGTGCATATGCTAATATTGCTAAGAACGCTTTATTAGCGTA
1201 TTATTGTTGGATGTCCGATATTACGTCTAAGGCATCAACATTTGTATCATTTGATCTTGATTATGTTGGATAAATTTTAATGTTCAGATAATTGAATATG
                                                                  BR1 >.
1301 TATATTTATTTCAAAGACTTTGGTTGAGAAGGAGTACAATTATTGTTGATACAGTCATGTACCGTTGATCGCACAATTTCGTTTAATTGTGCAATTGACA
               < BL1
1401 ATGTTATGCTTGATTGGGCTCTCTGAGCTCCAACAATTGACGCAGAATCTCCCGGGTCCAAAACACTGGTTCCCAGTCTATTTAAATCCTTGTATGGGTG
1501 TATTGCGCTCTCTATGTCCGAGTCCGCATCTACATGAGACATACCGATTGTACTTCTTGAGGCCCAAGACTCTCCTGGTTTTAATTCGATTGGGCCTTGT
1601 AGACCATACCTTGAGGATGATGCGGACCGGATCAATTTCCTTTCCCACCTCCCATAGTCGACGTGGGAGAAGTCGACGTCTTTATTGGAAAACTGTTTTG
1701 ATAATATTTTTACTGTTGGTGCCCGAAAGGGGATATCTACTGAATGTTTAGCCGTTGATAATTTCAGTTTTCCCTTGAATTTTGCGAAATGTGTCCTTTG
1801 ATGAACGTTTGTGTCTGAAACTCTATAATAGAGTTTCCATGGTATTGGGTCTTTCAATGAGAAAAATGACGAGGAAAAGTAGTGGAGATCTATGTTGCAC
1901 CTTATGGGAAATGTCCATGAAGCTTGTAATGATTCGTTGTCTGTCATTCTTTTGTCATGAATCTCCACTACGACCGATCCTGTTGCGTTTATAGGCACCT
2001 GTTGTCTATATTCTATGACGCAATGATCGATCTTCATACAACTGCGACTGAATCTTGCAGATAATTGAGAAGCTGTAGAAGGAAATTGAAGGATTATCTC
2101 AGTTAGATCATGAGACAATTGATATTCGTCTCTGTGTGACTCTACATAATTAAATGCATTTGGAGGATTTGCTAACTGAGAATCCATTTAAGAATAAATG
                                                                  < BL1
2201 GCCGCAGCGGAAGCGCAAAGATCGATAAGATCGATTTTAACTAGCGGAATGCCTAGACAGAGAAAAACAGTTTGGTCACACTCTCATACAACTGATAGAC
2301 CGACCAATATTATGGAATAGTTTATATTGATATAGATAAGGAATGTGTAAGCTTATATAGGCAGTTGTATGCCTTTGGATCCGCTTTTGAAAAAGAACAT
2401 ATTATTTAATTAATATGTTAGTGTGTTTTTTTTGAACATGATATATATATATCAGATATATATATTTTGATGAACTTAATATATTTATGGTATATTAAATAT
2501 TAAATGTCAGTTGGATGTTATTATTGAATTATATATTCTGAGCTTGAATTATTTAAGGACTAATAACCAAAACGATATCGTTTTGAAAGTATGTTG 2596
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Fig. 1B

Fig. 2. Complete nucleotide sequence of bean golden mosaic virus isolate from Dominican Republic. A, DNA A component; B, DNA B component. The common region (CR) is underlined and extends from nucleotide 1 through 208. Bold letters in the CR indicate a direct repeat (22 through 32 and 45 through 55) and a hairpin (stem loop) structure (154 through 187). Start (ATG) and stop (TAA, TAG) codons are double underlined. Arrows by the ORFs (genes) indicate the direction of transcription, e.g. < AL1 ORF is transcribed from the negative strand and thus is read from the 5' to the 3' end of the ORF. AR1 represents the coat protein gene. In tomato golden mosaic virus, the AL1 gene product is essential for replication of viral DNA.

```
   1 GTGATGGCATATTTGTAAATATGCGAGTGTCTTCCGAATGGGTTTGCGAGTGTCTCCAATTGAGGCTCCTCAAACTCTCGCATTCAATTGGAGACTGGAG
     < AL1
 101 TACAATATATACTAGAACCCTCAATCTCGTGAATTACGAGATTCACACACGTGGCGGCCATCCGATATAATATTACCGGATGGCCGCGCGCCCCCTTTA
 201 TTTCCGTACTTCTCCACGTGGCGCTTTAATTTAAATTAAAGATGTCTATTTTTGACTGACCAATGCTTTTGCATGTGAGGAGCTTAGTTATTTGTGTAAA
 301 ACTTGGCGACTAAGTTTTACCCTCGTTTATAAATTTAAATTAAATGCATGCCCATTCCACGTGTAAGTCCAGAATGCCTAAGCGTGATGCGCCTTGGCGT
                                                 AR1 >
 401 AATAATGCGGGAACCTACAAGGTTTCCCGTTCTGGCAATTATTCTCCAGGCGGTGGAATGGGCTCAAAATCCAACAAGGCCAATGCATGGGTTAACAGGC
 501 CCATGTATAGAAAGCCAAGGATATATCGGATGTACAGAAGCCCAGACGTGCCCAAGGGATGTGAAGGACCTTGCAAGGTCCAATCATATGAACAACGCCA
 601 TGATATATCTCATGTTGGTAAGGTTATGTGTATATCCGATATCACACGTGGGAATGGTATTACCCACCGTGTTGGTAAACGTTTTTGTGTGAAGTCTGTG
 701 TACATTTTAGGGAAGATATGGGAAAATATCAAGCTTAAGAACCATACTAATAGTGTTATATTTTGGTTGGTCCGTGACCGTAGACCATATGGAACCCCTA
 801 TGGATTTTGGTCAAGTTTTTAATATGTTTGACAATGAACCCAGTACTGCTACGGTCAAGAACGATCTTCGTGATCGTTATCAAGTTATGCATAGGTTCAA
 901 TGCAAAGGTGACTGGTGGTCAATATGCAAGCAACGAGCAAGCCTTGGTAAGGCGATTTTGGAAGGTGAACAACCATGTCGTCTATAACCACCAGGAAGCA
1001 GGAAAATACGAGAATCATACGGAGAATGCGTTATTGTTATATATGGCATGTACACATGCCTCTAATCCTGTATATGCGACATTGAAAATTCGGATCTATT
1101 TCTATGATTCGATAACCAATTAATAAAATTTATATTTTATTTCATGATTCTCAAGTACAGTATTTACATATGATTTGTCTGTTGCGAACTGAACAGCTCG
          AR1 >      < AL3
1201 ATTGACATTGTTTAATCCTATTACGCCTAACCTATGTAAATACAATAAAACCAAATGTCTAAATCTATTTAAATATGTCGTCCCAGAAGCTTGAATCGAT
                                                          < AL2
1301 GTCGTCCAGACTTGGAAGTTCAGGAATGCTTTGTGGAGATCCAGTGCTTTCCTGAGGTTGTGATTGAACCTGACTTGGATGTGGTATATCCTTGTCCGTG
1401 TGTATGCTGGATCTTCCACCCGCATGATCTTGAAATAAAGGGGATTTGGTACCTCCCAAATAAAAACGGAATTCTCTGCCTGATGCGCAGTGATGTTCTC
1501 CCCTGTGCGTGAATCCATGATCTGCGCACTTGATATGGTAAAATATGGAACAGCCGCAGTTCAGGTCAATGCGTCGTCGACGAATGGCTTTACGTTTGGC
          < AL3                                                                          < AL1
1601 AATCCTGTGCTGTGCTTTGATAGAGGGGGGCTGTGAGGGTGACGAAGATCGCATTATGAATAGTCCAGTTGTGTAAAGCTCGGTTTTCTTCTTTGTCGAG
1701 GAAGTCTTTATAACTGGAACCCTCACCTGGATTGCACAGCACGATTGATGGTATTCCTCCTTTAATTTGAACCGGCTTTCCATATTTACAGTTTGATTGC
                                                                  < AL2
1801 CAGTCCTTTTGTGCCCCAATTAGTTCTTTCCAGTGCTTTAACTTCAAATAATTGGGGCTTATGTCATCAATGACGTTGTATTCCACCTCGTTGGAATAGA
1901 CACGTGAATTAAAGTCCAAGTGACCGCTCAAATAATTATGTGGTCCTAATGCACGAGCCCACATTGTTTTTCCTGTTCGTGAATCACCTTCGACGATGAT
2001 ACTAATAGGTCTCTCCGGCCGCGCAGCGGAACCCCTTCCGAAATAGTCGTCAACCCATTCTTGCATAACAACCGGAACATTGATGAATGATGACAACGGA
2101 AATGGAGGAACCCATGGTTCCGGCACTTTGAAGAAGATCCGTTCGAGATTAGAACGGATGTTGTGATTTTGAAGGACGTAATCTTTCGGTTGTTCTTCCT
2201 TTAATATCGTCAAGGCAGATTCAATTGAATCTGCGTTTAATGCCTTTGCATATGAGTCGTTGGCAGTCTGCTGACCCCCTCTTGCAGATCTGCCGTCGAC
2301 TTGAAATTGTCCCCATTCGATTGTGTCTCCATCTTTGTCGATGTATGCTTTGACGTCTGAACTTGATTTAGCTCCCTGAATGTTCGGATGGAAATGTGCT
2401 GACCTGGTTGAGGATACCAGGTCGAACAATCTTTTATTTGTGCAGACGAATTTACCTTCGAACTGAATAAGCGCATGAAGATGGGGTTCACCATTCTCGT
2501 GACGTTCCTCACAGACTTTGATGAATTTTTTATTCGTCGCTGTATGAATCTTCTGAAGTTGCGAAAGAGCTTCTTCTTTCGGTATAGAGCAATGAGGATA
2601 AGTGAGGAAATAGTTTTTGGACTGAACTCTAAATCTATGAG 2641
                                  < AL1
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Fig. 2A

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   1 GTGATGGCAGTTTTGTAATAAGAAGGTGTCTCCATTCGAGTTCTTGAGTGTCTCCATTTGATCCCCCTCAAAACTCGCTCATGCAATTGGAGACTGGAGT
 101 ACAATATATACTAGAACCCTCAATCTCGTGAATTACGAGATTCACACACGTGGCGGCCATCCGATATAATATTACCGGATGGCCGCGCGCTCCCTCTTAT
 201 ATCCGTACGCCGCATTTCGATACCAGAAATGCCCTTCCCACGCTTTATACACGGTATTCTATACCGTTGGATAAAGCCTATCGCACTCTATCTTTTGAAT
 301 TTTGAATTATTGGCTTTGCCCTTGTGTCTAGTTATTTATGGATATGTCTGACACATCTCTGATATTGTACGACTCGACCAATCAAAATTCAAAATAGAAG
 401 TTTAGTTAAATCGTGTGTTAATATGTGATTCATATAAATAAGCATGTTTGATAAAGAAAAAACGTACCGTTTTTAATGACTATGATTTTATGTTAGCCAC
 501 ACGATTTAATTATGTATACGTCTAAGTATAAACGTGGTTCGTCTAACTATCAACGACGAGGTTATTCACGTAGCCAAGGTTTTCGACGAACGTCAATTGT
                  BR1 >
 601 TAAACGACATGATGGTAAGCGTCGACAACATCAATCCAGTAAGTCTAATGAAGATCCTAAATTGTTGGTGCAATGTATACGTGAAAATCAATTTGGTCCT
 701 GATTTTGTTATGGCTCATAATACTGCCATATCCACGTTTATTAATTATCCCCAATTGGGTAAGATCGAACCTAATCGATGTAGGTCGTATATGAAGTTGA
 801 AACGCTTGCGTTTCAAAGGGACGGTTAAAATTGAACGTGTACACTCTGATGTGAACATGGATGGATTATCTCCTAAGATTGAAGGCGTATTTAGTATTGT
 901 TATTGTGGTTGATCGGAAACCACATTTAAATCCCAGTGGATGTCTTCATACATTTGATGAACTATTTGGAGCAAGGATTCATAGCCATGGGAATCTTGCT
1001 GTGATGCCTTCTTTGAAAGACCGATTTTACATACGACACGTGTTGAAGCGTGTTTTATCGGTAGATAAAGACACGACTATGATAGACGTTGAGGGTTCCA
1101 CTTTGTTGTCTAATAAGCGTTATAATATGTGGTCTACGTTTAATGATTTTGATCATGACTCATGTAATGGTGCATATGCTAATATTGCTAAGAACGCTTT
1201 ATTAGTGTATTATTGTTGGATGTCGGATATTATGTCTAAGGCATCGACATTTGTGTCATTTGATCTTGATTATGTTGGATAAACGTCAATGTATAAGATA
                                                                  BR1 >
1301 AATAAATATGTTTATTTATTTCAAAGATTTTGGTTGAGAAGGAGTACAATTGTTGTTGATACATTCATGTACCGTTGATCGCACAATTTCGTTTAATTGT
                  < BL1
1401 GCAATTGACAATGTTATGTTTGATTGGGCTCTCTGAGCCCCAATAATTGACGCTGAATCTCCCGGGTCCAAAACACTGGTTCCCAGTCTGTTTAAATCCT
1501 TGTATGGGTGTATCGCGCTCTCTAAGTCCGAGTCCGCATCTACATGAGACATACCGATTGTACTTCTTGAGGCCCAAGACTCTCCTGGTTTTAATTCGAT
1601 TGGGCCTTGTAGACCATACCTTGATAATGATGCGGACCGGATCAATTTCCTTTCCCACCTCCCATAGTCGACGTGGGAGAAGTCGACGTCCTTATTGGAA
1701 AACTGCTTTGATAATATTTTAACTGTTGGTGCCCGGAAGGGGATATCTACTGAATGTTTAGCCGTTGATAATTTCAGTTTTCCTTTGAATTTTGCGAAAT
1801 GTGTCCTTTGATGAACGTTGGTGTCTGAAACTCTATAATAGAGTTTCCATGGTATTGGGTCTTTCAACGAGAAAAATGACGATGAAAAGTAGTGGAGATC
1901 TATGTTGCATCTAATGGGAAATGTCCATGAAGCTTGTAATGATTCGTTGTCTGTCATCCTCTTGTCATGAATCTCCACTACAACTGATCCTGTTGCGTTA
2001 ATAGGCACCTGTTGCCTATATTCTATGACGCAATGATCGATCTTCATACAGCTGCGACTGAATCTCGCAGATAATTGAGAAGCTGTGGAAGGAAATTGAA
2101 GGATTATCTCAGTTAGATCATGAGACAATTGATATTCGTCTCTGTGTGACTCTACATAATTAAATGCATTTGGAGGATTTGCTAACTGAGAATCCATTTA
                                                                  < BL1
2201 AGAAGAAATGGCCGCGCAGCTGAAGCGCAAAGATAGAGAAGCTCGACTCTAACTAGCGGAATGCTAAGAGAGAGAAACTCATTTGGTGACACTCGCATAC
2301 AACTGATAAACTGAACAACCAAGAGATTATGAAATAGTTTATATTGATATAGACAAGCATTGTGTATGCTTATATAGGCAGTTGTATGCCTTTATATCGC
2401 CTTTTGAAAAAGAACATATTATTTAGTTAATATGTTAATGTGTTTTATTTGAACATGATATATATATCGGATATATATATTTGAAGAATTTAATATATTT
2501 ATGGTATATTAAATGTTATATGACAGTTGAATGTTAATATCAAATTATTAATTTGGTTTATGAATTATTTGATGATTAATAAGCAAAACGATATCGTTTT
2601 GAGTCTAT 2608
```

Fig. 2B

Fig. 3. Comparison of the 3' end of the DNA B components, which is the most divergent domain, of bean golden mosaic virus (BGMV) isolates and tomato golden mosaic virus (MTGB).

```
        1                                                                                                   100
MTGB... ..................... .AtattatGaagAcCtGccT CGCAgAGgcAGcgtttcAct GaaaAtaaTAAgccaagaGa AtAGCTAtGAAAttcAAgCc
BGMVBBZ tatcaacTgaATaTAAGAAT gAATGGCCGCGCAGCGcAcT CGgttcGggAG....tT... ............TAtgatGc AgAGactAtAAAagagctgT
BGMVBPR ............TTAAGAAT AAATGGCCGCGCAGCGGAAT tGCtcAGAgAG....aTAaG GTCcACTC.AACTAa..tGG AtAGCTAAcAAAtAagAACT
BGMVBDR ............TTAAGAAg AAATGGCCGCGCAGCtGAAg CGCAAAGAtAG....agAaG cTCgACTCTAACTAg..cGG AatGCTAAGAgAgAgAAACT
BGMVBGA .......TccATTTAAGAAT AAATGGC..CGCAGCGGAAg CGCAAAGAtcGataagatcG aTt....tTAACTAg..cGG AatGCctAGAcAgAgAAAaa

        101                                                                                                 200
MTGB... tTGcTgccgGcAgCAacgAa ctgAaaatattAgctcAagA gAAtagctA..TGAAATtcA accctcgctGcagGcaAtGa ggaactgAAatactaAcAGA
BGMVBBZ tT.TcTtGcttAAgAacaAT ttgACaGAaAggaaG....g aA..GAGAAgtTGttATAtA TgATAaTGgG........cg tTccccaAtGtTTAaATAGA
BGMVBPR aT.aTTtGTGAAACACtCAT ACAACTGATAtACtG....A CAAgGAGAATgTGAAATAGA TTATAgTGAGATAGAgAAGc ATTGTtgtAaCTTATATAGA
BGMVBDR ca.TTTgGTGAcACtCgCAT ACAACTGATAaACtGAACAA CcAaGAGAtTATGAAATAGt TTATATTGAtATAGAcAAGc ATTGTgtAtGCTTATATAGg
BGMVBGA caGTTTgGTcAcACtCtCAT ACAACTGATAgACcGAcCA. .....AtAtTATGgAATAGt TTATATTGAtATAGAtAAGg AaTGTgtAAGCTTATATAGg

        201                                                                                                 300
MTGB... aAaTaaTcgttCaggAAAaa T........AAAAGAAgATA TTAagcctaatAatTtagtA (12nt)aaAcTTGtcaAgag ataATtatcatatgtcggcg
BGMVBBZ CAagTtTATtttcgTAcAaC TCtgT.....AAAGtAatcA aTgaTTAtgTA......... TGTtcaagtTggaccaAgtA atcATtTtTTActTtatTAa
BGMVBPR CAGTTGTATGCgaTTAAAgC TCtTTTTGAAAAAGAACATA TTATTTAagTA......... ........ATgatcAtgaaA TATATATcTTAtATATATAT
BGMVBDR CAGTTGTATGCCtTTAtAtC gCcTTTTGAAAAAGAACATA TTATTTAgtTAATATGTTAA TGTGttTTATTTGaAcATgA TATATATATcgGATATATAT
BGMVBGA CAGTTGTATGCCtTTggAtC cgcTTTTGAAAAAGAACATA TTATTTAatTAATATGTTAg TGTGttTTtTTTGaAcATgA TATATATATcAGATATATAT

        301                                                                                                 400
MTGB... tagaacTGgAaaTgggTacA TaTATAtAAaAccctAat.. ..................... ..................... .....................
BGMVBBZ ATggTGTttATTTAgAgTAA acTtatGTgTATctgtgtgT ATAaGTttGTgttAaag... ..................... .....................
BGMVBPR ATTgTaTGaATTggAcaTgA TTatTGtTATAcTAAATgTc gTAcGatgaTcaGAaGTTAA TATgGA.TTATATtgTTTtT T.ATGAATTATTTGAaGgTT
BGMVBDR ATTtgaaGaATTTAAtaT.A TTTATGGTATATTAAATgTT ATAtGacAGTTGaAtGTTAA TATcaAATTATTaATTTgGT TtATGAATTATTTGAtGATT
BGMVBGA ATTtTGatgAacTtAAtatA TTTATGGTATATTAAATaTT AaAtGTcAGTTGGAtGTTAt TATtGAATTATATATTcTGa gctTGAATTATTTaAgGAcT

        401                                434
BGMVBPR AATAAGCAAAACGAagcCGT TTTGAActTATtcgt
BGMVBDR AATAAGCAAAACGATATCGT TTTGAgtcTAT....
BGMVBGA AATAAcCAAAACGATATCGT TTTGAAagTATgttg
```

Nucleotide numbers at the start of the alignments:
MTGB - 2193; BGMVBBZ - 2273; BGMVBPR - 2196; BGMVBDR - 2198; BGMVBGA - 2188

PROGRESS REPORT
Phase I, year 2, 1988 - 1989.

Research project: Characterization of the main bean yellow mosaic virus isolates that limit bean production in Northern Africa, West Asia and China.

Staff: Vittoria LISA, Sara ANTONIAZZI, Giuseppina DELLAVALLE, Caterina PERRONE; electron microscopy: Vera MASENGA, R.G. MILNE; production of healthy plants: Giulia MORINO.

The work done from May 1988 to April 1989 had followed two main lines:
I. Identification of the viruses isolated from the bean samples collected in Turkey and, in smaller numbers, received from Iran and Bulgaria; characterization of the bean yellow mosaic virus (BYMV) isolates identified.
II. Continuation of the search for natural sources of resistance in Phaseolus vulgaris germplasm to the South American strain Orfeo-Inia of BYMV (BYMV-OI).

I A. Indexing for bean viruses in Turkey

From June 27 to July 5, 1988, V. Lisa and G. Dellavalle, together with Dr. J. Tohme, CIAT, surveyed bean cultivations in the localities indicated in Fig. 1, to collect samples from plants showing symptoms of virus disease. A total of 142 samples were collected. Symptoms on field samples and indications of the localities are given in Table 1. Due to unseasonal weather especially at Eskisehir and Erzurum, bean cultivation were late and showed generalized yellowings and poor growth, possibly due to cold and rain. Each sample, of 1 g average weight, consisted of a young leaf taken from a single plant. Samples were taken in small plastic bags. Later, the bags were discarded and the samples, wrapped in paper, were each put in a small bottle containing 5-7 g of $CaCl_2$. On arrival at our lab in Torino samples were moved to containers with fresh CaCl2 and kept at -20 C.

To isolate viruses, the dried field samples were ground in 0.05 M phosphate buffer ph7 and inoculated to test plants (P. vulgaris cv Saxa, Chenopodium amaranticolor and/or C. quinoa, Nicotiana clevelandii). A part of each dried sample was kept for further investigations, if necessary.

Viruses were identified by immunodiffusion tests, using SDS in the case of elongated viruses, on crude or concentrated sap from test plants, mainly bean. Electron microscopy was employed in doubtful cases. Sera to bean common mosaic v.(BCMV), BYMV, clover yellow vein virus (ClYVV) and cucumber mosaic virus (CMV) all prepared in this Institute, were routinely used.

The viruses identified are listed in Table 1. BCMV was the commonest, found in 55 samples; CMV was identified in 15 samples and BYMV in 10. BYMV was present in one locality only, Adapazari, in the

Sapanca area.

No virus was isolated from 67 samples, in spite of repeated attempts at transmission. Seventeen of the original dried samples, taken from plants apparently severely virus-infected, but negative in infectivity tests, were checked by negative staining with 2% uranyl acetate, and electron microscopy. All samples ("EM" in Table 1) were negative, except N. 131, in which rhabdovirus particles were seen (Fig.6). One attempt to transmit this virus to test plants by sap-inoculation failed.

B. Characterization of the BYMV isolates from Turkey

It was not possible in one case (sample 123) to separate BYMV from CMV. The other 9 isolate were maintained in bean cv Saxa, on which they caused symptoms varying from green-yellow mosaic to severe leaf curling, malformation and blistering. Fig. 2 shows symptoms on bean cv Saxa. All isolates caused malformation of pods. A limited host range study did not reveal relevant differences among the isolates.

To evaluate the pathogenicity on bean of the 9 isolates, they were inoculated to a selection of the bean cultivars used to differentiate BCMV pathotypes (Drijfhout et al., Neth. J. Pl. Path. 84, 1978, 13-26; Morales and Bos, AAB Descriptions of Plant Viruses No. 337, 1988). Seedlings were sap inoculated once,then again after one week. Three weeks later all plants were checked serologically for BYMV, by SDS immunodiffusion tests, and negative plants were further tested by back inoculation.

Results, referring only to systemic reaction of the plants, are given in Table 2. Although the isolates varied in pathogenicity, none infected all cvs, indicating that possible sources of resistance are available.

To obtain a serological comparison, two antisera, to a standard European isolate (BYMV-1V) and the South American strain (BYMV-OI , respectively, were titrated against the nine Turkish isolates. The slide precipitin test was used, with concentrated antigens (results in Table 3). All isolates were serologically closely related to both BYMV-1V and BYMV-OI, with minor differences among them.

C. BCMV and CMV isolates from Turkey

BCMV. Portyfive BCMV isolates were tested for presence of necrotic strains by inoculation to the necrosis-susceptible bean cvs Topcrop and Widusa. These plants were sap-inoculated twice with the isolates, and the reaction was evaluated by visual observation . Since most BCMV isolates were seed-transmissible in bean cv Saxa, isolates that had been passed trough the seed were used as inoculum when possible. Five isolates (" ***" in Table 1) gave apical necrosis (Fig. 3) on one or both differentials. Of these isolates, No. 111 was the severest, giving necrosis on both Topcrop and Widusa. The test was repeated, but the necrotic reaction was reproduced in one plant only (out of 4) for each cv. Five other isolates (" **" in Table 1) caused mild green mosaic on one or both cvs, while the remaining 35 isolates ("*" in Table 1) caused no symptoms.

On C. amaranticolor and C. quinoa the reaction varied from a large number of chlorotic-necrotic local lesions (Fig. 4) to absence of symptoms. Three isolates (No. 47, 61, 137) occasionally caused systemic symptoms in C. quinoa (Fig. 5).

CMV. Twelve CMV isolates out of 15 identified were maintained in tobacco cv White Burley or in petunia. A limited host range study indicated that only isolate 56 had unusual characteristics, causing only local infection (chlorotic ringspots) in tobacco White Burley, chlorotic local lesions on C. quinoa and not infecting Cucurbita pepo cv Genovese. All isolates induced mosaic in Saxa

bean.

D. Bean samples from Iran

Thirteen dried bean leaf samples collected from individual plants in the Iranian province of Ourmia (Fig. 1), West Azerbaijan, were sent to our Institute by Dr. R. Parvizy, Agricultural Research Centre, Ourmia. Viruses were isolated from 3 samples. Two samples contained BYMV (isolates VE 108 and VE 112) and one BCMV plus CMV. The two BYMV isolates were studied in the same way as those from Turkey, (Tables 2 and 3).

E. Bean samples from Bulgaria

Nine dried bean leaf samples were sent to our Institute by Dr. D. Kostova, Institute for Vegetable Crops, Plovdiv, Bulgaria. From them were isolated BCMV, CMV and alfalfa mosaic virus, the last in one sample. BYMV was not detected.

All virus isolates, from any locality, are in storage as dried leaf at -20 C.

We wish to acknowledge the helpful cooperation of the following researchers and Institutes during our survey in Turkey or in sending us infected bean material.

Dr. S. CALI - Ankara Plant Protection Res. Inst.
Bagdat Cad. 250, Giftlik mah., Yenimahalle, Ankara, Turkey

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P.K. 257, Erzurum, Turkey

Dr. M. AFRICAN - Sakaraya Agr. Res. Inst.
P.K. 39, Adapazari, Turkey

Dr. R. ERGIN - Ataturk Central Hort. Res. Inst.
P.O. Box 15, Yalova, Turkey

Dr. R. PARVIZY - Agricultural Research Centre
P.O. Box 365, Ourmia, Iran

Dr. D. KOSTOVA - Maritsa, Inst. for Vegetable Crops
Plovdiv, Bulgaria

II. Screening for natural sources of resistance to BYMV-OI

A. Since May 1988 we have tested 20 new lines of wild type and 3 cvs of P. vulgaris for resistance to BYMV-OI (Table 4).

To obtain quick and uniform germination and good growth of the wild type beans we used the following method, devised by Giulia Morino. The seeds surface was gently abraded with a small grater after the seeds had been soaked for 12 hours in water a 5 C. The seeds were then sown in 8 cm diameter clay pots, in a mixture of 70% of our standard compost and 30% Perlite.

Seedlings were sap inoculated once, then again after one week. Plants not showing virus symptoms about 20 days later received a third inoculation. The few plants still symptomless after the last inoculation were checked for latent infection by back inoculation to test plants. For seed production, virus-free plants were raised in an aphid-free glasshouse or screenhouse.

B. Plants of P. vulgaris and P. coccineus found free of virus in the 1987/88 tests were grown in screenhouses and seed collected. The cvs or lines are listed in Table 5. Seeds from these lines, except from the wild bean plant 1130/108, have been sent to CIAT.

C. Sixteen seedlings from the "resistant" P. vulgaris plant 15053/59, cv Anellino di Brescia (Table 5) were inoculated with BYMV-OI as described in IIA. Six plants became infected without showing symptoms. The other 10 were not infected and seeds of these are now ripening. This line, under our conditions, can be considered tolerant to BYMV-OI; in time, hopefully, a line may be selected showing stabilized resistance to the virus.

Fortyfive seedlings from the "resistant" plant of wild type bean 1130/108 were all susceptible to BYMV-OI, showing severe necrotic disease.

III Antiserum to BYMV-OI

The maximum titer of this serum was 1/1024 (progress report 1987/88) but in the rabbit the titer later fell to 1/256. The rabbit was therefore given 3 booster injections before bleeding twice. The new bleedings had homologous titers 1/1024 and 1/512, respectively, in slide precipitin test, and reacted up to 1/4 with healthy plant components. Aliquots of all bleedings, for a total of 20 ml, have been sent to CIAT. This serum is routinely used in the diagnostic work on BYMV from western Asia.

Table 1. Samples collected in Turkey, June 27 - July 5, 1988.

SAMPLE	LOCALITY	BEAN TYPE	SYMPTOMS ON THE FIELD PLANT	VIRUS(ES) IDENTIFIED
No. 1	Ankara (farmer)	local variety	dwarfing, yellowing	0
2	Eskisehir (Exp. Sta.)	Dalmason 3, 682-1	yellowing, necrosis	CMV
3	"	" "	yellowing	BCMV **(1)
4	"	" 4F-2406	green leaf curl	CMV
5	"	" 4F-2407	leaf curl, dwarfing, yellowing	0 EM: 0
6	"	" 4F-638/4	yellow vein banding	0
7	"	Barbunya, 4F-2379	mosaic	0
8	"	Ph. coccineus 4F-2189	vein banding	0
9	"	Barbunya, 4F-2386	green mosaic	0
10	"	" 4F-2395	yellow spots	BCMV * (1)
11	"	" 4F-1182/1	yellow mosaic	BCMV *
12	"	" 4F-2378	yellowing on older leaves	0
13	"	Horoz 1, 4F-2627	vein banding	BCMV *
14	"	" 4F-2792	vein banding, necrosis, dwarfing	0
15	"	" F6-90	yellowing, leaf curl	BCMV *
16	"	Fresh bean, 4F-527/4	yellowing, mosaic, deformation	BCMV *
17	"	" "	yellowing	0
18	"	" "	yellowing	BCMV *
19	Eskisehir (farmer)	Horoz, local variety	yellowing, black root	0
20	Eskisehir (Exp. Sta.)	Horoz 1, 4F-2666	aspecific yellowing	0
21	"	" "	" " "	0
22	"	" "	" " "	0
23	"	" 855	yellowing, leaf curl	0
24	Eskisehir (near Sabuncu)	unknown, loc. var.	dwarfing, leaf roll	BCMV *
25	"	" "	yellowing	0
26	"	" "	leaf curl	BCMV *

27	"	"	"	yellowing, dwarfing, leaf roll	0	EM: 0
28	"	"	"	*dwarfing, leaf roll*	0	
29	Eskisehir to Ankara(Exp.Sta)	unknown		yellowing, dwarfing, leaf curl	0	
30	"	"		leaf curl	0	
31	"	"		yellow mosaic (large patches)	0	EM: 0
32	"		WA/1635-3	yellow mottle	0	
33	"		"	" "	0	
34	"		"	yellowing, dwarfing	0	
35	"		"	bright yellow mosaic, dwarfing	0	EM: 0
36	Diyarbakir (towards Cermik)	Horoz,	loc. var.	yellow mosaic	0	EM: 0
37	"	"	"	" "	0	
38	"	"	"	green mosaic	BCMV *	
39	"	"	"	" "	BCMV	
40	"	unknown		dwarfing, leaf curl	0	EM: 0
41	"	"		dwarfing, yellowing	0	
42	"	"		top curl	0	
43	"	"		yellow spots (aspecific)	0	EM: 0
44	"	"		yellow leaf curl, mosaic	BCMV *	
45	"	"		yellow mosaic	0	EM: 0
46	"	"		yellow spots (aspecific)	0	
47	Malkaya (Cermik)	"	climbing	bright yellow spots	BCMV *	
48	void					
49	Malkaya	unknown	climbing	green mosaic	0	
50	above Malkaya	"	loc. var.	green mosaic	BCMV	
51	"	"	"	yellow vein banding	BCMV **	
52	"	"	"	" " "	BCMV *** (1)	
53	"	"	"	aspecific yellowing	0	
54	"	"	"	green mosaic	BCMV *	
55	Tuzlakoy	"	"	severe dwarfing, leaf curl	BCMV **	
56	"	"	"	yellow mosaic, dwarfing	BCMV, CMV	
57	"	"	"	yellow mosaic	BCMV	

58	"	"	"	green mosaic	BCMV	*	
59	"	"	"	yellowing, leaf curl	0		
60	Yayla	unknown	loc. var.	yellow mosaic	BCMV	*	EM: 0
61	"	"	"	yellow vein banding, ring pattern	BCMV	*	
62	"	"	"	green mosaic, blistering	0		EM: 0
63	"	"	"	" " "	BCMV	*	
64	"	"	"	leaf roll	BCMV	*	EM: 0
65	Illicar	"	climbing	bright yellow spots	BCMV	*	
66	"	"	"	" " "	BCMV	***	
67	Cobantasi	Dalmason 3		green mosaic, dwarfing	BCMV	*	
68	"	"		green-yellow mosaic, dwarfing	BCMV	*	
69	"	"		green mosaic, leaf curl	BCMV	*	
70	"	"		yellow mosaic, necrotic rings, leaf curl	BCMV	*	
71	"	"		severe green mosaic, dwarfing	BCMV	*	
72	Pasinler (Exp. Sta.)	Sekker,	loc. var.	aspecific yellowing	0		
73	"	"	"	yellowing, dwarfing	0		
74	"	"	"	yellowing, vein banding	0		
75	Pasinler (1st farm)	unknown,	climbing	aspecific yellowing	BCMV, CMV		
76	"	"	"	yellowing, dwarfing	0		
77	"	"	"	dwarfing	0		
78	Pasinler (2nd farm)	Lubja		green mosaic	BCMV	*	
79	"	"		" "	BCMV	*	
80	"	"		yellowing	BCMV	*	
81	Tortun - Narman	unknown		aspecific yellowing	0		
82	"	"		" "	0		
83	"	"		none	0		
84	"	"		green - yellow mosaic	BCMV	*	
85	"	"		yellowish mottle	0		
86	"	"		dwarfing, blistering	CMV		
87	"	"		yellow vein banding	BCMV	*	

88	"	"		yellow mosaic, vein banding	0		
89	"	"		dwarfing	0		
90	"	"		vein banding, leaf roll	BCMV	*	
91	Tortun - Narman	unknown		green mosaic, blistering	BCMV	*	
92	" "	"		green mosaic	BCMV		
93	" "	"		yellow mosaic	0		EM: 0
94	" "	"		leaf curl	0		
95	" "	"		yellowing, dwarfing	0		
96	" "	"		" "	0		
97	" "	"		yellow mosaic	0		
98	" "	"		yellowing, dwarfing	0		EM: 0
99	" "	"		green mosaic	0		
100	Samikale (Narman)	Barbunya	loc. var.	aspecific yellowing	0		
101	"	"	"	malformations	0		
102	"	"	"	vein banding	0		
103	"	"	"	leaf curl	BCMV		
104	"	"	"	blistering	0		
105	"	Sekker	"	necrotic spots	0		
106	"	"	"	necrosis, malformations	0		EM: 0
107	"	"	"	aspecific yellowing and dwarfing	0		
108	"	"	"	leaf roll	BCMV		
109	"	"	"	dwarfing, malformation	0		
110	"	"	"	" "	0		
111	"	"	"	yellow and green vein banding	BCMV	***	
112	Sapanca (Exp. Sta.)	unknown		green mosaic, bushy plant	BCMV, CMV		
113	"	"		necrosis	BYMV		
114	"	"		severe dwarfing, mosaic, curl	BYMV		
115	"	"		dwarfing, mosaic, blistering	BYMV		
116	"	"		halo blight, dwarfing	CMV		
117	"	"		necrosis	BYMV		
118	"	Horoz	4F-2629	green mosaic (small patches)	BYMV		

119	"	Eskisehir	855	halo blight, leaf curl	CMV		
120	"	Horoz	4F-2849	yellow mosaic, necrosis, leaf curl	0		EM: 0
121	"	"	4F-2666	mottle	0		
122	Sapanca (Exp. Sta.)	Navy	4F-2848	dwarfing, mosaic, necrosis	BYMV		
123	"	"	"	yellow mottle	BYMV, CMV		
124	"	"	"	mosaic, blistering, necrosis	BYMV		
125	"	"	"	top necrosis	BYMV		
126	"	Horoz	ped. 1313	yellow mosaic	0		EM: 0
127	"	"	" 1	bright yellow spots	BYMV		
128	"	"	" ?	necrotic lesion	0		
129	"	"	" 37	yellow vein banding, mottle	CMV		
130	"	"	" 61	yellow mosaic	CMV		
131	"	"	" 131	severe leaf curl, pod malformation	0		EM: Rhabdovirus
132	"	"	" 126	yellow mosaic, dwarfing, leaf curl	CMV		
133	"	"	" 186	large yellow ringspots	CMV		
134	Yalova (Exp. Sta.)	climbing bean		mottle	BCMV	*	
135	"	"	"	yellow mottle	BCMV	*	
136	"	"	"	mottle	BCMV, CMV		
137	"	"	"	mosaic	BCMV	*	
138	"	Sekker		yellow-green mosaic	BCMV	***	
139	void						
140	Yalova (Exp. Sta.)	Sekker		mosaic, blistering	BCMV	*	
141	"	"		mosaic	BCMV	**	
142	"	bush bean		yellowing, dwarfing	BCMV	***	
143	"	climbing bean		green mosaic	BCMV, CMV	*	
144	"	"	"	yellow spotting	BCMV	**	

(1) Reactions of bean cvs Top Crop and Widusa:
*: no symptoms;
**: mild green mosaic;
***: necrosis.

Table 2. Reaction of differential cvs to BYMV isolates.

BYMV Isolate	Differential cvs : RGC	RGB	GN123	Sanilac	Michelite	Pinto 114	Monroe	GN 31	Widusa	Jubila	Top crop	Amanda	Saxa
113 Turkey	mosaic	(1) 0	0	mosaic	mosaic	0	0	0	mosaic / necrosis	mosaic	mosaic	0	mosaic
114 Turkey	latent	mosaic	0	mosaic	mosaic	0	0	0	mosaic / necrosis	latent	mosaic	0	mosaic
115 Turkey	(1) 0	(1) 0	0	mosaic	mosaic	latent	0	0	mosaic / necrosis	mosaic	mosaic	0	mosaic
117 Turkey	mosaic	latent	0	necrosis	necrosis	0	0	0	mosaic / necrosis	mosaic	mosaic	0	mosaic
118 Turkey	(1) 0	mosaic	latent	mosaic	mosaic	0	0	0	mosaic / necrosis	mosaic	mosaic	latent	mosaic
122 Turkey	mosaic	mosaic	mosaic	mosaic	mosaic	latent	mosaic	0	mosaic	mosaic	mosaic	mosaic	mosaic
124 Turkey	latent	mosaic	latent	mosaic	latent	latent	0	0	mosaic	mosaic	mosaic	0	mosaic
125 Turkey	latent	mosaic	0	mosaic	mosaic	0	0	0	necrosis	mosaic	mosaic / necrosis	0	mosaic
127 Turkey	mosaic	latent	latent	(1) 0	mosaic	latent	0	0	mosaic	mosaic	mosaic	0	mosaic
VE 108 Iran	mosaic	mosaic	mosaic	mosaic	mosaic	mosaic	0	0	mosaic / necrosis	mosaic	mosaic	0	mosaic
VE 112 Iran	mosaic	mosaic	0	mosaic	mosaic / necrosis	mosaic	0	0	mosaic / necrosis	mosaic / necrosis	mosaic / necrosis	mosaic	mosaic

Key to symptoms. Mosaic = green mosaic; necrosis = apical necrosis; latent = symptomless infection; 0= no symptoms, and back-inoculation to test plant negative.

(1) = repetition of the test in progress.

Table 3. Serological reaction between sera to BYMV-1V and BYMV-0I and the BYMV isolates from Turkey and Iran. Antigens were concentrat from infected bean cv Saxa by extraction of sap with 0.5 M phosphate buffer pH 8 containing protectans clarification of the with freon 113, concentration of the virus by ultracentrifugation, and suspension of sediments in solution of 0.05 M Na-citrat 0.02 M Na_2SO_3, brought to pH 7.5 with citric acid. Tests done by the slide precipitin test.

Serum	Homologous titre	Titer(1) to: 113	114	115	117	118	122	124	125	127	VE 108	VE 112
BYMV-1V (A 194 II)	1024	512	512	256	256	256	512	512	512	512	512	512
BYMV-0I (A 256 I)	1024	256	256	256	256	256	512	256	256	512	512	512

(1): reciprocal value.

Table 4. Lines of wild type bean and cultivated varieties tested with BYMV-OI since May 1988

Line No.	Reaction	No. of plants infected/ No. of plants tested	
a - wild type bean *			
0190	green mosaic	12/12	
0406	" "	21/22	1 plant symptomless**
0580	necrosis	20/20	
0621	green mosaic	16/16	
0622	" "	14/15	1 plant resistant
0695	" "	15/16	1 plant symptomless**
0748	necrosis	8/14	6 plants resistant
1042	green mosaic	22/22	
1052	necrosis	6/6	
1057	green mosaic	20/22	2 plants symptomless**
1066	necrosis	12/12	
1073	"	10/10	
1074	green mosaic and/or necrosis	38/39	1 plant resistant but not survived
1075	necrosis	22/23	1 plant symptomless**
1076	"	14/14	
1091	green mosaic and/or necrosis	8/8	
1095	" " " "	15/15	
1102	necrosis	20/21	1 plant symptomless**
1105	"	10/11	as plant 1074
1130	green mosaic	16/17	1 plant resistant
b - cultivated varieties			
Anellino di Trento	green mosaic	27/27	
Rimini	no clear symptoms	7/7	tolerant?**
Taylor's hort.	necrosis	4/4	

* Gembloux collection. Seeds kindly provided by Dr. A. Allavena, Istituto Sperimental per l'Orticoltura, Montanaso Lombardo, Italy.
** Control for latent infection in progress.

Table 5. Seeds collected from plants "resistant" to BYMV-OI in tests conducted during 1987/88.

Specie	Accession No.	Cv.	No. of mother plants
P. vulgaris	15083/59	Anellino di Brescia	1
" "	1130/108	wild type	1
P. coccineus		Bianco di Spagna	8
" "		Corona	4
" "		Rampicante bicolore	5
" "	33028		11
" "	30034	Di Spagna	2
" "	30043	"	4
" "	30076	"	2
P. lunatus	60000		3

Fig. 1. Map of Turkey. In colour the localities surveyed. In yellow Sapanca, in West Turkey and Ourmia, Iran, where BYMV was detected.

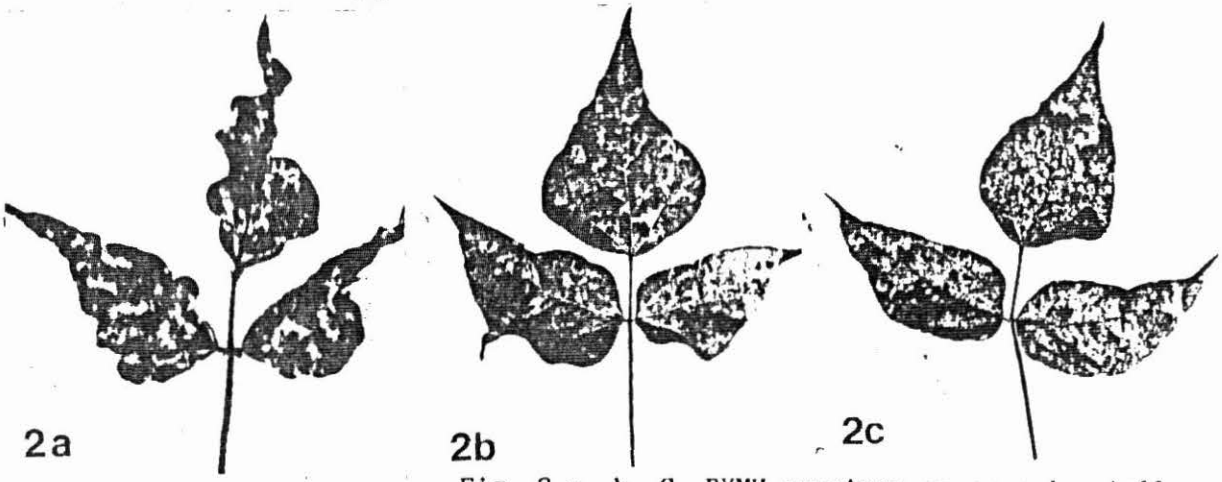

Fig. 2 a, b, C. BYMV symptoms on experimentally infected Saxa bean. Systemic infection from isolate 115 (a), 122 (b) and 124 (c). Plants belonged to the same batch and were grown in uniform conditions.

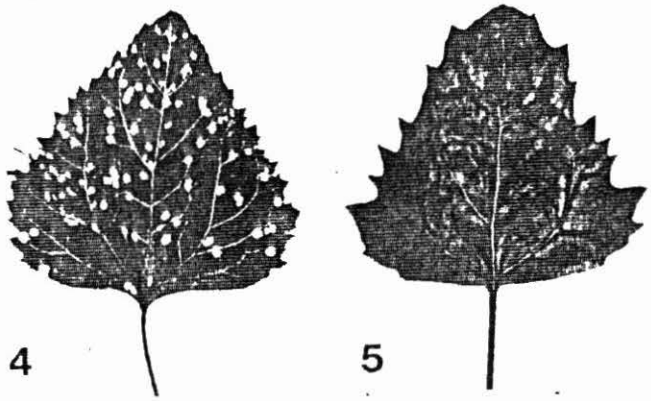

Fig. 3. BCMV isolate 66: apical necrosis on cv Widusa.

Fig. 4. BCMV isolate 52: local lesions on C. amaranticolor.

Fig. 5. BCMV isolate 137: systemic infection on C. quinoa.

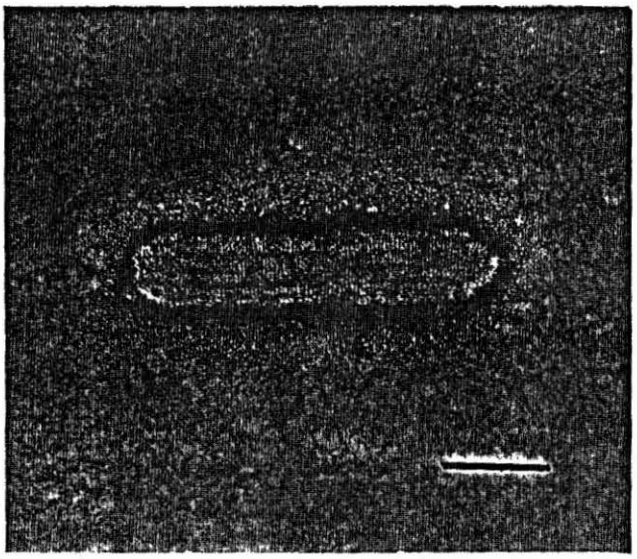

Fig.6 Rhabdovirus particle from sample 131, negatively stained in uranyl acetate. Bar = 100 nm