ANNUAL REPORT

VIROLOGY RESEARCH UNIT

December 1990

For Internal Circulation and Discussion Only

Centro Internacional de Agricultura Tropical

TABLE OF CONTENTS

VIROLOGY RESEARCH UNIT

ANNUAL REPORT 1990

EXECUTIVE SUMMARY

During 1990 the Virology Research Unit (VRU) further integrated the expertise of its senior and junior staff to conduct applied and strategic research across all CIAT's commodities. The application of advanced molecular virology techniques proved particularly valuable to solve standing research problems related to the control of bean, cassava and rice viruses as described below:

Bean Virology

While the VRU continued the characterization of the viruses which affect bean production and/or restrict the international exchange of bean germplasm in the tropics, considerable progress was made towards the control of whitefly-transmitted bean viruses in Latin America though a cooperative project with the University of Wisconsin entitled "Molecular Approaches for the Control of Bean Golden and Bean Dwarf Mosaic Viruses". Thanks to this project, four of the most representative isolates of bean golden mosaic virus (BGMV) and an isolate of bean dwarf mosaic virus (BDMV) were characterized and shown to be distinct according to their geographical origin (Brazil, the Caribbean, and Central America). General and isolate-specific geminivirus DNA probes have been now developed for use in epidemiological studies (pathogenic variability and identification of natural weed reservoirs).

From this work involving the cloning and sequencing of selected BGMV and BDMV isolates, and the demonstration that cloned DNAs were infectious in *Phaseolus vulgaris* (all isolates) and *Nicotiana benthamiana* (BDMV), different virus-derived resistance approaches, such as the coat protein-mediated and the dominant lethal schemes, will be pursued. In the meantime, the additional methodology developed has greatly enhanced our capacity to select highly tolerant parental bean genotypes possessing different mechanisms of resistance. As a result, a new group of BGMV/BDMV resistant lines have been developed in Central America and South America, some of which will soon be released as new cultivars.

Tropical Pastures Virology

The characterization of the viruses that affect tropical forage legumes was initiated only recently following the creation of the VRU in 1988. Consequently, the main efforts have been directed towards the detection of viruses in the main forage legume species collected and transferred by the Tropical Pastures Program to collaborating national programs. So far, three distinct viruses and several related strains have been isolated from the three main genera of forage legumes, *Arachis*, *Centrosema*, and *Stylosanthes*, with a view to implementing reliable virus detection methods for the safe exchange and utilization of tropical forage legume germplasm.

Cassava Virology

Progress was made over a broad range of cassava viruses and virus-like diseases. Cassava Colombian Symptomless and Cassava American Latent Viruses were added to the cassava viruses that can be routinely identified using the enzyme linked immunosorbent assay (ELISA). The molecular characterization of Cassava Common Mosaic Virus is progressing and the sequencing of over half of the virus is complete. The area with the most progress is the characterization of the agents of Caribbean Mosaic and Frogskin Diseases. The interpretation of the results indicates that the causal agent of Caribbean Mosaic Disease is a virus that is member of a new subgroup of the phyto-reoviruses. A similar but distinct virus is the causal agent of the mosaic symptoms associated with Frogskin Disease. Additional tests are in progress to determine if all the symptoms associated with this disease can be attributed to this virus.

Rice Virology

The molecular characterization of rice hoja blanca virus (RHBV) was begun this year with experiments to map the genome of RHBV. Several proteins have been indentified using *in vitro* translation analysis. A cDNA probe was synthesized and was used in hybridization analysis. While many of the rice isolates from the Cauca and Tolima valleys appear homologous using this probe, the Echinocola strain of RHBV is distinct. Isozyme assays of the vector of RHBV, *Sogatodes orizicola,* show that there is variation with the populations of this planthopper even within the colony maintained at CIAT. A better understanding of the types of variation of both the virus and the vector is needed in order to help prevent the periodic epidemics caused by RHBV.

VIROLOGY RESEARCH UNIT

ANNUAL REPORT 1990

Introduction

The Virology Research Unit (VRU) had an active year in which the two senior staff virologist achieved a higher degree of integration of research responsibilities across commodities. The main constraint encountered this year was the selection of junior staff possessing basic working knowledge of standard molecular virology techniques. Nevertheless, 1990 has been one of the most exciting years with respect to the successful application of molecular biology and genetic engineering techniques to CIAT's germplasm improvement activities.

The VRU has operated in close communication with the Leaders, scientists and, particularly the plant pathologist of the four commodity programs, to better define priorities and meet research objectives. Equally important, the VRU has maintained close links the the Biotechnology and Genetic Resources Units in the areas of molecular biology and the safe exchange of virus-free germplasm, respectively.

We expect to continue the applied and strategic research which all the CIAT-mandated commodities require for germplasm improvement and deployment to collaborating national programs.

can also serve as parents in national bean improvement programs.

The new BCMV resistant lines contain different combinations of recessive genes (bc2-2, bc3) which either used alone, as in the case of bc3, or in conjunction with the dominant 'I' gene, will provide complete resistance to both necrosis or mosaic inducing strains of the virus in Africa and Latin America. These lines encompass a wide variety of grain types and growth habits suitable for breeding programs on both continents. Some work is still needed to incorporate the resistance genes into climbing bean types and other elite bush lines with multiple disease resistance.

BEAN GOLDEN MOSAIC

Bean golden mosaic has now expanded into the main bean-growing regions of the Latin American lowlands, including Mexico, Central America, the Caribbean, and Brazil and Argentina in South America, where it has become endemic. The unarrested spread of this disease is predominantly the result of the continuous expansion of soybean plantings and, to a lesser extent, of other cash crops, such as tomato, tobacco, and cotton in Latin America.

The identification of new mechanisms of BGMV resistance in different non-black seeded genotypes (Bean Program Annual Report 1988,1989) led to the utilization of new sources of resistance and selection of advanced lines derived from these BGMV resistant genotypes.

Central America: The C. American BGMV breeding project is the oldest and, therefore, the most dynamic. Intensive crossing for BGMV has been conducted by National Program Breeders, outposted CIAT Staff, and CIAT breeders at headquarters.

The impact of the first black-seeded BGMV-tolerant cultivars released in Guatemala (ICTA-Quetzal) and, later, in Mexico (Negro Huasteco), has already been amply documented. These materials have been used since 1980 to further improve Central American bean cultivars for other desirable agronomic characteristics, such as earliness.

Currently, a third generation of BGMV-tolerant lines has been obtained using new sources of BGMV resistance. For instance, the red-seeded DOR 364 is finding acceptance in some areas for its superior BGMV resistance, while several other red-and black-seeded breeding lines present even higher levels of BGMV resistance, most notably DOR's 390, 438, 476, 482, 483 and 484.

Preliminary observations on populations derived from Race D sources (Pinto, Red Mexican, Ojo de Cabra, Great Northern, etc.) suggest that when these are combined with DOR 364, a reasonably acceptable agronomic type can be recovered combined with higher resistance. DOR 364 has demonstrated an unusual ability to combine with Type III genotypes and produce progeny with upright branches.

Caribbean Region

The Caribbean BGMV project has been centered in the Dominican Republic where this viral disease constitutes a major bean production constraint particularly in the localities of Azua and San Juan de la Maguana.

This year, the BGMV project continued as a cooperative effort between the Dominican National Program, the University of Wisconsin (CRSP-Title XII), and CIAT. 1989 and 1990 were marked by the high populations of *B. tabaci* which developed in crops, such as tomato and eggplant, grown in or near traditional bean productions areas of the Dominican Republic.

Last year we reported that populations were under selection to incorporate resistance into the Pompadour bean types from genetically diverse sources of BGMV resistance. In the past year, several such families were purified, which present a superior reaction to BGMV in the Guatemalan golden mosaic nursery. Although it is very difficult to improve the Pompadour type while maintaining acceptable grain color and size, a few families do present promising grain type, while most are intermediate products for use in the crossing program. It is expected that these lines can be distributed and tested under BGMV pressure in the Caribbean region next year.

Simultaneously, and through a cooperative project with the University of Wisconsin, some epidemiological studies, particularly the identification of virus reservoirs in nature and the variability of BGMV isolates in different regions of the D.R., were undertaken. The results of these studies indicate that the BGMV-D.R. isolates are not significantly variable and, therefore, results obtained in the molecular characterization of the type BGMV-DR isolate should be applicable when more advanced research in the area of genetic engineering is completed.

Regarding natural virus reservoirs, none of the weeds collected by Dr. D. P. Maxwell in the D.R. (University of Wisconsin-CIAT BGMV project), hybridized at high stringency with the BGMV-DR radioactive specific probes in nucleic

test parents. Regarding individual resistance traits, Red Mexican 36, Pinto UI 114, and PVA 1111 exhibited low foliar yellowing; Royal Red and Red Mexican 36 showed resistance to plant dwarfing; Redlands Greenleaf C and Pinto UI 114 had the lowest incidence of flower abortion; and Pinto UI 114, Great Northern 31, and Redlands Greenleaf C produced the most pods.

Mean square values due to general combining ability (GCA), specific combining ability (SCA), and reciprocal and maternal effects are given in Table 3. Values for the latter two were nonsignificant ($P>0.05$) for all four characteristics.The GCA mean square is indicative of additive genetic variance, and the SCA of nonadditive genetic variance. In a highly self-pollinating crop such as common bean, where true-breeding pure line cultivars are often sought, selection for a trait is effective only in presence of significant additive genetic variance. The nonadditive variance diminishes in successive generations of inbreeding and it is, therefore, of little or no value to breeders. Highly significant ($P<0.01$) mean square values due to GCA were recorded for all four resistance traits. Values due to SCA were nonsignificant ($P>0.05$) for foliar yellowing and flower abortion. Moreover SCA mean square values for other traits were also lower than GCA mean squares. Thus, for the four BGMV resistance traits evaluated here, additive genetic variance was found to be more important than nonadditive effects. This would suggest that the parental BGMV scores per se should be reliable for predicting the performance of their hybrid populations and derived recombinant lines. Thus, early generation bulk hybrid populations could be tested, and poor performing BGMV susceptible crosses discarded. Greater emphasis could then be given on selection of resistant genotypes within more promising populations. Moreover, it should be feasible to combine and select for different genes/mechanisms of BGMV resistance among and within hybrid populations, provided adequate pressure of the disease exists in breeding nurseries. Although it is not yet possible to predict how much the level of BGMV resistance could be increased by recombining various sources of resistance, certain CIAT breeding lines, such as A 429, A 774, A 775, and DOR 476 which combine genes from two or more resistance sources, exhibit higher levels of field resistance to BGMV than previously observed in individual parents. Since the parental sources of resistance to BGMV (Table 2) belong to different races of common bean, and there is F_1 hybrid incompatibility in some crosses between parents belonging to the small-seeded race Mesoamerica and those of large-seeded Andean race Nueva Granada, this information should be taken into account to combine different sources of BGMV resistance. For incompatible crosses, BGMV-resistant parents, which are noncarriers of the dosage-dependent lethal Dl-1 and Dl-2

Table 3. Mean squares from 8 x 8 diallel crosses of common bean for resistance to bean golden mosaic virus

Source	d.f.	Foliar yellowing	Plant dwarfing	Flower abortion	Pod formation
General combining ability	7	8.32**	3.54**	7.35**	7.89**
Specific combining ability	28	2.06	1.19**	1.71	2.18*
Reciprocal	28	1.42	0.26	0.69	0.63
Maternal	7	2.35	0.43	0.75	0.61
Error	63	1.58	0.54	1.47	1.29

*,** Significant at 0.05 and 0.01 probability levels, respectively.

genes (e.g., Pinto UI 114 and ICA Pijao), would be required as bridging parents. The Dl-1 and Dl-2 genes are found in some small-seeded and large-seeded germplasm of Middle and Andean American origin, respectively. These are major genes affecting root and shoot growth, respectively. In none of the crosses made in this study was the F_1 hybrid incompatibility phenomenon observed.

GCA effects of eight parents for four traits are given in Table 4. Alubia Cerrillos, the susceptible parent, had significant positive GCA effects for all BGMV-resistance traits, suggesting that all populations involving this parental genotype would usually be more susceptible to BGMV. Redlands Greenleaf C also had positive GCA effect for foliar yellowing. None of the GCA values for Great Northern 31 and PVA 1111 were significant. Only Royal Red had significant negative GCA effects for all four traits. Porrillo Sintetico had significant negative GCA values for foliar yellowing, flower abortion, and amount of pod formation. Thus, Royal Red and Porrillo Sintetico should be valuable sources of resistance to BGMV although they belong to two different races of common bean. Whether they possess different genes for BGMV resistance or not, is outside the scope of this study. Nonetheless, their utilization in breeding programs for bean golden mosaic resistance should be maximized.

Simple phenotypic correlation coefficients among the four resistance traits evaluated (Table 5), showed significant positive association among all traits. However, it is not known whether these associations were due to linkage of genes controlling different traits, pleiotropic effects of gene(s), or mostly developmental or environmental effects. In the former two cases, selection for any given trait should simultaneously result in positive correlated response (improvement) in other BGMV resistance traits.

From these results, it can be concluded that resistance to BGMV in common bean is controlled largely by additive genes and, thus, significant progress should be expected from selection among and within hybrid populations involving BGMV resistant parents of diverse origin.

Infectious Cloned Bean Dwarf Mosaic Virus

In cooperation with Dr. Douglas Maxwell of the University of Wisconsin and Dr. Dave Russel of Agracetus, infectious cDNA clones to BGMV and BDMV were produced. The technique is most efficient when using a particle gun to

Table 4. General combining ability effects for eight parents of common bean utilized in diallel cross analysis for resistance to bean golden mosaic virus

Parental Identification	General combining ability effect			
	Foliar yellowing	Plant dwarfing	Flower abortion	Pod formation
Alubia Cerrillos	0.78**	0.71**	0.91**	1.09**
Redlands Greenleaf C	0.56*	0.15	-0.14	-0.03
Red Mexican 36	-0.48*	-0.08	0.11	-0.01
Royal Red	-0.50*	-0.41*	-0.57*	-0.54*
Pinto UI 114	-0.30	-0.22*	-0.01	-0.11
Great Northern 31	0.30	-0.03	0.23	0.11
Porrillo Sintetico	-0.48*	-0.14	-0.59*	-0.45*
PVA 1111	0.10	0.03	0.05	-0.03
S.E. of GCA	0.31	0.18	0.30	0.28
Trait mean	3.95	2.44	6.32	6.57

*,** Significantly different from zero at P = 0.05 and P = 0.01, respectively.

Table 5. Simple phenotypic correlation coefficent among characters for resistance to bean golden mosaic virus in common bean obtained from 8x8 diallel crosses

	Plant dwarfing	Flower abortion	Pod formation
Foliar yellowing	0.34**	0.31*	0.28*
Plant dwarfing		0.53**	0.55**
Flower abortion			0.87**

*,** Significant at P= 0.05 and P= 0.01 levels, respectively.

inoculate bean seedlings. Infectious cDNA clones of BDMV were shot into 12 Top Crop seedlings using the particle gun acquired this year by the BRU, and all of the bean plants became infected. Dr. Maxwell has also provided cDNA clones that are known to be infective to the Guatemalan and Brazilian isolates of BGMV. The Brazilian isolate is interesting because the particles cannot normally be mechanically inoculated. The cDNA clones are infectious using the particle gun. One advantage is that germplasm accessions can be screened for BGMV tolerance or resistance to BGMV without using the vector *B. tabaci*.

CUCUMBER MOSAIC VIRUS

The existence of strains of cucumber mosaic virus capable of naturally infecting legumes, particularly beans, was known since 1941. Epidemics of these viruses in bean-producing regions occurred in N. America and Europe from 1941 through 1975. Since then, CMV has been found affecting beans in Africa, the Mediterranean region, and recently in China, according to surveys conducted through cooperative CIAT projects. This year, CMV was found for the first time in Latin America causing a major epidemic in Chile, which further complicates the already serious virus situation in that country.

The legume strains of CMV differ in their pathogenicity to *Phaseolus vulgaris*. Table 6 shows the pathogenic range of two strains of CMV, one from Turkey and the second one from Chile. As can be concluded, the Chilean strain of CMV has an extreme pathogenicity range causing infection in every bean genotype tested. On the other hand, genotypes, such as Red Mexican 34 and Pinto 114, can be selected as sources of resistance to the Turkish strain of CMV. A search for new sources of resistance or tolerance to the Chilean isolates of CMV is currently under way.

Table 6 Reaction of differential bean cultivars to a Turkish and a Chilean isolate of cucumber mosaic virus-legume strain

Bean Cultivar	CMV strain	
	Turkish	Chilean
Double White	S	L,N,M
Stringless Green Refugee	S	S
Redlands Greenleaf C	S	S
Puregold Wax	S	S
Imuna	S	S
Redlands Greenleaf B	S	S
Great Northern 123	S	S
Sanilac	S	L,S
Michelite 62	S	S
Red Mexican 34	-	S
Pinto 114	-	S
Monroe	L,-	L,S
Red Mexican 35	L,-	L,S
Great Northern 31	S	L,S
Widusa	S	L,N,M
Black Turtle Soup	S	S
Jubila	S	S
Topcrop	S	S
Improved Tendergreen	S	S
Amanda	S	L,N,M
Bountiful	S	S
Diacol Calima	S	S

L: Local lesions on inoculated leaves; S: systemic infection;
N: Systemic necrosis; -: no systemic infection.

TROPICAL PASTURES VIROLOGY

This year, the VRU finished the characterization of the main viruses previously detected in the three most important genera of tropical forage legumes, namely, *Arachis*, *Centrosema* and *Stylosanthes*. Once reliable virus detection methods were implemented, the VRU conducted a more detailed investigation on the extent of pathogenic variability of the viruses isolated from *Centrosema* spp., and their relationship to the viruses of *Arachis* and *Stylosanthes* spp.

Final charachterization of two potyviruses infecting *A. pintoi* and *Stylosanthes* spp.

The causal agents of the *A. pintoi* ringspot and *Stylosanthes* sp. chlorosis and leaf malformation diseases (Figures 1 and 2) were manually transmitted to seedlings of the bean cultivar Bountiful, inducing chlorotic local lesions on the inoculated primary leaves. Only the *Stylosanthes* sp. virus induced noticeable systemic symptoms in Bountiful bean, consisting of severe mosaic and leaf malformation.

An electron microscopy examination of symptomatic *A. pintoi*, *Stylosanthes* sp., and inoculated bean plants demonstrated the presence of filamentous flexous virus particles, approximately 750 nm in length and 15 nm in diameter (Figure 3A, B).

The virus isolated from *A. pintoi* was also manually-transmitted to the following legumes: *Arachis hypogaea* (21 different genotypes), Canavalia sp., *Cassia occidentalis*, *Centrosema brasilianum*, seven soybean cultivars (Clark, ICA L-121, Mandarin, Marshall, Ogden, Rampage, and York), three bean cultivars (Dubbele Witte, Widusa, and Black Turtle Soup), *Phaseolus lunatus*, pea, *Stylosanthes capitata*, *S. macrocephala*, *Vigna radiata*, and *V. unguiculata* 'Blackeye' (Table 7). Other species systemically infected by the *A. pintoi* virus were *Nicotiana benthamiana*, and *Physalis angulata*. The list of insusceptible and local lesion hosts is shown in Table 7.

The virus isolated from *Stylosanthes* sp. systemically infected: peanut, five bean cultivars (Dubbele Witte, Stringless Green Refugee, Redlands Greenleaf C, Black Turtle Soup, Great Northern 123), pea, *Stylosanthes macrocephala* and *Vigna unguiculata* (cv. Blackeye). The only non-leguminous species infected systemically was *N. benthamiana* (Table 7). The list of local lesion

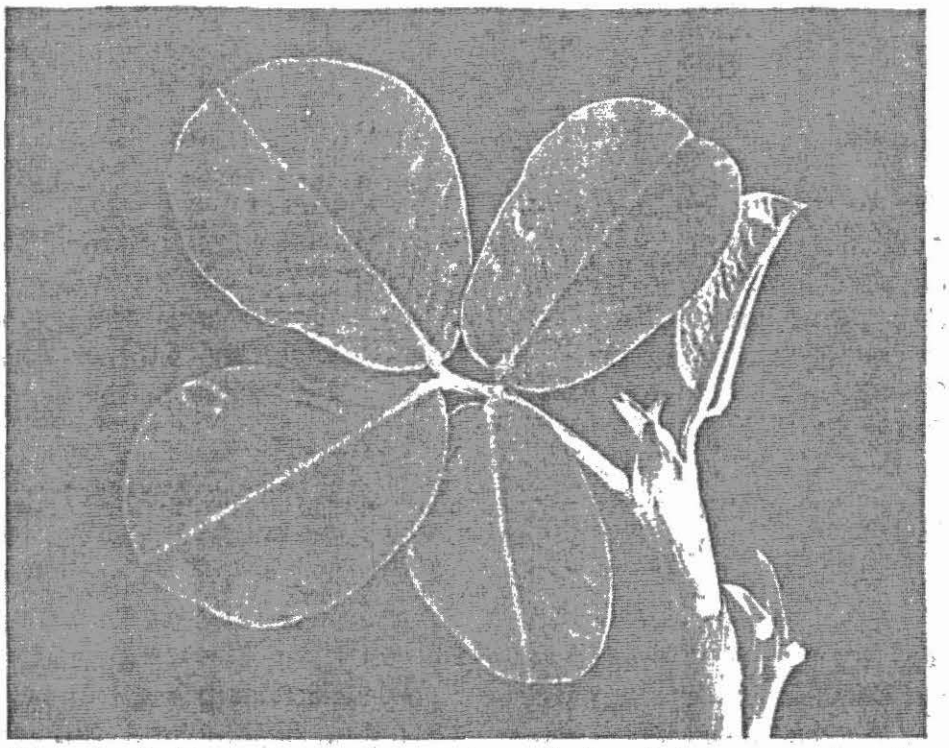


Fig. 1. Foliar ringspot symptoms induced by a strain of peanut mottle virus in the tropical forage legume, Arachis pintoi.

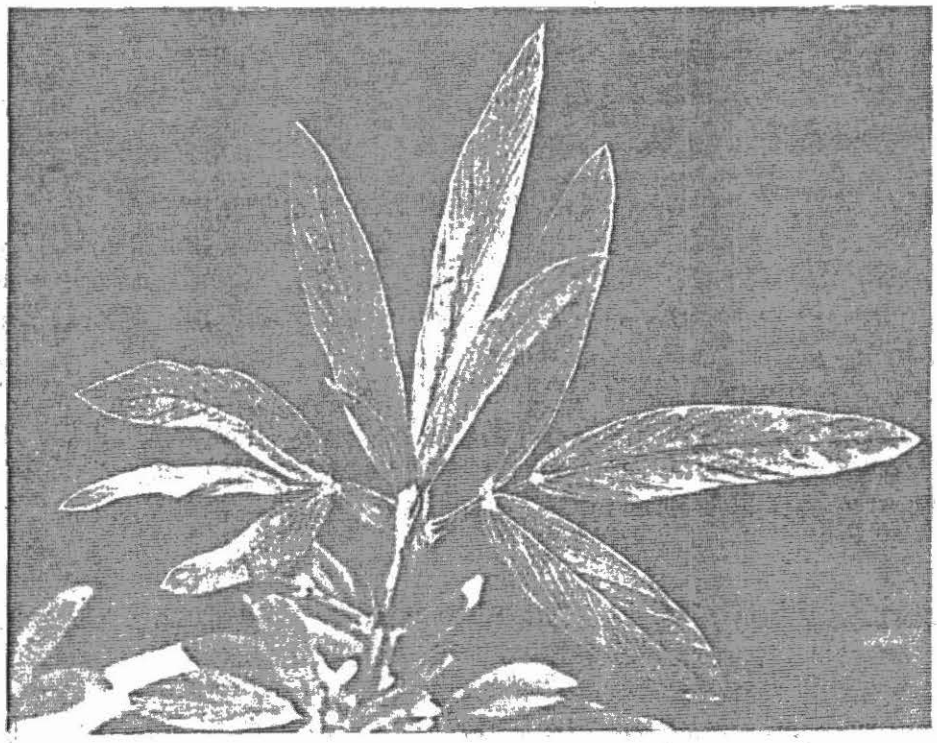


Fig.2 Foliar chlorosis and malformation symptoms induced by a strain of peanutmottle virus in the tropical forage legume, Stylosanthes macrocephala.

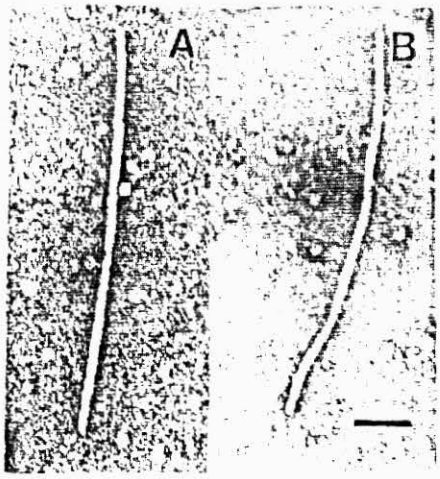


Fig. 3 Filamentous flexous virus particles observed in leaf extracts of Arachis pintoi (A) and Stylosanthes p. (B) plants affected by foliar ringspot and chlorosis, respectively.

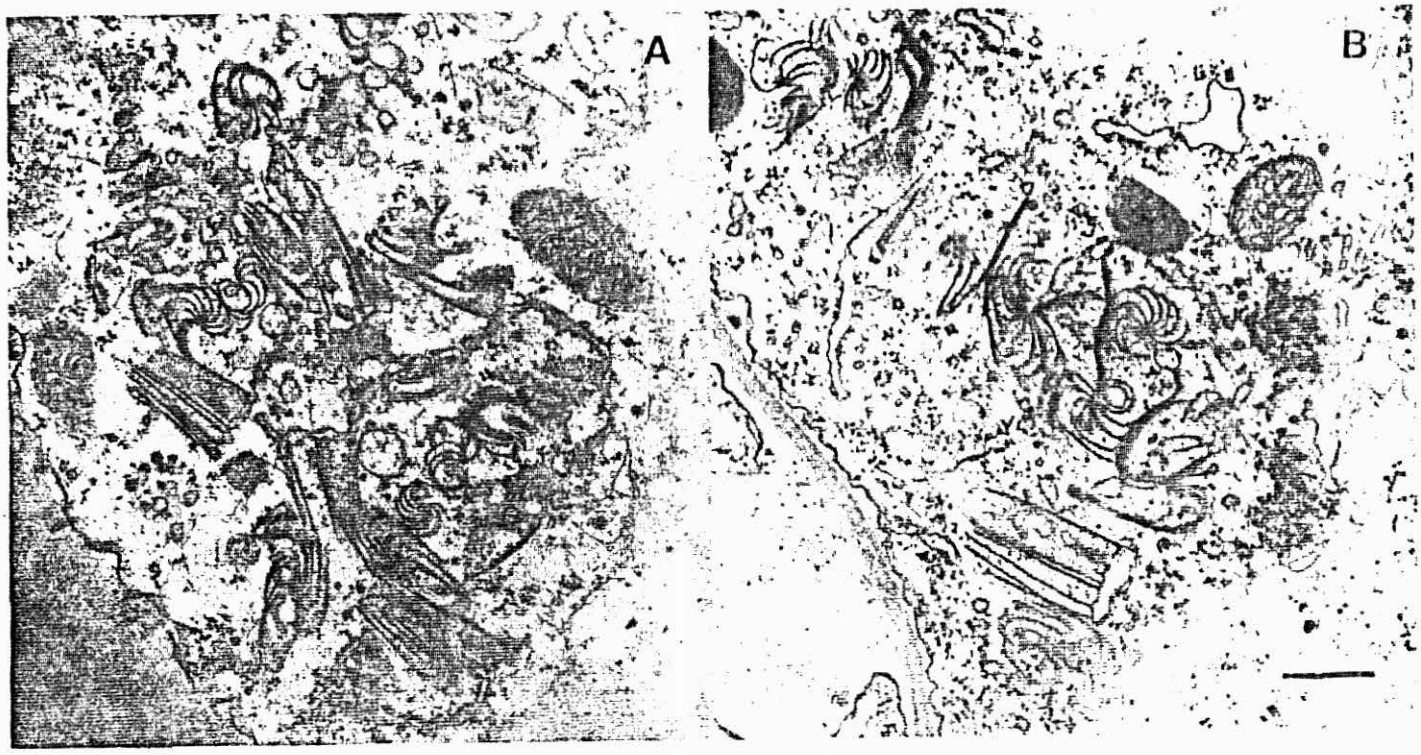


Fig. 4 Cytoplasmic cylindrical inclusions induced by (A) a potivirus isolated from Arachis pintoi, and (B) another potyvirus isolated from Stylosanthes spp., consisting of pinwheels (pw), scrolls (sc), and curved laminated aggregates (la).

Table 7. Comparative host range of two potyviruses isolated from the forage legumes Arachis pintoi and Stylosanthes sp., respectively, in Colombia, South America.

Plant Species	Local/Systemic Symptoms[x]	
	APV[y]	STV[z]
Leguminosae		
Arachis hypogaea	-/MT	-/MT
Arachis pintoi	-/RS	-/-
Canavalia sp.	-/MS	NT
Cassia occidentalis	-/MS	-/-
Centrosema brasilianum	-/MS	NT
Glycine max	LL/MT	-/-
Phaseolus vulgaris	LL/MS,N	LL/MS,N
Phaseolus lunatus	-/MT	NT
Pisum sativum	-/MS	-/MS
Stylosanthes macrocephala	-/MM	-/MM
Stylosanthes capitata	-/MM	-/-
Vigna radiata	-/SS	-/-
Vigna unguiculata	-/MS	-/MS
Other plant families		
Chenopodium quinoa	-/-	-/-
Chenopodium amaranticolor	LL/-	LL/-
Datura stramonium	-/-	-/-
Gomphrena globosa	-/-	-/-
Nicotiana benthamiana	-/MM	-/MM
Nicotiana glutinosa	-/-	-/-
Nicotiana tabacum	-/-	-/-
Physalis angulata	-/MM	-/-

[x] LL = local lesions; MM = mild mosaic; MS = mosaic; MT = mottle; N = Necrosis; NT = not tested; SS = symptomless; - = no infectionas determined by ELISA or electron microscopy test.

[y] APV = Arachis pintoi virus.

[z] STV = Stylosanthes sp. virus.

and insusceptible hosts can be found in Table 7.

Thin tissue sections of *A. pintoi* and *S. macrocephala* plants infected with the respective *Arachis* and *Stylosanthes* virus isolates, were shown by electron microscopy to contain cylindrical inclusions consisting of pinwheels, scrolls and short curved laminated aggregates (Figures 4 A, B).

The *Arachis* and *Stylosanthes* viruses were purified from their respective propagation hosts in quantities of 10 and 7.5 mg, respectively, per kg of infected tissue. Absorbance 260/280 values for these purified preparations ranged between 1.25 and 1.4, uncorrected for light scattering.

Electrophoretic analyses of these purified preparations in the presence of SDS, yielded single coat protein subunits of approximate molecular weight 34 kd, for both the Arachis and Stylosanthes viruses.

A precipitin reaction of identity was observed in Ouchterlony tests between peanut mottle virus (PMoV) and the *A. pintoi* virus in reciprocal tests. The PMoV antiserum also detected the *Stylosanthes* sp. virus in infected tissue extracts and purified preparations without the formation of spurs over adjacent PMoV precipitin reactions. Neither the *A. pintoi* virus nor the *Stylosanthes* virus was antigenically related to bean common mosaic virus, peanut stripe virus, soybean mosaic virus or watermelon mosaic virus-2 in any of the serological tests performed in this study.

ELISA tests of 3,451 *A. pintoi* seeds, using *A. pintoi* virus antiserum, did not detect the presence of this virus in any of the seeds. A similar assay of 4.880 *Stylosanthes* spp. seeds, using the *Stylosanthes* virus antiserum, also yielded negative results. In neither case, however, was the incidence of these viruses determined in the fields where the test seed was collected.

In SSEM tests, the antiserum to PMoV trapped approximately three and four times the average number of particles of the *Stylosanthes* and *A. pintoi* viruses, respectively, observed in untreated leaf dips (265 particles/1000 μm^2). No virus particle trapping occurred in these tests when soybean mosaic virus was included as control. Antisera to the *A. pintoi* and *Stylosanthes* viruses trapped 2,072 and 1,169 particles/1,000 μm^2, respectively, in homologous SSEM tests, and 1,169 and 790 particles 1,000 μm^2, respectively, in heterologous tests.

The commercial anti-potyvirus monoclonal antibody detected bean common

mosaic and soybean mosaic potyviruses but did not react with either the *A. pintoi* or *Stylosanthes* viruses in ELISA tests following standard overnight incubation periods (16 hr) of the IgG conjugate. However, when this incubation time was extended to 26 hr, a positive reaction was obtained with the *Stylosanthes* potyvirus.

Considering their particle morphology, the formation of cytoplasmic cylindrical inclusions in infected plant cells (7), and the molecular weight of their respective capsid protein subunit, we conclude that the two viruses isolated in this investigation from *A. pintoi* and *Stylosanthes* sp., are members of the potyvirus group (10). The serological relationship demonstrated here between these two viruses and a known member of the potyvirus group, peanut mottle virus (2), further supports this conclusion.

The failure of the anti-potyvirus monoclonal antibody to detect these legume potyviruses following standard ELISA procedures is puzzling, but this result has been confirmed in subsequent tests conducted by two independent laboratories in the United States, using the same potyviruses tested here (G.I. Mink, personal communication) and a strain of PMoV (F.W. Zettler, unpublished results).

The close serological relationship observed between the *A. pintoi* and *Stylosanthes* sp. potyviruses, and PMoV; their pathogenic reactions in several peanut genotypes; and the formation of cytoplasmic inclusions similiar to those induced by PMoV (6) lead us to propose that the two tropical forage legume potyviruses isolated in this study may be considered as strains of PMoV despite notable differences in their host range (particularly in the case of the *Stylosanthes* sp. potyvirus). The observation of differences in host range among PMoV strains, however, are not unique to this study (9,13,17) and are probably related to the lack of a universal set of differential peanut cultivars or diagnostic hosts tested with representative PMoV strains from different parts of the world.

Finally, we can not exclude the possible transmission of these PMoV strains in the seed of *A. pintoi* or *Stylosanthes* spp., since we ignore the actual incidence of these viruses in the localities where the seed tested in this investigation was produced, and PMoV has a relatively low seed-transmissibility in peanut (2). Moreover, *A. pintoi* is also vegetatively propagated (16) and, consequently, it is important to test for the absence of these potyviruses in vegetative and/or sexual seed of *A. pintoi* and *Stylosanthes* spp. before these promising tropical forage legumes become

widely distributed in the tropics.

Pathogenic variability of potyviruses isolated from *Centrosema* spp.

Last year, we reported on the detection of five potyvirus isolates (CP-1, CP-2, CP-3, SQ-15 and SQ-25) in field-grown *Centrosema* spp. Of these isolates, only one (SQ-15) reacted (Table 8) with antiserum produced to the first potyvirus previously isolated by the VRU from Centrosema spp., later identified as a strain of soybean mosaic virus (SMV-CE).

A comparative pathogenicity study of the above five isolates and the original SMV-CE potyvirus (Table 9) demonstrated that while the SQ-15 isolate is not the same virus as SMV-CE, it may be considered as yet another strain of soybean mosaic virus affecting *Centrosema* spp.

Regarding the SQ-25 isolate, Table 8 shows that this virus is serologically related to the *A. pintoi* and *Stylosanthes* spp. potyviruses described above. As shown in this table, a specific antiserum has been prepared to detect the CP-1 isolate. The remaining isolates, CP-2 and CP-3, though distinct from each other and the remaining *Centrosema* isolates, can be currently detected by antisera to watermelon mosaic virus-2 (wide reactivity with legume viruses) and by the anti-poty monoclonal antibody marketed by AGDIA, modifying the ELISA test to allow for longer conjugate incubation periods.

Table 10 shows the pathogenicity range of the six potyviruses isolated in four *Centrosema* species. It is thus quite evident that *Centrosema* spp. is susceptible to a significant number of distinct legume potyviruses. This finding clearly demonstrates the susceptible nature of most legumes to a wide range of viruses, and suggest that considerable attention should be given to the early detection and control (eradication) of these viruses from *Centrosema* germplasm.

Regarding the transmission of the above described *Arachis*, *Centrosema*, and *Stylosanthes* potyviruses via sexual seed, the VRU has preliminary evidence from tests conducted with infected bean cvars suggesting that the CP-3 isolate is highly seed-transmissible in *Phaseolus vulgaris*. The SMV-CE has already been reported as seed-borne in *Centrosema* spp. and *Phaseolus vulgaris*.

Table 8. Serological double inmunodifusion tests with five potyvirus isolates from Centrosema spp. using four legume virus virus antisera.

As/Ag	CP-1	SMV-CE	Arachis	Stylosanthes
CP-1	+	-	-	-
CP-2	-	-	-	-
CP-3	-	-	-	-
SQ-15	-	+	-	-
SQ-25	_	_	+	+

Table 9. Comparative pathogenicity study of five potyviruses isolated from Centrosema spp. and the Centrosema isolate of soybean mosaic virus in selected legume host.

Cultivar	Isolates					
	CP-1	CP-2	CP-3	SQ-15	SQ-25	SMV-CE
Bean cvar.						
Dubbele Witte	L,S*	S	S	S	L,S	S
Stringless Green Refugee	L,S	L,S	S	S	L,S	L,S
Redlands Greenleaf C	L	-	-	-	L	L
Redlands Greenleaf B	-	-	-	-	L	L
Michelite 62	L,S	L	L,S	L	L	L
Sanilac	S	L	L,S	L	L	-
Pinto 114	L	-	L	-	L	-
Monroe	L	L	L	L	L	L,S
Widusa	L,N	-	L,N	-	L,S	L,N,S
Black Turtle Soup	L,N	-	L,N	-	L,S	L,N
Jubila	L	L	L	L	L	L,N
Topcrop	L	-	L,N	-	L,N	-
Amanda	L	-	L	-	L	-
Bontiful	L,S	S	L,S	L,S	L,S	S
Diacol Calima	L,S	S	S	S	S	n.t.
Soybean cvar.:						
ICA-Linea 121	L,S	L	S	L,S	S	S
Clark	-	-	-	S	-	S
Rampage	-	-	-	L,S	-	S
Davis	-	-	-	-	-	-
York	-	L	-	-	-	-
Marshall	L,S	-	S	-	L,S	-
Ogden	S	L	-	-	S	-
Kwanggyo	-	-	-	-	-	-
Buffalo	-	L	-	-	-	-
coupea cvar.:						
Cabecita Negra	S	L,S	S	S	S	S

* L: local lesions on inoculated leaves; S: systemic infection
N: systemic necrosis; -: no infection; nt = not tested.

Table 10. Pathogenicity of six potyvirus isolates in selected *Centrosema* species.

Centro Isolate	*C.acutifolium*	*C.brasilianum*	*C.macrocarpum*	*C.pubescens*
CP-1	+ *	+	-	+
CP-2	+	+	-	+
CP-3	+	+	+	+
SQ-15	+	+	+	+
SQ-25	+	+	+	+
SMV-CE	+	+	+	+

* + = systemic infection; - = no systemic infection (E.M. and serology negative).

CASSAVA VIRUSES

Introduction

Progress was made over a broad range of cassava viruses and virus-like diseases. Cassava Colombian Symptomless and Cassava American Latent Viruses were added to the cassava viruses that can be routinely identified using the enzyme linked immunosorbent assay (ELISA). The molecular characterization of Cassava Common Mosaic Virus is progressing and the sequencing of over half of the virus is complete. The area with the most progress is the characterization of the agents of Caribbean Mosaic and Frogskin Diseases. The interpretation of the results indicates that the causal agent of Caribbean Mosaic Disease is a virus that is member of a new subgroup of the phyto-reoviruses. A similar but distinct virus is the causal agent of the mosaic symptoms associated with Frogskin Disease. Additional tests are in progress to determine if all the symptoms associated with this disease can be attributed to this virus.

Frogskin and Caribbean Mosaic Diseases.

Frogskin (FSD) and Caribbean mosaic diseases (CMD) are both virus-like disorders of unknown etiology that are present in Colombia. These two diseases were reported as distinct because the root symptoms associated with FSD are either absent or very mild in plants with CMD. There is significant yield loss caused by CMD in susceptible cassava, but there are many cassava cultivars that are tolerant of this disease. There are few reports of cassava clones that are resistant or tolerant to FSD. Secundina is the cassava clone used for the detection of both CMD and FSD, since both diseases produce mosaic symptoms on the leaves of Secundina. CIAT has *in vitro* culture clones and greenhouse grown stakes of Secundina that are disease-free and are available for indexing programs.

Virus-like particles were found in the leaves, petioles, and stems of cassava plants that were infected with either FSD or CMD (Fig. 5). These particles are approximately 80 nm in diameter and are similar in size and morphology to reovirus particles. Virus-like inclusion bodies were found only in the roots of the cassava cultivar MCol 113. This cultivar develops root symptoms but no leaf symptoms.

Double-stranded RNAs were purified from cassava plants infected with either CMD or FSD and run on both agarose and polyacrylamide gels. On agarose

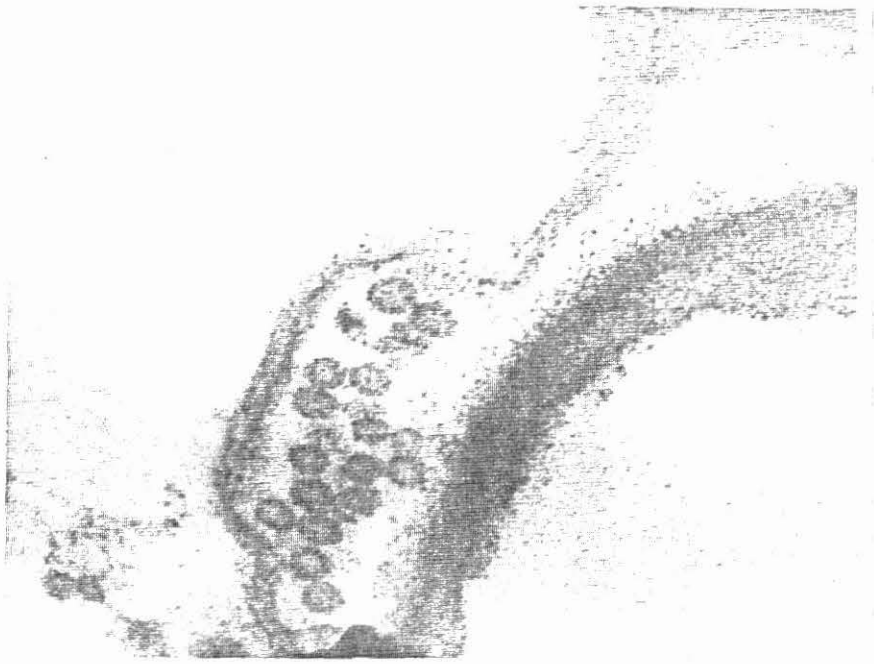


Figure 5. Virus-like particles found in a thin section of cassava leaves. The virus-like particles were found sometimes in groups as shown in the photograph, but more frequent scattered through the cytoplasm.

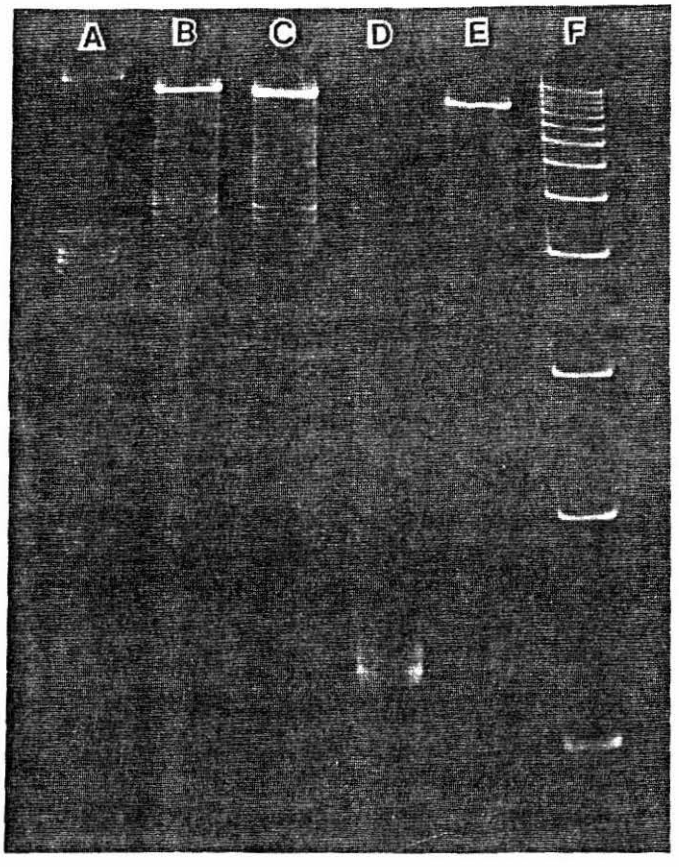


Figure 6. A polyarcylamide gel showing the ds-RNA segments associated with infected frogskin (FSD) and Caribbean mosaic (CMD) diseases. Lane A is a molecular wieght marker of ds-RNAs, lane B is CMD isolate 5, lane C is FSD isolate 29, lane D is FSD isolate 80, lane E is CMD isolate 86, lane F are ds-DNA markers.

gels, there appear to be three or four bands, but on polyacrylamide gels there are ten bands for FSD and nine bands for CMD (Fig. 6). The number and size of the ds-RNA bands are different between FSD and CMD. The relative molecular weights (M_r), the number of the ds-RNAs found in CMD and FSD infected plants are similar to genomes of the Fiji subgroup of the phyto-reoviruses (Table 11). Reoviruses have unique genomes that consist of either 10 or 12 segments of ds-RNA. Table 5 lists the sizes of the ds-RNA segments of selected phyto-reovirus and the ds-RNA segments found in CMD and FSD infected plants. The are genomic segments are often of similar or equal size in the characterized phyto-reoviruses. Since several of the bands in both FSD and CMD are similar in size only 3 or 4 bands are detected by agarose gels, and 9 or 10 bands are resolved using polyacrylamide gels. Polyacrylamide gel have greater resolving power. It is predicted that there are10 segment of ds-RNA, but only nine bands found in polyacrylamide gels of CMD. There are probably two genomic segments of equal M_r.

The whitefly *Bemisia tuberculata* has been suspected as the vector of FSD, since the results of experiments using field collected whiteflies. Until this year, the only virus-like disease which had been transmitted by whiteflies is the WF isolate. The WF isolate originates from whiteflies collected in the field and used to inoculate healthy cassava plants. The plants infected with the WF isolate showed mosaic symptoms on the indicator clone Secundina but did not show root symptoms typical of FSD.

Manipulating the acquisition times of the vector increased the efficiency of transmission. The isolate 29 of FSD was consistently transmitted although the rate of transmission varied from 10-60% (Table 12). The acquisition period of one day gave the highest rates of transmission. According to these results, the disease agent does not need to replicate in the vector, but the whiteflies do need a minimum acquisition period of one day before they are able to transmit the virus. Table 3 is a list of the virus-like diseases transmitted by *Bemisia tuberculata*.

The plants which developed the mosaic symptoms in the transmission tests were analyzed for the presence of ds-RNA species. Both the mother plants used as the source of inocula and the plants infected in the transmission experiments had similar ds-RNA patterns. Also leaf dips of the infected plants contained virus-like particles with diameters of 80 nm. There were no virus-like particles or ds-RNAs in the healthy control plants.

Table 11. Molecular weights of dsRNA segements of phytoreoviruses and *Fijiviruses compared with the ds-RNA segments of selected isolates of frogskin* disease (FSD) and caribbean mosaic disease (CMD).

Genomic Segment	WTV	RDV	FDV	RBSDV	FSD	CMD
1	2.90	3.10	2.90	2.75	2.70	2.75
2	2.40	2.50	2.50	2.24	2.60	2.60
3	2.20	2.20	2.48	2.19	1.80	2.30
4	1.80	1.80	2.48	2.14	1.75	1.80
5	1.78	1.76	2.12	2.00	1.35	1.30
6	1.10	1.05	1.80	1.82	1.30	1.25
7	1.05	1.02	1.45	1.37	1.25	1.20
8	0.83	0.78	1.21	1.17	1.20	0.71
9	0.57	0.70	1.12	1.11	1.15	0.67
10	0.55	0.67	1.08	1.06	0.68	
11	0.54	0.48				
12	0.32	0.48				

The size estimates for the phyreoviruses and the Fijiviruses are from Plant Viruses Volume 1: Structure and Replication Editor C.L. Mandahar pages 220-221. These viruses are wound tumor virus (WTV), rice dwarf virus (RDV), Fijivirus (FDV), and maize rough dwarf virus (MRDV). The estimate for the FSD is from isolate 29 and the estimate for CMD is from isolate 5. These were compared with ds-RNA markers which were isolate from mycoviruses and were provided by Dr. R.L. Bozarth of Indiana State University.

Table 12. Virus-transmission test of the frogskin disease isolate 29 using *Bemisia tuberculata* as the vector.

Source plant and disease	Acquistion [1] period	Transmission period	Number of plants with symptoms	Appearence of symptoms in days
Secundina FSD 29	24 hours	5 days	(3/5)[2]	13-15
Secundina FSD 29	24 hours	5 days	(2/10)	12-15
Secundina FSD 29	24 hours	5 days	(1/10)	12
Secundina FSD 29	24 hours	5 days	(1/10)	15
Secundina FSD 29	24 hours	5 days	(2/10)	12-14
Secundina FSD 29	24 hours	5 days	(1/10)	12

1. 25 *B. tuberculata* per plant were used for all of the experiments.

2. The number (infected/total number) of plants in the experiment.

Table 13. List of isolates of viral-like diseases that have been transmitted to cassava by the whitefly *Bemisia tuberculata.*

DISEASE	ISOLATE	SOURCE PLANT	RECEPTOR PLANT	RESULTS DATE
WF-mosaic	3	WF from field[1]	Secundina	Transmission 1986
FSD	29	Secundina	Secundina	Transmission 1990
CMD	80	Secundina	Secundina	Transmision 1990
CMD	86	Secundina	Secundina	Transmission 1990

1. This isolate originated from whiteflies that were collected from the field. Later experiments showed that this isolate was transmitted by *B. tuberculata.*

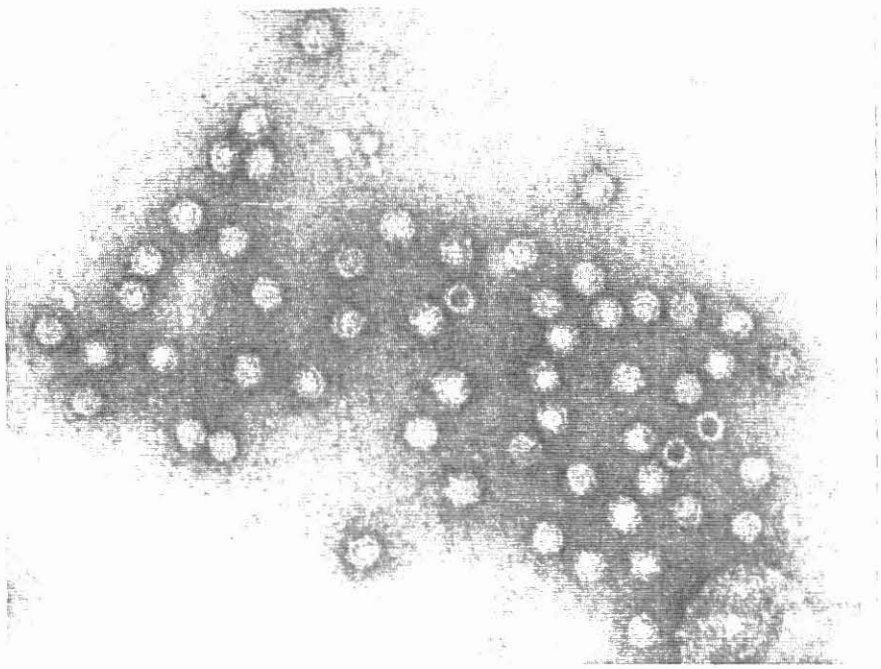


Figure 7. Virus-like particles that were found in *B. tuberculata* that were fed on plants infected with isolate 29 of frogskin disease. Similar particles were found in *B. tuberculata* that were fed on plants infected with Caribbean mosaic disease. No virus-like particles were found in whiteflies that fed on healthy plants.

Bemisia tuberculata were fed on plants infected with either CMD or FSD and examined using insect dip preparations for virus-like particles. There were virus-like particles of 80 nm in diameter in these samples. These were similar in size and morphology to the virus-like particles found in plants infected with CMD or FSD. The structure of the virus-like particles extracted from *B. tuberculata* are more distinct than the structure of the particles found in the plants. Figure 7 shows the morphological structures of these virus-like particles. Whiteflies that fed on healthy plants contained no virus-like particles. Using blind tests the whiteflies that had fed on the infected plants were consistently identified.

In conclusion, viruses of similar in morphology and genomic structure to the phyto-reovirses are associated with CMD and FSD. Both of these viruses are transmitted by the whitefly *B. tuberculata.* Additional experiments are needed to determine that all of the symptoms associated with CMD and FSD are present in the plants infected by the whiteflies. Currently some of the plants infected in the transmission experiments are being grown in a small field plot in a screen house to determine the type of root symptoms caused by the virus transmitted by the *B. tuberculata*. Additional experiments will be conducted in field trials.

Based on the ds-RNA patterns, it appears that the virus that associated with CMD is distinct from the virus associated with FSD. These viruses appear to be member of a new subgroup of phyto-reoviruses based on their morphology and genomic structure. The main difference between the Fiji subgroup of the phyto-reoviruses and the viruses associated with CMD and FSD are the type of vectors. The vectors of phyto-reoviruses are leafhopper or planthoppers, and all except wound tumor virus (WTV) infect only monocotyledon plants. The vector of viruses associated with CMD and FSD is B. tuberculata and the only known host for these viruses is cassava a dicotyledon plant.

Most of the phyto-reovirus are unstable viruses that are difficult to purify. Still knowing the type of virus that one is trying to purify usually makes the task easier. The development of a rapid assay method to these viruses will be a priority during the next year.

Potexviruses in cassava.

Cassava Colombian Symptomless Virus (CCSpV) was originally isolated from cassava infected with CMD. There are no symptoms produced in cassava infected only with CCSpV, and it is only present in some of the characterized isolates of CMD or FSD. An antiserum to CCSpV provided by Dr. Harrison of SCRI, and this antiserum is being used to screen germplasm for the presence of this virus. CCSpV is serologically related to CsXV but distinct from Cassava X Virus (CsXV). Although neither virus causes any appearent disease in cassava, CIAT germplasm is being screened by ELISA for both of these viruses to help assure virus-free germplasm.

The molecular characterization of cassava common mosaic virus (CCMV) is continuing and the sequence of approximately half of the genome is complete. Figure 8 is a diagram of the predicted molecular organization of the virus. Based on homology at the RNA and protein sequences, CCMV is most closely related to potato virus X (PVX). The Cassava Trans project, based in Dr. Roger Beachy's laboratory in Washington University, has successfully demonstrated that coat protein mediated cross protection of CCMV is effective in *Nicotiana benthamiana*. The major technical limitation of its deployment as a resistance gene in cassava is the transformation of cassava.

Cassava Vein Mosaic Virus.

A survey of viruses infecting cassava was conducted in the northeast section of Brazil. The states surveyed include parts of Bahia, Perambuco, and Cerea. Much of this area is semi-arid and is the area for the selection and production of the germplasm for the IFAD project. Based on the observations of symptoms, cassava vein mosaic virus is widespread throughout these areas of Brazil. Typical infection rates were 20-30%, but some fields had 100% infection rates. The loss yield in cassava infected with CVMV is unknown, but cassava does produce good yields even though the virus is prevalent. This virus disease is probably similar to CCMV which causes yield losses of 20-30% in infected plants. More information on the losses caused by this virus is needed to determine the importance of this viral disease. Also the development of rapid diagnostic techniques for the detection of CVMV is needed to facilitate the exchange of clean germplasm.

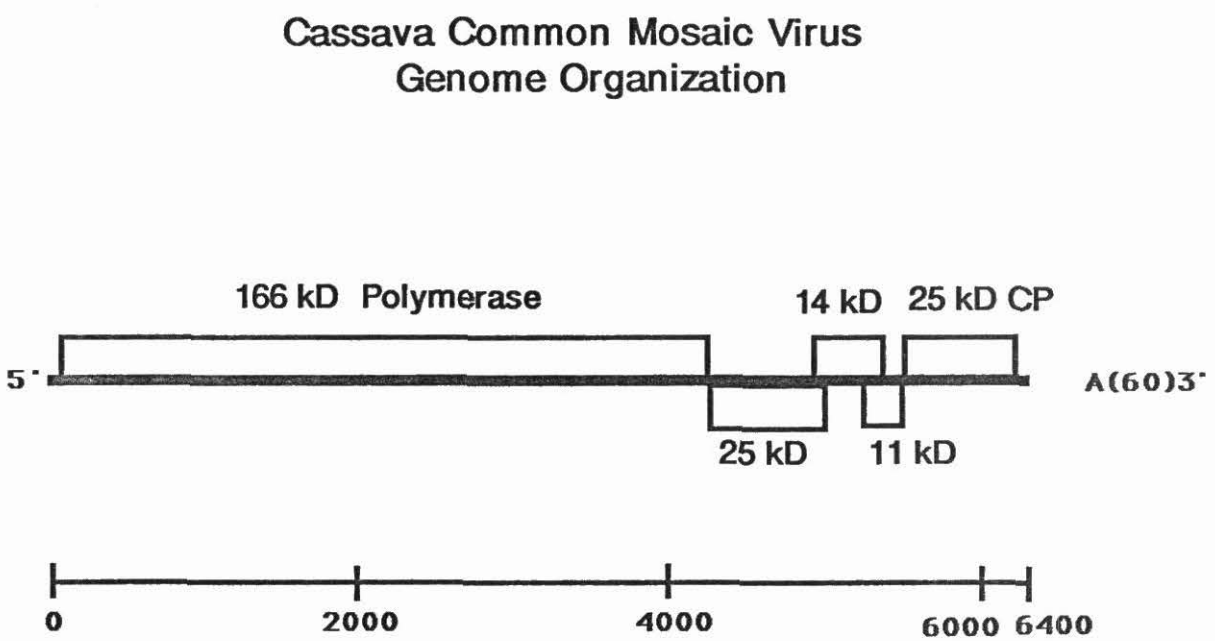


Figure 8.

The size in kilodaltons (kD) of the four putative proteins and there position on the genomic map are shown in the diagram. The 166 kD protein shares homology with other polymerase genes and the 25 kD CP is the coat protein.

Cassava American Latent Virus.

A new nepovirus of cassava was reported by Dr. B. Walter of CIRAD (Colmar, France). The virus was isolated from samples that were infected with CCMV which were collected around Manaus Brazil and in French Guyana. These are humid lowland areas which are isolated from the Andean regions of South America. The virus does not produce symptoms or appearent disease in cassava. It is not known if the virus causes any yield losses or if the virus is seed transmitted.

Since nepoviruses are frequently seed transmitted, a survey of the seeds being sent to IITA in Nigeria was conducted using an antiserum to the virus that was provided by Dr. Walter. For the seedlots that will be sent to IITA in 1991 both seedlots and motherplants were tested for the virus. The results which were all negative, and it appears that this virus is not present at CIAT.

More work needs to be done to determine the distribution, seed transmission, and effect on yield of this virus. CALV (like CCSpV and CsXV) is a minor problem that causes no obvious disease. Additional surveys will be done at CIAT, but all tests to date have been negative and the virus probably is not present at CIAT headquarters.

Rice Hoja Blanca Virus

Introduction

A cooperative project with Dr. Anna-Lisa Haenni of the Institut Jacques Monod to characterize the genome of Rice Hoja Blanca Virus (RHBV) was begun this year. The goals of the project include the identification of viral encoded proteins, the assignment of functional roles to these products, and the determination of the transcription and translation strategies of RHBV. A cDNA library of the RHBV genome will be made and the primary structure of the genome will be determined by sequencing the cDNA clones. The molecular characterization of plant viruses has led to the development of novel methods to control plants viruses. The most recent example is the coat protein mediated cross protection method which has been shown to either reduce or delay symptoms and to increase the yields for more than 10 groups of plant viruses. Several other types of molecular mechanism that interfere either with the ability of the virus to replicate or to move systemically throughout the host are being tested are being tested as ways to control viral infections. Basic information on the structure of the RHBV genome is essential before projects such as coat protein mediated cross protection can be initiated.

Another goal of the project is to develop methods to determine the relationship of the different isolates of RHBV. One potential method is the use of cDNA probes in hybridization studies. Currently there is little information on different isolates of RHBV because the antisera available react equally with all the isolates tested. Also RHBV has a limited host range which is another traditional method of classifying isolates of a virus. Additional studies have been initiated to determine if there are biotypes of the vector which is the planthopper *Sogatodes orizicola.* A better knowledge of the variation within the RHBV isolates and the vector is needed to understand the complex relationship that is the basis for the periodic epidemics of RHBV.

In vitro translation studies.

The RHBV-RNA was purified and translated *in vitro* using a rabbit reticulocyte lysate system. At least four protein products are translated from the total RNA. One of the proteins is precipitated by an antiserum to the 17 kD inclusion body protein. This protein appears to be translated from the RNA 4

of RHBV. The in vitro experiments are still in progress and more information on mapping the translation products to specific species of RNA is needed to develop a genomic map.

Hybridzation experiments.

Hybridization studies were begun using the first-strand synthesis products of total RHBV-RNA. The cDNAs were generated using random primers and were labeled with $^{[32]}$P-dATP. Both RHBV-RNA transferred to nitrocellulose filters and dot blots from infected rice were detected by this method. A major advantage of the dot blot technique is the easy of preparing samples in the field with the minimum of equipment. Once the filters are prepared the sample is stable for many months. Rice showing symptoms of RHBV was collected from four different sites in the Tolima valley. The RHBV-cDNA hybridization probe reacted similarly with the isolates from the different localities (Fig. 9) under conditions of high strigency. When the hybridization conditions are highly strigent, the assay detects only isolates that share areas of homology. These results mean that a wide range of the RHBV isolates both at CIAT and in the Tolima valley are very similar. The RHBV cDNA probe does not react with the Echinocola strain of RHBV (Fig. 10) under conditions of high stringency. The Echinocola strain of RHBV and isolates of RHBV that infect rice are closely related serologically. This type of hybridization probes is useful in distinquishing closely related strains within the RHBV complex. Based on these results, this technique can supplement serology in the detection of RHBV isolates and distinquish differences within the RHBV complex. The development of probes that are more specific and potentially more useful for strain identification will depend on the successful cDNA cloning of RHBV.

Vector studies.

Isozymes were used to analyze differences among the vector (*Sogatodes orizicola*) of RHBV. Several isozyme patterns were found even within the colony of *S. orizicola* that is kept at CIAT using the esterase enzyme. Therefore the population of Sogatodes is heterogeneous for this trait. Variation in the esterase isozyme patterns were found in the planthoppers collected from Tolima Fig. 11). Normally only a small percentage of the leafhoppers in the field are efficient vectors the virus. Even the colony needs to be continually screened to maintain a high percentage of vectors within the planthopper population. Efforts will continue to find a phenotypic marker that correlates with the ability to vector RHBV. A phenotypic trait that is

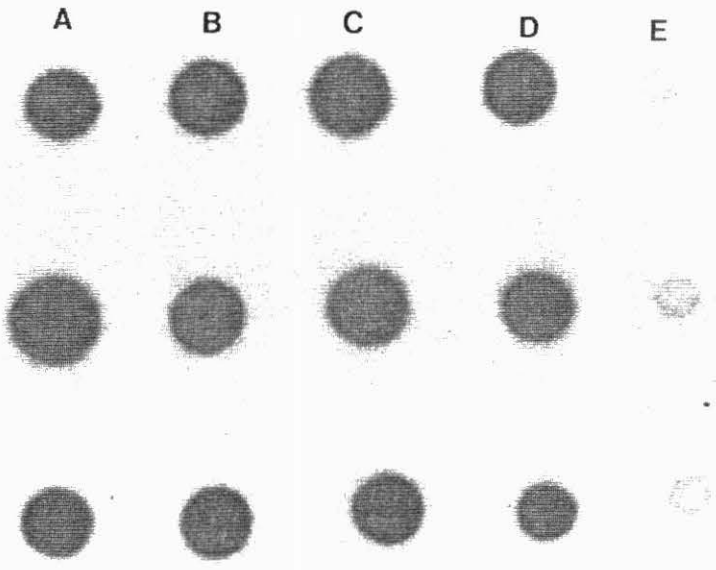


Figure 9. A dot blot hybridization using a first-strand cDNA probe prepared from RHVB-RNA using random primers. Three samples of rice which showed symptoms were collected from four sites in the Tolima. Group A were collected from Mpo de Ambalema, Finca Las Brisas and are the cultivar Oryzira III, Group B were collected from Mpo Armero, Hda Santvario and are the cultivar IR-22, Group C were collected from Mpo de Ambalema, Finca La Plazuela and are the cultivar Linea 2, Group D were collected form Mpo de Ambalema, Finca La Plazuela and are the cultivar LLano 5, Group E are the healthy controls. The probe hybridized with all the samples and indicates that they all share homologous regions.

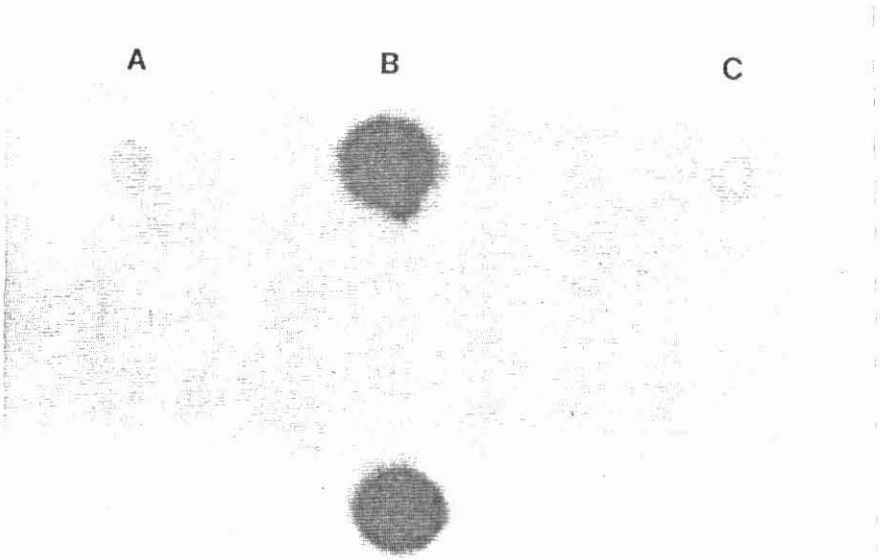


Figure 10. A dot blot hybridization using a first-strand cDNA probe prepared from RHBV-RNA using random primers. Duplicate samples were prepared for each of the isolates. Group A is healthy rice, Group B are infected with the homologous strain of RHBV, and Group C is the Echinochloa strain of RHBV. The filters were hybridized and washed under conditions of high strigency. Only the homologous RHBV strain reacted with the probe.

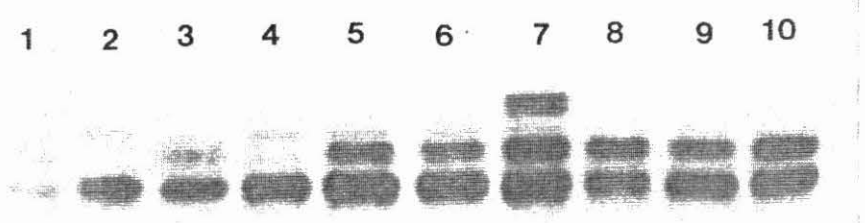


Figure 11. Esterase isozyme variation within a population of *Sogatodes orizicola* from one locality in Ambalema, Tolima. All samples were winged adults. 1-3 are males and 4-10 are females. In the males there are three patterns and in the females there are three patterns. Isozyme diversity within the populations appears to be common and are one method to study the heterogenity of the RHBV vectors.

with the ability to transmit virus could make the identification of vectors easier than the current method. The development of rapid assays for the ability to vector virus would allow easier determination of the status of the field populations and lead to better models to predict epidemics. If a threshold number of vectors could be determined this could lead to recommendations for insecticide use that are more efficacious.

PUBLICATIONS

In Refereed Journals

Morales, F., Niessen, A.I., Castaño, M., and Calvert, L., 1990. Detection of a strain of soybean mosaic virus affecting tropical forage species of *Centrosema*. Plant Disease 74: 648-650.

Morales, F.J., Niessen, A.I., Ramirez, B.C., and Castaño, M. 1990. Isolation and partial characterization of a geminivirus causing bean dwarfing mosaic. Phytopathology. 80: 96-101.

In Books

Thottappilly, G., Russel, H.W., Reddy, D.V.R., Morales, F.J., Green, S.K., and Makkouk, K.M. 1990. Vectors of Virus and Mycoplasma Diseases: An Overview. In. Insect Pests of Tropical Food Legumes. S.R. Singh Ed. J. Wiley & Sons, New York pp. 323-342.

Submitted in 1989 to Refereed Journals

Morales, F. J., and Singh, S.P. 1991. Genetics of resistance to bean golden mosaic virus in Phaseolus vulgaris. Euphytica: in press.

SPECIAL AWARDS IN 1990

Premio Nacional de Fitopatologia

In both the professional (Miss Ana C. Velasco) and student categories (Miss Maritza Cuervo).

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. (U. of W.) Gilbertson (UC-Davis), F. Morales (CIAT).

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).

Rockefeller Rice Biotechnology

TITLE: "MOLECULAR CHARACTERIZATION OF RICE HOJA BLANCA VIRUS."

Institutions: Institut Jacques Monod, CIAT-VRU.

Principal Investigators: A.L. Haenni, B. C. Ramiez (IJM) and L. Calvert (CIAT).

VIROLOGY RESEARCH UNIT

PERSONNEL

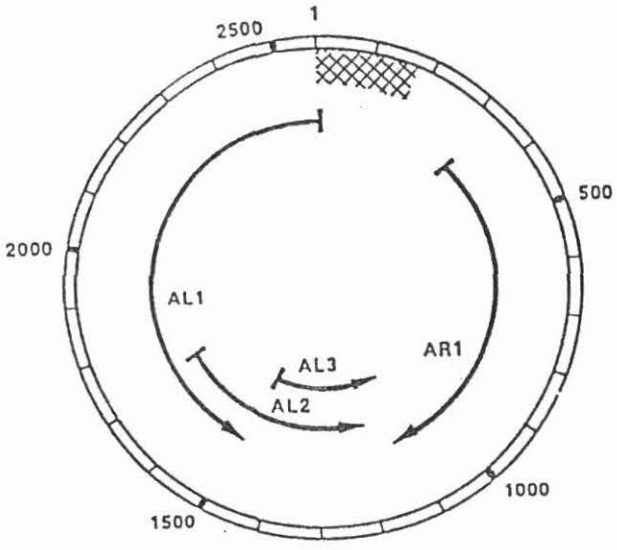
Francisco J. Morales	Senior Staff Head-Virologist
Lee A. Calvert	Senior Staff Virologist
Mauricio Castaño	Research Associate II
Elena Reichel	Research Associate II
José Alejandro Arroyabe	Assistant I
Ana Cecilia Velasco	Assistant II
Luis Miguel Constantino	Assistant II
Maritza Cuervo	Assistant II
Catalina Yumi Masuda	Assistant II
Ivan Lozano P.	Technician I
Guillermo Guzmán	Technician II
Armando Bedoya	Technician II
Carlos Julio Alvarez	Technician III
Maria Consuelo Martínez	Technician III
Rosa Marina Jiménez	Worker II
Cesar Tulio Rodríguez	Worker II

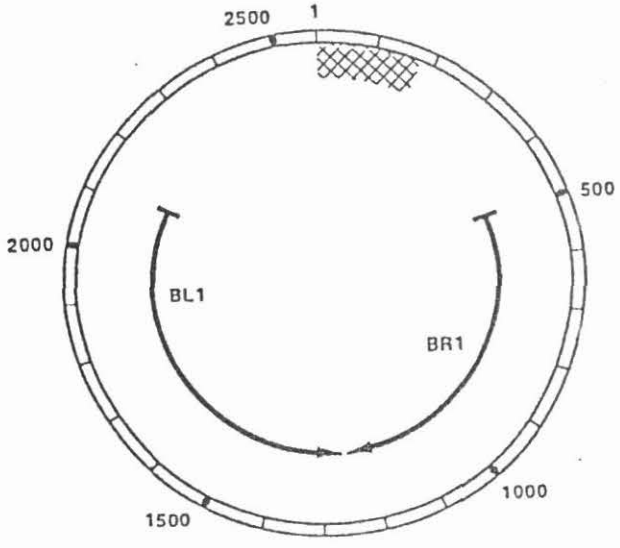
Apolinar Umaña	Worker II
Gloria López	Secretary V
Bertha Cecilia Ramirez *	Research Associate II

(*) Left 1990

APPENDIX

MOLECULAR APPROACHES FOR CONTROL OF BEAN GOLDEN MOSAIC VIRUS





A

COLLABORATIVE PROJECT

BY:

BEAN/COWPEA CRSP/USAID

PSTC/USAID

CESDA, Dominican Republic

University of Nebraska-Lincoln

University of Wisconsin-Madison

MUCIA Indonesia Program/World Bank Funds

CIAT

AGRACETUS

EMBRAPA, Brazil

University of Puerto Rico

CSRS/USDA

90crsp/10-11-90

BEAN/COWPEA CRSP FY 90 Annual Report
Maxwell/Wisconsin-Saladin/Dominican Republic-Morales/CIAT

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

October 1, 1989-September 30, 1990

Personnel:

US:

Department of Plant Pathology
and
Institute for Molecular Virology
University of Wisconsin-Madison
Madison, WI 53706

Dr. Douglas P. Maxwell, US-PI
Dr. Paul G. Ahlquist
Dr. Robert L. Gilbertson (funded by CRSP)
Dr. Josias C. Faria
Mr. Stephen F. Hanson
Ms. Elisabeth S. Hidayat
Ms. R. Teresa Martinez
Ms. Denise Smith
Ms. Eunice Zambolim
Ms. Amy O. Loniello (funded by CRSP)
Ing. Maria Rojas
Ms. Mercedes Otoya

Agracetus, Inc.
8520 University Green
Middleton, WI

Dr. David R. Russell

CIAT:

CIAT, Cali, Colombia

Dr. Francisco J. Morales
Dr. Lee Calvert

University of Puerto Rico
Mayaguez, Puerto Rico
Dr. James S. Beaver

University of Nebraska
Lincoln, NE
Dr. Dermont Coyen

DOMINICAN REPUBLIC:

CESDA
SEA
Dominican Republic

Ing. Freddy Saladin, HC-PI
Lic. Aridia Figueroa

1990 ANNUAL REPORT

TABLE OF CONTENTS

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

EXECUTIVE SUMMARY

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

Bean golden mosaic is a major factor reducing yields of beans in many areas of Latin America. Some fields typically experience 100% losses. In the HC of the Dominican Republic, bean golden mosaic geminivirus (BGMV) was so serious in October 1989 that growers were required to destroy the bean crop to reduce the potential of infection of the next bean crop.

This project has undertaken to molecular characterize the geminiviruses causing bean golden mosaic and related geminiviruses that infect beans. Past results have shown that the isolate of BGMV from Brazil is distinct from the isolates from the Caribbean and Central America. This year, an isolate of bean dwarf mosaic geminivirus (BDMV) was cloned and sequenced and because of the sequence divergence (30%) between this isolate and the isolates causing golden mosaic symptoms, it was considered a separate bean-infecting geminivirus. Also, full-length clones of the component B of bean calico mosaic geminivirus from Mexico were obtained. Cloning of the geminivirus from a common weed, Rhynchosia minima from Puerto Rico, was initiated. The sequence information from the various bean-infecting geminiviral isolates was used to develop isolate-specific probes for the detection of BGMV-BZ, BGMV-GA/BGMV-DR, and BDMV. The general probe and these isolate specific probes have been used to evaluate the presence of geminiviruses in samples of weeds and crop plants from Dominican Republic, Costa Rica, Puerto Rico, Honduras, El Salvador, Egypt, South Africa, Argentina, Florida-USA, and Utah-USA. The general geminiviral probe detected the new geminivirus in tomatoes from Florida and in some ornamemtals from Florida and Utah. Several weeds were found to be infected with geminiviruses, but generally not those infecting beans. Initial studies were started on the use of polymerase chain reaction techniques for measuring variability of bean-infecting geminiviruses. The first step toward the development of a virus-derived scheme for resistance was acheived in collaboration with Agracetus, Inc., Middleton, WI. Cloned DNAs for the four geminiviral isolates were found to be infectious on beans when radicles were inoculated by electric discharge particle acceleration methods (particle gun). It is now possible to initiate mutational analyses studies to determine functions of various parts of the viral genome. A dominant lethal scheme involving the putative replicase gene is being persued as a possible strategy for creation of transgenic beans resistant to BGMV. Agracetus, Inc. has initiated a major program to develop the technology to transform beans using electric discharge particle acceleration technology.

Complimentary DNA clones of the protein coat region of bean common mosaic polyvirus were produced and the coat protein gene is being sequenced.

In collaboration with CIAT, restriction fragment length polymorphism analysis of Xanthomonas campestris pv. phaseoli isolates from various geographical regions showed that there was considerable variability between isolates when a plasmid (p2) or a chromosomal (p7) probe was used.

Training included the following individuals who were not funded by CRSP: Dr. Josias C. Faria, a visiting scientist, from Brazil (DNA sequencing), Ms. Eunice Zambolim, a research specialist, from Brazil (molecular characterization of bean common mosaic virus), Ms. Mercedes Otoya, a research specialist, from CIAT (molecular characterization of the bacterial common blight pathogen), Ms. E. Hidayat, a graduate student, from Indonesia (development of an isolate-specific probe for BDMV), Ms. Teresa Martinez, a research specialist, from the Dominican Republic (molecular characterization of bean calico mosaic geminivirus), Ms. Denise Smith, a graduate student, from USA (mutational analysis of the component B), Ms. Maria Rojas, a graduate student, from Costa Rica (genetic variability of geminiviruses), and Mr. Steve Hanson, research specialist, from USA (site-directed mutagenesis of AL1 gene and sequence of BDMV). CRSP provided funding for an Assistant Scientist, Dr. Robert L. Gilbertson, and partial support for an undergraduate student, Ms. Amy O. Loniello.

Team members presented papers at the Bean Improvement Cooperative Conference in Toranto, Canada, and the Annual Meeting of the American Phytopathological Society, Grand Rapids, MI. Invited workshops on the application of DNA probes for detection of plant pathogens were given at the 82nd meeting of the Quebec Society for Plant Protection, Quebec City, Quebec, Canada and the American Phytopathological Society Meeting. Invited papers on CRSP research were presented at the Advanced Research Workshop on Beans held at CIAT, Cali, Colombia.

FY 90 ANNUAL REPORT
Molecular Approaches for the Control of Bean Golden Mosaic Geminivirus

I. PROGRESS

A. Specific Research Contributions

1. Research results from FY 90 disseminated and in use

...Molecular characterization of four isolates of bean-infecting geminiviruses has shown that the golden mosaic inducing isolates in the Caribbean and Central America are distinct from those in Brazil and that separate breeding programs for disease resistance should be developed for these two regions.

...Bean dwarf mosaic geminivirus (BDMV) DNAs A and B were sequenced and sequence comparisons with other geminiviruses showed that BDMV is a distinct bean-infecting geminiviruses and is not closely related to other geminiviruses.

...General and isolate-specific geminiviral DNA probes have been developed. These probes were used to detect geminiviruses in bean, weeds, and other crop plants from Argentina, Brazil, Costa Rica, Dominican Republic, Egypt, El Salvador, Honduras, Puerto Rico, South Africa, and USA. Agdia, Inc., a diagnostic laboratory, in Indiana has recently made arrangements with the Wisconsin Alumni Research Foundation to evaluate our general geminiviral probe for use in their commercial laboratory. Our results indicate that weeds are not a major source of BGMV inoculum in the Dominican Republic. Use of our general probe showed that tomatoes in Florida and Costa Rica were infected with a whitefly-transmitted geminivirus. Also, we found that several ornamentals were infected with geminiviruses in Florida and Utah.

...In collaboration with Agracetus, Inc., clones of the four geminiviral isolates were found to be infectious by inoculating bean radicles with cloned DNA using electric discharge particle acceleration methods (particle gun). It was not possible to infect beans with cloned DNA by standard mechanical inoculation methods. This makes it possible to initiate mutational analysis studies to develop virus-derived schemes for the creation of transgenic plants with resistance to BGMV.

...Sequence aligments of the putative replicative gene for the sequenced geminiviruses indicated that there may be an ATP binding site in this protein; and thus, this catalytic site is an appropriate target for evaluation of the dominant lethal scheme for creation of transgenic beans resistant to BGMV and other geminiviruses, e.g. the tomato geminivirus causing problems in Florida, Mid-East and Central America.

...Scientists at Agracetus, Inc. have had some initial success transforming bean tissue using a modification of the methods they employ for soybean transformation.

...Polymerase chain reaction methods were used to show that the cloned BGMV-DR DNAs are representative of the BGMV in the Dominican Republic.

...Clones of BGMV-GA were used by Agracetus, Inc. to explore the possibility of constructing a minichromosome to assist in the transformation of soybeans.

...Bean germplasm was evaluated for the Dominican Republic and CIAT. It was found that two landraces (Pompadour G and Pomedadour J) from the Dominican Republic, which were thought to be resistant, were susceptible and did not give typical golden mosaic symptoms when infected with BGMV-DR. Also, there was a correlation between symptom development to BGMV-DR and moderate levels of field resistance to BGMV.

...Several bean cultivars immune to BDMV were detected and these will be used in an inheritance study of factors controlling disease resistance to geminiviruses.

2. Other research-related results

a. Germplasm conservation and use

Pompadour J and Pompadour G were collected by F. Saladin as part of the University of Nebraska CRSP and these were evaluated in the Dominican Republic, Puerto Rico and Wisconsin. This seed has been provided to CIAT.

b. Seed production-not part of this project

c. Impact of other CRSP-produced technologies

Some interest has been expressed by researchers in the use of the pathovar specific DNA probes for the detection of Xanthomonas campestris pathovar phaseoli. These probes will be provided to CIAT , University of Nebraska, and University of Puerto Rico for future studies on variability in X. c. pathovar phaseoli.

d. Project impact on production of beans-not applicable at this time.

e. How results address needs of small-scale farmers and women.

Technology is being developed which will provide better control strategies for one of the major constraints to bean production in Latin America. This will have a positive impact on

yield and quality of beans. Every effort is being made to provide technical training for women. Nine women have received training in biotechnology during FY 90.

f. Other research results of importance

...The coat protein region of the bean common mosaic polyvirus, an RNA virus, was cloned.

...Restriction fragment length polymorphism analysis of 26 isolates of Xanthomonas campestris pv. phaseoli and xcp var. fuscans indicated that considerable genetic variability exists in this pathovar. This should be considered when cultivar are evaluated for disease resistance.

3. Changes in national production of beans in HC

The University of Nebraska CRSP project is coordinating a baseline survey of bean production in the Dominican Republic to use in an assessment of the impact of PC 50, which is being increased for future release to growers.

B. Institutional Development and Training

It should be noted that this project has not had funds for training of HC personnel and funds have been available for one postdoctoral fellow or scientist. Other funds have been sought for training personnel and nine individuals have received training this year. Also, there has not been a host country budget for this project.

1. Cite changes since FY 89

Since FY 89, Ms. Maria Rojas (funded on a USAID scholarship from Costa Rica) has started a M.Sc. degree program, Ms. Ann Batista, a Ph.D. student in Molecular Biology did a rotation in our laboratory, Ms. Eunice Zambolim (funded by the Brazilian Government) is receiving special training in molecular virology of viruses, Ms. Mercedes Otoya from CIAT received three-months training on RFLP analysis,and Ms. Mary Bett (funded by a State research assistantship) is a Ph. D. graduate student who started a rotation in our laboratory in August. Considerable effort was devoted to bring Lic. Aridia Figueora from the Domininican Republic to our laboratory for three-months, but she could not get a VISA because she was on the immigration list.

2. Over life of project

A PSTC/USAID grant provides funds for Ms. R. Teresa Martinez from the Dominican Republic to receive training in virology and vegetable crop pest management. Efforts are underway as indicated by a letter from Director Metz to have her hired at CESDA. She is funded by a PSTC/USAID grant until December 1991.

3. Project training to be completed by end of extension period

Ms. R. Teresa Martinez from the Dominican Republic (2.5 years, funded by PSTC/USAID). Ms. Mercedes Otoya from CIAT (3 months, funded by CIAT). Ms. Denise Smith from USA will complete an M.Sc. (funded by State and Hatch). Ms. Elisabeth S. Hidayat from Indonesia will complete an M.Sc. (funded by the World Bank). Ms. Eunice Zambolim from Brazil (one year funding by Brazilian government) will have received training in molecular virology of RNA plant viruses. Dr. Robert L. Gilbertson (funded by CRSP) from USA will have received 2 years training and he has accepted a faculty position at University of California-Davis. He is also Co-PI on the Malawi CRSP project. It is expected that Dr. Wayne McLaughlin from the University of West Indies will receive three months training in the summer of 1991.

C. Progress toward Objectives Listed in Log Frame

1. How long has this CRSP project been engaged in the lines of research addressing these objectives?

An intensive research program on BGMV was initiated in January 1987 when Dr. Gilbertson went to the University of Florida to receive training on methods for mechanical transmission of geminiviruses.

2. Original time estimates for achieving objectives

..Cloning and sequencing BGMV-BZ, BGMV-GA, BGMV-DR and BDMV-CO: This has been achieved.

..Development of general and isolate-specific probes for above listed bean-infecting geminiviruses (four): This has been achieved and manuscript has been accepted for publication in Plant Disease.

..Develop a BGMV/protoplast system for virus replication studies: This has not been achieved and may not be necessary since the full-length clones can be used to infect plants using the particle gun.

..BGMV DNA modified by site-directed mutagenesis to form a dominant lethal: These experiments are underway for site direct mutagenesis of the putative ATP binding site in the AL1 gene product.

3. Estimated time remaining to achieve the objectives in log frame

The research is on schedule as a result of an extremely hard effort by personnel involved in the project and the

expenditure of nonCRSP funds at about 300% of the CRSP funds. It is estimated that the first dominant lethal scheme for plant virus resistance will be evaluated in a model system by the end of this extension period. Transgenic bean plants with the viral coat protein gene will be available for evaluation in the five-year extension period.

4. Relation of research in log frame to other research

Our project is the major research project in the world on bean-infecting geminiviruses and it is not duplicated anywhere. Our efforts compliment those at CIAT as CIAT scientists they have done the initial characterization of the geminiviral isolates, BGMV-GA, BGMV-DR, and BDMV-CO, and we have then done the molecular characterization of these isolates. All our research efforts are coordinated with Dr. Francisco Morales, CIAT virologist. He collects plant samples on his trips to Africa and South American and then we probe these samples with our isolate-specific geminiviral DNA probes. This allows CIAT personnel to better understand the range of genetic diversity in bean-infecting geminiviruses and allows them to plan their breeding programs. Our Wisconsin team also works closely with Dr. Judy Brown, University of Arizonia in the characterization of bean calico mosaic geminivirus. She completed the host range and transmission studies and our group cloned part of the virus. Our studies on germplasm evaluation and inheritance studies are closely coordinated with the Univeristy of Nebraska/DR CRSP project and University of Puerto Rico/Honduras CRSP project. We have also provided geminivirus identification for scientists in Egypt, Costa Rica, and South Africa. These scientists did not have access to someone who was willing to help them with geminivirus identification.

The most closely related geminiviruses research project is at Washington University, St. Louis and directed by Dr. R. Beachy. His research team has recently obtained a detectable level of resistance to tomato yellow leaf curl geminiviruses in transgenic Nicotiana benthamiana plants expressing viral coat protein.

5. Research contributions

Our characterization of bean-infecting geminivirus has provided clear evidence that breeding programs need to be designed for specific regions, eg. Brazil or the Caribbean and Central America. Also, the use of our isolate-specific probes have shown that weeds may not be the main source of inoculum in the Dominican Republic. Also, our general probe for whitefly-transmitted geminiviruses is being developed commerically by Agdia, Inc. The research on dominant lethals may provide the basis for a new approach for the control of geminiviruses and other viruses. Stimuation of research on the

development of a bean transformation system using biolistic approaches may have considerable impact on future development of beans. Once transgenic beans are available with resistance to geminiviruses, it is expected that this techology could be applied to cultivars for many regions and these resistant cultivars will have a significant impact on bean yields in Latin America (potentially increasing yields by 10-20%).

D. Evidence of Biological/Social Integration

1. Attention to relevant WID issues

Our project has made a major effort in the training of women in biotechnology and this past year, nine women have received training on this project. Only two men have received training.

2. Other issues

In our field trip to the Dominican Republic, R. Teresa Martinez discussed the growing of beans which were developed by transformation technology with several individuals at various levels, e.g., seedsman, experimental station workers, growers, and administrators. There was not a concern as long as the transgenic plants were better than current beans.

E. Collaboration with Other Groups

Since we started working on molecular characterization of bean common mosaic potyvirus (an RNA virus), we received virus isolates from Dr. M. Silbernagel (Washington State CRSP) and Dr. J. Kelly (Michigan State CRSP). Also, Dr. Roy French, USDA scientist at the University of Nebraska, has been very helpful. Our efforts on RFLPs of the common bacterial blight pathogen involved Dr. M. Pastor-Corrales, bean pathologist from CIAT. He supplied the isolates for the study and a paper will be published jointly with him and his technican, M. Otoya. We also have started to process samples for Ing. Roger Meneses, CATIE, Costa Rica for the detection of geminiviruses in vegetables.

II. FUNDING/FISCAL MANAGEMENT IN FY 90

A. Problems Regarding Funding in HC

Since no HC funds have been available, this has created a research program in which very little responsibility has been associated with the HC. Funding level by CRSP has been extremely low for the expected output in the area of molecular biology research and training.

B. Adequancy of Current Management, etc.

Last November, a three person review panel visited our

geminivirus team at the University of Wisconsin and reviewed all aspects of the project. It is pleasing to note that our project received the highest rating along with several other projects by the EEP.

C. Activity Towards Buy-ins and/or Other Funding

Dr. Maxwell participated in the CRSP effort to establish a research project on beans with the Agricultural Research Center, Giza, Egypt. This is likely to result in a project funded by USAID with emphasis on biological nitrogen fixation. Recently, a trip to Costa Rica was made to discussion geminivirus research with people at CATIE, the Universidad de Costa Rica, and the USAID Mission. It is hoped that Costa Rica will become a HC in the next extension period. Additional funding for this project was obtained from the following sources in FY 90: Hatch (support for half-time specialists, $10,000), University of Wisconsin Graduate School (3 months of a visiting scientist, $5,000), State funds (one research assistantship, $12,500, and one half-time specialist, $10,000), USAID Scholarship from Costa Rica (one research assistantship, $12,000), PSTC/USDA grant ($39,000 for visiting scientists, and trainee), CNPQ from Brazil (one trainee, about $6,000), and World Bank (one research assistantship, $18,000). It is also important to note that Agracetus, Inc. donated all the time and resources for sequencing the component A of BDMV and for the particle gun experiments with the viral cloned DNAs and they have make a major commitment to bean transformation (estimated contribution over $20,000). This is a total of $132,500 in direct costs or $190,800 including indirect costs. The CRSP budget for last year was about $78,000 for direct and indirect. Thus, there is about 2.5 times as much support from nonCRSP sources and this does not include faculty salaries.

III. STATUS IN FY 90

A. Appropriateness of Activities to Goals of Golbal Plan

The Golbal 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, Inc., University of Puerto Rico, University of Nebraska, and University of Wisconsin-Madison. 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 and that other bean-infecting geminiviruses are present in Colombia and Mexico. This would indicate that breeding programs for resistance need to be continued for these different strains and geminiviruses. The general geminiviral DNA probe has been used to detect geminiviruses in weeds and other plants, e.g. tomatoes.

The isolate-specific probes for the different geminiviruses can be used to determine if weeds are a main source of inoculum. This information can then be used in designing better control strategies.

Major effort will be devoted to using recombinant DNA technology to develop beans resistant to BGMV. Considerable progress was made when we found that the full-length clones of the four geminiviral isolates were infectious. This will allow studies on genome function and the development of virus-derived schemes for creation of transgenic beans. Additionally, Agracetus, Inc. is developing a bean transformation using modifications of the soybean technology which they have developed. 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, PSTC, CIAT, CESDA-Dominican Republic, Agracetus, Inc., University of Puerto Rico, University of Nebraska, and University of Wisconsin, 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 in FY 90

If funds for Dr. Robert L. Gilbertson, an Assistant Scientist, are assigned to training, then 60% of the direct costs of the CRSP are for training. Our project has never had an adequate training component and other resources have been sought. Thus for FY 90, funds for three M.Sc. students, three trainees and one visiting scientist were obtained from other sources. See section Part II, C. for additional details.

The training program for FY 90 was essentially as expected in FY 89 except Maria Rojas joined our program form Costa Rica and we didn't know for certain if this was going to work out.

C. Balance of US vs. Overseas Activities

Because of the highly technical nature of the research; which involves techniques associated with molecular biology and special resources, the major research effort has been at the University of Wisconsin and Agracetus, Inc. This year more effort was devoted to collecting field samples in the Dominican Republic than in the past; and it is expected that additional experiments on whitefly transmission of geminiviruses in weeds to beans can be undertaken in the coming year. The field research for the evaluation of germplasm is conducted in the Dominican Republic in cooperation with the University of Nebraska and University of Puerto Rico.

D. Level of Cooperation Between US and HC Personnel

Drs. Maxwell and Gilbertson and Ing. Teresa Martinez visited the Dominican Republic in February to collect samples and arrange for future research. In April, Ing. Freddy Saladin, HC-PI, visited the University of Wisconsin and plans were discussed for future research and budgets. Since funds for a HC budget have not been available, only minimal effort has been devoted to this process. Our project serves as the major research project on bean-infecting geminiviruses for Latin America and thus research priorities are decided by the team which includes CIAT personnel. In July, Dr. James Beaver, Dr. Morales (CIAT), Dr. Silbernagel (Washington State University), and Dr. Pastor-Corrales (CIAT) visited our laboratory to discuss future research. These visits have provided opportunities for the international scientists to interact directly with the geminivirus team and scientists from Agracetus, Inc.

E. Relative Contributions Towards Objectives

1. USAID/CRSP, University of Wisconsin, and HC contributions to budget

USAID/CRSP:

Direct costs (Funds for assistant scientist, supplies, and travel)	$53,884
Indirect costs	$23,709
TOTAL	$78,000

US, University of Wisconsin:

Specialist	$10,000
Research Assistantship	$12,500
Graduate School	$5,000
TOTAL	$27,500

Salary for Drs. Maxwell and Ahlquist provided by the University of Wisconsin and are part of the matching funds ($18,877). Other sources of funds discussed in section II.C. (page 9).

HC:

HC funds were used for support of HC-PI and Aridia Figueroa (about 5-10% of their time was devoted to CRSP activities for this project).

2. Other funding

Funds spent at UW-Madison (direct costs):

Hatch (used for a half-time specialist)	$10,000

PSTC/USAID (used for support of trainee, supplies and travel)	$36,000
CIAT (used for support for Ms. M, Otoya, CIAT for 3 months)	$ 6,000
USAID Scholarship from Costa Rica (M.Sc. graduate student)	$12,000
CNPQ, Brazil (trainee from Brazil)	$ 6,000
World Bank-Indonesia program	$18,000
Gift funds (travel)	$ 2,000
TOTAL	$90,000

Estimate of funds provided by Agracetus, Inc.

Scientists at Agracetus sequenced BDMV component A and provided the primers needed for sequencing component B. They also provided the resources that we used to show that our viral clones from four isolates were infectious.

Estimated value: $20,000

Importance of these funds: Non CRSP funds provide about 65% of the financial resources needed for us to achieve the current level of training and research activities. The contribution of Agracetus, Inc. can not be over emphasized. Without their help, we would not have known that our viral clones were infectious and a considerable effort (1-2 yr) may have been devoted to this activity rather than four months. We are now in a position to start a mutational analysis of the viral genome.

Also, Agracetus, Inc. has started an effort to transform beans with the coat protein gene from BGMV-GA. They were not interested in bean transformation until we started working with them in August 1989.

F. Support of USAID Mission

We have always visited USAID mission personnel during our trips to the Dominican Republic and had a useful exchange of information. Unfortunately, the USAID personnel had to refuse permission for Lic. A. Figueroa to come to our lab for training because she is on an immigration list. This is a USAID policy.

G. Institutionalization in Host Country/US

1. Faculty recognition

Dr. Maxwell has been appointed to serve on the College's Internation Agricultural Program Commitee and his CRSP project

activities will be featured in the International Ag Newsletter from UW-Madison. Ag Administration and Departmental faculty have been very understanding of Dr. Maxwell's need to devote time to this CRSP project. As a Departmental Chair, this has caused some stress on other faculty and office staff.

2. Integration of commodity resarch programs with CRSP

Dr. Maxwell had approved a new HATCH project on "Develoment of transgenic Phaseolus vulgaris plants with resistance to geminiviruses" and he also contributed to the preparation of the new W-150 regional project on Genetics and Breeding Beans for Improved Disease Resistance and Yield.

Internationally, Dr. Maxwell was appointed to the newly formed Steering Committee of the Advanced Bean Research Network in September 1990 at an international workshop at CIAT, Cali, Colomiba. He will be representing the CRSP Technical Committee.

3. Internal project management

Dr. Maxwell does all the administrative management of the project and Drs. Gilbertson, Ahlquist and Maxwell have formulated research direction policies. Dr. Gilbertson has been the day-to-day advisor for the research on BCMV, Xcp and PCR technologies.

Dr. K. Shapiro, Director of International Programs for the College, was recently assigned the position of Institutional Representative. He has been extremely supportive and has provided some administrative personnel to help with paperwork associated with USAID trainees. He also visited our research facilities and discussed the CRSP research program in considerable detail. He has also been supportive of Dr. Maxwell's travels to Egypt and Costa Rica.

4. Student/Professor relationships

Dr. Maxwell has had a challenging year as "mentor" to so many Latin women and U.S. women students. He devotes as much time as he can to their special educational and social needs. An undergraduate student, Amy O. Loniello, supported by CRSP won a research award scholarship for her research proposal on characterization of bean calico mosaic geminivirus and she also received the first D. J. Hagedorn Fellowship in plant pathology. (Dr. Hagedorn started this B/C CRSP project in Brazil in 1982 and he established a scholarship fund when he retired.)

H. Other Comments

Dr. Maxwell will resign as Departmental Chair on June 30, 1991 so that he can devote more time to the research and training activities associated with this CRSP project.

Much of the success of this project has been the result of Dr. Robert L. Gilbertson's efforts. He departed October 1, 1990 for UC-Davis and he will be a Co-PI on the Malawi CRSP project. Our team will miss him in many ways.

IV. PUBLICATIONS AND PRESENTATIONS IN FY90

A. Publications

Gilbertson, R. L., S. H. Hidayat, R. T. Martinez, S. A. Leong, J. C. Faria, F. J. Morales, and D. P. Maxwell. 1991. Differentiation of bean-infecting geminviruses by nucleic acid hybridization probes and aspects of bean golden mosaic in Brazil. Plant Disease (In Press).

Gilbertson, R. L., J. C. Faria, S. F. Hanson, F. J. Morales, P. Ahlquist, D. P. Maxwell, and D. R. Russell. 199-. Infection of beans (Phaseolus vulgaris) with cloned geminviral DNA mediated by electric discharge particle acceleration. Phytopathology (submitted for publication).

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 Dis. 74:322-327.

Gilbertson, R. L., J. C. Faria, S. F. Hanson, P. G. Ahlquist, F. J. Morales, D. P. Maxwell, and D. R. Russell. 1990. Infection of beans (Phaseolus vulgaris) with cloned geminviral DNA mediated by electric discharge particle acceleration. Ann. Report Bean Improvement Cooperative 33:165-166.

Gilbertson, R. L., J. C. Faria, S. F. Hanson, E. Hendrastuti, P. G. Ahlquist, F. J. Morales, and D. P. Maxwell. 1990. Relationships among bean-infecting geminviruses. Ann. Report Bean Improement Cooperative 33:72-73.

Gilbertson, R. L., E. Hendrastuti, R. T. Martinez, and D. P. Maxwell. 1990. The application of nucleic acid probes for the detection of plant pathogens. Proceedings of the 82nd Annual Quebec Society for Plant Protection (In Press).

Gilbertson, R. L., and D. P. Maxwell. 1990. Common bacterial blight of bean. Chapter for Plant Diseases of International Importance. (In Press).

B. Presentations

Faria, J. C., R. L. Gilbertson, S. F. Hanson, P. Ahlquist, and D. P. Maxwell. 1990. Variabilidade do virus do

mosaico dourado do feijoeiro e uso de sondas para a sua characterizacao. Brazilian Bean Research Meeting, May 1990.

Gilbertson, R. L., J. C. Faria, S. F. Hanson, E. Hendrastuti, P. G. Ahlquist, F. J. Morales, and D. P. Maxwell. 1989. Relationships among bean-infecting geminviruses. Bean Improvement Cooperative Meeting, November, 1989, Toronto, Ontario, Canada.

Gilbertson, R. L., E. Hendrastuti, R. T. Martinez, and D. P. Maxwell. 1990. The application of nucleic acid probes for the detection of plant pathogens. 82nd Annual Quebec Society for Plant Protection, June, 1990, Quebec, Canada.

Gilbertson, R. L., E. H. Hidayat, and D. P. Maxwell. 1990. DNA probes for detection of plant pathogens. Workshop at the American Phytopathological Society, August, Grand Rapids, MI.

Gilbertson, R. L., J. C. Faria, S. F. Hanson, F. J. Morales, P. Ahlquist, D. P. Maxwell, and D. R. Russell. 1990. Infection of beans with cloned geminiviral DNA mediated by electric discharge particle acceleration. Annual Meeting of the American Phytopathological Society, August, Grand Rapids, MI.

Faria, J. C., R. L. Gilbertson, F. J. Morales, D. R. Russell, P. G. Ahlquist, S. F. Hanson, and D. P. Maxwell. 1990. Sequence of infectious clones of two mechanically transmissible isolates of bean golden mosaic geminviruses. Annual Meeting of the American Phtyopathological Society, August, Grand Rapids, MI.

Maxwell, D. P. 1990. Biotechnology and International Agriculture. Biotechnology Workshop for Industry, March 1990,. Milwaukee, WI.

Maxwell, D. P. 1990. Molecular biology of bean-infecting geminviruses. Advanced Research Workshop, September 1990, CIAT, Cali, Colombia.

Gilbertson, R. L. 1990. Biotechnology and implications for research on bean diseases of beans. Advanced Research Workshop, Septemer 1990, CIAT, Cali, Colombia.

APPENDIX

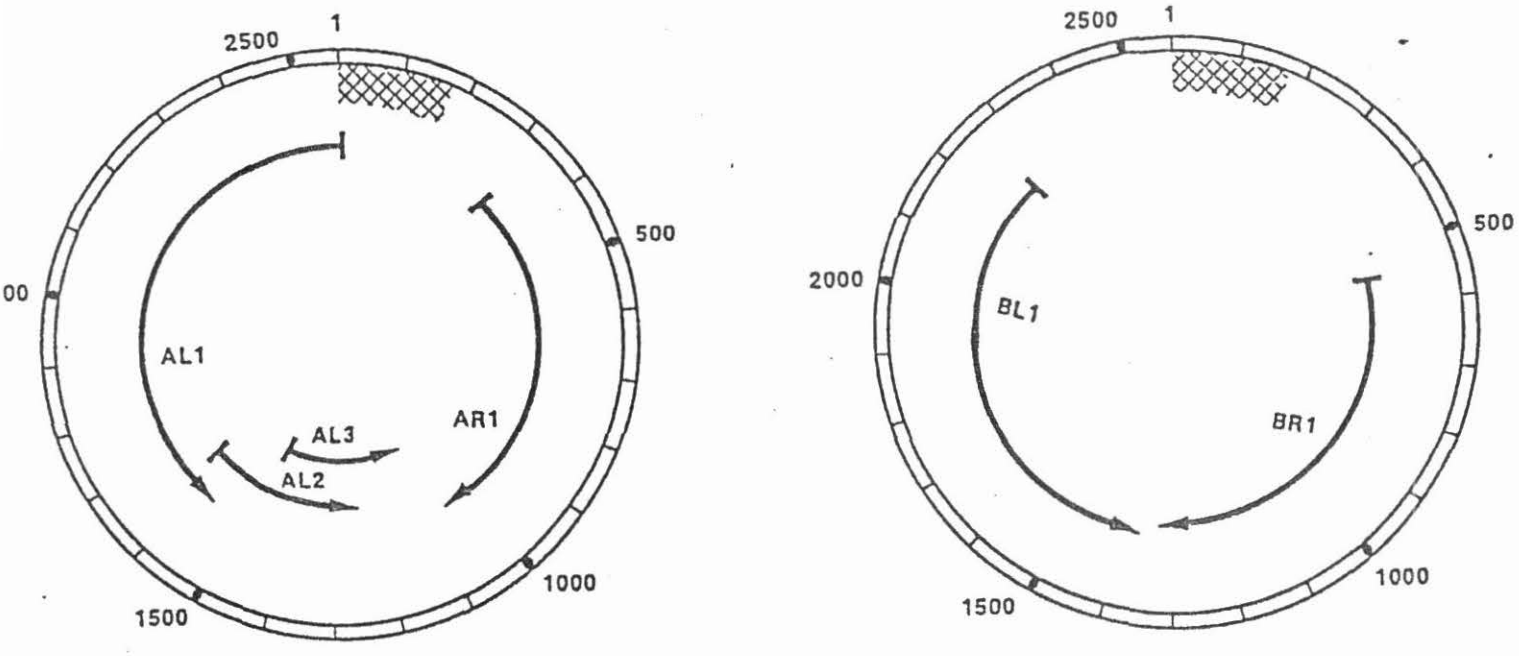


Fig. 1. Schematic map showing common region (cross hatched region starting in position number 1), open reading frames (genes, e.g. AR1), for the replicative forms (component DNA A, left circular DNA molecule, and component DNA B, right circular DNA molecule) of bean dwarf mosaic geminivirus isolate from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

Fig. 2. Sequence of bean dwarf mosaic geminivirus DNA A.

```
   1  TGGCATTTTT GTAATAAGAG CTGGTACTCC AGTTGAGTTA CTCCAATTCC
  51  CCCCTCTCAA AACTATCTCA TTCTATTGGA GTATTGGAGT TACTTATATA
 101  CTAGAACCCT CAATCTGGTT TCGGAACACG TGGCGGCCAT CCGTATAATA
 151  TTACCGGATG GCCGCGCTTC GGAGTACGCT CTCTCTCTCC CTTTAATTTG
 201  AATTAAAGCG CACTGCTTTC GTCTCAGCCA ATCATATTGC GCCTGACGAG
 251  CTTAGATATT TATAACAACT TGGGCCCTAA GTTGTTGGTT GTACGATATA
 301  AATTAAAGGT AACCCGGCCC ACTGTCTTTA ACTCAAAATG CCTAAGCGCG
 351  ATGCCCCATG GCGCTCTATG GCGGGAACGA CAAAGGTCAG TCGCAATGCC
 401  AATTACTCTC CCCGTGGGGG AATTGGGCCA AAGATGACAA GGGCCGCAGA
 451  GTGGGTTAAC AGGCCCATGT ACAGGAAGCC CAGGATCTAT CGAACGCTAA
 501  GGACGCCTGA CGTCCCACGA GGTTGTGAAG GCCCATGTAA GGTGCAGTCT
 551  TATGAACAGC GTCACGATAT TTCACATGTT GGGAAGGTAA TGTGTATCTC
 601  TGATGTCACA CGTGGTAATG GCATTACTCA CCGTGTTGGC AAGCGTTTTT
 651  GTGTTAAGTC TGTGTATATT CTAGGAAAGA TTTGGATGGA TGAGAATATC
 701  AAGCTCAAGA ACCACACGAA CAGTGTTATG TTCTGGTTGG TCAGGGACCG
 751  TAGACCGTAT GGAACGCCCA TGGATTTCGG CCAGTTGTTC AACATGTTTG
 801  ACAACGAGCC CAGCACTGCC ACGGTTAAGA ACGATCTTCG CGATCGTTTT
 851  CAAGTTATGC ATAAGTTCTA TGGGAAAGTC ACAGGTGGAC AGTATGCGAG
 901  CAATGAACAG GCAATCGTCA AGCGTTTTTG GAAGGTCAAC AATCATGTGG
 951  TTTACAATCA TCAAGAGGCT GGCAAGTATG AGAATCATAC GGAGAACGCC
1001  TTATTATTGT ATATGGCATG TACACATGCC TCTAATTCTG TGTATGCAAC
1051  TCTGAAAATT CGGATCTATT TTTACGATTC GATCATGAAT TAATAAAGTT
1101  TGAATTTTAT TGAATGATAT TCTATTACAT GAGTTACATA CGATCTGTCT
1151  GTTGCGAATC GAACACCTCT AATGACATTG TTAATGGATA TAATTCCTAA
1201  TTGATCTAAA TACATAATAA CTAAATGTTT AAATCTATTT AAATAAGTCG
```

Sequenc of BDMV DNA A continued.

1251 ACCCAGAAGC TGTCGTCGAT ATCGTCCAGA CTTGGAAGTT CAGGAAGGCT
1301 TTGTGGAGAT GCAACGCTCT CCTCAGGTTG TGGTTGAACC GTATCTGTAC
1351 GCTGTATACC CTGCTGTTGG TGTACAACGG TTCCTCTACT CTGTATATCT
1401 TGAAATAGAG GGGATTTTCT ATCTCCCAGA TATACACGCC ATTCTCTGCT
1451 TGAAGTGCAG TGATGAGCTC CCCTGTGCGT GAATCCATGT CCCGTACAAC
1501 CTATGTGTAT GTAGATGGAG CAACCGCATT CCAGGTCAAT GCGGCGTCTC
1551 CTGATCGCCC GCCGCTTGGC TTGCCTGTGT GCCTTCTTGA TAGAGGGTGG
1601 AGTCGAGAGT GATGAAGATT GCATTCTTCA ACGTCCAATT CCTTAGCGCT
1651 GTATTTTCCT CTTTGTTTAG GAAATCTTTA TAGCTGGCAC CCTCACCAGG
1701 ATTGCATAGC ACGATTGATG GGATCCCTCC TTTAATTTGA ACAGGCTTGC
1751 CGTACTTGCA ATTTGACTGC CAATTCTTTT GGGCCCCAAT CAATTCCTTC
1801 CAGTGCTTTA ACTTTAGATA ATGCGGTGCG ACGTCATCAA TGACGTTATA
1851 CTCCACTTCG TCTGTGAACA CTTTGGAATT GAAGTCTAGG TGTCCACTTA
1901 AATAATTATG TGGGCCTAGT GCTCGAGCCC ACATCGTCTT CCCTGTTCTC
1951 GAATCACCTT CTACTATGAG ACTTACTGGT CTTTCTGGCC GCGCAGCGGA
2001 ACCTCTCCCG AAATAATCGT CTGCCCACTC TTGCATCTCG TCTGGAACGT
2051 TAGTAGACGA AGAGAGGGGA AACGGAGGAA CCCATGGTTC CGGAGCCTTT
2101 TTGAATATGC TGGTGGCGTT AGCGACCAGG TTGTGATGCT GAAGGAAGAA
2151 ATGTTGCGGT TGTTCTTCCT TTATTATGCG CAGAGCTTGC TCTGCGGATC
2201 CTGCATTTAA CGCCTTTGCG TATGTATCGT TAGCAGACTG CTGACCTCCT
2251 CTAGCAGATC TGCCGTCGAT TTGAAACACT CCCCATTCGA CGGTGTCGCC
2301 GTCTTTGTCG ATGTAGGACT TGACGTCGGA GCTGGATTTA GCTCCCTGAA
2351 TGTTCGGATG GAAATGTGCT GACCTGGTTG GGGAAACCAG ATCGAAGAAT
2401 CTGTTATTCG TGCATTGGTA TTTACCTTCG AACTGGATGA GGACGTGCAG
2451 ATGAGGTTCC CCATCTTCGT GTAATTCCCT GCAAATCTTG ATGAATTTCT

Sequence of BDMV DNA A continued.

2501 TGTTAACTGG AGTTTTTAGG TTTTGGATTT GGGAAAGTGC TTCCTCTTTA

2551 GTAAGAGAGC ACTGTGGATA TGTGAGGAAA TAGTTCCTCG ATTGAACTCT

2601 AAATTTCTTA GGCGG

Fig. 3. Sequence of bean dwarf mosaic geminivirus DNA B.

```
   1  TGGCATTTTT GTAATAAGAG ATGGTACTCC AGTTGAGTTA CTCCAATTCC
  51  CTCCCCTCAA AACTATCTCA TTCTATTGGA GTATTGGAGT TACTTAAATA
 101  CTAGAACCCT CAATCTGGTT TCGGAACACG TGGCGGTCAT CCGTATAATA
 151  TTACCGGATG GCCGCGCTTC GGAGTACGCT CTCTCTCCTC TCCCCTGGTG
 201  CGTTCCTGGT CCCCTGCCAC CTGCCACTCT CCTAGTGGAT GGTCGCTCGT
 251  CTTTTCCTGC GAGTTGTGGG CCGTAGTTTG AATGATTAAT CTTTAATTTA
 301  AATTAAAGAT GACTTTTACA TGTCGCGCGA TCTCATTTGA ATCTTGAATA
 351  ATTGTCTCGC GGTTCATGAC TACGGCCCAC TGTACTATAT AATGGACGTG
 401  GCTGATTTTA GACCATGCTG CTATGTCTAT TTATTCAATT TGAACAACCA
 451  TTTTCTATAA ATGAGGACGG ACCATACATT CCATTCAAGC TGACTCAGCT
 501  GTCAACGACG TGAAAATATC TATTTGTATT GGTATTATCG CCTAATAATG
 551  TATGGTTTGC GGAATAAACG TGGTTCATCG TTCAGCCATC GCCGATTTTA
 601  TTCACGTAGC AGTTTTTTAA ATCGCTTGTC CGCTAATAAG CGTCATGATG
 651  GCAAACGTCG AGCTATGAAT CCTAGTAAGC CCATTGACGA GCCCAAGATG
 701  TCAGCCCAAC GCATACATGA GAACCAGTAT GGGCCTGAAT TTGTAATGGC
 751  CCATAATTCA GCCATTTCTA CGTTTATCAG CTACCCCAGC AAGGGCAAGA
 801  TGGAACCCAA CCGATCGAGG TCCTATATTA AGTTGAAACG ACTTCGTTTC
 851  AAAGGGACTG TCAAGATTGA TCGTGTTCAA CCAGATATGA ACATTGACGG
 901  TTCTGCCCCA AAAGTGGAAG GAGTGTTCTC TCTGGTGGTT GTTGTGGATC
 951  GTAAACCCCA CTTGGGTGCG TCTGGATGCC TGCATACATT CGACGAGCTG
1001  TTCGGTGCAA GGATCCATAG CCATGGTAAT CTCAGCATAA CACCCTCTTT
1051  GAAAGACCGA TTCTACATAA GACACGTGTT CAAACGTGTA TTGTCCGTGG
1101  AGAAGGATAC GATGATGGTT GACGTGGAAG GATCTACATC GCTCTCTAAC
1151  AGGCGATATA ATTGTTGGTC CACTTTTAAG GATCTTGACC ATGAGTCATG
1201  CAAGGGTGTT TATGACAACA TCAGCAAGAA CGCCCTCCTA GTATATTACT
```

Sequence of BDMV DNA B continued.

1251 GTTGGATGTC AGATACTATG TCAAAGGCAT CTACTTTTGT ATCGTTTGAC

1301 CTTGATTATA TCGGTTGATT AATGATAATT GTAATAAAAA GCTATTATTG

1351 AACTTTCAAT TCCTCAACAA AGAAATTATT GCAACGATTT GGGCTGATAA

1401 GCTTACAGTT ACTATTTATA CACTCCTGGA CAGTGTTTTT CACTAGCTCG

1451 TTTAATTGCC CCATCGACAT AGTAATGTTG GATTCCGCTC TCTGGGCCCC

1501 TACAATTGAG GCAGACTCCC CTGGGTCTAA GACGCTTGTT CCAAGCCTGC

1551 TGAGATGCCT ATATGGATGC ATTGCGTTTT CCACCTCTGA GTCGGCATCG

1601 GAGTTGCTGA GCCCAATTGT ACTCCGTGAA GCCCATGATT CACCCGGCTT

1651 GATCTCTATT GGGCCTGGTA GTCCAATCCT TGACATGGAT GCGCATCTTA

1701 TGGGTTTCCT TTCCCATCTT CCGTAGTCGA CATGTGAAAA GTCGACATCT

1751 TTATCTGTGA ACTGTTTCGA CAGGATCTTT ACTGTCGGTG CCCGGAAGGG

1801 TATATCCACG GAGTGTTTCG CCGTCGATAG TTTCAGTTTC CCTTTGAACT

1851 TGGCGAAGTG GGTCCTCTGA TGAACATTCG TATCGCAAAC CCTATAATAG

1901 AGTTTCCATG GAATTGGGTC TTTCAACGAG AAGAACGAAG CCGAGAAATA

1951 ATGTAGATCT ATGTTGCATC TGATTGGAAA TGTCCAGGAA GCCTGCAGCG

2001 ATTCGTTGTC TGTCATTCGC TTGTCGTGGA TCTCGACAAT GACCGACCCT

2051 GTCGCGTTAA TCGGCACTTG TTGCCTGTAC TCTATGACGC AGTGGTCGAT

2101 CTTCATGCAG CTACGGCTCA GCCTAGCTGT TAACTGCGAC GCCGTGGAAG

2151 GGAATTGCAG AATTATCTCA GTGAGGTCAT GAGAAAGTTG ATATTCGTCC

2201 CGATGGGACT CTATGTAGTT AAATGCGTTG GGAGGATTGA CCAATTGAGA

2251 ATCCATATAA AGAATAATGG CCGCGCAGCG GAACCGATTG CTGAAGTTGA

2301 ACTGGTGAAG AAGAATTTAG GGCTGTAATT GAAGAACAAA TGATGAACTG

2351 TTCTTGAATG TGGAGAGGGT TTCTGGGAAA CTCAGAAAGT TTGTAAAGAA

2401 ATTGATGAAC AGTTGTTGAA CTTCTGCTGA ATATGAGATG TTTTTTGAGA

Sequence of BDMV DNA B continued.

2451 AAGAGTAGAA AGCTGAAGAG GAATTACTTG TTTATACTCT GCTAGATCTG

2501 TTAGGGTTTA TATAGAAGGT TAGAATCTGT GTTTATAGTT GAGAGCTTCC

2551 ACGAGAAGTC TATAATAGAA GAGAA

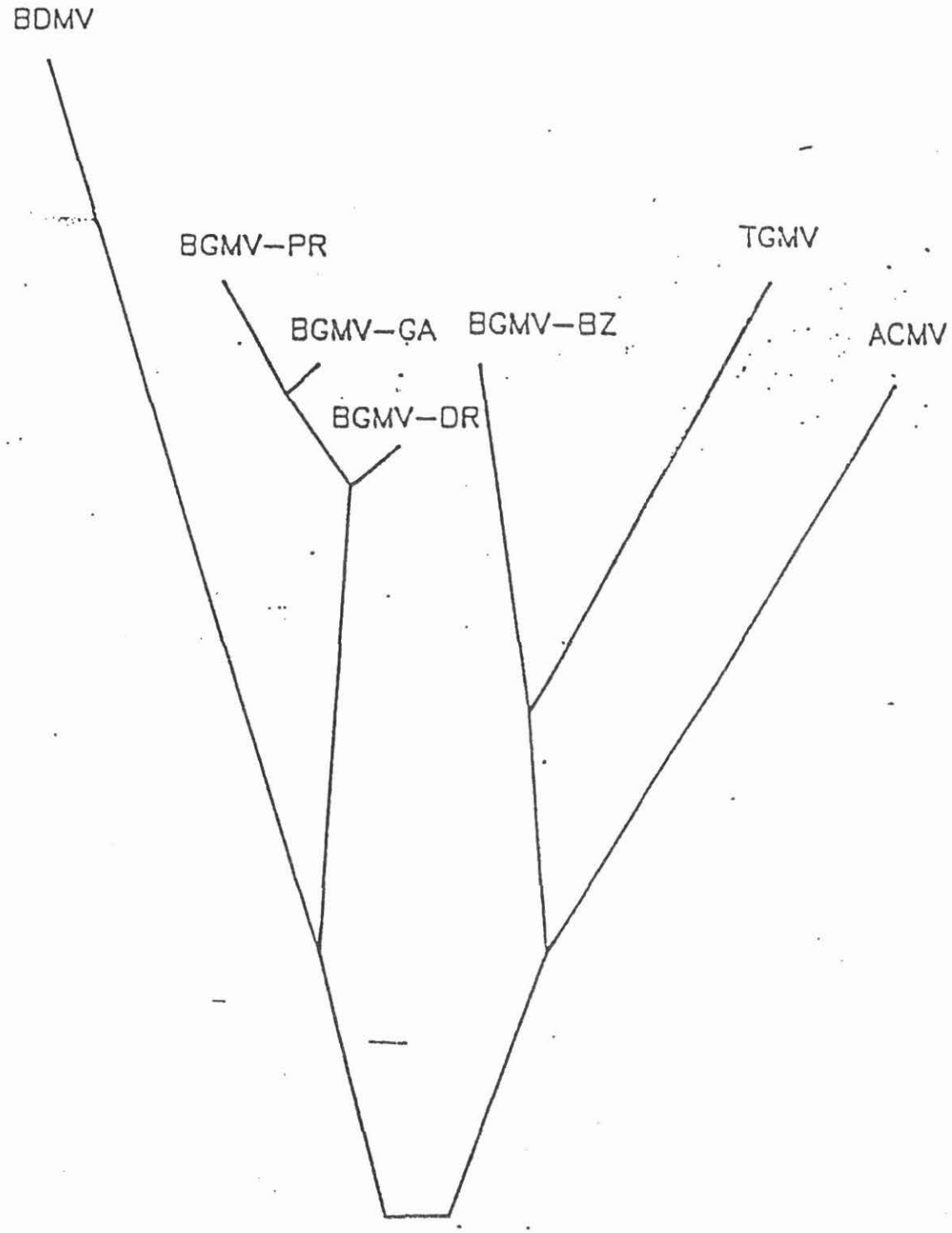


Fig. 4. Phylogenetic bree derived from computer comparions of the common region for BDMV, BGMV-PR, BGMV-DR, BGMV-GA, BGMV-BZ, tomato golden mosaic geminivirus (TGMV), and African cassava mosic geminivirus (ACMV). Clearly, BGMV-BZ, BDMV, and BGMV-GA/BGMV-DR form three distinct bean-infecting geminiviruses.

Genetic Analysis of Viral Gene Functions

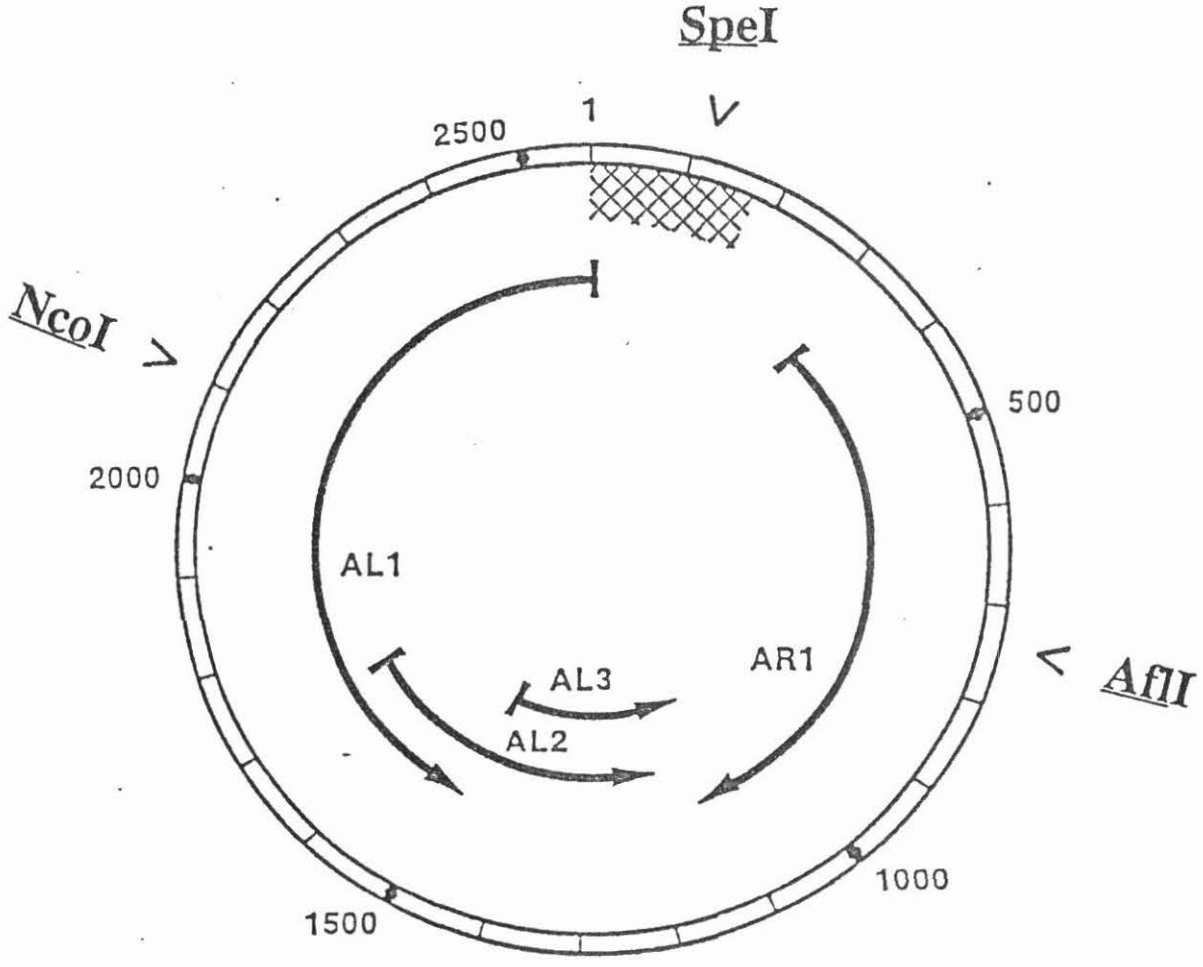


Fig. 5. Mutational analysis for BGMV-GA DNA A mutations at three restriction sites, SpeI (common region), AflI (AR1, coat protein gene), and NcoI (AL1, replicative product gene). Mutant DNA A molecules were inoculated with wild-type DNA B molecules by electric discharge particle acceleration methods. With the common region mutant for DNA A (added one nucleotide), symptoms were delayed and attenuated. The mutation in the coat protein changed one amino acid and this resulted in delayed symptom expression. The frame shift mutation in the replicative gene resulted in no symptoms and no viral DNAs were detected. Wild-type DNA A and B molecules resulted in typical symptoms in 10-14 days.

Research project: CHARACTERIZATION OF THE MAIN BEAN YELLOW MOSAIC VIRUS ISOLATES THAT LIMIT BEAN PRODUCTION IN NORTHERN AFRICA, WEST ASIA AND CHINA.

Progress Report
Phase II, year 1, 1989 - 1990

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

The work done from May 1989 to April 1990 has followed two main lines:

1. Identification of the viruses isolated from bean samples collected in Bulgaria or received from Iran and Turkey, and typing of the bean yellow mosaic virus (BYMV) isolates identified;

2. Continuation of the search for natural sources of resistance to BYMV in Phaseolus sp.

In November 1989 the Institute moved from the old site to a new building. The consequent interruption of work caused a delay in developing the program. For this reason the characterization of BYMV isolates found in 1989 is not yet complete.

1. Indexing for bean(*) viruses in Bulgaria, Iran and Turkey.

A total of 270 bean samples have been checked and viruses isolated from 122.

To isolate viruses, the dried-leaf field samples were ground in 0.05 M phosphate buffer containing 0.001 M Na-EDTA, 0.005 M Na-DIECA, 0.005 M Na-thioglycolate and a small amount of activated charcoal. This method proved effective to isolate viruses from some samples negative with phosphate buffer alone. The slurry was inoculated on test plants (P. vulgaris cv Saxa, Chenopodium amaranticolor, C. quinoa, Nicotiana clevelandii). Viruses were identified serologically: DAS-ELISA, using the antiserum to the Italian strain "1 V", was used to identify BYMV; immunodiffusion tests, with SDS in the case of elongated viruses, were used for alfalfa mosaic (AMV), bean common mosaic (BCMV), cucumber mosaic (CMV), and clover yellow vein (ClYVV) viruses. All sera belonged to the collection of the Institute.

To identify the necrotic strains of BCMV, an antiserum was prepared to an African necrotic isolate of BCMV (close to "NL 3"), provided by Dr. F. Morales, CIAT. This serum was used roùtinely in SDS tests. Electron microscopy and immunoelectron microscopy were used to clarify doubtful cases.

(*) the term "bean" is used throughout to indicate P. vulgaris.

1a. Indexing in Bulgaria.

A survey was done by V. Lisa in July 17-24, 1989, on invitation from the Maritza Institute for Vegetable Crops, Plovdiv.

Samples were collected in the experimental fields of the Maritza Institute at Plovdiv, in the Institute of Introduction and Plant Genetic Resources at Sodovo (both in Central Bulgaria, South-East of Sofia) and in the fields of the Institute for Wheat and Sunflower "Doubroudia" at Tolbuhin, in the North East, close to the Roumanian border.

Fifty samples were collected and viruses isolated from 39. In detail:

	CMV	BCMV	AMV	No virus
Plovdiv (B1-B15)	15	6	1	0
Sodovo (B16-B29)	12	4	0	2
Tolbuhin (B30-B50)	2	9	2	9

CMV was the commonest virus, isolated from 29 samples, followed by BCMV found in 19. Four of the BCMVs were of necrotic type, as determined by serology and reaction on the bean cvs Widusa, Jubila and Top Crop. Mixed infections of two or three viruses were common, especially at Plovdiv and Sodovo. BYMV was not detected.

1b. Indexing in Iran.

Seventy samples were sent to the Institute, 53 from Prof. I. Izadpanah, College of Agriculture, Institute of Plant Protection, Shiraz University, Shiraz, South Iran, and 17 from Dr. R. Parvizy, Agricultural Research Centre, Ourmia, North West Iran. The samples from Shiraz were from different legumes: 12 from broad bean (VE 117-VE 128), 1 from Vicia sp (VE 129), 1 from pea (VE 131), 22 from bean (VE 166-VE 188).

The samples from Ourmia were all from bean; 38 samples were virus-infected and three different viruses were identified. In detail:

Shiraz area
BYMV in 9 samples, all from broad bean;
CMV in 10 " " " bean;
BCMV in 8 " " " " ;

Ourmia area
BYMV in 1 samples;
CMV in 7 " ;
BCMV in 4 " ;

Four of the BCMV isolates from Shiraz were of the necrotic type, as determined by serology and reaction on differential bean cvs. The characteristics of the BYMV isolates are given in section 1d. and in Tables 1 and 2.

1c. Indexing in Turkey.

One hundred fifty bean samples were collected by Dr. Z. Onceler, Transitional Region Agricultural Research Institute, Eskisehir, Turkey, in a wide area around Eskisehir, Central Western Turkey. The samples, dried over

calcium chloride, were brought to Italy by Dr. Onceler and 100 of them were analyzed by him during a two month stay in our Institute. Viruses were isolated from 45 out of the 150 samples tested.
Three different viruses were identified:

BYMV in 9 samples
BCMV in 33 "
CMV in 3 "

Mixed infection of two or more viruses occurred rarely. The 9 BYMV isolates all came from one locality (Gurleyik, North West of Eskisehir). The properties of the BYMV isolates so far studied are given in section 1d and in Tables 1 and 2.
BCMV was the commonest virus detected; all isolates were of the "common mosaic" type, as determined by serology and reaction of differential bean cvs. CMV was found in 3 samples only, all collected in one locality close to Eskisehir.

1d. Characterization of the BYMV isolates.

Twenty BYMV isolates were studied in the period in question, 19 from Iran and Turkey and one from Yemen, kindly supplied by Dr. D. Walkey, I.H.S., Wellesbourne, U.K.. This last isolate (VE147) was from broad bean. Eight isolates, from Iran, Turkey and Yemen, have been characterized for pathogenicity towards differential bean cvs (Table 1) and 7 isolates for serological behaviour in comparison with sera to two known BYMV strains (Table 2). Characterization of the other isolates is in progress.

After isolation on test plants and prior to characterization, the BYMVs were transferred to bean cvs possessing the I gene of resistance to BCMV, to separate BYMV from possible BCMV contaminants. Fortunately in 1989 no mixed BYMV-CMV infections were found.

The 10 BYMV isolates from Iran (9 from broad bean collected in the Shiraz area - our identification No. VE 117, 118, 119, 121, 122, 123, 125, 126, 127 - and one from bean, Ourmia area, VE 151) showed unusual behaviour on test plants. The original dried leaf material infected only Chenopodium amaranticolor and C. quinoa, with local lesions. Several attempts to transfer the isolates from Chenopodium to beans, using different anti-inhibitor treatments failed. The isolates could be transmitted to Vicia faba and Trigonella foenum-graecum using 2% polyvinylpyrrolidone (PVP) or activated charcoal in the extraction buffer, and from these plants to bean. The isolates so far tested, all from broad bean, infected a restricted number of differential bean cvs, as compared either with the Turkish or with the Iranian isolates studied in 1988-1989. The Yemen isolate had a restricted host range, like those from Iran. None of the BYMVs so far studied infected bean cv GN 31 (Table 1).

No relevant serological difference was found between the 7 Iranian or Turkish isolates and the two standard BYMV strains from Italy and Chile (Table 2).

Samples were repeatedly requested from different Countries in North Africa (Algeria, Tunisia, Morocco) but until now without success.

2. Search for source of resistance to BYMV in P. coccineus and P. vulgaris.

As in the past, the highly pathogenic strain BYMV-OI, from Chile, was used to assess the degree of susceptibility of P. coccineus and P. vulgaris lines to the virus. Seedlings were inoculated at least twice and DAS-ELISA was used to detect infection. Plants negative in this test were further tested by back inoculation to test plants.

The serum to BYMV-OI prepared in 1989 was found to react also with BCMV, possibly due to a contamination of the virus culture used to prepare the serum. The virus culture was restored from the original dried leaf material and a new serum prepared, with titer 1/512 in slide precipitin test.

2a. Behaviour toward BYMV-OI of seedlings derived from plants found free of virus in the previous years (see Reports for 1987/88 and 1988/89).

P. coccineus:
Thirty two seedlings of cvs Bianco di Spagna, Corona, Rampicante Bicolore and line 30028, were found to be resistant to the virus.

P. vulgaris:
Line 15083/59, from cv Anellino di Brescia: 41 seedlings from the 10 plants found free of virus in 1989 were either susceptible (mild mosaic) or tolerant (symptomless infection), or not infected (2 plants). Seeds will, if possible, be collected from these last plants.
Line 695/9, from the wild type bean no. 695 of the Gembloux collection: 19 seedlings checked, all susceptible with severe infection. This line will be discarded.

2b. New material.

Two P. vulgaris lines were kindly supplied by Dr. D. Walkey, I.H.S., Wellesbourne, U.K.:
line JP7Q, highly susceptible to the virus;
line Col Al 63, tolerant. Ten seedlings from the original accession reacted with symptomless infection. Seeds from these plants yielded seedlings that reacted to BYMV-OI with either mild mosaic, symptomless infection or no infection (2 plants). Seeds have been collected from these last 2 plants.

If the tolerant reaction to BYMV-OI of line 15083/59 and of Col Al 63 is confirmed, these lines will be checked with the BYMV isolates found in the screening in West Asia.

Acknowledgements

We are grateful for the kind and helpful cooperation of the following researchers and Institutes during the survey in Bulgaria or in sending us infected samples:

Drs. D. Kostova and I. Poryazov
Maritza Institute for Vegetable Crops
Plovdiv, Bulgaria.

Prof. I. Izadpanah
College of Agriculture, Shiraz University
Shiraz, Iran.

Dr. Z. Onceler
Transitional Agriculture Research Institute
P.O.B. 17
Eskisehir, Turkey

Dr. R. Parvizy
Agricultural Research Centre
P.O.B. 365
Ourmia, Iran

Dr. D. Walkey
I.H.S.
Wellesbourne, U.K.

Table 1 Reaction of differential bean cvs to BYMV isolates.

	VE 117 IRAN		VE 118 IRAN		VE 123 IRAN		VE 125 IRAN		VE 127 IRAN		Tu 72/89 TURKEY		Tu 79/89 TURKEY		VE 147 YEMEN	
	loc	syst	loc	syst	loc	syst	loc	syst	loc	syst	loc	syst	loc	syst	loc	syst
Dubbele Witte	nll	nec	0	0	nll	nec	nll	nec	nll	mos	c/nll	mos	c/nll	mos	nll	mos
Redland Greenleaf C	0	0	lat	0	0	0	0	0	0	0	cll	mos	cll	0	0	0
Redland Greenleaf B	0	0	0	0	0	0	0	0	0	0	0	mos	0	mos	cll	0
Great Northern 123	0	0	0	0	cll	0	0	0	nll	0	nll	mos	0	0	0	0
Sanilac	nll	*mos*	lat	mos	nll	mos	nll	nec	nll	mos	cll	mos	cll	mos	nll	mos
Michelite	nll	mos	0	lat	nll	mos	0	mos	c/nll	mos	nll	mos	cll	mos	nll	*mos*
Pinto 114	0	0	0	0	nll	0	0	0	nll	0	0	lat	cll	0	0	0
Monroe	nll	0	0	0	nll	0	0	0	nll	0	nll	nec	cll	0	nll	0
Great Northern 31	nll	0	0	0	nll	0	0	0	nll	0	nll	0	cll	0	nll	0
Widusa	nll	nec	0	lat	0	lat	0	0	cll	mos	nll	nec	cll	mos	nll	mos
Black Turtle Soup 1	n/cll	*mos*	cll	mos	nll	mos	-	-	nll	mos	c/nll	nec	c/nll	mos	cll	mos
Black Turtle Soup 2	n/cll	nec	cll	nec	cll	mos	nll	mos	c/nll	mos	c/nll	nec	cll	mos	nll	*mos*
Jubila	cll	0	lat	0	0	0	0	0	cll	0	c/nll	mos	cll	mos	0	0
Top Crop	cll	0	0	0	cll	0	0	lat	0	0	c/nll	mos	cll	mos	0	0
Amanda	0	0	0	0	0	0	0	0	0	0	cll	mos	0	0	0	0
Saxa	0	0	0	0	0	0	0	0	0	0	c/nll	mos	cll	mos	cll	mos

Key to symptoms. loc = local infection; syst = systemic infection; cll = chlorotic local lesions; nll = necrotic local lesions mos = green mosaic; nec = vein or apical necrosis; lat = symptomless infection; 0 = no symptoms, no virus detected by ELISA test or back inoculation to test plants; - = not tested.

Table 2. Serological comparison of BYMV isolates from Iran and Turkey with standard BYMV strains. Homologous and heterologous titres were determined by slide precipitin test; antigens concentrated by ultracentrifugation.

SERA	Homologous titre(*)	Heterologous Titres(*) to					
		VE 118 IRAN	VE 123 IRAN	Tu 72/89 TURKEY	Tu 79/89 TURKEY	Tu 100/89 TURKEY	Tu 101/89 TURKEY
A 194 II (Italian strain BYMV-IV)	1024	1024	512	1024	1024	1024	1024
A 256 III (Chile strain BYMV-01)	512	512	256	512	512	512	512

(*) Reciprocal value.