ANNUAL REPORT 1991

VIROLOGY RESEARCH UNIT

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VIROLOGY RESEARCH UNIT

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EXECUTIVE SUMMARY

Bean Virology

Viruses remain major constraints to bean production in the tropics, due to the introduction of new crops and exotic viral pathogens into traditional bean growing regions. Some of the newly introduced crops are also preferred plant hosts to insect vectors or endemic viruses. Last, the incorporation of certain genes for virus resistance in improved bean cultivars, is creating unexpected pathogenic problems with some of the more recently introduced viruses. As examples, we can cite the introduction of exotic strains of cucumoviruses (aphid- and seed-borne viruses of the cucumber mosaic virus group) adapted to leguminous hosts. These viruses are now widely distributed in Latin America and have already been detected in bean-production countries of Africa and Asia, particularly China. These cucumoviruses possess a very broad pathogenicity spectrum in *Phaseolus vulgaris*. Also, a temperate (northern) virus, alfalfa mosaic virus, was detected this year for the first time in Chile.

Bean golden mosaic virus (BGMV) continues to spread in Latin America as a result of the expansion of export crops, such as soybean, tomato, tobacco, and others, which act as preferred hosts to the whitefly vector of the virus. This factor, together with the occurrence of dry, warm conditions, often result in excessive virus pressure, and significant yield losses even in fields planted to BGMV-tolerant cultivars.

The deployment of monogenic dominant resistance to bean common mosaic virus (BCMV) has been a productive strategy in Latin America for over two decades. Recently, however, several Central American bean cultivars, which possess this type of resistance, have been suffering severe attacks by beetle-borne viruses of the comovirus group. These viruses, referred to as the 'severe mosaic' virus complex, consistently induce necrosis and considerable plant distortion in dominant (I) gene cultivars, while BCMV-susceptible bean genotypes seldom exhibit these severe symptoms. We will, therefore, implement a new breeding strategy for BCMV and comoviruses in general, as of next year.

Regarding the genetic control of bean viruses, the VRU, has been actively cooperating with bean breeders in the selection and utilization of new germplasm sources of virus resistance for breeding purposes. Additionally and for the first time, a bean plant has been genetically transformed by incorporation of three different genes (GUS gene, herbicide (Bar) resistance, and the coat protein gene of bean golden mosaic virus. We are awaiting permission to use transformed bean seeds in BGMV screening tests to evaluate the viral coat protein-mediated plant resistance approach engineered by Agracetus (a private biotechnology company), the Plant Pathology Department of the University of Wisconsin, and the Virology Research Unit of CIAT, with USAID funding.

Cassava Virology

The VRU has the responsibility to investigate the diseases of cassava that are caused by viruses or virus-like agents. There are four main diseases that are caused by the following viruses or virus-like agents. They are the frogskin and Caribbean mosaic disease complex (virus-like agents), cassava vein mosaic virus (CVMV), cassava common mosaic virus (CCMV), African and Indian cassava mosaic viruses (ACMV & ICMV).

Progress has been made on the identification of phytoreovirus-like agents associated with FSD and CMD. Virus-like particles and viroplasm-like bodies have been found in affected plants. Consistently nine ds-RNA segments are found in the affected plants. The hybridization studies provide evidence that the ds-RNAs associated with FSD and CMD are either identical or closely related. The whitefly *Bemisia tuberculata* appears to be the vector of these phytoreovirus-like agents. A cDNA probe has been developed to identify rapidly the ds-RNAs associated with this disease complex. The research on FSD and CMD are continuing and the investigations are centered on confirming the association of the phytoreovirus-like agents and the entire complex of disease symptoms.

The sequencing of CCMV is nearly complete. The virus is 6400 bases in length and it is most closely related to potato virus X (PVX). Most of the sequencing of CCMV was done at the VRU in CIAT and this is perhaps the first plant virus to be sequenced in Latin America.

Cassava vein mosaic virus is most prevalent in the northeastern states, especially in the hot semiarid zones where it is not unusual to find more than 50% of the plants infected with CVMV. Since the virus is not present in Colombia, all work on this virus had to be done in Brazil. A cDNA clone to CVMV has been obtained, and this will facilitate efforts at molecular characterization and the development of rapid diagnostic tests.

African cassava mosaic virus is the most destructive cassava virus in the world. Efforts are being made to find new sources of resistance through the CIAT-IITA cooperative project. The identification of ACMV resistant germplasm adapted to tropical America will be a safeguard against the possible establishment of ACMV in this hemisphere.

Beside the major diseases there are seven other known viruses that infect cassava most of which are symptomless viruses that are not known to cause disease. Diagnostic methods are available at CIAT to the four viruses that cause diseases and to the three symptomless viruses found in Latin America. These diagnostic methods help assure the safe movement of cassava germplasm, and research will continue to develop even more sensitive detection methods.

The control of viral diseases requires either the identification of resistant germplasm or the implementation of cultural practices that mitigate disease losses. Most viral diseases are controllable with the current technology, and continued development of rapid diagnostic

technics together with the deployment of resistant germplasm should further reduce the losses caused by viruses.

Rice Virology

The genomic characterization of RHBV is progressing with the completion of the *in vitro* translation experiments, and the successful cDNA cloning and sequencing of RHBV. A 20K protein encode by the RNA-3 and a 17K protein encoded by RNA-4 were identified by the *in vitro* translation experiments. The 17K protein was shown to be the inclusion body protein.

A complementary DNA (cDNA) library to RHBV has been prepared and approximately 3,000 bases of the RHBV genome has been sequenced. Non-structural proteins on RNA 3 and 4 have been identified and share 50-60% direct amino acid homology with the equivalent proteins encoded by RStV and MStV.

Analysis of the *in vitro* studies and the sequence indicates that RHBV has a genomic organization that is similar to other members of the tenuiviruses. The genomic organization of RNA-3 and 4 appear to be ambisense. Each species encodes one protein from the ss-RNA that is encapsidated in the virions. They also encode a protein on the complementary strand, which makes both species protein encoding (sense) strands. From the *in vitro* data, it appears that the RNA-1 and 2 encode proteins on only one strand and that the negative sense strand, are the encapsidated ss-RNA species.

The cloning and sequencing of the RHBV RNA-2, 3, and 4 are expected to be completed during the next year. This will nearly complete the primary molecular characterization of RHBV, and will be a basis for a better understanding of the diversity, epidemiology, and effective control strategies of RHBV.

Tropical Pastures Virology

Potyviruses are ubiquitous pathogens of tropical forage legume species, such as *Centrosema* spp., *Arachis pintoi*, and *Stylosanthes* spp. Consequently, CIAT has developed reliable serological techniques to detect the various potyviruses detected to date in these species. Using these techniques, potyvirus-free seed or asexual reproductive material has already been produced under screenhouse conditions. This year, a survey of *A. pintoi* field plots planted with virus-free reproductive stock produced at CIAT, showed that it is possible to establish and maintain a virus-free forage legume field, as concluded from the absence of potyviruses in these fields. We expect to plant similar trials with four *Centrosema* species next year.

A viral disease was detected for the first time in a forage grass, *Brachiaria brizantha*. The causal agent was shown to be an aphid-borne potyvirus, which induces streaking and ring formation in susceptible accessions. This virus can also establish latent infections in some *Brachiaria* sp. accessions. A preliminary survey suggested that this potyvirus has a wide geographical distribution, and current research seeks to establish its relationship with previously reported potyviruses of *Brachiaria* spp., such as sugarcane mosaic and maize dwarf mosaic viruses.

VIROLOGY RESEARCH UNIT

ANNUAL REPORT

Introduction

The Virology Research Unit (VRU) conducts research on the viral diseases that affect the adaptation, yield potential, nutritional quality, or germplasm exchange of the different plant species investigated by CIAT's Commodity Programs.

One of the main goals of the VRU has been to assist plant breeders in the identification and selection of sources of resistance to economically important viruses, for use in plant improvement projects. Additionally, the VRU interacts with plant breeders, geneticists, and molecular biologists to study the genetics of pathogenicity and plant resistance to selected plant viruses. An example of this research is the paper published this year, entitled "Genetics of Resistance to Bean Golden Mosaic Virus in *Phaseolus vulgaris* L.", which was widely requested by a considerable number of agricultural researchers from around the world.

With regard to molecular biology projects, the VRU is pleased to report for the first time on the genetic transformation of *Phaseolus vulgaris* by the introduction of foreign genes, using a 'ballistic' (particle gun acceleration) method. These transformed bean plants express GUS activity, herbicide resistance, and the coat protein gene of bean golden mosaic virus (viral coat protein-mediated plant resistance). This is the result of a cooperative project on bean golden mosaic virus involving the University of Wisconsin, Agracetus (a biotechnology company), and the VRU.

Finally, considerable progress was made in the application of advanced molecular virology techniques for the detection, characterization, and control of rice and cassava viruses. This work implied the creation of a well organized molecular virology laboratory within the VRU. These advanced techniques will soon be applied to the characterization of tropical forage viruses.

BEAN VIROLOGY

Bean Common Mosaic Virus (BCMV)

Incorporation of monogenic dominant resistance into BCMV-susceptible landraces.

The deployment of the dominant I gene continues to be a valid strategy in most of the main bean-producing areas of Latin America. Among the advantages it has, we can cite 1) the availability of several sources of the I gene, 2) the simple inheritance of the gene, 3) the elimination of BCMV from the seed in I-gene cultivars, and 4) the relative simplicity of the screening procedures implemented at CIAT.

As disadvantages, we can cite: 1) dependency on a single gene which can be overcome by some existing BCMV strains, 2) genetic linkage between certain seed colors and susceptibility to BCMV, and 3) darkening and modification of the seed color/pattern in some commercial seed types. Moreover, it is becoming apparent that the necrosis I gene is interacting with viruses other than BCMV both in the same group (potyviruses) or different groups, as will be discussed later on under 'bean severe mosaic'. Last but not least, the incidence of BCMV in the American and African highlands in on the increase due to various environmental factors which favor the development of large aphid vector populations at these altitudes.

Incorporation of recessive resistance genes to control BCMV

The majority of the BCMV strain-specific resistance genes have not been actively used in breeding for common mosaic resistance projects due to 1) the existence of BCMV strains which can overcome these genes, and 2) the relatively simple manipulation of the dominant necrosis gene. However, due to the high incidence of necrosis-inducing strains of BCMV in East Africa, some recessive genes, namely bc-2^2 and bc-3, are being incorporated into E. African landraces.

An international BCMV-Black Root (IBRN) nursery has been available and distributed for some time (see page 6, Virology Research Unit 1990 Annual Report, or page 129, Bean Program 1990 Annual Report). This nursery has been very useful as a source of recessive genes for resistance to BCMV, and to detect the presence of necrosis-inducing strains in a bean-production region, as it occurred this year in the Mexican highlands, where the presence of necrotic BCMV strains was confirmed this year in the unprotected dominant I gene control genotypes included in the IBRN. Such, findings indicate the need to deploy recessive, BCMV-resistant genes in these areas.

Viruses of the Cucumber Mosaic Virus group infecting *Phaseolus vulgaris*

It has been known for some time that the so-called "legume strains of cucumber mosaic virus" have been attacking *Phaseolus vulgaris* in every major continent of the world where

beans are grown. Currently, major epidemics have occurred in Europe, Turkey, China, and Chile, and the virus(es) has been detected in East Africa, thanks to cooperative projects between CIAT and advanced research institutions in Germany and Italy.

In Latin America, Chile is the country with the highest incidence of these bean cucumoviruses, and the country where the most virulent strains have been detected so far in the world. A new collection of 481 Chilean landraces evaluated in Chile this year by the VRU, in collaboration with INIA's scientists, was shown to have a 93.4% incidence of viruses, particularly BCMV and cucumoviruses. It must be remembered that cucumoviruses are seed-borne in bean seed and are also efficiently transmitted by several aphid species under field conditions.

Although some sources of resistance have been identified against the Turkish strains of cucumoviruses, notably Red Mexican 35, Pinto 114, and Monroe (Table 1), no resistant genotypes had been identified for the Chilean or Chinese strains.

Table 1. Screening of differential bean genotypes for their reaction to selected bean cucumoviruses from Chile, China and Turkey.

	Bean Cucumovirus isolates				
	Chile		China	Turkey	
Bean cvar.	INIA	Parral	(39 & 59)	56	114
Double White	L,N	N,S	S	S	S
Stringless Green Refugee	S	S	S	S	S
Redlands Greenleaf C	S	S	S	S	S
Puregold Wax	S	S	S	S	-
Imuna	S	S	S	S	-
Redlands Northern 123	S	S	S	S	-
Sanilac	S	S	S	S	S
Michelite 62	S	S	S	S	-
Red Mexican 34	S	S	S	S	-
Pinto 114	S	S	S	-	-
Monroe	L,S	L,S	S	L,-	L,-
Red Mexican 35	L,S	S	S	L,-	L,-
Great Northern 31	L,S	S	S	S	-
Widusa	L,N	N,S	S	S	-
Black Trutle Soup	S	S	S	S	-
Jubila	S	S	S	S	S
Topcrop	S	S	S	S	S
Improved Tendergreen	S	S	S	S	S
Amanda	L,N	N,S	S	S	S
Bountiful	S	S	S	S	S
Diacol Calima	S	S	S	S	S

L = Local lesions; S = Systemic infection; N = Systemic necrosis; - = No systemic infection.

As mentioned before, this year we identified 32 bean genotypes in the new collection of Chilean landraces, which seemed to be free of viruses. Additionally, 6 accessions were identified as virus-free in INIA's and CIAT's Chilean bean germplasm collections also evaluated in Chile this year. Seed of the new collection of Chilean landraces has just arrived at CIAT and it is currently being processed by Colombian quarantine authorities. Once it is cleared, the VRU will be screening.

Bean Severe Mosaic

The name Bean Severe Mosaic is currently being proposed to refer to a group of beetle-borne comoviruses, (isometric RNA viruses of the COwpea MOsaic VIRUS group) which cause severe mosaic, malformations, and sometimes necrosis in systemically infected bean plants. These viruses have now been detected in Mexico (Sinaloa), Guatemala, Honduras, El Salvador, and Venezuela.

The main concern in relation to these comoviruses, is their apparent interaction with the dominant I gene, causing necrosis in bean genotypes that possess this type of monogenic dominant resistance to BCMV. However, there are other genes that are also reacting in a hypersensitive manner with these comoviruses. These genes are currently being identified to exclude them from future breeding -for-bean-severe-mosaic-resistance projects. The following is a technical report (prepared for publication) on the current knowledge on these bean comoviruses.

Various comoviruses are natural pathogens of the common bean (*Phaseolus vulgaris* L.) in the American Continent. The main comoviruses reported to date are: bean rugose mosaic virus (BRMV), bean curly dwarf mosaic virus (BCDMV), and cowpea severe mosaic virus (CSMV) in Latin America, and bean pod mottle virus (BPMV) in the United States (5, 11, 12, 13). Each of these comoviruses belongs to a different but related serogroup (1, 10). The endemic nature of comoviruses in common bean-producing regions of Central and South America, is probably related to the traditional association of bean and maize plantings, since the latter species also supports large populations of some of the main beetle vectors of comoviruses (2).

Since 1985, severe epidemics of comoviruses have taken place in important Central American (El Salvador, Guatemala, and Honduras) and South American (Venezuela) bean-growing regions. Moreover, we reported in 1985 (3), that disease severity was greater in improved bean cultivars possessing monogenic dominant resistance to BCMV (7, 8, 9). This investigation was thus conducted to confirm the above observation, considering the wide distribution of bean cultivars carrying the dominant necrosis gene (I), in the American continent. The genetics of resistance to BCMV has been recently summarized by Drijfhout (8).

Materials and Methods

Virus isolates, maintenance, and transmission tests. The viruses used in this study were isolated from symptomatic leaf samples collected in bean fields in the States of Aragua (Venezuela) and Goias (Brazil), and in the Province of Francisco Morazán (Honduras). These virus isolates will be referred to as the Venezuelan (CV-VZ), Brazilian (CV-BZ) and Honduran (CV-HD) isolates, respectively. Under field conditions, symptom expression in systemically-infected bean plants, varied from mosaic and rugosity for CV-BZ and CV-VZ, to mosaic, plant malformation and necrosis for CV-HD. These isolates were maintained in the bean cultivar Bountiful, inside separate insect-proof cages in a glasshouse with a maximum light intensity of 1,100 $\mu E\ m^{-2}\ s^{-1}$, average temperature of 27 °C, and 75% relative humidity. All mechanical inoculation tests were conducted in insect-proof facilities, using extracts from infected plants diluted five-fold in sterile distilled water. The inoculation materials and the test plants were incinerated following their use and final evaluation.

A local isolate of bean southern mosaic virus (BSMV) was also used in this study for comparative purposes.

Test plants. A total of 53 common bean genotypes (six test plants minimum/ genotype) were mechanically inoculated as described above, with the three selected virus isolates. The following bean genotypes: Dubbele Witte, Stringless Green Refugee, Redlands Greenleaf-C, Puregold Wax, Imuna, Redlands Greenleaf-B, Great Northern 123, Sanilac, Michelite 62, Red Mexican 34, Pinto 114, Monroe, Great Northern 31, Red Mexican 35, and IVT 7214, possess the recessive alleles (I^+I^+) of the necrosis gene described for BCMV (7, 8, 9). Conversely, cultivars Widusa, Black Turtle Soup, Jubila, Topcrop, Improved Tendergreen, and Amanda, possess the dominant alleles (II) of the necrosis gene (7). Three bean genotypes, IVT 7233, Orfeo-INIA and MCM 5001, which possess specific recessive genes (bc-2^2 or bc-3) that protect the dominant I gene against necrotic strains of BCMV (7), were also tested. All of the above bean cultivars, with the exception of Orfeo-INIA and MCM 5001, are used to differentiate BCMV strains (7, 8, 9), and are maintained at the Virology Research Unit of CIAT.

Cultivars Bountiful (G 06715), Commodore (G 05652), Mexico 309 (G 05692), Potomac (G 07475), Tendergreen (G 5497 and 09391), Tenderlong 15 (G 01594), Beka (G 08666, 10067, and 11211), Columbia Pinto (G 05050), Great Northern 59 (G 08501), Guatemala 416 (G 03717), ICA-Tui (G 04454 and 05728), La Vega (G 03769), Plentiful (G 01741 and 09292), Porrillo 1 (G 03090 and 04461), Sensutepeque (G 03773), Surecrop Wax (G 09384), Sutter Pink (G 2944), and Rico 23 (G 03827, 4469, and 4791), previously tested (12) with bean curly dwarf mosaic virus (BCDMV), were also inoculated with the three selected comoviruses. These accessions are maintained at the germplasm bank of the Genetic Resources Unit of CIAT. As indicated above, some of these cultivars are represented by more than one accession in the germplasm bank, some of which may be genetically different.

A set of nine cultivars: Alubia-Cerrillos (G 07930), Diacol-Calima (G 01853), Red Kidney (G 06054), ICA-Pijao (G 05773), Porrillo Sintetico (G 04495), Bayo Gordo (G 13707), Cacahuate 72 (G 05481), Arbolito Retinto (G 18247) and Honduras 46 (G 04791), was also included for evaluation with the test viruses to increase the number of different bean genotypes evaluated.

Finally, the bean cultivars Kentucky Wonder (G 00148), Iguaçu (G 04120), Red Mexican (G 05507), Cornell 49-242 (G 05694), and Moruna 80 (G 17703) were tested as reported local lesion assay genotypes for cowpea severe mosaic and bean southern mosaic viruses (4).

Serological tests. The three virus isolates were serologically assayed by immunosorbent electron microscopy (ISEM) as described by Derrick (6). Antisera to cowpea mosaic virus (CpMV), CpSMV, BRMV, BPMV, and quail pea mosaic virus (QPMV), are maintained at the Virology Research Unit of CIAT. The antisera to BPMV and QPMV were originally obtained from the Department of Plant Pathology, University of Arkansas, and the antisera to CpMV and CpSMV, from the Department of Plant Pathology, University of Florida. The antiserum to BRMV was produced at CIAT.

Results

As data in Table 2 indicates, none of the first group of 24 bean genotypes evaluated was immune to any of the three viruses inoculated. With few exceptions, these viruses induced chlorotic local lesions (Fig. 1A) and, less frequently, ringspots (Fig. 1B) on the inoculated primary leaves. Symptom severity in I^+I^+ genotypes varied from mild to severe mosaic and foliar malformation, (Fig. 2) according to the virus isolate. The most virulent virus was CV-HD, followed by CV-VZ and, finally, CV-BZ. Symptom severity was clearly greater in bean genotypes possessing monogenic dominant resistance to BCMV, particularly when inoculated with CV-HD or CV-VZ. CV-HD consistently induced necrosis on the leaves and stems of I gene cultivars, as previously observed under field conditions in Honduras and other Central American countries. CV-VZ also induced necrosis in cultivars Widusa and Topcrop but not in the rest of the I gene cultivars tested. CV-BZ did not induce necrosis in any of these test bean genotypes. The presence of BCMV strain-specific (7) recessive genes, such as $bc\text{-}2^2$ (IVT 7233 and Orfeo-INIA) and bc-3 (MCM 5001), further increased symptom severity (Fig. 3), as determined by the more severe malformation, defoliation and necrosis observed in plants inoculated with CV-VZ and, particularly, CV-HD.

Table 2. Reaction[a] of three groups of common bean cultivars possessing the recessive or dominant alleles of the necrosis gene[b], or the necrosis gene protected against necrotic strains of bean common mosaic virus (BCMV) by specific recessive genes, to the inoculation of selected comovirus isolates from Brazil, Honduras, and Venezuela.

Common bean cultivar	BCMV strain-specific recessive genes[c]	Comovirus isolated		
		CV-BZ	CV-VZ	CV-HD
Cultivars with recessive alleles (I^+I^+) of the necrosis gene				
Dubbele Witte	none	cl/ep,m	cl/m	-/ep,m,fm
Stringless Green Refugee	none	cl/m	cl/m,fm	cl/m,fm
Redlands Green Refugee	bc-1	cl/m	cl/m,fm	-/m,fm
Puregold Wax	bc-1	cl/m	cl/m	ep/m
Imuna	bc-1	cl/m	cl/m,fm	cl/ep,m
Redlands Greenleaf B	bc-1^2	cl/ep,m	cl/m,fm	cl/m,fm
Great Northern 123	bc-1^2	cl/mm	cl/mm	cl/ep,m,fm
Sanilac	bc-2	cl/m	cl/m,fm	-/m,fm
Michelite 62	bc-2	cl/m	cl/m,fm	cl/ep,M,FM
Red Mexican 34	bc-2	rs/m	rs/mm	cl/mm
Pinto 114	bc-1,bc-2	rs/ep,m	rs/mm	-/mm
Monroe	bc-1^2,bc-2^2	cl/m	cl/m,fm	cl,ep,n/m,clf
Great Northern 31	bc-1^2-bc-2^2	rs/m	rs/m	cl/mm
Red Mexican 35	bc-1^2-bc-2^2	rs/m	rs/m	cl/mm
IVT 7214	bc-3	cl/mm	cl/mm	cl/mm
Cultivars with dominant alleles (II) of the necrosis gene				
Widusa	none	cl/m	cl/ep,N,fm	cl/M,FM,N
Black Turtle Soup	none	cl/im	cl/M,FM	cl/M,FM,N
Jubila	bc-1	cl/m	cl/M,FM	cl/M,FM,N
Topcrop	bc-1	cl/m	cl/m,fm,m	cl/M,FM,N
Improved Tendergreen	bc-1	cl/m	cl/M,FM	cl/M,FM,N
Amanda	bc-1^2	cl/m	cl/M,FM	cl/M,FM,N
Cultivars with dominant alleles (II) of the necrosis gene protected against necrotic BCMV strains by recessive genes				
IVT 7233	bc-2^2	cl/m	cl/M,FM,n	cl/M,FM,N
Orfeo - INIA	bc-2^2	cl/m	cl/M,FM	rs/ep,M,N
MCM 5001	bc-3	rl/ep,m	rl/M,FM	rs/ep,M,N

[a] Predominant (local/systemic) symptoms: cl = chlorotic lesions, rl = reddish lesions, rs = ringspots, ep = epinasty. m = mosaic, mm = mild mosaic, fm = mild moderate foliar malformation, M = intense mosaic, FM = severe foliar malformation, n = moderate necrosis, N = severe necrosis.

[b,c] According to E. Drijfhout (7, 8).

[d] *Comovirus* (CV) isolates from Brazil (BZ), Venezuela (VZ), and Honduras (HD).

Table 3 shows the comparative response of 18 bean genotypes to CV-HD and, as previously reported, to BCDMV from El Salvador. In general terms, the results of this evaluation demonstrate that, while there are similarities, there are notable differences between the symptoms reported for BCDMV from El Salvador, and those caused in this by the test CV-HD virus from neighboring Honduras. The main difference observed was the lack of a consistent interaction between BCDMV and the dominant alleles of the necrosis gene. On the contrary, only the I gene cultivars, Commodore, Potomac, and accession G 09391 of Tendergreen, reacted with leaf and stem necrosis to CV-HD. The rest of the inoculated bean genotypes, which possess the recessive alleles (I^+I^+) of the necrosis gene, only showed mosaic and foliar malformation, with the exception of one Kentucky Wonder accession (G 05513), which reacted with top necrosis in one test plant.

The results of the inoculation of the nine additional bean genotypes, including cranberry, red kidney, and white and black-seeded cultivars, further confirmed the enhanced symptom expression (severe mosaic, rugosity, and plant malformation) induced by the three test viruses on the dominant I gene cultivars ICA-Pijao and Porrillo Sintetico. As observed above for other I gene bean genotypes, only CV-HD induced foliar and stem necrosis in ICA-Pijao and Porrillo Sintetico. Bean cultivars Alubia Cerrillos, Diacol-Calima, Red Kidney, Bayo Gordo, and Cacahuate 72, devoid of the dominant alleles of the necrosis gene, only expressed mosaic symptoms. The remaining I^+I^+ bean genotypes, Arbolito Retinto and Honduras 46, reacted with top necrosis (Fig. 4) to the mechanical inoculation of the three test viruses (Fig. 4).

The five bean cultivars reported to possess hypersensitive genes to BSMV and CpSMV, reacted to BSMV with necrotic ringspot lesions, 1-2 mn in diameter, on the manually inoculated primary leaves (Fig. 5), confirming their hypersensitivity to this sobemovirus. However, these bean cultivars became systemically infected by the three test viruses. Arbolito Retinto, included for comparative purposes, did not show any local lesions on the BSMV-inoculated primary leaves but developed severe mosaic and rugosity. This bean genotype, reacts with concentric ring-shaped lesions (Fig. 5B) to the inoculation of the viruses selected in this investigation, suggesting its potential use as a diagnostic host.

Table 3. Comparative reaction of selected bean genotypes previously evaluated for their reaction to bean curly dwarf mosaic comovirus (BCDMV) from El Salvador, to a bean comovirus isolate (CV-HD) from Honduras .

Bean cultivar	G accession[1]	Alleles of necrosis gene[2]	Reaction to CV-HD[3]
Cultivars reported to react with top necrosis to BCDMV[4]			
Bountiful	6374	I^+I^+	M
Commodore	6715	II	M,D,N
Mexico 309	5652	I^+I^+	M,FM,N
Potomac	5692	II	M,FM,N
Tendergreen	7475	I^+I^+	m
	9391	II	M
Tenderlong 15	5497	II	N
Kentucky Wonder	5513	II	M,TN
Kentucky Wonder	00148	II	M,N
Cultivars reported to react with mosaic, dwarfing and vein necrosis to BCDMV[4]			
Beka	1594	I^+I^+	M
Beka	8666	I^+I^+	M
Beka	10067	I^+I^+	M
Columbia Pinto	11211	I^+I^+	M
Great Northern 59	5050	I^+I^+	m
Guatemala 416	8501	I^+I^+	m
ICA-Tui	3717	II	M,D,N
ICA-Tui	4454	II	M,D,N
La Vega	5728	II	M,D,N
Plentiful	3769	I^+I^+	m
Plentiful	9292	I^+I^+	m
Porrillo 1	1741	II	M,D,N
Porrillo 1	4461	II	M,D,N
Sensutepeque	3090	I^+I^+	m
Surecrop Wax	3773	II^+	M,D,N
Sutter Pink	9384	I^+I^+	m

[1] G = identification number given by the Genetic Resources Unit of CIAT.

[2] Evaluation previously conducted by the Virology Research Unit of CIAT.

[3] Main symptoms induced by the Honduran comovirus: m = mild mosaic, M = intermediate to severe mosaic, D = dwarfing, N = necrosis, FM = foliar malformation, TN = top necrosis.

[4] Main symptoms induced by bean curly dwarf mosaic virus (BCDMV) according to J.P. Meiners *et al.* (12)

Serological tests. The three test viruses were shown to be comoviruses serogically related to CpSMV, QPMV, and BRMV, in that order of relatedness, as determined by a nine-, seven-, and two-fold increase in the number of virus particles trapped in ISEM tests, respectively, relative to the untreated control (ca. 700 particles/1,000 μm^2). The CpSMV and QPMV antisera, and the antiserum previously produced at CIAT against a bean comovirus from El Salvador, reacted more strongly with CV-HD than with CV-VZ or CV-BZ.

Discussion

Besides the extreme pathogenic range of the three test comoviruses in *P. vulgaris*, it was clear from this investigation, that two of these comoviruses, CV-VZ and CV-HD, interact with the dominant alleles of the necrosis gene. These comoviruses cause severe mosaic, plant malformation and, particularly in the case of CV-HD, necrosis of the stem and leaves of dominant I gene plants. Although cultivars Great Northern 31, Red Mexican 35 and Pinto 114, and the line IVT-7214, which possess recessive genes against necrotic BCMV strains, developed only mild mosaic when inoculated with the test comoviruses, these genes failed to protect II bean genotypes IVT 7233, Orfeo-INIA, and MCM 5001, against the severe symptoms induced by the three comoviruses tested.

Regarding the recent report by Costa (4), on the hypersensitive response of certain I gene bean cultivars to CpSMV and BCMV, we found notable differences in this investigation. First, the CpSMV isolate used by Costa only induced pin-point local lesions on inoculated leaves, but no systemic infection of the bean cultivars he listed as carriers or possible carriers of the dominant alleles of the necrosis gene. Secondly, some of the 'hypersensitive' bean genotypes tested by Costa, such as Columbia Pinto, Pinto UI 115, Red Mexican, Red Mexican UI 37, and Sanilac, do not possess the dominant alleles of the necrosis gene. And last, the five cultivars which reacted with local lesions to CpSMV in Brazil, namely Kentucky Wonder, Iguaçu, Red Mexican, Cornell 49-242, and Moruna 80, were systemically infected by the three comovirus isolates selected for this study. In fact, none of the dominant I gene bean cultivars inoculated in this investigation with CV-BZ, CV-HD, or CV-VZ, developed pin-point local lesions on the inoculated primary leaves. Instead, they reacted with enlarged local lesions unlike those associated in Costa's publication with dominant factors, which condition hypersensitivity to both BCMV and BSMV. Therefore, we conclude that while Costa's CpSMV isolate also interacts with the dominant alleles of the necrosis gene, it cannot infect dominant I gene bean cultivars systemically, as it is the case with the three comoviruses investigated here.

It seems unlikely that any of the three comoviruses tested in this investigation is the same as the BCDMV isolate described in El Salvador as a strain of QPMV, although CV-HD is closely serologically-related to BCDMV. Also, it is not clear whether the three comoviruses selected in this investigation belong to the QPMV or CpSMV serogroups. The high affinity of the CpSMV antiserum for the three bean comoviruses tested by ISEM, suggests these isolates may belong to the CpSMV serogroup. Moreover, from an examination of the literature, it is apparent that most of the Latin American comoviruses belong to the CpSMV

serogroup (10). Unfortunately, when BCDMV was originally reported as a strain of QPMV, its serological relationship with CpSMV was not determined (12).

The main conclusion of this investigation is that we are increasingly encountering in Latin America, some comoviruses capable of infecting all common bean cultivars tested to date, causing severe mosaic, plant malformation, stunting, and, sometimes, necrosis and plant death in cultivars possessing dominant hypersensitive resistance to BCMV. The implications of this pathogenic interaction are significant, considering that the majority of the improved common bean cultivars grown in Latin America, the United States, and Europe, possess monogenic dominant resistance to BCMV, and that some of the chrysomelid vectors of these comoviruses are also found in bean-growing regions in temperate countries.

We have been collectively referring to the mosaic, plant malformation and necrosis syndrome observed in Latin America, as bean severe mosaic, and to the comoviruses that induce them, as bean severe mosaic virus strains.

Figure Legends

Fig. 1 Local chlorotic lesions (A) and ringspots (B), induced on the manually-inoculated primary leaves of Topcrop and Red Mexican 35 bean plants, respectively, by a bean comovirus isolated in Venezuela.

Fig. 2 Mosaic and foliar malformation symptoms observed on IVT 7233 bean plants systemically infected by a bean comovirus isolated in Honduras.

Fig. 3 Severe mosaic and plant malformation symptoms expressed by MCM 5001 bean plants inoculated with a bean comovirus from Honduras.

Fig. 4 Systemic necrosis in an Arbolito Retinto bean plant inoculated with a bean comovirus from Brazil.

Fig. 5 Necrotic ringspot lesions (A) caused by bean southern mosaic virus on the manually-inoculated primary leaves of a Red Mexican bean plant, and concentric anular local lesions (B) induced by a bean comovirus from Brazil on the primary leaves of an Arbolito Retinto bean plant.

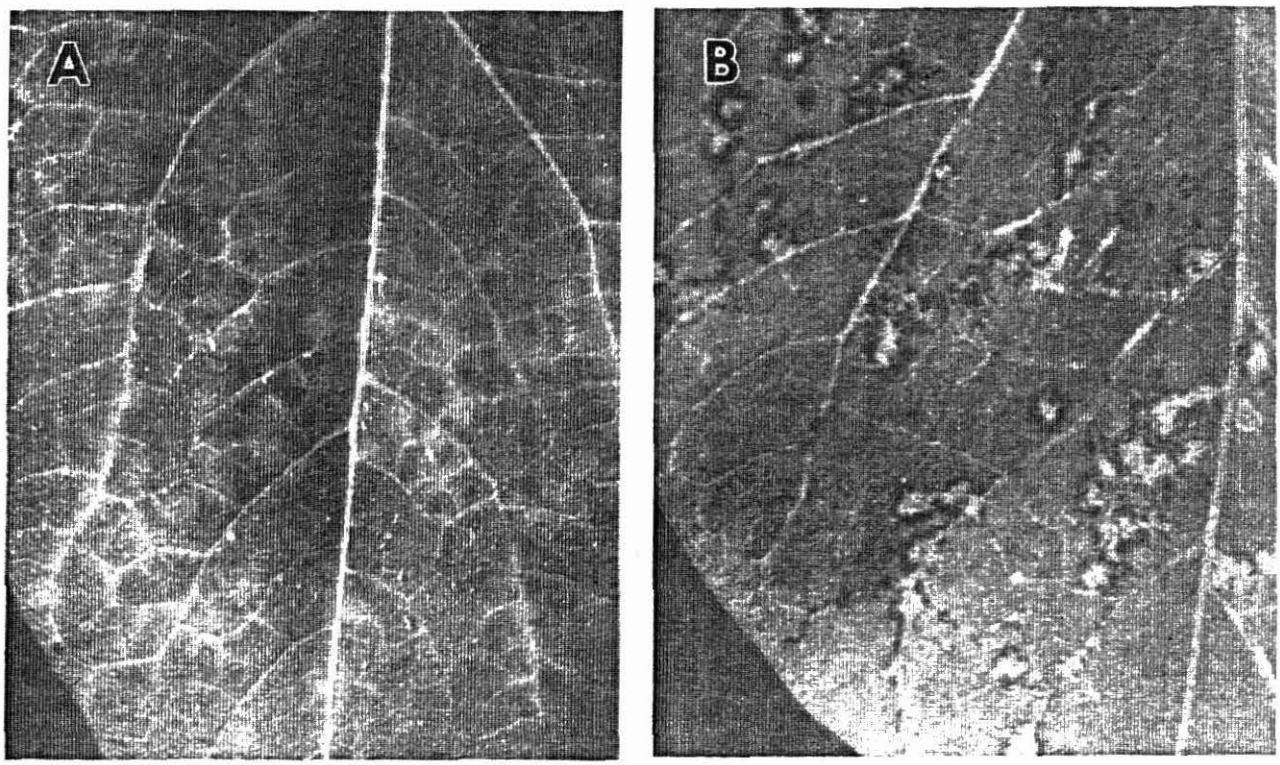

FIG. 1

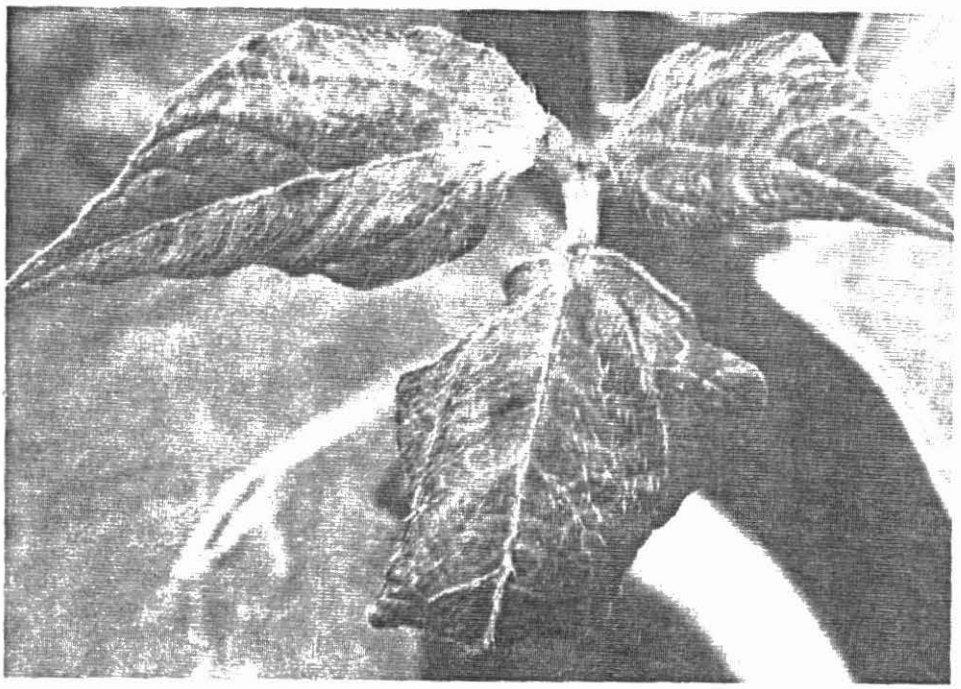

FIG. 2

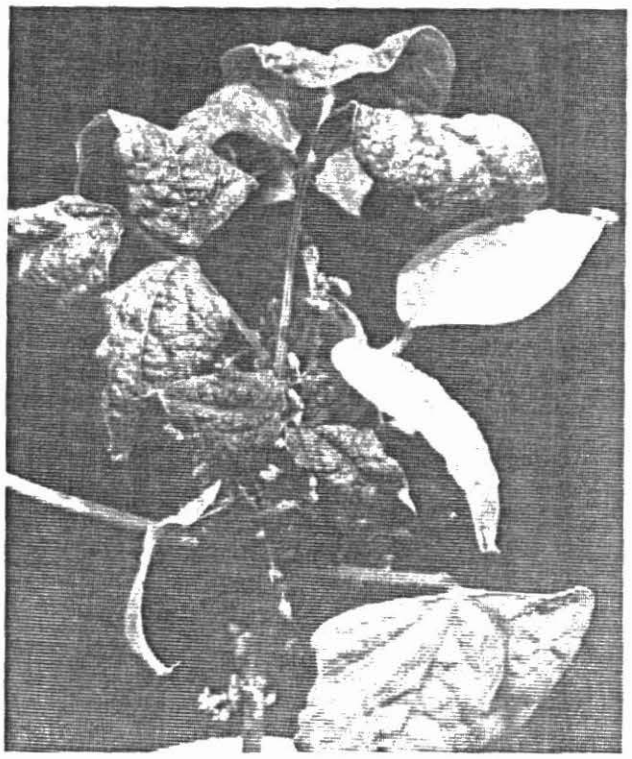

FIG. 3

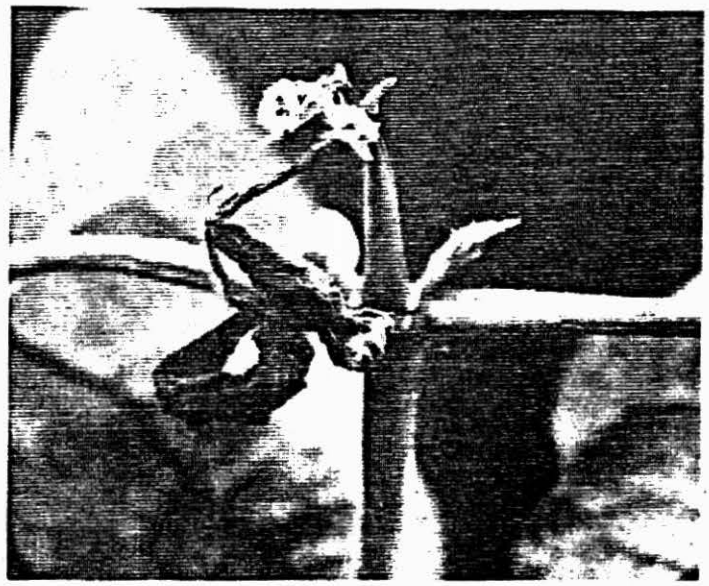

FIG. 4

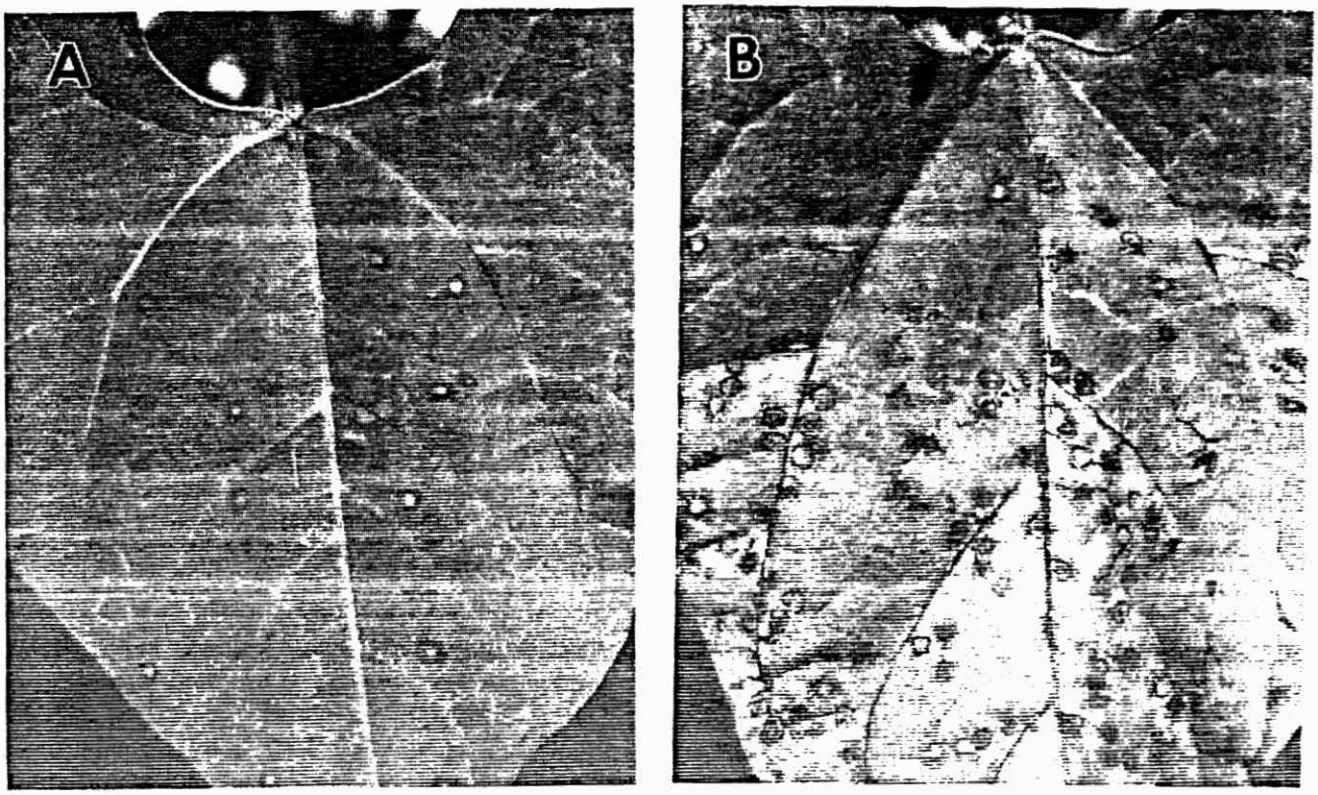

FIG. 5

Bean Golden Mosaic

First development of transgenic bean plants

Last year, we presented the 1990 research highlights of the Bean/Cowpea CRSP/USAID PSTC collaborative (University of Wisconsin Agracetus-CIAT-VRU) project entitled "Molecular Aproaches for Control of Bean Golden Mosaic Virus". The major breakthrough came about when, in collaboration with Agracetus, Inc., clones of three bean golden mosaic virus (BGMV) isolates were shown to be infectious following inoculation of bean radicles with cloned DNA, using electric discharge particle acceleration methods. This made possible the pursue of virus-derived schemes for the creation of transgenic plants with resistance to BGMV, based on the initial success obtained by Agracetus in the transformation of soybean.

This year, for the first time, unequivocal evidence for the development of transgenic bean (*P. vulgaris* 'Seafarer') plants was presented thanks to this collaborative project. The transformed beans express beta-glucuronidase (gus) activity, BAR herbicide resistance, and mRNA for the coat protein of BGMV.

In greenhouse tests, transgenic bean plants exhibit the same morphological traits of non-transformed 'Seafarer' beans. The BGMV coat protein gene (AR 1) is about 800 bp and it represents 15% of the total viral genome. We are awaiting permission from APHIS to move transgenic bean seed to Puerto Rico and CIAT, Cali, Colombia to screen these transgenic plants for their reaction to BGMV inoculated by whiteflies and mechanical means.

In the meantime, a second virus-derived resistance scheme, the dominant lethal scheme, involving mutational changes in the putative viral replicase gene, is being pursued as an alternative to the coat-protein-mediated resistance approach described above. The reason for pursuing this objective before the coat-protein-mediated resistance approach is fully tested, is the close serological relationship that exists between whitefly-transmitted geminiviruses, which may diminish the expression and cross-protective effect of the coat protein gene.

The importance of this report, however, is the demonstration that *Phaseolus vulgaris* can be transformed by 'ballistic' methods in the absence of a breakthrough in the *in vitro* regeneration of bean plants.

CASSAVA VIROLOGY

The VRU has the responsibility to investigate the diseases of cassava that are caused by viruses or virus-like agents. The development of methodology to assure the movement of virus-free cassava germplasm is a research priority. This is especially important because there are diseases of cassava of unknown etiology.

The control of viral diseases requires either the identification of resistant germplasm or the implementation of cultural practices that mitigate disease losses. Most viral diseases are controllable with the current technology, and continued development of rapid diagnostic technics together with the deployment of resistant germplasm should further reduce the losses caused by viruses.

Frogskin and Caribbean Mosaic Diseases

Frogskin disease (FSD) is a virus-like disease of unknown etiology that was first reported in 1971 from southern Colombia. There are no leaf symptoms produced in most cassava clones affected by FSD, but there can be very severe root symptoms. The malady was named frogskin disease because the root periderm and corky layers enlarge to form characteristic raised lip-shaped fissures on the roots. The roots often show a zone of constriction where these fissures are prominent. In severely affected plants, the roots do not fill with starch, and there are yield losses of more than 90% in areas where the disease is endemic. A few cassava clones develop mosaic symptoms on the leaves and are stunted when they are affected by FSD.

Caribbean mosaic disease (CMD) is a virus-like disease of unknown etiology that was reported in 1981 from the northern coastal regions of Colombia. There are foliar mosaic symptoms and significant yield loss in susceptible cassava varieties affected with CMD. Yield losses caused by CMD in susceptible cassava clones can be severe, but other clones are tolerant of this disease and produce normal yields.

These two diseases have been reported as different, because the root symptoms associated with FSD are either absent or mild in plants affected with CMD. While the root symptoms are apparently different, these diseases share many similarities. Both disease agents are transmitted by grafting and the cassava variety Secundina can be used in indexing programs for the detection of both CMD and FSD. The mosaic symptoms on the leaves of Secundina are expressed most prominently when the plants are kept in an area where the maximum temperature is kept below 30 C. Constant temperatures (above 28 C) suppress the leaf symptoms for both diseases. Neither FSD nor CMD can be mechanically inoculated and the only known host for both diseases is cassava. Insect vectors have been suspected for both these diseases, since they spread rapidly in the field.

Identification of a phytoreo virus-like agent associated with FSD and CMD

Isometric virus-like particles (70-80 nm in diameter) are found in thin sections of leaves, petioles, stems, and roots of CMD affected plants. Similar virus-like particles are also found in the same tissues of plants affected with FSD. These particles have been found in plants affected with all the FSD and CMD isolates that have been tested (Fig. 1A). Viroplasm-like bodies have also been found in FSD and CMD affected plants. These viroplasm-like bodies are often associated with the chloroplasts of the cells (Fig. 1B).

Double-stranded RNAs were purified from cassava plants infected with either CMD or FSD and run on both agarose and polyacrylamide gels. On agarose gels, there appear to be three or four bands, but on polyacrylamide gels there are nine bands that are consistently present in plants affected with FSD or CMD (Fig. 2). The ds-RNA segments are estimated to be 4000, 3800, 3400, 2600, 1900, 1800, 1700, 1100, and 1000 bases in length (Fig. 3).

Radioisotopic labeled cDNA probes have been prepared from the isolated ds-RNA, and these probes were used in hybridization analyses to determine the relatedness of the various FSD and CMD isolates. From the limited studies to date, there appears to be a fairly high degree of similarity between the isolates. For example, the FSD isolate 14 hybridizes with the CMD isolate 80 (Fig. 2). This is further evidence that ds-RNAs associated with FSD and CMD are either identical or closely related.

The partial purification of the phytoreo virus-like particles has been attempted, but they are very labile. The particles have been banded on a cesium sulfate gradient, and then visualized with a TEM. Some particles are complete, but the majority of particles appear to have degraded into a 50 nm structure typical of the core virions of phytoreoviruses. To determine if the isolated particles contained the ds-RNAs associated with CMD and FSD, a dot blot assay was performed using a radioisotopically labeled cDNA probe prepared from the ds-RNA. The dot blots were positive indicating that the fraction containing the virus-like particles also contained the ds-RNA. This is evidence that these phytoreovirus-like particles contain a ds-RNA genome.

The virus-like particles and the ds-RNA bands that are present in cassava affected with FSD or CMD, are similar to those reported for in phytoreoviruses. Neither FSD nor CMD has been mechanically transmitted despite many attempts, and this is consistent with the causal agent being a phytoreovirus since these viruses are not mechanically transmitted.

Vector transmission experiments

Whiteflies have long been suspected as the vector of FSD. For most of the transmission experiments to date, we have used the indicator clone Secundina. The mosaic symptoms in the leaves were used as the marker to determine that the transmission was successful. Table 1 is a list of the FSD and CMD isolates for which transmission by *Bemisia tuberculata* has been shown. The acquisition period of one day gives the highest rates of transmission.

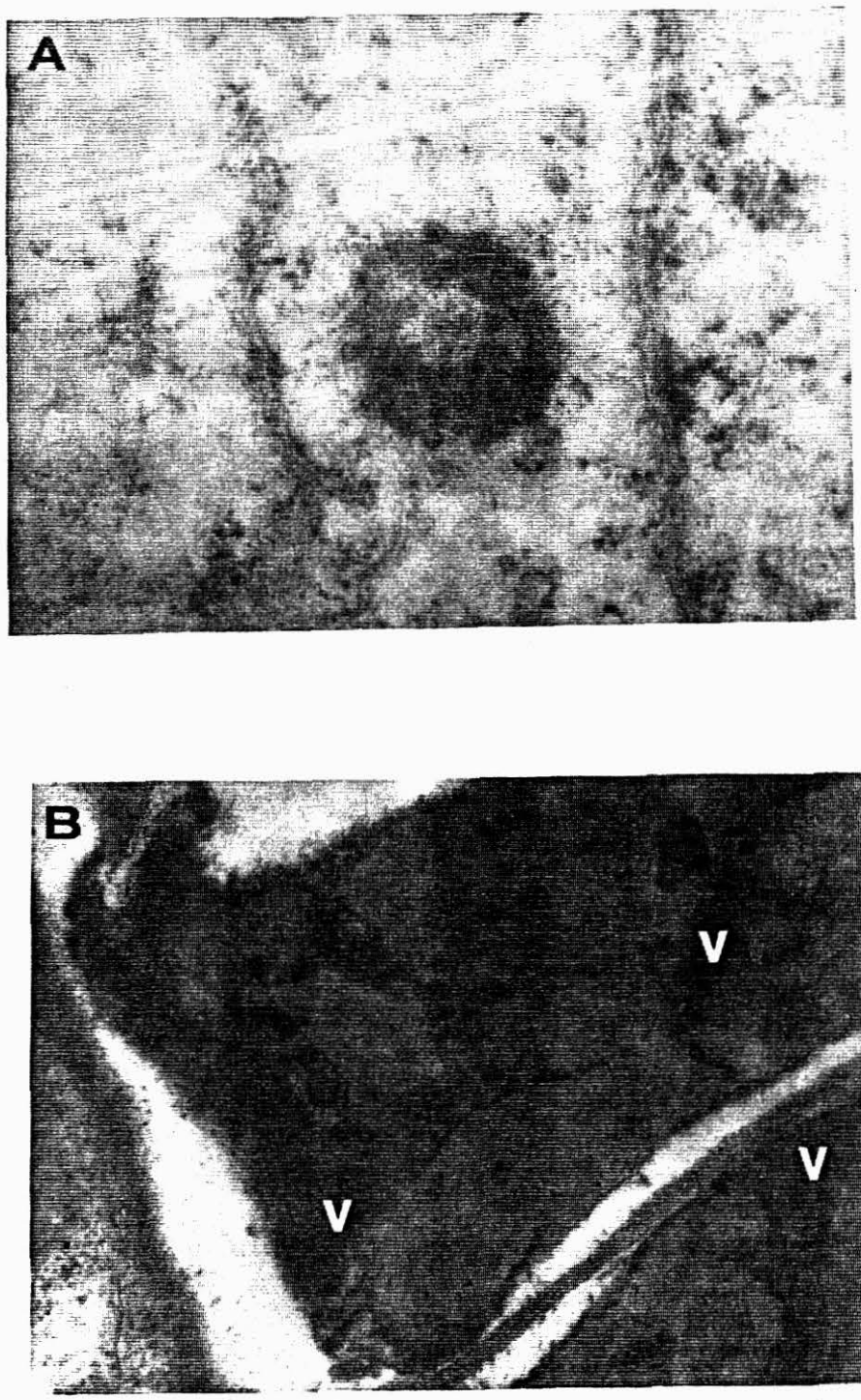

Figure 1. A: A group of virus-like particles found in a thin section of a leaf of the cassava clone secundina affect with the mosaic symptoms associated with FSD.

B: Viroplasm-like bodies found in a similar section as the virus-like particles, V indicates the viroplasm-like bodies.

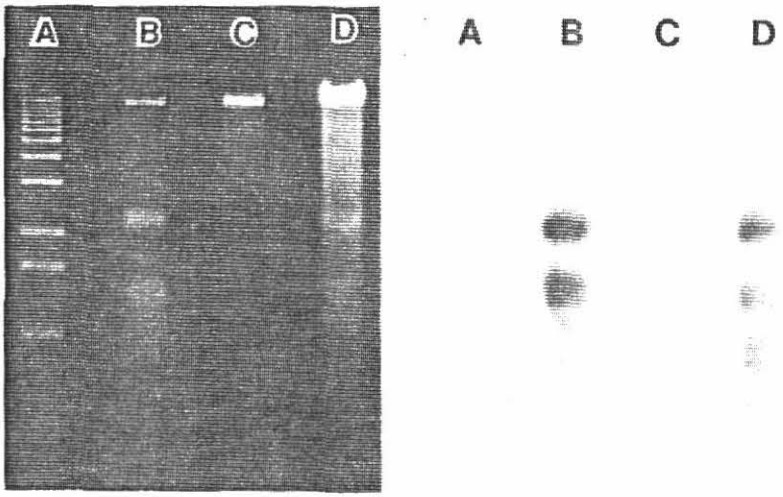

Figure 2. 1. An agarose gel showing the dsRNA segments extracted from plants infected with Frogskin (FSD) and Caribbean mosaic (CMD). 2. blot hybridization using a first-strand cDNA probe prepared from FSD-dsRNA (Isolate 14). A: dsDNA markers; B: FSD isolate 14; C: FSD isolate 24; D: CMD isolate 80.

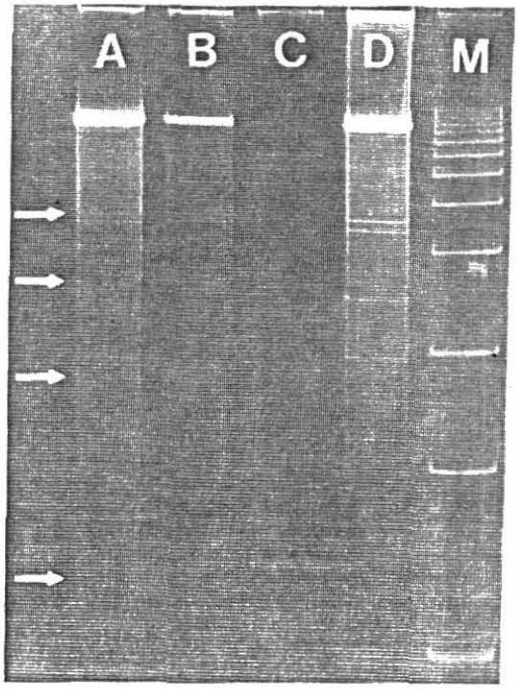

Figure 3. A polyacrylamide gel showing the dsRNA segments isolated from plants *infected* with FSD or CMD. A: FSD isolate 24, B: CMD isolate 5, C: FSD isolate Tolima, D: FSD isolate 29, M: dsDNA markers.

There is a latent period during which the virus is not transmitted by the vector. The minimum period of inoculation that has resulted in transmission is three days.

Additional experiments must be done to more accurately characterize the transmission of the FSD agent, but the preliminary results indicate that the agent is transmitted persistently, presumably in a circulative manner. There is no evidence at this time, that the agent multiplies within the vector.

Table 1. List of isolates of FSD and CMD 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	1986
FSD	29	Secundina	Secundina	1990
CMD	80	Secundina	Secundina	1990
CMD	86	Secundina	Secundina	1990
FSD	24	M Col 72	Secundina	1991
FSD	Tolima	Secundina	Secundina	1991

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

Plants that developed mosaic symptoms were transplanted in soil inside a screenhouse. The plants were grown for six to eight months and then the roots were harvested and inspected for symptoms of FSD. Plants infected with FSD isolate 29 and CMD isolates 80 and 86 all showed mild but distinct FSD symptoms on the roots (Fig. 4).

The plants that developed the mosaic symptoms in the transmission tests were analyzed for the presence of ds-RNA species. In most cases, both the mother plants used as the source of inocula, and the plants infected in the transmission experiments had similar ds-RNA patterns. The exception has been the FSD isolate 29, which loses some of the ds-RNA bands during the transmission experiments. Only the nine bands, that have been consistently associated with FSD and CMD, are present in the plants infected during transmission experiments with isolate 29 or the other isolates. This suggests that FSD isolate 29 may be co-existing with a second virus.

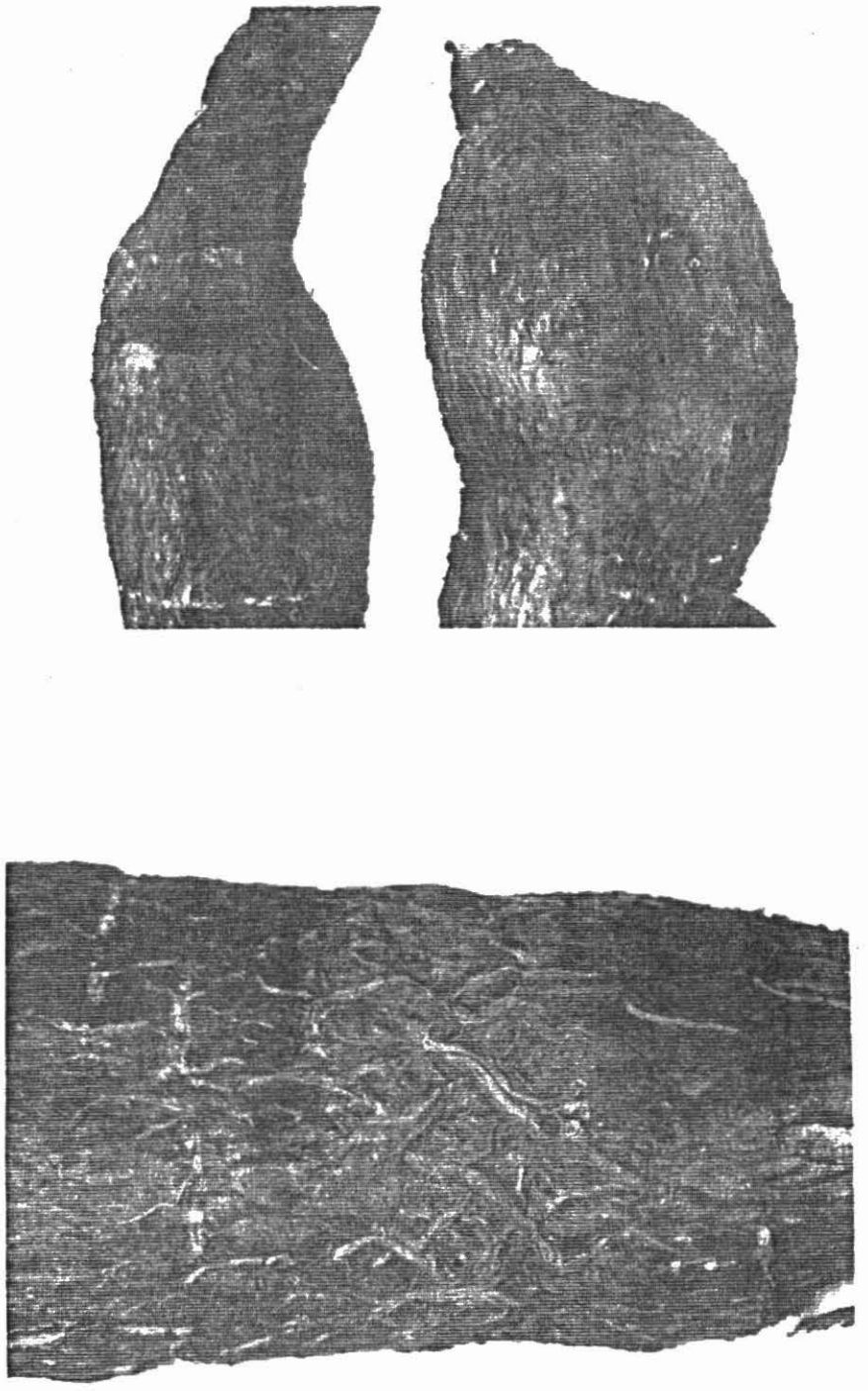

Figure 4. Roots showing mild symptoms typical of FSD. There roots were harvested from plants that were infected with the phytoreovirus-like agent using *Bemisia tuberculata* as the vector. The plants were grown in a screenhouse to prevent other sources of infection.

The virus-like particles of 80 nm in diameter, similar to those found in FSD and CMD infected plants, have been found in the newly infected plants from all isolates that have been tested. Also, virus-like particles have been found directly in *B. tuberculata* individuals fed on infected plants. No virus-like particles were found in *B. tuberculata* individuals fed on healthy plants.

Control strategies

The damage caused by FSD can be limited by using clean stakes. In heavily infested fields, the old crop should be removed for at least a month and then the field should be planted with clean stake material. The use of the indicator clone Secundina can facilitate the selection of clean stake material. Since the disease is not mechanically transmitted no special care is needed when handling stakes. The key for sustainable yields is the continued selection of clean stakes. There are no leaf symptoms in most cassava clones; therefore, the selection of the stake material must be made at the time of harvest. The roots must be inspected carefully for the symptoms of FSD. Many of the root symptoms are mild, but these must be recognized and stakes from those plants eliminated to mitigate the losses caused by FSD.

Quarantine implications and diagnostic tests

FSD is the most serious viral disease that is endemic in Colombia. While progress has been made on the identification of a phytoreovirus-like agent associated with the disease, there are still some unanswered questions concerning the etiology of FSD. Therefore extra care must be taken to assure that the germplasm exported from CIAT is free of FSD agents. Currently, two tests are being used to detect FSD. The first method, that has been used for many years, is to graft the test plant to the indicator clone Secundina. The second method is to extract ds-RNA from the plants and confirm their presence either in a polyacrylamide gel or with a dot blot assay and hybridization probe. If either test is positive the clone is assumed to be infected. Only clones that have no history of FSD will be considered for export.

Cassava Common Mosaic Virus

Cassava common mosaic virus (CCMV) is a member of the potexvirus group. The particle morphology is a semiflexuous rod with the approximately dimensions of 15 X 495 nm. The viral particles contain a single coat protein with a relative molecular weight (Mr) of 25,000, and a ss-RNA genome of approximately 6400 bases. Nuclear inclusions typical of the potexvirus group can be found in cassava and *Nicotiana benthamiana*. Cassava plants infected with the virus have mosaic symptoms on the leaves, and may suffer yield losses of more than 20%. CCMV occurs throughout Tropical America from Mexico to Paraguay and is considered in terms of total yield loss to be the most destructive viral disease in cassava in the Americas.

Molecular characterization

The sequencing of CCMV is nearly complete. The virus is 6400 bases in length, and it is most closely related to potato virus X (PVX). CCMV has a genomic organization typical of other members of the potexviruses whose genomic sequence is known. Most of the sequencing of CCMV was done at the VRU in CIAT, and this is perhaps the first plant virus to be sequenced in Latin America.

The cDNA cloning and sequencing of CCMV coat protein was an essential element for the Cassava Trans project, that is based in Dr. R. Beachy's laboratory in Washington University. The coat protein gene of CCMV was successfully introduced into *Nicotiana benthamiana*, and these plants show almost complete immunity to CCMV. Coat protein mediated cross protection will be one method to develop resistant germplasm to CCMV. The major technical limitation is the transformation of cassava.

Quarantine implications and control.

Antisera against CCMV are available, and the virus is readily detected by ELISA. All germplasm from the Americas is routinely screened for the presence of CCMV before exportation from CIAT.

There are no reports of a vector for CCMV, and the leaf symptoms caused by CCMV are very distinct. The disease caused by CCMV is controlled by selecting plants without leaf symptoms and using these stakes for propagation. If the area is heavily infested the cutting tools should be disinfected between plants.

Cassava Vein Mosaic Virus

Cassava vein mosaic virus (CVMV) is a member of the caulimovirus group, that has isometric virions approximately 50 nm in diameter, and a ds-DNA genome of approximately 8000 bases. The only known host for CVMV is cassava, and the vector is unknown. Symptoms (Fig.) include a chlorosis of the veins that can either appear as a chevron pattern or coalesce to form a ringspot pattern. Some leaves also show a mosaic pattern over the entire leaf. There is often leaf distortion and sometimes the young leaves show epinasty. The symptoms are variable but are expressed better at high temperatures. The virus is reported in many States in Brazil. CVMV is most prevalent in the northeastern States, especially in the hot semiarid zones where it is not unusual to find more than 50% of the plants infected with CVMV. CVMV is not known to occur outside Brazil, and there is very little information on the yield losses caused by this virus.

Quarantine implications and diagnostic tests

The initial characterization of CVMV was done by Drs. E. Kitajima and A. Costa (U. of Brazil). An antiserum to the virus was made by Dr. M. Lin around 1980, but it is not known

if it is still viable. Infected plants can be recognized by the distinctive symptoms and can be confirmed by TEM since the particles are large isometric particles of 50-55 nm. Since symptoms are not always present, and the virus can infect *in vitro* cultured plantlets, a rapid and reliable diagnostic test is needed. DNA clones to CVMV have been prepared by Dr. R. Shepherd of the U. of Kentucky, and he has provided one of these to the VRU. A polymerase chain reaction (PCR) test for the rapid detection of CVMV should soon be available at CIAT. There is a need to prepare a new antiserum or transfer to a PCR detection method to EMBRAPA/CNPMF to facilitate screening of cassava germplasm for CVMV in Brazil.

African and Indian Cassava Mosaic Viruses

African cassava mosaic geminivirus (ACMV) is found throughout tropical Africa, and a similar virus, Indian cassava mosaic geminivirus (ICMV), is found throughout India and Sri Lanka. These viruses cause the most destructive viral diseases of cassava. The symptoms of the disease include mosaic, yellowing, distorted leaves and stunted growth. The vector of these viruses is the whitefly, *Bemisia tabaci*. There are varieties that are resistant to these viruses, and in these plants symptom expression is erratic, and it is presumed that distribution of the virus is restricted in these varieties.

Quarantine implications

Recently ACMV was introduced into the island Praia of Cape Verde. The origin of the germplasm in Cape Verde was Brazil, and the losses were nearly 100% since the germplasm was not resistant to the virus. Similar losses can be expected if ACMV and an active vector are introduced into tropical America. These geminiviruses have not been reported in Asia outside India and Sri Lanka; therefore, much of Asia faces a similar threat. *In vitro* cultures of cassava received from IITA (Nigeria) at CIAT have all been tested for the presence of these geminiviruses at the Scottish Crop Research Institute (SCRI) by Dr. Harrison. Monoclonal antisera to these viruses have been produced at the SCRI. There are antisera and cDNA probes to ACMV available at CIAT that can be used for the detection of these geminiviruses.

While *B. tabaci* is common throughout the Americas, normally it does not colonize cassava. Recently a biotype of *B. tabaci* has become dominant in Florida, Puerto Rico, and the Dominican Republic, and this biotype is able to colonize cassava. Since this biotype is a possible vector of ACMV, there is the need to monitor the distribution of this new biotype of *B. tabaci*.

Control strategies

The use of resistant clones of cassava is the most effective method of mitigating the losses caused by these geminiviruses. There are resistant clones in Africa and in India. The clones, that have been received at CIAT from IITA with resistance to ACMV, have severe

agronomic and disease problems when grown in Colombia. These have been used in a breeding program and are one source of resistance. Another means of identifying resistance germplasm is through the joint CIAT-IITA program described in section 23. A third possible source of resistance could be obtained through the Cassava Trans project which is working on coat mediated cross protection for both CCMV and ACMV. With the spread of the new biotype of *B. tabaci*, the threat of a introduction into tropical America of ACMV has increased. Therefore additional emphasis will be placed on identifying ACMV resistant germplasm that is adapted to tropical America.

Latent Viruses of Cassava in Latin America

There are several latent viruses that are known to infect cassava in Latin America. These latent viruses were discovered while working on other virus or virus-like pathogens of cassava. None of these viruses are known to cause disease in cassava or to have any effect on the yield of cassava. There is only limited information on the distribution of these viruses. CIAT is committed to sending germplasm that is free of pathogens. Therefore, the primary concerns of the investigations of these viruses are methods of detection that allow certification that germplasm does not contain these latent viruses.

Cassava X and cassava Colombian symptomless viruses

Cassava X (CsXV) and cassava Colombian symptomless (CCSpV) viruses are both potexviruses that were discovered during attempts to identify the causal agent of FSD or CMD. The discovery of these viruses occurred because they are mechanically transmitted to diagnostic hosts. Subsequent tests have shown that these viruses are not present in most of the plants affected with FSD or CMD. Neither virus is known to cause symptoms or disease in cassava, either alone or in combination with other viruses.

Quarantine implications and diagnostic tests. There are antisera to both these viruses and all in vitro germplasm that originates from Colombia is checked for the presence of these viruses by ELISA before being shipped internationally.

Cassava American latent virus

Cassava American latent virus (CALV) is a member of the nepovirus group and was discovered by Dr. B. Walters, who isolated it from cassava that was infected with CCMV. The virus was isolated from samples that were from Guyana and Brazil (Manaus). While the vector of the virus is unknown, it is probably a nematode. It is also possible that this virus is seed transmitted. There are no known cassava plants in the field that are infected with this virus, which hinders our ability to test if the virus is seed and/or nematode transmitted.

Quarantine implications and diagnostic tests. Dr. Walter has made an antiserum for CALV available. This antiserum has been used to test cassava in Colombia and Brazil. This has been useful in certifying seedlots as free of CALV. Thousands of seeds and hundreds of plants grown at CIAT headquarters, were assayed for CALV, and all the tests were negative. CALV does not appear to be present in the Cauca valley; therefore, the further testing of materials grown at CIAT is not warranted. Approximately 200 plants (about 18%) of the germplasm collection at EMBRAPA/CNPMF at Cruz das Almas were tested for CALV and all plants tested negative. CALV does not appear to be present at the CNPMF farm in Cruz das Almas, but some additional testing may be done for the IFAD project. The distribution of this virus is not known, and the only reported source of the virus is from the humid tropical regions of the Amazon. The virus has not been found at the main sites were true cassava seeds are produced for export. Therefore the risk that this virus is contaminating these cassava seedlots is extremely low.

Conclusions and Future Research Objectives

There are four main diseases caused by virus or virus-like agents. These are FSD, CVMV, CCMV, and ACMV (ICMV). While the losses that are caused by these diseases can be mitigated through cultural practices, there is the need to continue efforts to identify and distribute cassava clones that are tolerant or resistant to these diseases.

The research on FSD is continuing and the investigations are centered on confirming the association of the phytoreovirus-like agents and the entire complex of disease symptoms. Hybridization assays have been developed to detect the ds-RNAs associated with the disease. The development of this rapid diagnostic test allows for the more effective screening of cassava germplasm. Also a major limitation to screening germplasm for resistance to FSD has been the lack of rapid diagnostic method for the disease; therefore, using this assay more emphasis will be placed on looking for resistance.

Additional research is also needed in the Northeast of Brazil to determine the losses caused by CVMV. The vector of this virus needs to be determined to understand the epidemiology of this disease. The control of this virus should be a part of the integrated pest management strategies developed for this area of Brazil.

There are excellent detection and control measures for CCMV. Some additional research is needed to identify resistant germplasm, but this research needs to be done in areas where the disease is causing disease losses. Screening CCMV-resistant material from the Cassava-Trans project can be done at CIAT.

The greatest need for ACMV is the identification of additional resistant germplasm. As resistant germplasm is identified in the CIAT/IITA collaborative project, it needs to be transferred back to CIAT so that there are adequate sources of ACMV resistance in germplasm that is adapted to tropical America. A similar project is needed for those areas of tropical Asia in which the virus is not present.

Beside the major diseases there are seven other known viruses that infect cassava. Although most of these viruses do not appear to cause disease, they are of quarantine significance because of their limited distribution. Diagnostic methods have been developed to both the viruses that cause diseases and to the symptomless viruses. These diagnostic methods help to assure the safe movement of cassava germplasm, and one research objective will be the development of even more sensitive detection methods.

RICE VIROLOGY

The only known virus in rice in Latin America is rice hoja blanca virus (RHBV), and the major emphasis of the research during the last year has been the molecular characterization of RHBV. The molecular characterization is a collaborative project with the laboratory of Anna-Lisa Haenni at the Institut Jacques Monod. The molecular characterization of RHBV includes the identification of viral encoded proteins, the assignment of functional roles to these proteins, and the determination of the genomic organization of RHBV. These goals are being achieved through *in vitro* translation studies, and the cloning and sequencing of the genome of RHBV. RHBV is a member of the tenuiviruses RHBV. Recently, the partial molecular characterization of maize stripe virus (MStV) and rice stripe virus (RStV) have been reported, and RHBV appears similar with these other tenuiviruses.

RNA and *in vitro* translation analyses.

The RHBV-RNA was purified from RHBV virions and seven species have been identified (Fig. 1). Four of these species are single-stranded (ss-) and three are double-stranded (ds-) RNA. There is no evidence of a fifth species of ss-RNA as has been reported for MStV and RHBV appears similar to RStV with four species of ss-RNA. The length of RHBV-RNA 1-4 are estimated to be 8,800, 3,800, 2,200, and 1,800 using a ss-RNA ladder in glyoxal or formaldehyde gels. The size estimates using the ds-RNA were somewhat larger at 4,200, 2,700, and 2,300 bases for RNA 2-4, respectively. The exact sizes will be determined from the complete sequence.

Total RHBV-RNA was purified and translated using a rabbit reticulocyte *in vitro* translation system. Two major protein products of M_r 17,000 and 20,000 were translated from total RNA. The 17K product was identified by immunoprecipitation using a antiserum specific to inclusion body protein. This protein is similar in size to the native inclusion body protein.

The ss-RNA species of 2, 3, and 4 were purified by extraction from agarose gels and translated as individual species. The 17K protein was translated by the RNA-4, and the protein of 20K is translated by the RNA-3. There were no translation products from the experiments using RNA-2, and the RNA-2 appears to be minus RNA. The quantity of RNA-2 was significantly less than the quantity isolated for RNA-3 and 4, and we cannot rule out the possibility that the lack of products were because of insufficient RNA. Not enough of the RNA 1 could be gel purified to attempt an in vitro translation experiment, but all translation products from the complete translation are accounted for by the translation of RNA-3 and 4.

Complementary DNA cloning and sequencing.

Complementary DNA clones of RHBV were prepared using a modification of the methodology of Gubler and Hoffman using random primers to begin the reaction.

Complementary DNA to RNA-2, 3, and 4 of RHBV have been identified from the library of clones.

Approximately 500 bases of the RNA-2 species has been sequenced, and it contains one continuous open reading frame. The RNA-2 of RHBV is approximate 4,000 bases in length, therefore additional cloning and sequence analysis must be done before we will know the genomic organization of the RNA-2.

Within the library of cDNA clones there are many clones complementary to RNA-3. Approximately 1400 bases of RNA-3 has been sequenced and there is an open reading frame that encodes for a non-structural protein of approximately 20K (Fig. 2). The 20K product is at the 5' terminus of the ss-RNA species and is believed to be the product encoded using the *in vitro* translation system. This protein shares about 55% direct amino acid homology with the corresponding proteins encoded by RStV and MStV. The is also a large intergenic region that contains a high A-T content. This is also consistent with the intergenic regions of RStV and MStV. The cloning and sequencing of the RNA-3 will soon be completed, but based on the current sequence data and the *in vitro* translation results, the RNA-3 of RHBV appears to be ambisense and has a genomic organization which is comparable to RStV and MStV. The coat protein gene is the other expected protein encoded by the RNA-3.

A group of cDNA clones that are homologous with the RNA-4 of RHBV have been identified. Approximately 1000 bases of RHBV-4 has been sequenced and most of the putative amino acid sequence for the 17K non-structural protein that forms the inclusion bodies has been determined. This protein shares a high degree of homology with the inclusion body proteins of MStV and RStV. There is a large non-translated intergenic region, and the RHBV-RNA4 appears to be ambisense.

Approximately 3,000 bases of the RHBV genome has been sequenced during the last year. Analysis of the *in vitro* studies and the sequence indicates that RHBV has a genomic organization that is similar to other members of the tenuiviruses. The similarities will be exploited to rapidly finish the complete cloning and sequencing of the RNA-2, 3, and 4.

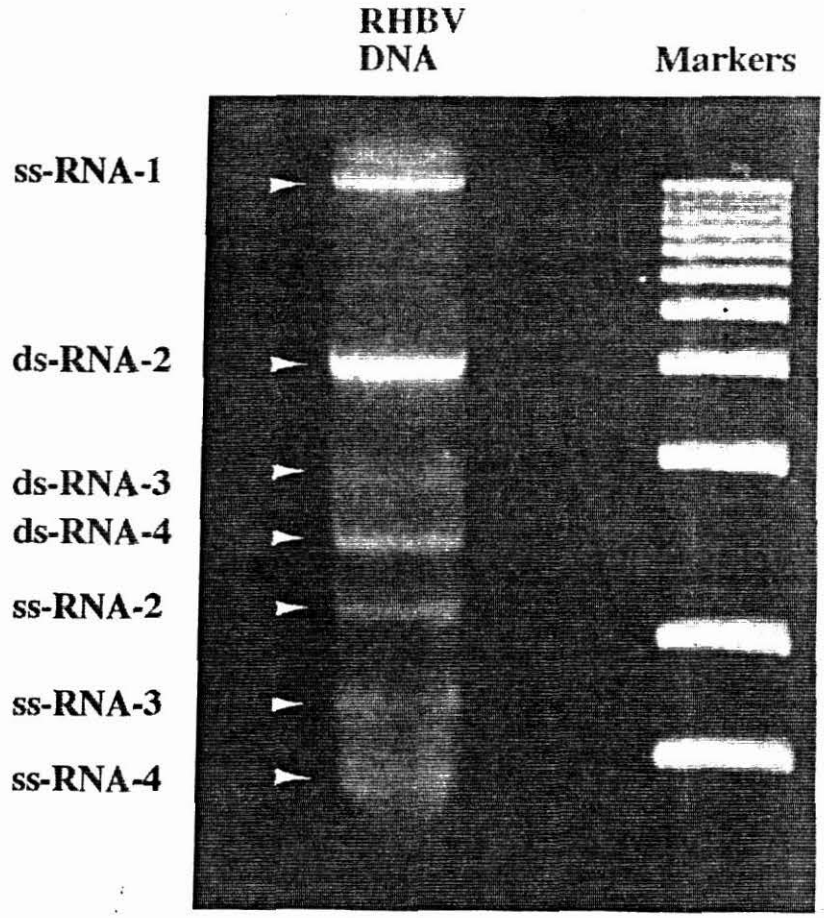

Figure 1. Both single and double stranded RNAs are associated with purified virions of RHBV. The approximate sizes of the ss-RNA 1-4 are 8,800, 3,800, 2,200, and 1,800 bases, respectively.

TROPICAL PASTURES VIROLOGY

The initial objective of the Virology Research Unit (VRU) in the area of tropical pastures virology, has been the detection and characterization of the viruses that affect yield, quality, propagation, and/or free germplasm exchange of tropical pasture species investigated by the Tropical Pastures Program (TPP). This task, undertaken soon after creation of the VRU in 1988, has demanded a great deal of original research considering the almost complete lack of previous information on viral pathogens that affect forage species in tropical America.

TROPICAL FORAGE LEGUME VIROLOGY

For the past three years, emphasis has been given to those forage species under evaluation by the TPP that exhibited virus-like problems. These species were in the legume genera *Arachis, Centrosema,* and *Stylosanthes*. No virus-like problems were apparent at that time in forage grasses.

The first genus to be investigated was *Centrosema*, particularly *C. macrocarpum, C. brasilianum,* and *C. pubescens*. The first virus to be detected, isolated and characterized in 1989 was a strain of soybean mosaic virus (SMV-CE). A rapid serological detection technique was developed for detection of this strain. In 1990, a field survey was conducted on the distribution of SMV-CE at CIAT-Palmira and CIAT-Santander de Quilichao. Results of this survey revealed (Ann. Rept. 1990) the presence of other viruses of the same group affecting *Centrosema* spp. To date, five different potyvirus isolates have been identified in *Centrosema* spp. and only one of these isolates is related to SMV-CE. These results suggest that *Centrosema* spp. is highly susceptible to different aphid-borne.

A study was conducted during 1991 on the differential reaction of four species (*C. acutifolium, C. brasilianum, C. macrocarpum,* and *C. pubescens*) to six selected potyvirus isolates. With the exception of *C. macrocarpum*, these potyviruses have a broad spectrum of pathogenicity in the *Centrosema* species tested (Table 1) when inoculated mechanically.

Table 1. Pathogenic spectrum of selected potyvirus isolates in four *Centrosema* species inoculated by mechanical means.

Centrosema spp.	Potyvirus isolates					
	CP1	CP2	CP3	SQ15	SQ25	CeMV
C. acutifolium	+	+	+	+	+	+
C. brasilianum	+	+	+	+	+	+
C. macrocarpum	+	-	-	-	+	+
C. pubescens	+	-	+	+	+	+

(+) = systemic infection; (-) no infection as determined by electron microscopy.

Finally, samples were taken from symptomatic *Centrosema* spp. plants at CIAT and Santander de Quilichao, to assess the relative incidence of the *Centrosema* potyviruses detected to date. At CIAT, 66.6% of the samples (mostly *C. pubescens*, followed by *C. macrocarpum* and *C. plumieri*) assayed by electron microscopy contained a potyvirus. In Santander de Quilichao, 100% of the samples of *C. acutifolium, C. brasilianum,* and *C. macrocarpum* contained potyviruses, although again, these percentages only represent the incidence of potyviruses in suspected or clearly diseased plants. A serological assay of these samples revealed that there was more than one potyvirus consistently associated with most plants tested. It is thus clear that various legume potyviruses can affect *Centrosema* spp.

Arachis pintoi. A viral disease of *A. pintoi* was first detected in 1988. The characteristic symptoms of this disease consist of ring-shaped (eyespot) lesions on leaves. A potyvirus was isolated, and was later (1989) characterized as a strain of peanut mottle virus (PeMV). A complete description of this virus appeared in the TPP and VRU annual review of 1990.

The *A. pintoi* virus was first detected in the municipality of Ginebra, Valle, in farmers' fields. A survey conducted this year in the municipalities of Florida (Valle) and Santander de Quilichao (Cauca), showed that the virus is also present in these areas at a very high incidence (50-100%). This survey was also extended to cover the municipality of Chinchina (Caldas), where the *A. pintoi* strain of PeMV was also found, again, at a high incidence. Finally, the *A. pintoi* collection maintained at the Agricultural Research Center for the Cerrados of Brazil (CPAC), located near Brasilia, was also surveyed. Here, the characteristic ringspot symptoms were also observed in *A. pintoi*, suggesting that the virus is widely distributed.

The distribution of the *A. pintoi* potyvirus seems to be facilitated by the vegetative propagation of this forage legume. Also, PeMV is seed-transmitted in its original host, *Arachis hypogaea* (peanut), which suggests that this virus could also be seed-borne in *A. pintoi* sexual seed. We have serologically assayed thousands of *A. pintoi* seed for the presence of this virus, with negative results. However, we can not rule out the possibility that this virus may be transmitted in a very low proportion of the sexual seed of *A. pintoi* (less than 1%), just like PeMV in peanut.

***Stylosanthes* spp.** A virus survey conducted in 1989, in the localities of CIAT-Santander de Quilichao and CIAT-Palmira, revealed the presence of yet another potyvirus, distinct from those of *Centrosema* and *A. pintoi* but serologically related to the *A. pintoi* potyvirus. This virus infects some accessions of *S. capitata* and *S. macrocephala* but, so far, it has not systemically infected various accessions of *S. guianensis* inoculated by mechanical means. In general terms, this potyvirus does not seem to drastically affect *Stylosanthes* and its distribution appears limited. Should it become important, however, there are various resistant accessions or sources of resistance.

Control of potyviruses affecting tropical forage legumes

Potyviruses are typically transmitted by aphids in a non-persistent manner. That is, the virus can be acquired from an infected virus source and transmitted to a healthy plant within seconds. Under these circumstances, chemical control of the aphid vector is obviously not feasible. Fortunately, aphids do not usually carry non-persistent viruses for very long distances, relative to other insect vectors, such as leaf-hoppers, and persistent viruses. This means that if aphids cannot acquire these potyviruses in the vicinity, there will be little chance for introduction or secondary spread. Hence, the importance of using virus-free germplasm when exotic forage species are introduced in new regions. Thus, CIAT can make impact by providing virus-free reproductive plant material for vegetative or sexual propagation of tropical forage legumes. As an example, of the various *A. pintoi* fields surveyed, only those in the municipality of Miranda, Cauca, planted by 'Semillas Pance' in cooperation with CIAT, were found free of viruses. The origin of this *A. pintoi* was vegetative planting material obtained from virus-free lots at CIAT, and seedlings produced in screenhouses and certified as virus-free by the Virology Research Unit.

In the past two years, the VRU has produced certified virus-free seed of *Centrosema brasilianum, C. acutifolium, C. pubescens,* and *C. macrocarpum*. Additionally, we also have also certified *Arachis pintoi* and *Stylosanthes* spp for vegetative propagation.

Furthermore, the VRU has produced specific and general virus-detection tests for the various potyviruses identified so far, in order to produce virus-free germplasm and satisfy quarantine requirements.

TROPICAL FORAGE GRASS VIROLOGY

This year, virus-like symptoms were observed, in a plant of *Brachiaria brizantha* (CIAT 16328) maintained under open screenhouse conditions at CIAT. These symptoms consisted of chlorotic and yellow streaks with the appearance of elongated rings later on (Fig. 1). An electron microscopy assay of this plant, revealed the presence of yet another potyvirus (Fig. 2).

A survey of this virus was conducted at CIAT-Palmira, and Carimagua, in the eastern plains of Colombia.

In laboratory tests the potyvirus isolated was transmitted by mechanical means, and by aphids (*Myzus persicae*) in a non-persistent manner. Also, sexual seed was collected from infected *Brachiaria* spp. plants, to investigate whether this potyvirus is seed-borne or not.

A host range study was also initiated with *Brachiaria brizantha, B. decumberns*, and *B. ruziziensis*, and selected maize, sorghum, rice, and sugarcane cultivars. The results obtained indicated that only *Brachiaria* species were susceptible. Therefore, this potyvirus does not seem to be a strain of either maize dwarf mosaic virus or sugarcane mosaic virus, which are the only potyviruses previously reported to infect *Brachiaria* spp. However, this is still preliminary work which requires a more in-depth study considering the scant knowledge there is on potyviruses of grasses in tropical America.

A similar potyvirus was recently detected in a *Brachiaria* sp. plant sent through tissue culture from Tanzania and kept under phytosanitary containment at CIAT. However, a serological test demonstrated that this is not the same potyvirus originally detected at CIAT, although both potyviruses are antigenically related. It may well be that we are dealing with a potyvirus of wide distribution in the tropics which had not been previously detected due to the lack of virology research with these grass species, and the lack of distinguishable virus symptoms in most of the *Brachiaria* accessions studied so far.

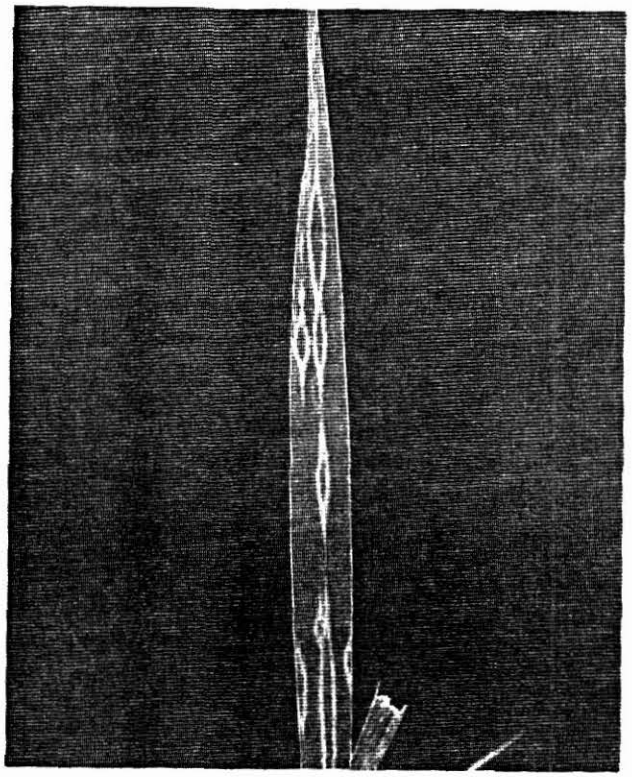

Figure 1. Streaking and elongated ring symptoms in *Brachiaria brizantha* infected by a Potyvirus.

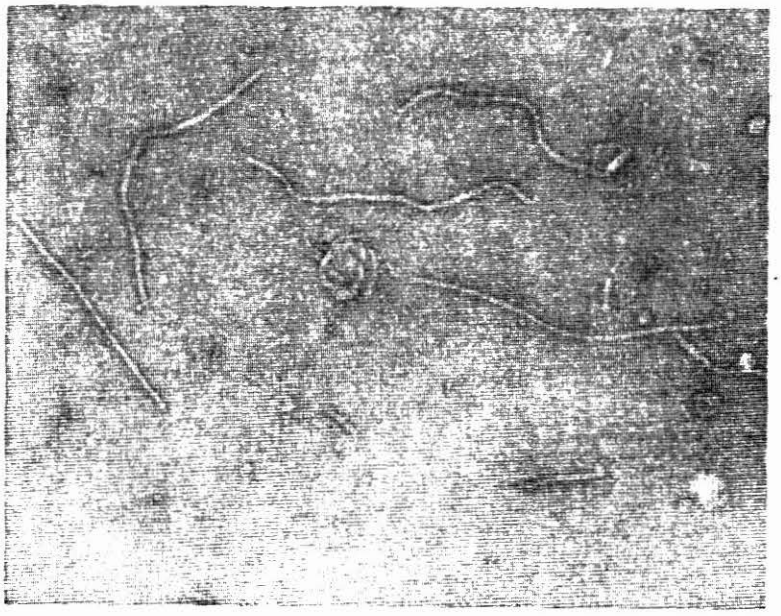

Figure 2. Potyvirus particles observed by electron microscopy of leaf extracts of symptomatic *Brachiaria brizantha*.

1991 PUBLICATIONS

In Refereed Journals

Morales, F. J., and Singh, S.P. 1991. Genetics of resistance to bean golden mosaic virus in *Phaseolus vulgaris*. Euphytica 52:113-117.

Gilbertson, R. L., Hidayat, S. H., Martinez R. T., Leong, S. A., Faria, J. C., Morales, F. J., and Maxwell, D. P. 1991. Differentiation of bean-infecting geminivirus by nucleic acid hybridization probes and aspects of bean golden mosaic in Brazil. Plant Dis. 75:336-342.

Morales, F. J., Castaño, M., Velasco, A. C., Arroyave, J., and Zettler F. W. 1991. Natural infection of tropical forage legume species of *Arachis* and *Stylosanthes* by potyviruses related to peanut mottle virus. Plant Dis. 75:1090-1093.

Gilbertson, R. L., Faria, J. C., Hanson, S. F., Morales, F. J., Ahlquist, P., Maxwell, D. P., and Rusell, D. R. 1991. Cloning of the complete DNA genomes of four bean-infecting geminiviruses and determining their infectivity by electric discharge particle acceleration. Phytopathology 81:980-985.

Submitted in 1990 to Refereed Journals

Morales, F. J., and Castaño, M. Increased disease severity induced by some comoviruses in bean genotypes possessing monogenic dominant resistance to bean common mosaic potyvirus. Plant Dis.

CURRENT COOPERATIVE RESEARCH PROJECTS WITH ADVANCED 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, (U. of W.)
R. 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. Ramirez (IJM),
L. Calvert (CIAT).

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